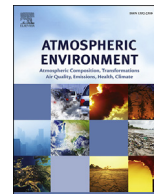




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## Morphological characteristics of bioaerosols from contrasting locations in southern tropical India – A case study



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### H I G H L I G H T S

- Investigating morphological characteristics of bioaerosols using Scanning Electron Microscope over tropical India.
- Morphological characteristics of bioaerosols were distinct for contrasting environment in India.
- Surrounding environmental factors affected the characteristics of bioaerosols.

### A R T I C L E I N F O

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### A B S T R A C T

Bioaerosols, which are ubiquitous in the earth's atmosphere, are poorly characterized in terms of their physical and chemical properties. Improved knowledge of their physical and chemical properties is essential to have a better understanding of their dispersion and long-range transport in the atmosphere and at the same time to assess their role as potential Ice Nuclei (IN). In the present work, possibly for the first time we report the morphological characteristics of bioaerosols from marine urban and high altitude continental regions in Southern India. The samples were collected using polycarbonate filter paper and analyzed using Scanning Electron Microscope (SEM) coupled with Energy-dispersive Spectra Detector (EDX/EDS). The observed bioaerosols exhibited great variability in their morphological features over this region of the world. At these contrasting environments, we found that fungal spores constituted the major fraction of the total observed bioaerosols. Pollen grains, plant and insect fragments, and lot of other non-identified bio-particles were also observed constituting the remaining fraction. Further, the classification of fungal spores exhibited strong variability over this region. For example, fungal spores of both Ascomycota and Basidiomycota class were seen in abundance in marine environment, while Ascomycota especially Cladosporium were seen in abundance in high altitude continental environment. Our findings also suggest that increase in diversity of bioaerosol particles at marine site appeared to coincide with precipitation. It appears that vast diversity in the morphological features of bioaerosols exists over this region, which should further be studied using advanced online techniques for better quantification under contrasting environments. However, the diversity observed in morphological characteristics of bioaerosols at these two contrasting locations is limited and restricted to these two sites and season of the year, and should therefore not be generalized over a large spatio-temporal scale; emphasizing the need for more similar studies covering larger geographical area.

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## 1. Introduction

Bioaerosol particles, also referred as bioaerosols, a subset of aerosol particles, broadly describe the solid airborne particles originating from biological organisms including microorganisms (viruses, bacteria, fungal spores, protozoa, algae, etc.), together with fragments of biological materials such as animal dander, plant debris, pollens, etc. (Després et al., 2012; Artaxo and Hansson, 1995; Coz et al., 2010; Elbert et al., 2007; Després et al., 2007; Huffman et al., 2010, 2012; Pöhlker et al., 2012; and reference therein). These bioaerosols may be present in the ambient atmosphere either as single particle, cluster, agglomeration, and even in association with other particles. Bioaerosols exist in variety of shapes like spherical, ovoidal, rod, spiral, etc., and exhibit distinct morphological characteristics (Matthiasmaser and Jaenicke, 1994). Their surface features also show significant diversities in surface ornamentation; such as smooth, wrinkled, reticulated, spiny, wedged, etc. (Grand and Moore, 1970; Grand and Vandyke, 1976). These surface features help them to withstand the adverse conditions and stresses in the atmosphere and also in their transport and dispersal. In addition the surface structure and characteristics play important role in heterogeneous ice nuclei formation at various temperatures (Morris et al., 2004, 2014). Though not as abundant in terms of number concentration as compared to non-bioaerosols, they constitute a significant component of atmospheric aerosols and play an important role in agriculture, public health, and climate by mediating the cloud properties and precipitation serving as cloud condensation and ice nuclei (Xu et al., 2011; Griffiths and Decosemo, 1994; Adhikari et al., 2004; Ariya and Amyot, 2004; Morris et al., 2011; Ariya et al., 2009; Moehler et al., 2007; Sun and Ariya, 2006; Iannone et al., 2011; Delort et al., 2010; Bowers et al., 2009; DeLeon-Rodriguez et al., 2013).

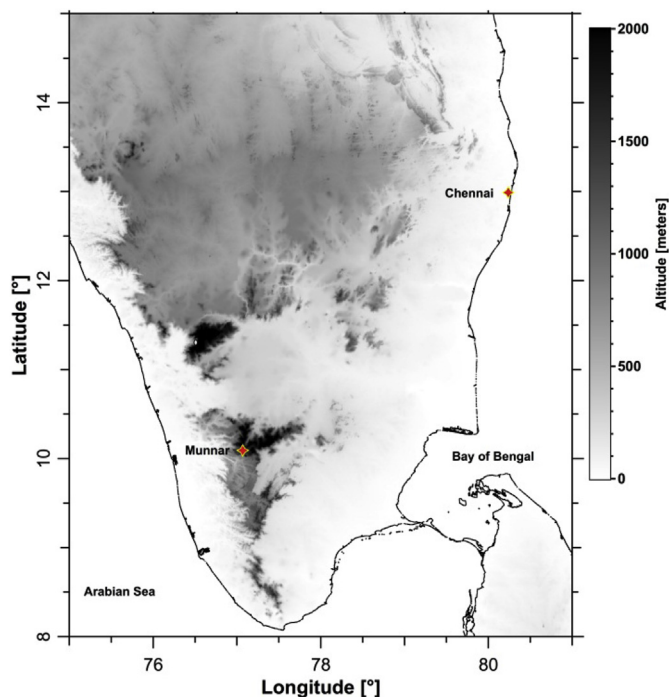
Since last century numerous studies were conducted in different parts of the world to understand the abundance and diversity of bioaerosols using different sampling and measurement techniques. During last decade the technology has been improved in terms of various advanced bio-chemical approaches, molecular techniques such as DNA analysis, and ability of the instruments to characterize the number size distribution of bioaerosols in high time and size resolution. These new techniques have greatly improved our understanding to better quantify the abundance and diversity of bioaerosols and their role in climate and human health (Huffman et al., 2010, 2012; DeLeon-Rodriguez et al., 2013; Fisher et al., 2012; Fröhlich-Nowoisky et al., 2012). Despite of such advancement sources, transport, abundance, and properties of bioaerosols remain poorly characterized across the globe.

Thus, over the Indian region, bioaerosols in general have received less attention than non-bioaerosols. Investigating and understanding the biodiversity of bioaerosols over India is important due to its unique and systematic climate pattern, and vast differences in geographical regions and vegetation. In the present study we investigate the morphological characteristics and diversity of bioaerosols in contrasting environment over Indian region giving first glance of bioaerosols over this part of the world.

## 2. Materials and methods

### 2.1. Sampling sites

The samples for the present study were collected over the period of 4 months at two contrasting locations (Fig. 1) a) Chennai, and b) Munnar. Chennai, a marine urban site located on the South-Eastern coast of India (12.99 N, 80.23 E; 14 m asl) has a tropical hot and humid climate with three seasons, namely summer, monsoon, and winter. The sampling of bioaerosols for scanning electron



**Fig. 1.** Locations of the measurement sites in southern tropical part of Indian peninsula. Chennai (yellow star) located on the Eastern coast can be classified as urban marine site, where sampling was performed on the campus of IIT Madras (12.99 N, 80.23 E; 14 m asl) situated 4.5 km areal distance away from the coast. Munnar as indicated by yellow star is South-West of Chennai (10.09 N, 77.06 E; 1605 m asl) located in the Western Ghats can be classified as high altitude fairly pristine site unperturbed by the local and regional emissions. Please refer to Tab. 1 for detailed sampling schedule. The map is color coded by altitude in meters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microscopy (SEM) analysis was performed within the campus of Indian Institute of Technology Madras (IITM), which is covered with dense trees spreading over more than 670 acres with vast varieties of flora and fauna. 36% trees, 24% herbs, and rest by shrubs, climbers, grass, and palm covers these 670 acres. The majority of the sampling in Chennai was carried out during October–November 2013, which is marked by the North-Eastern monsoon rainfall. The sampling in IITM was carried out on the roof-top of Mechanical Sciences Block (MSB), which is located around 5–7 km from coast and received mild sea breeze during afternoon hours. Samples were collected at a height of 1–1.5 m from the surface of the roof-top making sure air sampled was above canopy as roof-top site has well-mixed dynamics minimizing the local influences (Khattab and Levetin, 2008).

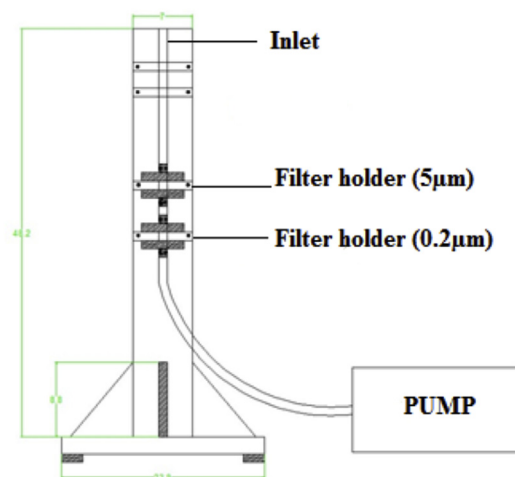
The other site, Munnar (10.09 N, 77.06 E; 1605 m asl), is a high altitude site situated in the Western Ghats (WG) range of mountains, which runs almost parallel to the Western coast of Indian peninsula. The mountain range is situated extremely close to the coast of Arabian Sea that the aerial distance from observational site to sea is less than 90 km signifying the presence of steep mountains making it geographically interesting location. WG has been declared as UNESCO World Heritage Site and is also one of the eight “hottest hotspots” for biological diversity in the world (Myers et al., 2000). This site is relatively clean due to its unique topography with wet temperate climate and is generally unaffected by local and regional pollution resulting from long-range transport. This site experiences a well-defined, systematic, and cyclic monsoon season, which is associated with the seasonal movement of Inter-Tropical Convergence Zone (ITCZ), which moves northward during

June–September causing majority of the rainfall during this period. No rainfall was observed during our measurements, which were carried out on 9th and 10th of December 2013. The measurements in Munnar were carried out on the premises of College of Engineering located on a hill-top. The site is not highly frequented by human and vehicular activities, and is open to East to West direction to valley of tea plantation, forest with vast varieties of flowering plants and trees, whereas the Northern side has a mountain with dense vegetation. Thus observational site at Munnar can be categorized to an extent as natural habitat and was relatively clean as compared to Chennai. The details about the sampling schedule for all the samples presented in this study are given in Table 1.

## 2.2. Aerosol sampling

The bioaerosol sampling at both the locations was carried out using a two stage filtering method using 25 mm diameter Nucleopore® Polycarbonate filter paper with the pore sizes of 5  $\mu\text{m}$  and 0.2  $\mu\text{m}$  in the two successive stages as shown schematically in Fig. 2. The filters were placed in a two identical Pall® 25 mm easy pressure syringe filter holders made up of polyoxymethylene with an average volumetric sample flow rate of 4 L/min. The samples were collected for approximately 60 min ensuring suitable and uniform coverage of the particles on the filter paper. Samples were sealed in an air-tight container and stored at 4 °C until analyzed using SEM. All the samples in Chennai were collected on pre-gold coated coarse and fine filter papers, whereas samples from Munnar were post-gold coated for SEM analysis. During the image investigations it was noticed that the quality of the images were better for the samples with post-gold coating. Here we present a detailed analysis on images from coarse mode filter samples for both the observational sites as fine mode particle images were very poor in resolution due to limitations associated with resolution of SEM instrument. Thus fine mode images were excluded from reporting in this study, as robust scientific conclusion was not possible.

In Chennai the sampling was performed during October–November 2013 where November was marked by scattered but heavy rainfall as a part of retreating North-Eastern monsoon. A total of seven days of samples were collected in Chennai including four



**FRONT VIEW (All dimensions are in cm)**

**Fig. 2.** A schematic diagram showing the sampling setup as viewed from the front side. Two cascaded filter holders were used for the sampling with pore size of 5  $\mu\text{m}$  (upper filter holder) and 200 nm (lower filter holder) with 25 mm diameter. The sampling was performed with average volumetric flow rate of 4 lpm.

during rainy days. However, only two days sampling was carried out in Munnar during the month of December 2013, which marks the winter season for this location (Table 1). Since this is a generalized attempt to characterize the morphological properties of bioaerosols from Indian region, an area of scanty studies related to the morphological analysis of bioaerosols, other advanced analysis like derivation of collection efficiency, shape factor, cut-off diameter, size distribution, etc. are excluded from the scope of present study.

## 2.3. SEM analysis

The scanning electron microscopy images of bioaerosols were obtained using three different scanning electron microscopes. 1. Quanta FEG 200 located at Sophisticated Analytical Instrument

**Table 1**

Overall summary of the samples collected from Chennai (Urban Marine: MA) and Munnar (High Altitude: HA) for SEM analysis along with sample ID, sampling date, sampling duration, nominal weather condition, volume sampled, SEM system used, total bioaerosol investigated, relative percentage contribution of C, N, and O obtained using DEX/EDS, and type of bioaerosol identified.

Sample ID	Sampling day	Sampling time duration (IST)	Nominal weather conditions	Air volume ( $\text{cm}^3$ )	SEM system used for analysis	Total bioaerosols investigated (images shown in paper)	Chemical composition of bioaerosols by EDAX in At%				Type of bioaerosol observed -
							C	N	O	Na/Mg/P/K/S/Cl	
MU – C1	21.10.2013	15:45–16:45	Rainy day; Monsoon	30,000	SEM 1	10(1)	83	6.33	10.42	0.307	Type – Pollens, fungal spores
MU – C2	28.10.2013	16:10–15:10	Rainy day; Monsoon	30,000	SEM 1	12(4)	80.61	7.34	12.45	0.197	Type – Fungal spores
MU – C2	28.10.2013	16:10–15:10	Rainy day; Monsoon	30,000	SEM 1	8(0)					Type – Fungal spores
MU – C3	5.11.2013	09:40–10:40	Clear day; Monsoon	30,000	SEM 1	4(1)	77.73	7.3	13.87	0.79	Type – Fungal spores
MU – C4	11.11.2013	12:40–13:40	Clear day; Monsoon	24,000	SEM 2	3(1)	80	4	13	0.54	Type – fungal spores, Plant fragments.
MU – C5	18.11.2013	12:05–13:05	Rainy day; Monsoon	24,000	SEM 2	1(0)	83.84	0.815	15.09	0.36	Type – Fungal spores
MU – C6	25.11.2013	07:20–08:30	Rainy day; Monsoon	30,000	SEM 3	7(7)	72.2	0	26.23	0	Type – Fungal spores, Protista, Insect fragments
MU – C7	29.11.2013	12:15–01:15	Overcast; Monsoon	30,000	SEM 3	6(2)	60	0	38.035	0	Type – Fungal spores
HA – M1	9.12.2013	14:20–15:20	Breezy & sunny; Winter	30,000	SEM 1	10(8)	82	5.32	11.2	0.1	Type – Fungal spores, Plant fragments.
HA – M2a	10.12.2013	09:10–10:10	Breezy & sunny; Winter	30,000	SEM 1	9(7)					Type – Fungal spores, Plant fragments.
HA – M2b	10.12.2013	12:40–01:40	Calm & sunny; Winter	30,000	SEM 3	7(2)					Type – Fungal spores

Facility (SAIF), which serves as a central instrumentation facility for IIT Madras, and 2. Quanta FEG 200 with similar specification to that of SAIF SEM located in Department of Metallurgical and Materials Engineering at IIT Madras, and 3. Hitachi S 4A00 located in the extension laboratory of Chemical Engineering Department of IIT Madras. All the three SEMs were equipped with energy-dispersive spectra detector (EDX/EDS), which was used to characterize the elemental composition of the morphologically identified bioaerosols. The procedure adopted to quantify bioaerosols is partially based on the method suggested by Matthias-Maser and Jaenicke (1991, 1994). However, the primary identification was purely based on the morphological characteristics of particle to classify it as a particle of biological origin and further confirmation of their organic nature was obtained by EDX results. Before loading the samples in the microscope the filter papers were cut in small squares of 1 cm<sup>2</sup>, thus part of the total filter paper was used for analysis. Any bioaerosol is expected to contain Carbon (C), Oxygen (O), and Nitrogen (N) elements as major components. During SEM analysis, when the sum of the relative percentage contribution of C, N, and O exceeded 90%, the aerosol was classified as of biological origin (Coz et al., 2010). Since the emphasis of this study is to investigate morphological characteristics of bioaerosols, other particles of non-biological origin were excluded from the study.

### 3. Results and discussions

The observed bioaerosol particles in the two contrasting environments exhibited varied morphological characteristics, and were identified and classified in to different categories broadly based on their size, shape, and morphological features obtained using SEM supplemented by EDX analysis results. Bioaerosols exhibit typical morphological and elemental composition, conspicuously separate from all other non-bioaerosols. Some of the non-bioaerosols, however, can exhibit high degree of morphological similarity to known bioaerosols. The EDX results were used to differentiate and exclude non-bioaerosols from further analysis and discussion in this study. The primary classification and identification of the bioaerosols obtained in this study were based on the similarity in morphological characteristics with those reported in earlier studies. Images that resembled already reported species were confirmed to be of organic in nature by EDX analysis. The images, which could be classified as bioaerosols based on EDX information, but did not find any similarity in morphology from previously published studies, were classified as unidentified bioaerosols.

The observed bioaerosols were identified as different types of fungal spores, pollens, plant and insect fragments etc. and were observed in abundance at both sites. The statistical analysis was performed on the absolute count of the bioaerosols observed at two contrasting locations and related statistical distribution is shown in Fig. 3. Please note that the statistics shown here do not represent the concentration or any other similar parameter but represent the absolute count of bioaerosols investigated using the SEM analysis (Table S1). A total of 51 and 26 bioaerosol particles were investigated from Chennai and Munnar respectively. It is evident from Fig. 3 that fungal spores were the most abundant biological kingdom at both locations constituting around 55% of the combined investigated bioaerosols from Chennai and Munnar. In Chennai 28 out of 51 (~55%) and in Munnar 14 out of 26 (~56%) examined bioaerosols contributed to fungal spores. A complete overview of characteristics of fungal spores observed at both the locations is shown in Table 2. The next abundant types of bioaerosol at both the locations were the pollens where a total of 9 and 4 pollens were identified in Chennai and Munnar respectively. In addition, small proportions of plant and insect fragments and Protista were identified. In Chennai around 60% of the total fungal

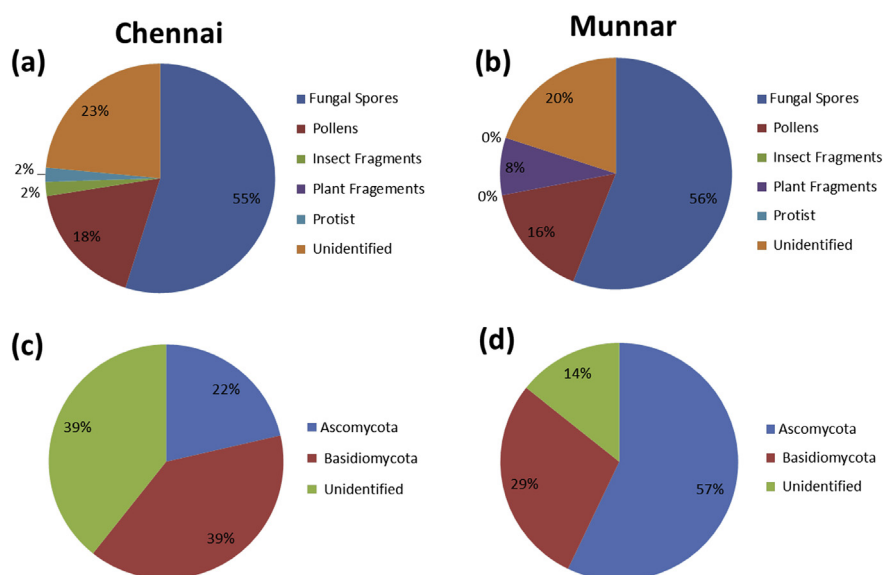
spores were identified and further classified, whereas in Munnar nearly 86% of the fungal spores were successfully identified. All the identified spores were further broadly classified into the phyla of Ascomycota and Basidiomycota. Basidiomycota accounted ~65% of the total identified spores in Chennai and spores in Munnar were dominated by Ascomycota contributing ~75% of the identified spores. Bacteria (found in the size range of 0.5–2.5 µm), which one would expect widely, were not observed at any of the sampling sites in coarse mode fraction. The absence of bacteria in coarse mode may be due to their low concentration in the air and possible limitation in identifying them using SEM. However, the presence of bacteria in the fine mode may not be ruled out, as the images from fine mode filter were inconclusive due to poor resolution.

#### 3.1. Fungal spores

Fungal spores and conidia were the most abundant type of bioaerosols observed during the entire sampling period at chosen contrasting locations with large variability in their size and morphological characteristics. The size range of the observed fungal spores varied roughly between 3 and 10 µm. Majority of the spores appeared single while some were either aggregated or appeared in the form of chains during the investigation. Spores shown in Fig. 4 are categorized as Basidiospores based on their distinct morphological characteristics observed in Chennai and Munnar. They mostly appeared in ovoid, sub globular or elongated shapes with a smooth surface and a small protruding opening (apiculus) at one end. The spores shown in Fig. 4a,b with globular to sub-globular outline were abundant in Chennai and were most likely to be spores of *Cantharellus* genus (Grand and Moore, 1970). Whereas the spore shown in Fig. 4c with small spike like protuberance on the surface appeared most likely to be of *Hydnaceae* family (Grand and Vandyke, 1976) belonging to either *Phelledon* or *Bankera* genus. This spore, observed in Chennai, was typically characterized by the presence of numerous un-branched blunt projections on its surface without any dichotomous branches (Grand and Moore, 1970; Grand and Vandyke, 1976; Baird and Khan, 1987) and typical size range was around 3 µm.

Fig. 4d shows a spore of *Bolitaecae* family (Grand and Moore, 1970) from Munnar, which was rod shaped (~8 µm) and resembled similar surface characteristics to that of spores observed in Chennai (Fig. 4a,b). Fig. 4e,f shows Basidiospores observed in Munnar with slightly wrinkled surface and apiculus at one end, and general morphology further suggested these spores to be of the class *Agaricomycetes* (Ertz et al., 2008). The spore shown in Fig. 4e appeared to have structured skin with wobbled shape and could belong to the species of *Cantharellus floccosus* (Bigelow, 1978). Whereas fungal spore shown in Fig. 4f showed much closer resemblance to spores of species *Gomphus floccosus*, which also has a wrinkled surface, but more uniform, organized, and with rough structure as compared to *Cantharellus* (Grand and Moore, 1970).

The Ascospores observed in Chennai and Munnar are shown in Fig. 5. Among the Ascospores, the spores of *Cladosporium* genus were found to be abundant in Munnar (Fig. 5d–f) and few in Chennai (Fig. 5a). The genus of *Cladosporium* is probably largest genera belonging to *Dematiaceae* *Hyphomycetes* and their characteristics coronate scar structure and conidia in acropetal chain (Fig. 5d,e) were clearly visible. They mostly appeared as chain of conidia (Fig. 5a,d) or conidium (Fig. 5e,f) in the size range of 5–8 µm with conspicuous or reticulate ornamentation. The conidium observed in Fig. 5d has two secondary conidia attached to the conidiophore, which generally found to be smoother than the conidium. The scar left due to the detachment of secondary conidia on conidium is clearly visible in Fig. 5f. The surface features, shape, and ornamentation clearly shows that even at same genus level



**Fig. 3.** Statistical distribution of total absolute number of bioaerosols investigated at Chennai and Munnar. Percentage distribution of various types of biological aerosols ((a) & (b)) and fungal spores ((c) & (d)) observed during sampling. For the statistical distribution of absolute number refer to Table S1.

morphological features of the fungal spores may exhibit variations depending up on their surrounding environmental conditions. In addition to the *Cladosporium* few other species of Ascomycota fungi were also observed at both the observational sites. The Ascospores, which were noticed in Chennai, are shown in Fig. 5b,c. Fig. 5b shows an ellipsoidal Ascospore with narrowly rounded ends and transverse striation on its surface belonging to genus of *Daldinia* (Stadler et al., 2002; Suwannasai et al., 2005) and has a high degree of match with species *Eschscholzii*. In any given genera high diversity of morphological stomatal characteristics poses a challenge in confident recognition. However, detailed microscopic characteristics have helped to make distinction at generic level, which includes apical apparatus in the ascus, position, size and shape, etc. The fungal spores, which appeared in the form of chain, shown in Fig. 5c were abundant and found consistently on more than two occasions in Chennai during the investigations. They showed roughly spherical structure with tiny and unevenly distributed protuberances on their surfaces and average size was 3–4  $\mu\text{m}$ . A similar kind of fungal chain with six spores with size range of around 2–3  $\mu\text{m}$  attached together was also observed in Munnar but had different surface features (Fig. 5g). Nevertheless, both these spores were possibly spores of an unidentified Ascomycota fungus. The observed fungal spore shown in Fig. 5h from

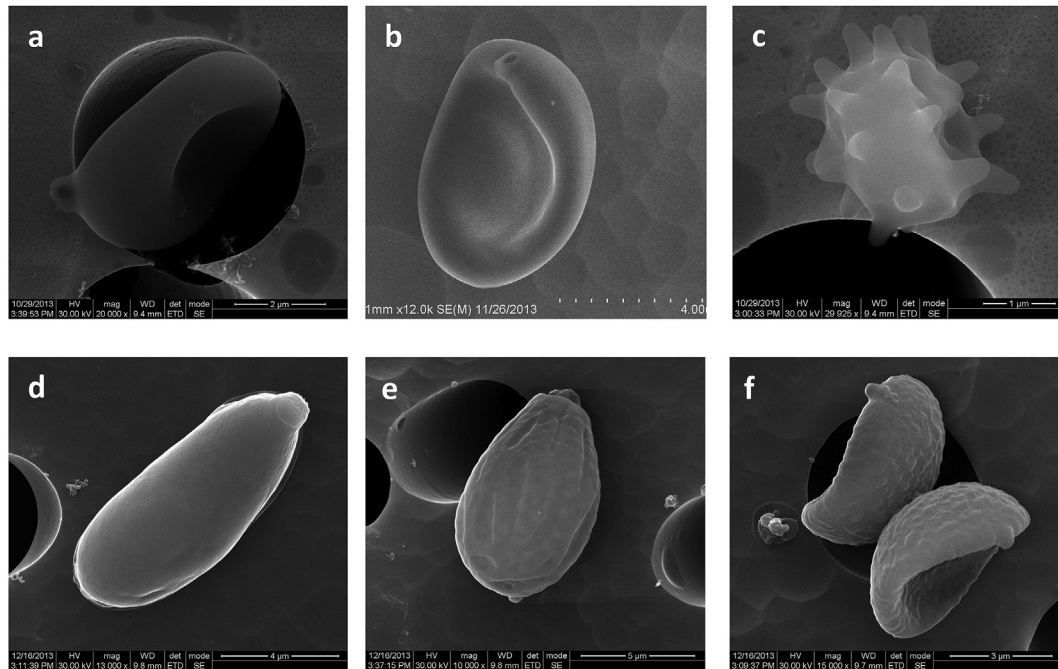
Munnar resembles *Alternaria* spore and may belong to *A. Alternate* species, which is usually seen in association with *Cladosporium* spores (Hjelmroos-Koski et al., 2006; Rodriguez-Rajo et al., 2005). Unlike the *Cladosporium* spore, however, it had an elongated shape with expanded bottom with length of around 8–10  $\mu\text{m}$  and width of around 2–3  $\mu\text{m}$ . The scars, although not prominent, could be noticed on both the ends of the spore, which might have resulted due to detachment from parent body. Wiest et al. (1987) have characterized this spore as a possible pathogen for a number of hosts including humans where it can cause upper respiratory tract infections and asthma with weakness in immunity. In temperate climate they were found to be peaking in late summer and autumn (Weber, 2001) with strong possibility of dispersion through air medium during dry periods (Ohollaren et al., 1991). This is consistent with the fact that climate and vegetation in Munnar appeared to be more favorable for *Alternaria* spore.

Interestingly few spores of slime mold and smut spore were also observed during the sampling in Chennai and Munnar respectively as shown in Fig. 6, which are generally characterized by the vast variety of their teliospores. Fig. 6a shows two spherical spores of around 3  $\mu\text{m}$  each from Chennai, which belonged to *Calonema* genus of the slime mold. Slime mold is known to creep in moist soil, on decaying wood, ingesting bacteria, and decaying vegetation

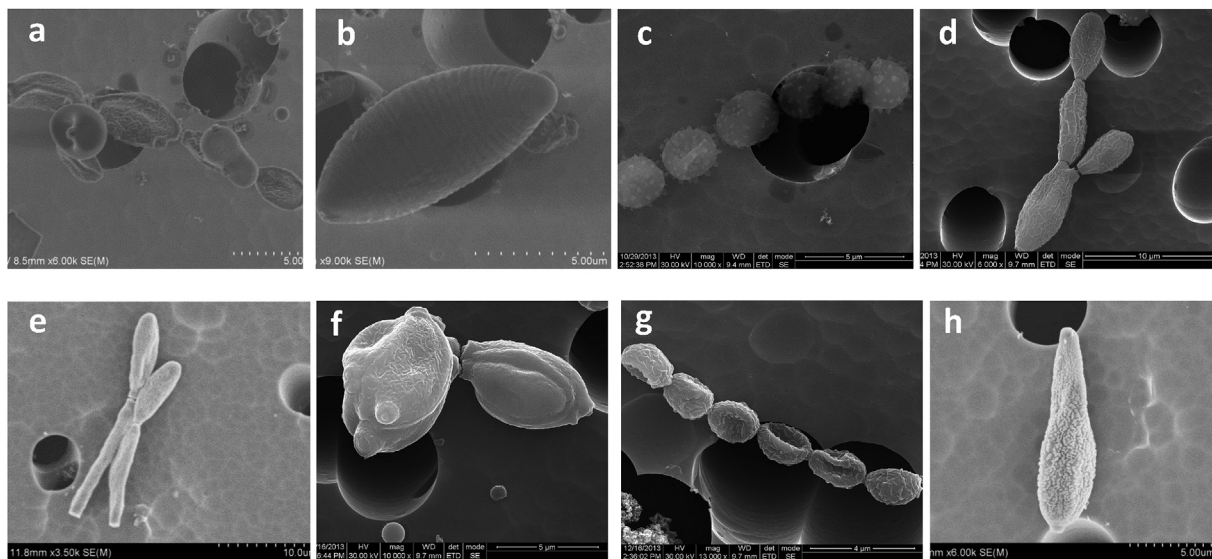
**Table 2**

Overview of two distinct classes, Ascomycota and Basidiomycota, of fungal spores emission observed in contrasting environments in southern tropical India. The classification is carried out based on morphological characteristic of observed spores supplemented by EDS/EDX analysis to confirm the biological nature of the particle under investigation. For more details please refer to Section 2.3 in the manuscript.

Location	Phyla	Spore type	Average size	Morphological features	Figure number	References
Chennai	Basidiomycota	<i>Cantharellus</i>	4–6 $\mu\text{m}$	Globular to sub-globular shape, smooth surface	4a,4b	Grand and Moore, 1970
		Hydnaceae	3 $\mu\text{m}$	Unbranched blunt projections on the surface	4c	Grand and Vandyke, 1976
	Ascomycota	<i>Daldinia</i>		Ellipsoidal with transverse striations	5b	Stadler et al., 2002; Suwannasai et al., 2005
Munnar	Basidiomycota	<i>Cladosporium</i>	5–8 $\mu\text{m}$	Reticulate ornamentation with digitate projections	5a	Gravesen, 1979
		Boltaceae	6–8 $\mu\text{m}$	Rod shaped, smooth surface	4d	Grand and Moore, 1970
		<i>Cantharellus floccosus</i>	4–6 $\mu\text{m}$	Ovoid shape, wrinkled surface with apiculus	4e	Bigelow, 1978
	Ascomycota	<i>Gomphus floccosus</i>	4–6 $\mu\text{m}$	Round shape, rough wrinkled surface with apiculus	4f	Grand and Moore, 1970
		<i>Cladosporium</i>	5–8 $\mu\text{m}$	Reticulate ornamentation with digitate projections	5d, 5e, 5f	Gravesen, 1979
		<i>Alternaria</i>	8–10 $\mu\text{m}$	Elongated shape with expanded bottom	5h	Hjelmroos-Koski et al., 2006



**Fig. 4.** Scanning electron microscopic images of Basidiomycota spores observed at Chennai (a–c) and Munnar (d–f). Spores were observed in varying size and shapes. Scale is varying for each panel and is shown at the bottom.

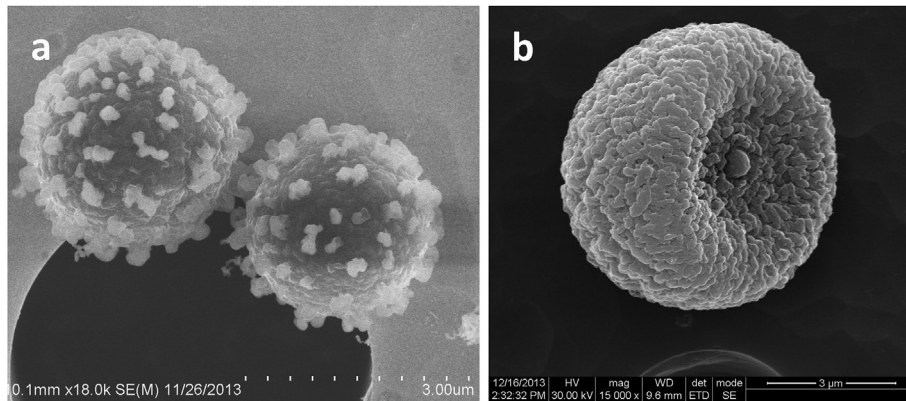


**Fig. 5.** Images of Ascomycota spores observed in Chennai (a–c) and Munnar (d–h). Scale is varying for each panel and is shown at the bottom.

specifically in highly humid atmosphere (Torres et al., 2003). Such reserve is plentiful given the nature and ecosystem of the IITM campus. *Calonema*, as described by Torres et al. (2003), is also characterized by the capillitium of tubular thread like structures with sub parallel veins, which are branched and anastomosed in a reticulate pattern continuous with peridial inner surface at the base of sporotheca. Based on this very characteristic feature this spore belonged to genus of *Calonema*. Please note that this genus does not belong to the kingdom of fungi but instead falls under the kingdom of Protista. On the other hand the spore observed in Munnar, shown in Fig. 6b, appeared to be smut spore with verrucose ornamentation and was 4–6  $\mu\text{m}$  in size. The scar left due to the detachment of spore from sterigma, which connects it with

basidium, was also distinctly visible on the surface.

Several other fungal spores with different morphological features were also found during our investigation (Fig. 7). Based on morphological characteristics, shape, size etc., all these investigated bioaerosols were different type of spores. However, further classification to any phylum or class level was not possible due to ambiguity in the physical characteristics. The reporting of these bioaerosols can facilitate the review by the bioaerosol community for positive identification. Fig. 7a,b shows fungal spores observed in Chennai with sharp densely packed spikes and small projections on their surface. The spores in Fig. 7c,d from Munnar were ovoid in shape with an opening at one end with size range of 4–6  $\mu\text{m}$ .

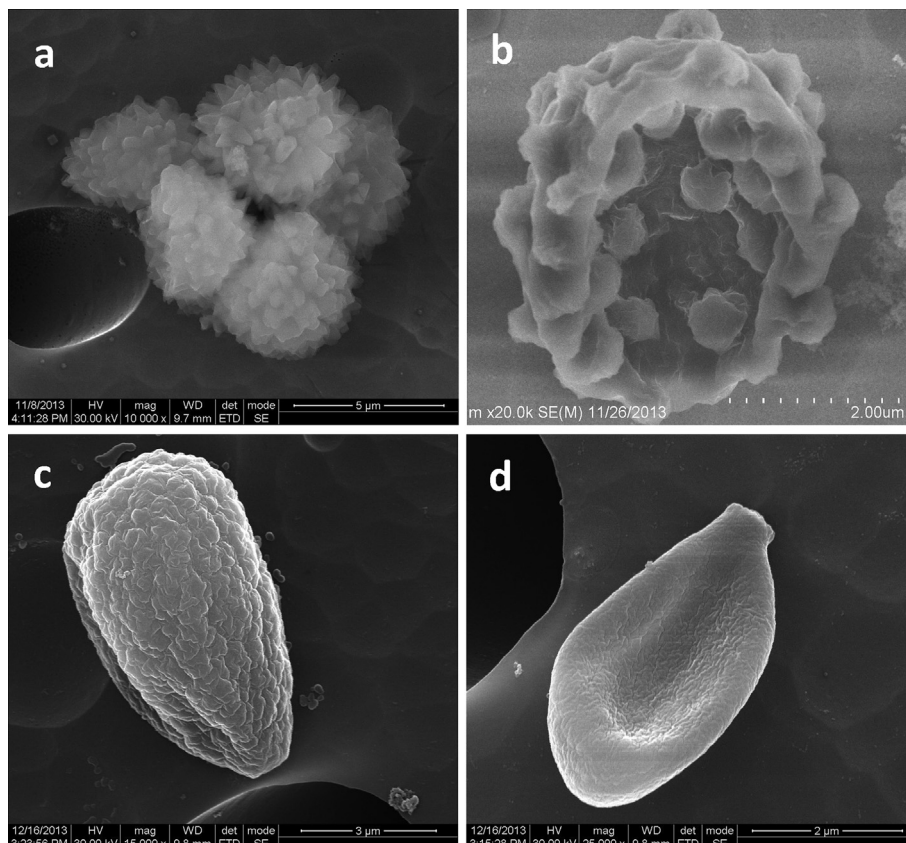


**Fig. 6.** Spore of (a) slime mold observed in Chennai (b) smut spore observed in Munnar. Scale is varying for each panel and is shown at the bottom.

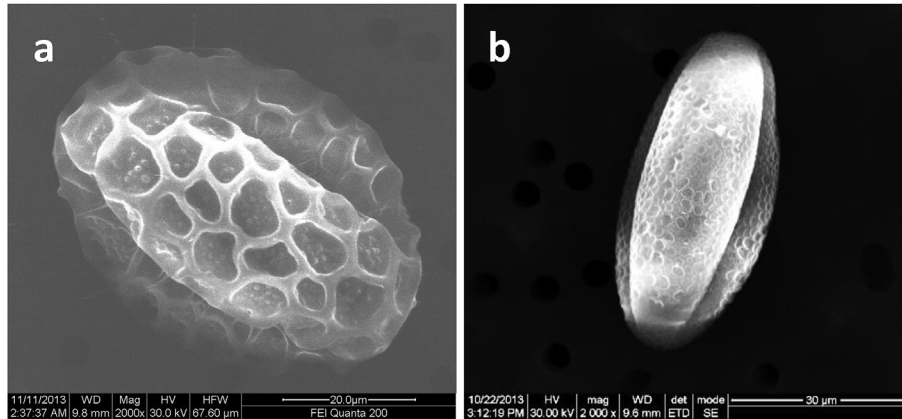
### 3.2. Pollen

In addition to fungal spores few types of pollens were also observed at Chennai and Munnar. Broadly the pollen grains were separated from fungal spores based on their size and to an extent shape with size in the range of 10  $\mu\text{m}$ –100  $\mu\text{m}$  (Huffman et al., 2010). In the present study during sampling at both the locations, pollen was mostly observed on days with high wind and clear sky. As shown in Fig. 8a,b two different types of pollens with distinct morphological and surface characteristics were observed in Chennai and ranged between 50 and 60  $\mu\text{m}$ . Both pollen grains were of tricolpate type, which are broadly characterized with three distinct colpi or furrows, but their surface features appeared to be entirely

different. The pollen grain shown in Fig. 8a exhibited a clear defined reticulate exine sculpturing with thick muri and wide lumina with some grainy structures inside. On the other hand Fig. 8b shows pollen with micro reticulate exine sculpturing, which appeared to be slightly spasmodically arranged when compared to the pollen grain shown in Fig. 8a. The pollen observed in Munnar shown in Fig. 9a–c appeared to be of entirely distinct morphology. Unlike the pollens observed in Chennai, these were relatively smaller in size and were all monocolpate with size range of 8–15  $\mu\text{m}$ . The pollen grain in Fig. 9a has an oval shape, which sharpens toward the top and bottom edges with a rough grainy surface and small depressions on the surface. Fig. 9b appeared to be grass pollen with smooth surface having powdery grains on it. The image shown in



**Fig. 7.** Exemplary images of few unidentified spores observed in Chennai (a–b) and Munnar (c–d). Scale is varying for each panel and is shown at the bottom.



**Fig. 8.** Exemplary images of pollens observed on IITM campus in Chennai. Scale is varying for each panel and is shown at the bottom.

**Fig. 9c** is presumed to be Orchid pollen with a cylindrical shape having tiny projections all over its surface with small opening at one end. Orchid pollens are known to be non-floating, sticky, and remain in its pollen pocket unless a pollinator, mainly insects, whips it away.

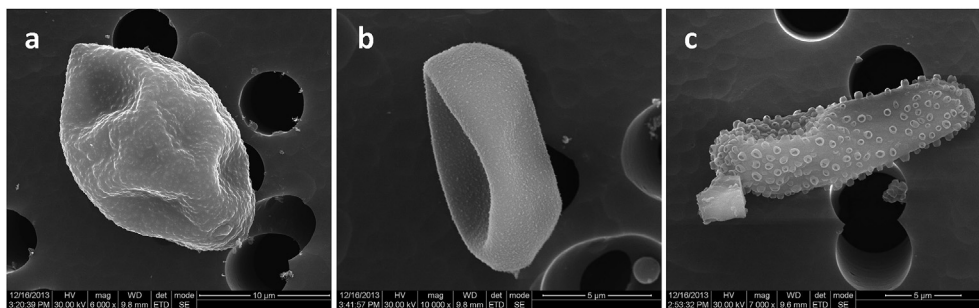
### 3.3. Insect and plant fragments

Apart from fungal spores, pollen, and unidentified bioaerosols, the few plant and insect fragments were also identified in few of the samples from Chennai and Munnar. **Fig. 10a** shows an insect scale from Chennai, which has a flat elongated shape with a small projection at the base that connects the scale to the insect body. The observed insect scale was about 50  $\mu\text{m}$  in length and 9  $\mu\text{m}$  in width with longitudinal parallel ribs on its surface. This was apparently the scale of Lepidoptera (butterfly) wing, which is responsible for its signature color on their wings. Though surrounded by vegetation, no plant fragments could be distinctly identified in any of the

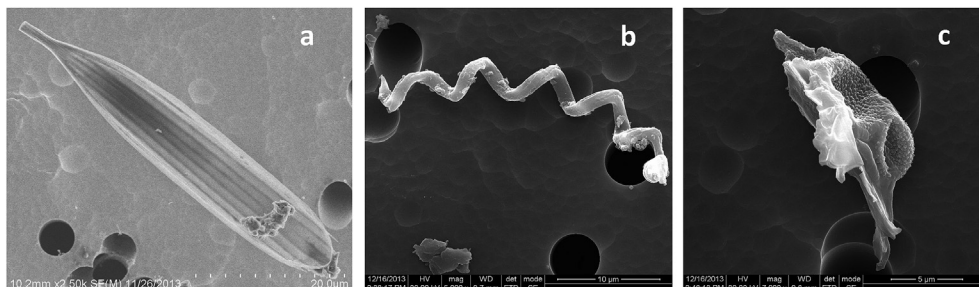
samples collected at Chennai. On the other hand a few plant fragments were identified from Munnar. **Fig. 10b** shows a long spiral fragment of some plant parts with sheared ends, which was nearly 40  $\mu\text{m}$  in length. Another irregularly shaped object, which was highly likely to be pollen part or flower fragment was observed and shown in **Fig. 10c**. It has some surface features, which can be closely related to that of pollen and appeared to be separated or damaged up on detachment. Although there are no reported studies on the effect of insect and plant fragments on human health and climate, they constitute a significant fraction of bioaerosol and thus cannot be neglected during the investigations of bioaerosol characteristics.

### 3.4. Unidentified

Despite all the possible efforts to categorize the investigated bioaerosols all of them could not be attributed to certain category or type. Out of the total bioaerosols investigated in Chennai nearly 23% remained unidentified whereas in Munnar unidentified



**Fig. 9.** Exemplary images of different pollens observed at Munnar in southern India. Scale is varying for each panel and is shown at the bottom.



**Fig. 10.** Exemplary images of insect scale and plant fragments. (a) Possibly the scale of a butterfly wing observed on IIT campus in Chennai, (b) spiral plant fragment, and (c) mostly likely damaged pollen or a plant fragment observed in Munnar. Scale is varying for each panel and is shown at the bottom.



bioaerosols constituted around 20% of the total bioaerosols investigated. Fig. 11 shows these bioaerosols, which were not assigned to any class or group.

#### 4. Summary and interpretations

We presented a case study of morphological characteristics of bioaerosols from an urban marine site at Chennai, and a relatively pristine high altitude site Munnar in Southern India using SEM. The assignment of investigated bioaerosols to specific class and type based on the limited database and reports available related to the morphological characteristics was difficult exercise. Spores contributed major fraction of the bioaerosols whereas pollens, insect scale, and plant fragment were observed on few occasions. All the observed fungal spores presumably were directly emitted in the atmosphere from the sources like ground, off plants, moist wooden surface, insects, mushrooms, etc. (Wittmaack et al., 2005).

The fungal spores, which were observed in abundance at both the locations, appeared to be highly diverse in characteristics. Morphological features showed that the fungal spores observed in Chennai were predominantly Basidiomycota spores largely constituted mushrooms, puffballs, rust, smuts, etc. are potential allergens and plant pathogens (Levetin and van de Water, 2008; Trejo et al., 2013; Hasnain et al., 2005; Horner et al., 1995, 1998). Previous studies in different parts of the world indicated that Basidiospores constitute a significant portion of fungal bioaerosols with different concentrations under distinct environmental conditions (Levetin and van de Water, 2008; Trejo et al., 2013; Hasnain et al., 2005; Calderon et al., 1995; Morales et al., 2006).

Although sampling in Chennai was marked by intermittent rain events, Basidiospores were observed throughout the sampling

period independent of rain. Basidiospores are predominantly released in the air through wet discharge mechanism resulting from high relative humidity in addition to precipitation (Elbert et al., 2007; Almaguer et al., 2013). Due to its close proximity to the coast sampling location experiences high relative humidity all-round the year and surrounded dense vegetation makes this location as a potential source of fungal bioaerosols and explains high abundance of Basidiospores. Hasnain et al. (2005) reported from three coastal cities of Saudi Arabia that, presence of Basidiospores was very common in the ambient atmosphere with high relative humidity. The abundance and diversity of other non-Basidiospore bioaerosol was much higher during the rainy days compared to non-rainy days in Chennai. This indicates that precipitation plays an important role in the discharge of these spores as reported by previous studies (Hirst, 1953; Huffman et al., 2013; Prenni et al., 2013; Schumacher et al., 2013). Pollens and insect fragments were observed in samples collected during clear and windy afternoons suggesting that dispersal of pollens is largely related to temperature and wind velocity. Brochosomes, the secretions of leafhopper, which were reported in previous studies (Wittmaack et al., 2005; Coz et al., 2010), were not detected in any of the coarse samples in Chennai and Munnar whereas their presence in fine mode filter samples cannot be ruled out.

The fungal spores from Munnar were dominated by conidia of *Cladosporium* genus. Previous studies reported that *Cladosporium* was a dominating fungal aerosol in temperate regions (Hjelmroos-Koski et al., 2006; Rodriguez-Rajo et al., 2005). Study conducted by Rodriguez-Rajo et al. (2005) in North-Western Spain reported *Cladosporium* concentration showed an increase with increasing index of continentality as well as altitude. A similar trend was observed in this study where more *Cladosporium* spores were

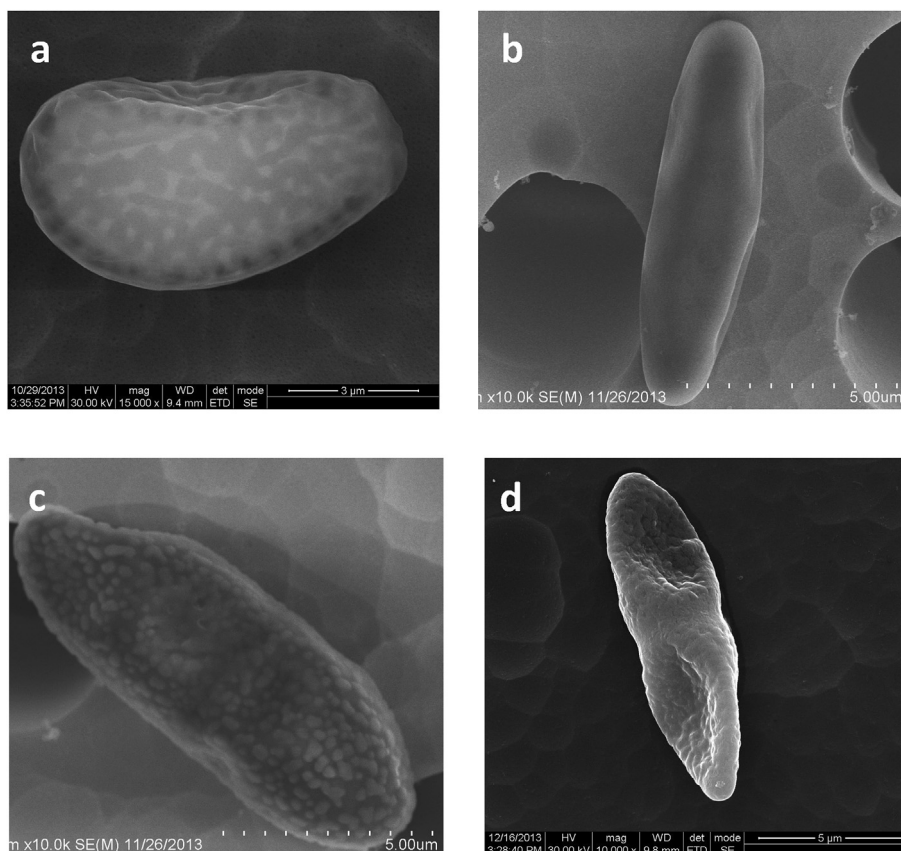


Fig. 11. Unidentified biological aerosols on IIT campus in Chennai (a–c) and Munnar (d). Scale is varying for each panel and is shown at the bottom.

detected at high altitude continental site of Munnar. Though the *Cladosporium* spores occur in abundance, they exhibit poor ice nucleation ability as compared to the bacteria (Iannone et al., 2011) and limited information regarding *Cladosporium* to act as ice nuclei makes it difficult to address their role and ability as potential ice nuclei. Apart from *Cladosporium*, spores of *Alternaria* and few other fungal classes were clearly identified. *Alternaria* is reported to be observed in association with *Cladosporium* spores its concentrations, however, were usually reported to be much lower compared to *Cladosporium* (Rodriguez-Rajo et al., 2005). In addition to fungal spores few pollens and some plants and insect fragments were also observed. The pollen observed in Chennai and Munnar showed distinct morphological features, which primarily may be due to difference in relative humidity levels.

The present study was broadly aimed at investigating the morphological characteristics and assessment of diversity of bioaerosols in contrasting environment over India where similar studies were not reported. This study could be of potential interest and distinct as this may be maiden study about morphological characteristics of bioaerosols found in particulate matter at two contrasting locations in India. Similar studies can be extended over larger spatial and temporal scale (covering all seasons in a year) supplemented by other biological and chemical techniques to better understand the characteristics, diversity, sources, and properties of bioaerosols over this climatically important region.

## 5. Conclusions

We examined the morphological characteristics and diversity of bioaerosols over two contrasting regions of Southern India. The observed bioaerosols included a variety of fungal spores, pollens, plant and insect fragments, etc. and exhibited a variations in their morphological characteristics. Fungal spores constituted the dominant fraction of observed bioaerosols, which were mainly classified in to Ascomycota and Basidiomycota. This study, investigating the morphological characteristics of bioaerosols will help us to have an improved understanding on region specific diversity, dispersion, and long-range transport. A better understanding of their chemical and physical characteristics is also required to assess their role in climate and atmospheric processes. Please note that the diversity observed in morphological characteristics of bioaerosols at these two contrasting locations is limited and restricted to these two sites and seasons of the year, and should therefore not be generalized over a larger spatio-temporal scale; emphasizing the need for more similar long-term studies covering larger geographical area. We suggest advanced observational and analytical techniques to obtain better understanding about bioaerosol abundance and seasonal variation, sources, long-range transport, and their effect on climate and health.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atmosenv.2015.09.071>.

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