Research Article

On distinguishing the natural and human-induced sources of airborne pathogenic viable bioaerosols: characteristic assessment using advanced molecular analysis

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Abstract

Ambient air consists of bioaerosols that constitute many microbes from biosphere due to natural and anthropogenic activities. Size-dependent ambient measurements of bioaerosols at two seminatural and three anthropogenic coastal sites in southern tropical India were taken during the summer 2017. All the five sites considered in this study considerably contributed to the bioaerosol burden with larger contribution from the dumping yard site followed by the marshland site, wastewater treatment plant, composting site, and Indian Institute of Technology Madras. The colony-forming units concentration for all the sites ranged from 17 to 2750 m^{-3} for bacteria and 42–2673 m^{-3} for fungi. *Firmicutes* and *Actinomycetes* were the dominant phyla observed in 698 bacterial OTUs obtained, and *Ascomycota* and *Zygomycota* were the dominant phyla observed in 159 fungal OTUs obtained in the study. Further, the study revealed the presence of pathogenic and ice-nucleating bacteria and fungi in the bioaerosols that can largely affect the well-being of the human population and vegetation in this region. Moreover, the statistical analysis revealed high bacterial abundance and diversity at the grit chamber of wastewater treatment plant and high fungal abundance and diversity at the dumping yard. Further, principal coordinate analysis of the sites studied inferred that the marshland, wastewater treatment plant, and the dumping yard sites shared similar microbial community composition indicating the existence of similar source materials and activities at the sites. Further, this study evidently brings out the fact that urban locations may play an important role in anthropogenic contribution of both pathogenic and ice-nucleating microorganisms.

Keywords Tropical India · Anthropogenic bioaerosols · Bioaerosol diversity · Bioaerosol sources · Marine urban

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1 Introduction

Bioaerosols are generally defined as the solid airborne particles of biological origin suspended in the gaseous medium and are ubiquitous in the Earth's atmosphere with an aerodynamic diameter of up to 100 µm [10, 17, 79, 90]. They can either be naturally released from the biosphere to the atmosphere or are released as a result of anthropogenic (human induced) activities [64]. They are widely known for their ability to cause infection and bio-precipitation [58]. These bioaerosols, depending up on their size and the surrounding environment, can contribute up to 25–50% of the total PM_{10} (particulate matter with aerodynamic diameter ≤ 10) mass burden [26, 43, 69, 94] and are known to have the potential effects on visibility, climate, and can negatively impact the ecosystem health [83]. Further, it is important to note that the PM_{10} which is of non-biological origin as a pollutant has a stringent standard in terms of "threshold limit" of mass, which is considered harmful for human being. On the contrary, human exposure to bioaerosol and resulting negative health impacts are independent of mass burden of bioaerosols. This means that the health implications associated with airborne bioaerosols are uniform over polluted and non-polluted regions.

Studies on natural and anthropogenic bioaerosols have shown that they carry microorganisms (bacteria and fungi) that cause respiratory diseases and significant opportunistic pathogens that affect the organs like eyes, skin, and urinary tract [4, 7, 26, 41, 51, 93]. Further, many researchers worldwide have documented the presence of various microbial toxins in the bioaerosols that cause lethal diseases to human and animals [13, 44, 86]. Similarly, inhalation of anthropogenic bioaerosols with improved allergenic properties can cause more severe respiratory diseases and ingestion of food contaminated by these aerosols leads to lethal enteric diseases compared to the naturally liberated bioaerosols [26, 37, 42, 59]. Hence, it is important to properly characterize the bioaerosols and to improve our understanding on their role in the impairment of climate and health.

However, while the rural areas are mostly dominated by the bioaerosols of the natural origin, the bioaerosols released due to intense anthropogenic activities can dominate urban region. The potential sites responsible for emission of anthropogenic bioaerosols in urban region may include biological wastewater treatment, composting, reuse of solid waste, landfills, industrial activities [19, 45], and transport of solid/biological waste. Among these, the wastewater treatment plant and composting sites are considered as potential sources of pathogenic and non-pathogenic bioaerosols [24, 63]. Even though the viability of the bioaerosols depends on the humidity, temperature, radiation, osmosis, other environmental factors, and the physical properties of bioaerosols, the studies investigating their type and diversity are very limited. Further, these limited studies focusing on investigating the characteristic properties of atmospheric bioaerosols are dominantly focused on bioaerosols of natural origin and advanced analysis of bioaerosols emitted from anthropogenic activities in an urban cluster is even more less understood. Thus, it is imperative, under the constantly urbanizing environment in southern Asia, to carry out a thorough study about identifying the type and diversity of the bioaerosols released from the natural and anthropogenic activities. The major objective of the present study focuses on the size-dependent qualitative and quantitative characterization of marine urban ambient air bioaerosols from five locations, which could be a potential source of atmospheric bioaerosols in the marine urban southern tropical Indian city, Chennai.

2 Materials and methods

To assess and characterize the marine urban bioaerosols, atmospheric air samples were collected from five sites in Chennai city (latitude: 13.08° N, longitude: 80.27° E, altitude: 13 m above mean sea level) located at southeastern part of India which is constantly engaged with a spectrum of anthropogenic activities. Sampling sites comprised of two seminatural and three major anthropogenic sites (Fig. 1) with varying activities. Seminatural sites are the sites with very minimal anthropogenic influence compared to anthropogenic sites chosen in the study, for example, Indian Institute of Technology Madras is a densely vegetated region with more natural activities and very minimal human activities. Sites were categorized as follows (NS—seminatural site and AS—anthropogenic site): NS1 from Indian Institute of Technology Madras (12.9912° N and 80.2363° E), a densely vegetated region with minimal anthropogenic activity, NS2 a marshland site (12° 56′ 15.72′ N and 80° 12′ 55.08′ E) with sparse vegetation and relatively high human activity, AS1 (a–c) from central part of Chennai (which includes grit chamber (a), surface aerator (b), and diffused aerator (c) of wastewater treatment plant located at 13.03° N and 80.19° E), AS2 from northern Chennai (composting site located at 13.21° N and 80.26° E), and AS3 from southern Chennai (waste dumping yard located at 12.96° N and 80.24° E).

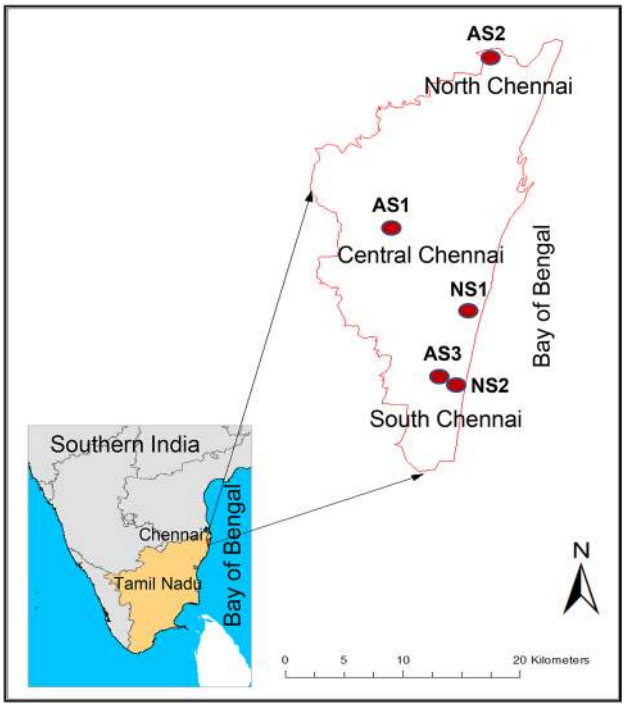


Fig. 1 Seminatural (NS) and anthropogenic (AS) sampling sites studied in the southern tropical marine urban city—Chennai: NS1—seminatural habitat [Indian Institute of Technology Madras (IITM)], NS2—human influenced marshland, AS1—wastewater treatment plant located at central Chennai, AS2—composting site located at northern Chennai, and AS3—dumping yard located at southern Chennai (the scale shown in the figure maps the Chennai region)

2.1 Collection of ambient air samples

Viable bacterial and fungal bioaerosols were collected in triplicates with an Andersen six stage impactor at a flow rate of 28.3 L min^{-1} for 2 ± 1 min [54, 60] using trypticase soya (TS) agar plates and potato dextrose (PD) agar plates, respectively. Post-sampling, the bacterial plates were incubated at 37 °C for overnight to 1 day and the fungal plates were incubated at 28 °C for 2–3 consecutive days. Further, the colony-forming units (CFU) m^{-3} were enumerated and colonies were picked for further DNA analysis. Two-stage SEM sampler was operated in tandem at a flow rate of 5 L min^{-1} for 30 min (with 0.2- and 5-µm pore-sized filter papers) to investigate the morphological details of the bioaerosols using Quanta FEG 200 SEM imaging equipped with EDX/EDS as described by Valsan et al. [83].

2.2 Collection of source samples

To obtain the closure and contribution details of the source materials (the materials pertinent to the anthropogenic activities observed in the site for, e.g., wastewater from wastewater treatment plant (WTP)) to anthropogenic bioaerosols in the study regions, wastewater, compost, and debris were collected from the respective sites. The samples were transported to the laboratory in sterile vials, serially diluted, and plated (10^{-10} dilution) on TS and PD agar following the robust sampling–transport–storage protocol. Bacterial plates were incubated at 37 °C for 24–48 h, and fungal plates were allowed to grow at 28 °C for 4–7 days. Then, the colonies were enumerated and picked for further DNA analysis (Table 1).

2.3 DNA analysis and molecular identification of the viable microorganisms

Chromosomal DNA of the colonies picked from the plates was isolated using the commercially available bacterial genomic DNA isolation kit, Helini, India and quick-DNA™ Fungal/Bacterial Microprep kit, Zymo Research, USA. Amplified using PCR with reaction mix (50 µl): 3 µl DNA, 25 µl Red dye master mix (Ampliqon, Denmark), 0.5 mM primers, 0.2 mM dNTPs, and water. Reaction conditions (Surecycler 8800, Agilent) were as follows: 3 min at 95 °C, 30× (1 min at 95 °C, 1 min at 57 °C (bacteria)/54 °C (fungi), and 1 min at 72 °C), and 3 min at 72 °C using the bacterial 16S rDNA primers (27f GAGTTGATCACTGGCTCAG and 1429r ACGGCTTACCTTGTTACGACTT) and fungal ITS primers (ITS-5f GGAAGTAAAAGTCGTAACAAGG and ITS-4r TCCTCCGCTTATTGATATGC). Then, the amplicons were sequenced using Sanger sequencing method by employing ABI3730xl96 capillary systems (Applied Biosystems), Eurofins, Bangalore, India.

The raw sequences were demultiplexed and trimmed to remove the primer, tag, and short sequences using Chromas software version 2.6. Trimmed sequences were analyzed using online tool NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/blast.cgi) and MOTHUR software version 1.39.5 [73, 74]. The operational taxonomic units (OTUs) were clustered with a 97% similarity (0.03) cutoff, and the chimeric sequences were removed using the UCHIME. Each OTU obtained represented the different genera of microbes present in each corresponding sample. The nucleotide sequences obtained in the study were submitted to NCBI-BankIt (https://www.ncbi.nlm.nih.gov/) and are available at GeneBank (Table S1).

2.4 Statistical analysis

Alpha diversity was visualized based on the observed OTUs. Five variables related to the diversity and abundance for each of the five-different taxonomic assemblage studied were calculated. Species richness and % abundance were calculated from the OTUs obtained for each site. Shannon's diversity index (*H*) was calculated using the

Table 1 Description of meteorological parameters, source CFU concentration, and diversity indices of the bioaerosols and source samples collected from study region

Sampling sites	Temperature (°C)	Humidity (%)	Precipitation	Source CFU concentration (log_{10} CFU)		Bacterial alpha diversity					Fungal alpha diversity				
				Bacteria	Fungi	OTUs	Chao 1	Shannon index	Simpson index	Shannon's evenness, Eh	OTUs	Chao 1	Shannon index	Simpson index	Shannon's evenness, Eh
NS1	30.2	62	NR	9.51/g	9.68/g	67	156	1.44	0.31	0.34	29	761	1.51	0.33	0.45
NS2	36.9	48.9	NR	9.91/ml	9.98/ml	137	1080	1.95	0.24	0.40	23	448	2.3	0.15	0.73
AS1a	33.6	66	30 min before sampling	9.83/ml	10.12/ml	85	242	1.76	0.78	0.4	25	199	2.6	0.88	0.81
AS1b	44.5	30.2	NR	9.73/ml	10.03/ml	127	399	1.34	0.63	0.28	9	93	1.47	0.67	0.67
AS1c	44.5	30.2	NR	9.52/ml	9.80/ml	37	99	1.53	0.74	0.42	16	129	1.6	0.65	0.58
AS2	34	60.5	NR	9.62/g	10/g										
AS3	35.3	44.6	NR	10.03/g	10.08/g										

NR no rain

equation $H = -\mathrm{SUM}[(\mathrm{pi}) * \ln(\mathrm{pi})]$ where pi is the number of individual species, Shannon's evenness of equitability (E_h) was calculated using the equation $E_h = {}^{H}/_{H_{max}}$ where H_{max} is the maximum possible diversity, and Simpson's diversity index (1-*D*) was calculated using the $1 - \sum n/N^2$ where *n* is the total number of species in the community and *N* is the number of different species in the community.

Beta diversity was analyzed using principal coordinate analysis (PCoA) with the Brays–Curtis distance using the skbio.stats.ordination module of Python, which contains several ordination methods, including PCoA [15, 50]. The Brays–Curtis distance was used as it is invariant to changes in units, unaffected by additions and removal of samples, and PCoA recognizes differences in total abundances when relative abundances are similar.

The type and diversity reported here for various sites are the representative of very peculiar scenario corresponding to the very nature of the site under specific condition and season. Therefore, the type, diversity, and concentration observed should not be generalized over a larger spatiotemporal scale. For the further improved understanding of the mechanism, long-term sampling in the close vicinity of these potential sources is highly recommended.

3 Results

3.1 CFU-based size-dependent concentration of the bacterial and fungal bioaerosols

Figure 2 explains the CFU distribution of the bacterial and fungal bioaerosols collected from the five sites. Bacterial CFU distributions were observed in all size ranges starting from 0.6 to 7 µm and > 7 µm, whereas the fungal CFUs were observed in the size range starting from 1 to 7 µm and > 7 µm. In Fig. 2, the seminatural site NS1 has shown a CFU concentration of 17 ± 2 to 443 ± 3 m^{-3} of air for bacterial and 87 ± 6 to 722 ± 36 m^{-3} of air for fungal bioaerosols, and CFU concentration of NS2 has varied from 1179 ± 773 to 2375 ± 1025 m^{-3} for bacteria and 470 ± 295 to 1048 ± 127 m^{-3} for fungi, which is indicative of the predominance of fungal bioaerosols at NS1 and bacterial bioaerosols at NS2. Among the anthropogenic sites studied, AS1a exhibited a CFU concentration ranging from 119 ± 52 to 810 ± 254 m^{-3} for bacteria and 137 ± 129 to 1113 ± 104 m^{-3} for fungi. AS1b (surface aerator) has shown a CFU concentration of 125 ± 47 to 1702 ± 385 m^{-3} for bacteria and 42 ± 27 to 446 ± 275 m^{-3} for fungi, and AS1c (diffused aerator) has shown a CFU concentration of 30 ± 37 to 292 ± 255 m^{-3} for bacteria and 48 ± 45 to 446 ± 253 m^{-3} for fungi. Further, AS2 has shown a CFU concentration

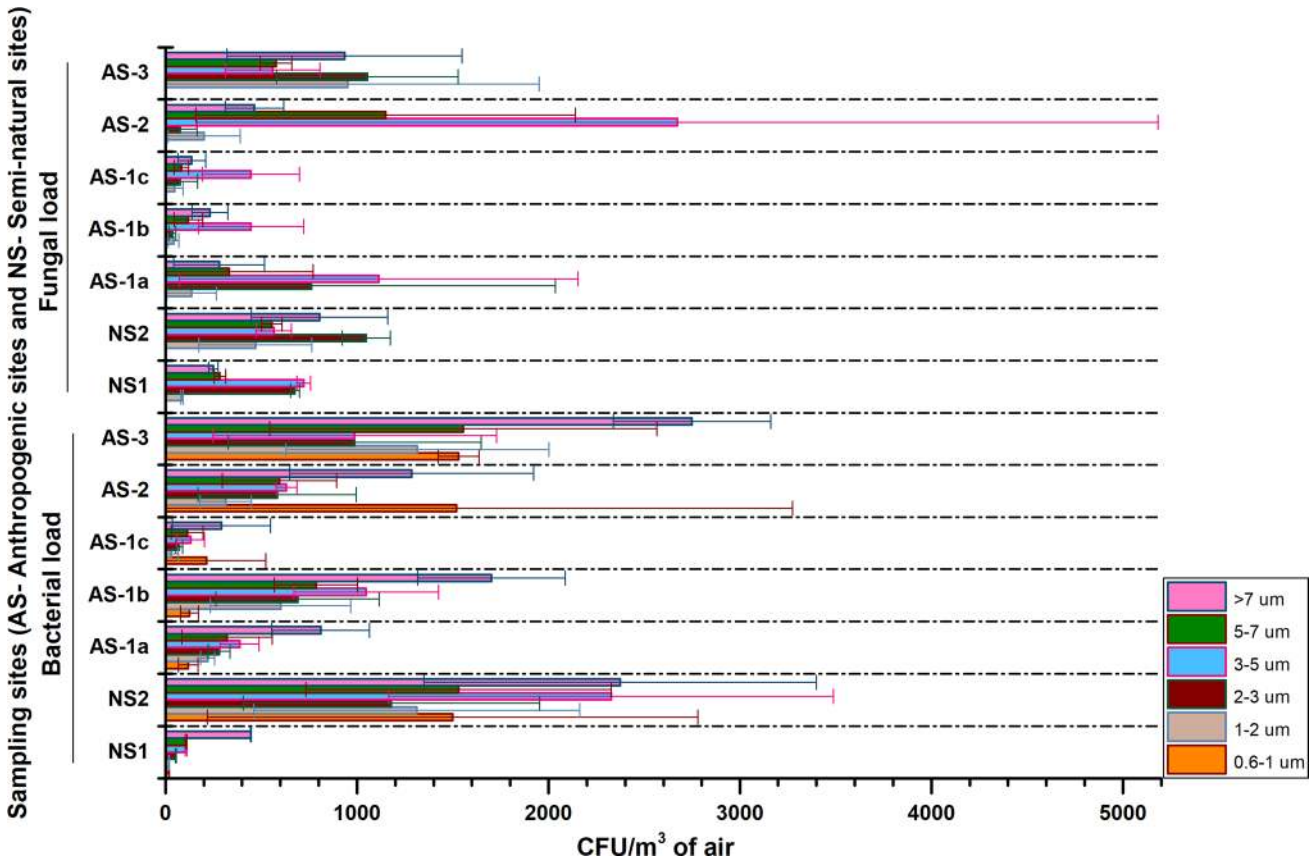


Fig. 2 Size-dependent CFU concentration and distribution of the bacterial and fungal bioaerosols: NS1—Indian Institute of Technology Madras (IITM); NS2—human influenced marshland site; AS1a—grit chamber, b—surface aerator, and c—diffused aerator of wastewater treatment plant; AS2—composting site; AS3—waste dumping yard

of 315 ± 134 to 1581 ± 1756 m^{-3} for bacteria and 77 ± 89 to 2673 ± 2510 m^{-3} for fungi and site AS3 has shown the presence of a very high CFU concentration ranging from 988 ± 660 to 2750 ± 412 m^{-3} for bacteria and 560 ± 248 to 1054 ± 473 m^{-3} for fungi demonstrating its potential role in the emissions of anthropogenic bioaerosols in the study region.

Regardless of the geographical location of the site and prevailing meteorological conditions (Table 1), bacterial bioaerosols (Fig. 2) have shown a high variability in distribution throughout the study period indicating the influence exhibited by the source materials. Whereas, the fungal bioaerosols (Fig. 2) have shown a demarcated high CFU concentrations in the size range of 2–5 µm at all the sites investigated with an interesting observation of noticeable CFU concentration at size range of 1–2 µm for fungal spores. Furthermore, Fig. 2 infers that the site AS3 has significantly contributed to the emissions of high concentration and diverse composition of bacterial bioaerosols, and sites NS2, AS2, and AS3 have played an imperative role in the emissions of fungal bioaerosols. The CFU concentrations of source samples (Table 1) have shown that the site AS1a has acted as a potential source for anthropogenic atmospheric fungal bioaerosols and AS3 for the bacterial bioaerosols.

Accordingly, Fig. S1, comprising of the morphological details (SEM images) of the bioaerosols, corroborates the fact that the observed fungal spores were dominated in the size range of 3–5 µm with the presence of fungal spores beyond this characteristic size range. Further, the presence of fungal spores and bacterial cells of varying size observed during the SEM analyses was in consistent with the size-dependent CFU observations. The fungal spores of varying shapes including, spherical and undulated, conidiophore, ruptured sporangiophore, and branched hyphal segments were observed during SEM analysis. Similarly, rods in single, rods in aggregation, and rods in association to dust particles and debris for bacterial bioaerosols were observed, implying the diverse nature of the microbes and their segments present in the bioaerosols.

3.2 Microbial composition at phylum and family level of the sites with dissimilar activities

Figure 3a–c explains the family and the phyla composition of bacterial and fungal bioaerosols from the locations using the results obtained from OTU analysis. Collectively, about 3208 viable bacterial bioaerosols were obtained contributing 698 OTUs, which comprised of major phyla *Actinomycetales, Firmicutes, Alpha-proteobacteria, Beta-proteobacteria, Gamma-proteobacteria, Bacteroidetes, Chloroflexi,* and *Spirochetes* (Fig. 3a). Further, seminatural locations have exhibited the dominance of *Firmicutes* (83% at NS1 and 82% at NS2) followed by the dominance of *Actinomycetales* (14%) at NS1 and *Gamma-proteobacteria* (9%) at NS2. It is also important to note that while at NS2 unidentified bacterial families contributed ~ 11% this contribution was found to be very high (~ 38%) at NS1. Similarly, *Firmicutes* have contributed to almost 78%, followed

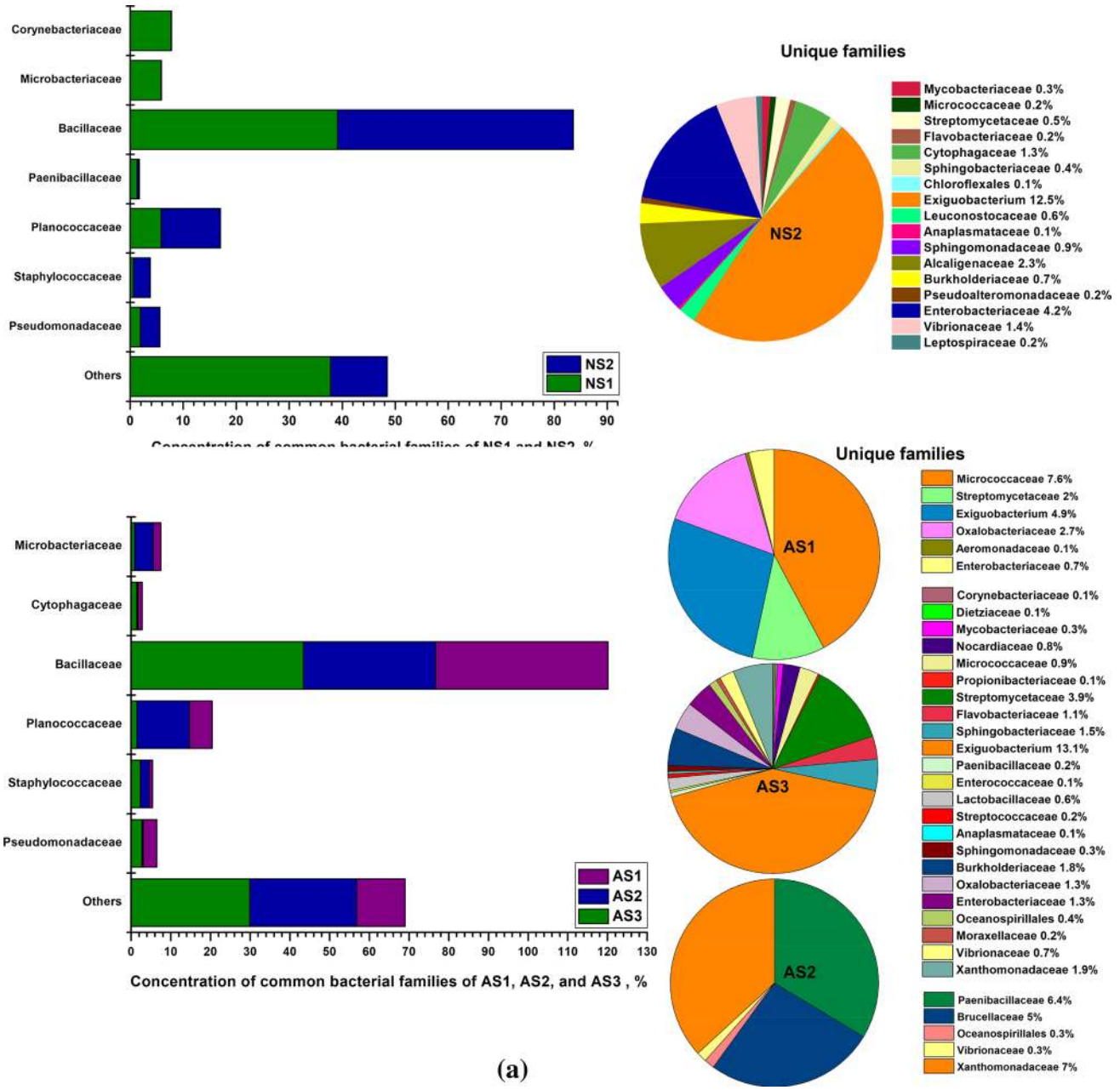


Fig. 3 Taxonomic community composition of bioaerosols and source materials based on the OTUs: **a** bacterial bioaerosols community composition; **b** fungal bioaerosols community composition; **c** bacterial and fungal community composition of the source samples (concentration in CFU/ml for liquid source samples and CFU/mg for solid source samples)

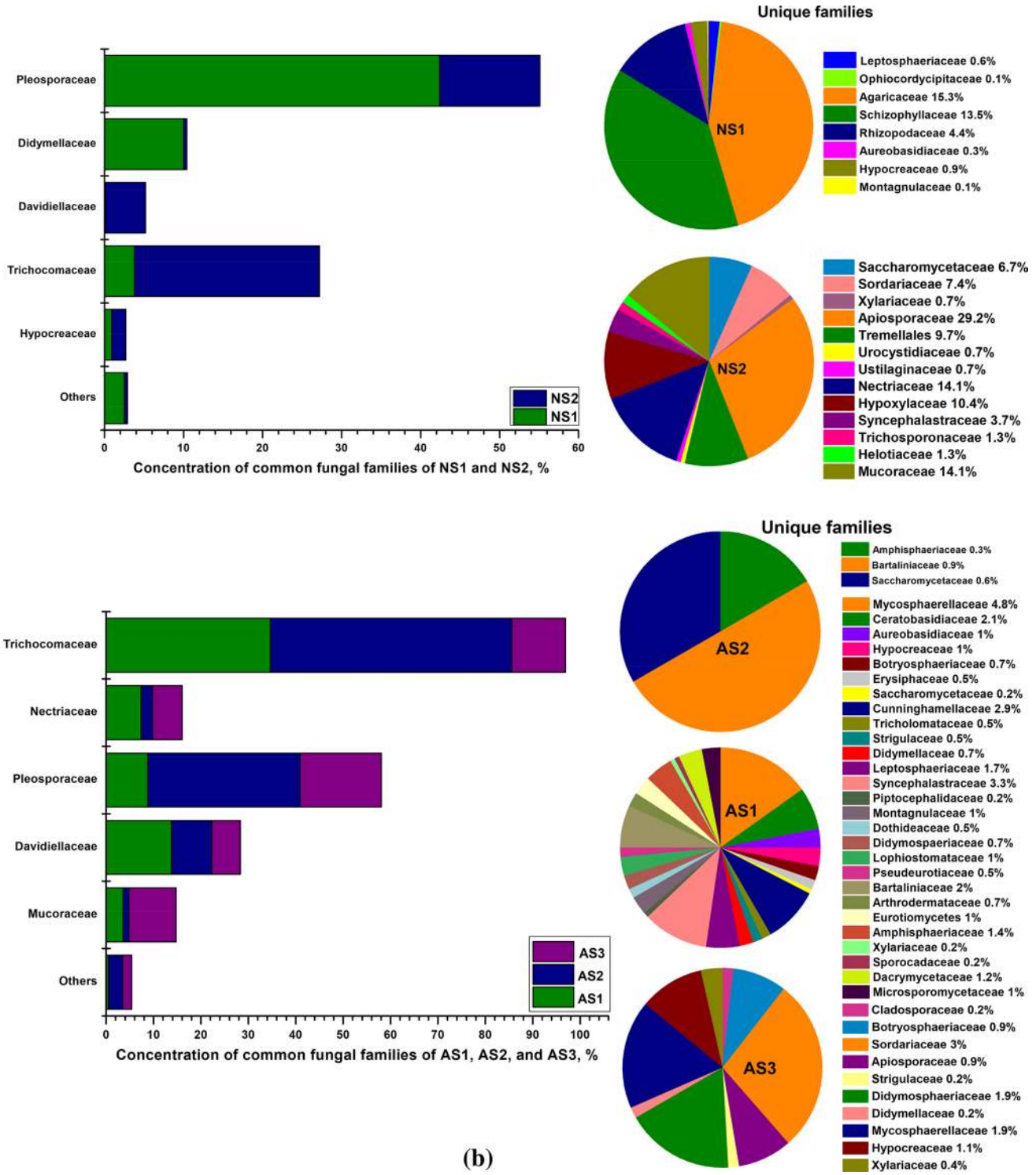
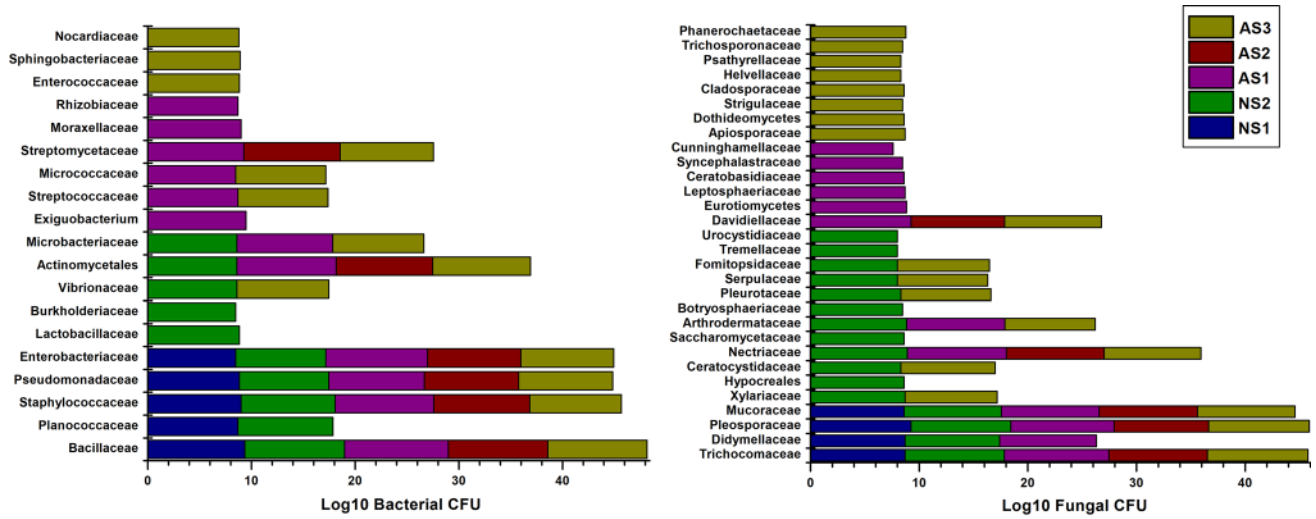


Fig. 3 (continued)

by *Actinomycetales* ~ 12%, and then followed by other phyla with the presence of major families like *Bacillaceae, Planococcaceae*, *Staphylococcaceae*, *Pseudomonadaceae*, *Exiguobacterium*, *Oxalobacteraceae*, *Enterobacteriaceae*, *Micrococcaceae*, *Streptococcaceae*, and *Cytophagaceae* at AS1. AS2 exhibited the dominance of *Firmicutes* contributing to about ~ 73%, *Gamma-proteobacteria* ~ 8%, *Actinomycetales,* and *Alpha-proteobacteria* contributing to 5% each, comprising the major families *Bacillaceae*, *Paenibacillaceae*, *Planococcaceae*, *Staphylococcaceae*, *Brucellaceae*,



(c)

Fig. 3 (continued)

Pseudomonadaceae, *Vibrionaceae*, *Xanthomonadaceae*, and *Microbacteriaceae*. AS3 has shown the presence of *Firmicutes* (~ 75%), *Actinomycetales* (9%), *Gamma-proteobacteria* (~ 8%), and *Bacteroidetes* (~ 4%) comprising the major bacterial families *Bacillaceae*, *Exiguobacterium*, *Paenibacillaceae*, *Planococcaceae*, *Staphylococcaceae*, *Burkholderiaceae*, *Xanthomonadaceae*, *Pseudomonadaceae*, *Vibrionaceae*, *Streptomycetaceae*, *Microbacteriaceae*, *Mycobacteriaceae*, and *Corynebacteriaceae* (Fig. 3a). Further, it was also interesting to note that the site AS3 has exhibited a highest bacterial diversity compared to the other sites.

Analogously, Fig. 3b indicates the presence of 2411 fungal bioaerosols contributing to 159 OTUs comprising the phyla *Ascomycota*, *Basidiomycota*, *Mucoromycota*, *Zygomycota*, and *Zoopagomycota*. Seminatural site NS2 has shown the presence of high fungal diversity with combined (NS1 and 2) dominant families, which includes *Pleosporaceae*, *Didymellaceae*, *Trichocomaceae*, *Agaricaceae*, *Mucoraceae*, *Davidiellaceae*, *Leptosphaeriaceae*, *Ophiocordycipitaceae*, *Schizophyllaceae*, *Rhizopodaceae*, *Aureobasidiaceae*, *Hypocreaceae*, *Montagnulaceae*, *Saccharomycetaceae*, *Sordariaceae*, *Xylariaceae*, *Apiosporaceae*, *Tremellales*, *Urocystidiaceae*, *Ustilaginaceae*, *Hypocreaceae*, *Nectriaceae*, *Hypoxylaceae*, *Syncephalastraceae*, *Trichosporonaceae*, *Helotiaceae*, *Helvellaceae*, *Acarosporaceae*, *Botryosphaeriaceae*, etc. Interestingly, *Ascomycota* was found to be the dominant phyla at both the locations indicating their abundant contribution in atmospheric bioaerosols, confirming their ubiquitous presence. Whereas for the diversity in family, *Pleosporaceae* (~ 42%) was found to be dominant at NS1 and *Trichocomaceae* (~ 23%) was found to be the dominant family at NS2. AS1 has shown the predominance of *Ascomycota* (~ 83%), *Zygomycota* (~ 9%), and *Basidiomycota* (~ 4%) comprising the diverse fungal families *Pleosporaceae*, *Trichocomaceae*, *Mucoraceae*, *Cunninghamellaceae*, *Nectriaceae*, *Didymellaceae*, *Leptosphaeriaceae*, *Syncephalastraceae*, *Montagnulaceae*, *Bartaliniaceae*, *Amphisphaeriaceae*, *Davidellaceae*, and *Ceratobasidiaceae*. AS2 has shown the highest dominance of *Ascomycota* (~ 93%) comprising the major families *Trichocomaceae*, *Nectriaceae*, *Pleosporaceae*, *Davidiellaceae*, *Bartiliniaceae, Saccharomycetaceae,* and *Mucoraceae*. AS3 has exhibited the predominance of *Ascomycota* (~ 79%) and *Zygomycota* (~ 14%) comprising the major families *Trichocomaceae*, *Davidellaceae*, *Nectriaceae*, *Botryosphaeriaceae*, *Pleosporaceae*, *Sordariaceae*, *Mucoraceae*, *Apiosporaceae*, *Didymosphaeriaceae*, and *Mycosphaerellaceae*. Moreover, Fig. 3b shows the presence of highest fungal diversity at site AS1 indicating the potential contribution of the site to the fungal bioaerosols.

Furthermore, it is inferred from Fig. 3a, b that each site has demonstrated its own community composition specific to the site, type of source material, and the characteristic meteorological conditions. Further, source material characterization (Fig. 3c) shows that the source played an imperative role in disseminating the specific microbes to the atmospheric air, which contributed to the bioaerosols concentration in each site (Tables 1, S1, and S2). The source has apparently contributed to about 52.6% of the total families observed in airborne bacterial bioaerosols at NS1, whereas 30.9% of the total families contributed to the airborne bacterial bioaerosols at NS2 (Fig. 3c). Similarly, the contribution was 58.3% at AS1, 25% at AS2, and 42.9% at AS3 for the bacteria.

Fungal diversity of source sample has contributed to 33.9% of total fungal families observed in the fungal bioaerosols at NS1, whereas it was 33.5% for NS2, 30.8% for AS1, 27.8% for AS2, and 23.1% for AS3, respectively (Fig. 3c). The predominant bacterial and fungal families that were obtained from the source samples include *Corynebacteriaceae*, *Bacillaceae*, *Planococcaceae*, *Staphylococcaceae*, *Pseudomonadaceae*, *Paenibacillaceae*, *Micrococcaceae*, *Microbacteriaceae*, *Exiguobacterium*, *Xanthomonadaceae* and *Pleosporaceae*, *Didymellaceae*, *Trichocomaceae*, *Agaricaceae*, *Mucoraceae*, *Davidiellaceae*, respectively, indicating the activities and the nature of source materials pertinent to the site.

Hence, the study clearly infers that the waste dumping yard has played a significant role in contributing the highest burden of atmospheric anthropogenic bioaerosols in the study region with the presence of a spectrum of diverse microbial population. Further, it was secondly dominated by the seminatural marshland site, thirdly by the wastewater treatment plant, then by the composting and the Indian Institute of Technology Madras (IITM) sites with a comparatively less diverse microbial population and bioaerosols concentration.

In concurrence with the findings obtained from OTU analysis, NCBI-BLAST analysis (Table S2) has also shown the presence of bacterial and fungal species that could potentially act as human/plant pathogens, opportunistic pathogens including ice-nucleating organisms, insect and animal pathogens, and some edible mushrooms. Furthermore, Table S2 infers that the investigated sources had a potential role in contributing the pathogenic bioaerosols over such an urban setup.

3.3 Composition of the potentially pathogenic microbes

Based on the CFU concentrations, as shown in Fig. 2 and OTU analysis, it was evident that the bioaerosols were comprised of potential human pathogens that could affect the whole respiratory tract in case of bacterial and majorly tracheobronchial region for fungal bioaerosols (Figs. S2 and S3). The deposition of these pathogens in various regions of the respiratory tract is strongly dependent on the size of the particles. Figure S2 shows that the site AS3 has exhibited highly diverse bacterial community composition followed by NS2 compared to the other sites. Similarly, Fig. S3 shows the presence of high fungal diversity at AS1a, NS2, and AS3.

Further, Fig. S2 infers that *Bacillaceae* was the predominant bacterial family observed in all size ranges of all the sites studied showing the abundant contribution of the family to the airborne bacterial bioaerosols burden. Besides, NS1 has also shown the dominance of the bacterial families like *Corynebacteriaceae, Paenibacillaceae,* and the unidentified families. NS2 has shown the presence of some environmental and enteric bacterial families like *Exiguobacterium*, *Cytophagaceae*, *Pseudomonadaceae*, *Enterobacteriaceae,* and *Vibrionaceae*. AS1a has shown the dominance of the family *Micrococcaceae* and AS1b has shown the presence of *Micrococcaceae* and unidentified families. AS1c has shown the predominance of *Micrococcaceae* and *Staphylococcaceae*. AS2 has exhibited the dominance of *Enterococcaceae*, *Planococcaceae*, *Pseudomonadaceae*, and *Brucellaceae*. AS3 with the highest bacterial diversity has shown the presence of *Exiguobacterium*, *Planococcaceae*, *Pseudomonadaceae*, and *Vibrionaceae* showing the presence of some enteric bacterial species along with the pathogenic species.

Figure S3, which details the fungal diversity, explicitly shows the overall predominance of *Pleosporaceae* over all the size ranges for the sites studied. Further, followed by the presence of sac fungi, white rot fungi, and edible/gilled mushrooms of families like *Schizophyllaceae*, *Psathyrellaceae*, *Agaricaceae* at NS1. NS2 has shown the dominance of human pathogens along with the environmental strains that include *Tremellales*, *Cunninghamellaceae*, *Aureobasidiaceae*, *Trichocomaceae*, *Nectriaceae*, *Mucoraceae*, *Leptosphaeriaceae*, *Hypocreaceae*, and *Montagnulaceae*. Similarly, AS1a has shown the presence of *Mycosphaerellaceae* and *Mucoraceae*, AS1b has shown the dominance of *Trichocomaceae* and *Arthrodermataceae* and AS1c has shown the presence of *Trichocomaceae, Amphisphaeriaceae*, *Bartaliniaceae*, *Nectriaceae*, and *Microsporomycetaceae*. Furthermore, AS2 has shown the dominance of *Trichocomaceae*, *Nectriaceae*, *Amphisphaeriaceae*, and *Davidiellaceae*. AS3 has shown the dominance of *Mucoraceae*, *Trichocomaceae*, *Sordariaceae*, *Botryosphaeriaceae*, *Didymosphaeriaceae*, *Hypocreaceae*, *Mycosphaerellaceae*, *Xylariaceae*, *Psathyrellaceae*, *Saccharomycetaceae*, and *Nectriaceae*. The presence of these families over all the size ranges of the study region clearly indicates the existence of human pathogenic fungi, environmental yeast like fungi, and common plant fungi.

Further, it is evident from Fig. S2 that the bacterial bioaerosols from sites NS1, NS2, AS1a, and AS1b have shown the highest diversity and dominance over all the size ranges posing major risk to the whole respiratory system. Further, AS1c has exhibited a highest diversity at 3–7 µm and > 7 µm possibly targeting the tracheobronchial and airway region. AS2 was found to pose a risk to the alveolar and tracheobronchial region with highest diversity at 0.6–5 µm and AS3, targeting the whole respiratory tract, exhibited a very high diversity and concentration of bacterial bioaerosols over all the size ranges.

Likewise, Fig. S3 infers that the sites NS1 and AS1a have exhibited a highest fungal biodiversity in the size range of 2–7 µm and > 7 µm possibly risking the tracheobronchial

and airway region; AS1b and AS1c posing possible risk to the airway and tracheobronchial region (3–7 µm and > 7 µm) and AS2 possibly affecting the alveolar and tracheobronchial region. AS3 and NS2 being the site with maximum fungal diversity have posed a possible risk to the whole respiratory tract.

3.4 Dynamics of microbial diversity of the sites with dissimilar activities

Alpha diversity and microbial community distribution over the study region were quantified based on the Chaos1 (*Richness*), Shannon's diversity index (*H*), the Gini–Simpson's diversity index (1-*D*), and Shannon's evenness of equitability index (E_h) (Table 1). Apparently, in accordance with the results observed with CFU concentrations and OTUs, AS3 was found to exhibit highest bacterial diversity and AS1a was found to exhibit highest fungal diversity. Further, Shannon index (*H*) infers that the bacterial communities have exhibited highest abundance of $H = 2.29$ for the site AS3 and the least being NS1 with *H* value of 1.44. Furthermore, Site NS2 has exhibited an abundance *H* of 1.95, AS1a with 1.76, AS1b with 1.34, AS1c with 1.53, and AS2 with 1.78. Similarly, Gini–Simpson's bacterial diversity index (1-*D*) shows that the bacterial bioaerosols of site NS2 exhibited a least similarity of 0.24 among the bacterial communities of the site compared to the other sites, which has shown a Gini–Simpson's index of 0.31 for NS1, 0.78 for AS1a, 0.63 for AS1b, 0.74 for AS1c, 0.78 for AS2, and 0.77 for AS3. Accordingly, the Shannon's evenness *(E_h)* explicitly shows that AS1c and AS3 have shown an evenness of 0.42 explaining the even distribution of the bacterial species in the sites compared to the other sites of the study region.

Likewise, Shannon index *(H)* for fungal diversity has clearly inferred that the site AS1a has exhibited the highest abundance ($H = 2.6$) for the fungal bioaerosols compared to the other sites with a *H* value of 1.51 for NS1, 2.3 for NS2, 1.47 for AS1b, 1.6 for AS1c, 1.26 for AS2, and 2.29 for AS3. Gini–Simpson's indices infer that the fungal bioaerosols have exhibited a diverse complex community compared to the bacterial community. Moreover, Gini–Simpson's fungal diversity index (1-*D*) has inferred the presence of more diverse fungal population at NS2 with a least similarity value of 0.15 compared to the highest similarity observed at AS1a with 0.88. Further, Shannon's evenness *(E_h)* shows that the site AS1a has exhibited an even distribution of the fungal population with an E_h value of 0.81 at this site compared to the other sites of the study region.

Figure 4 explains the principal coordinate analysis (PCoA) of the bacterial (Fig. 4a) and fungal (Fig. 4b) communities obtained from the study region. PCoA was plotted based on the Bray–Curtis dissimilarity indices to further corroborate the above finding and differentiate the inter-community composition (beta diversity). Figure 4 infers that the bacterial community of the sites NS2, AS1, and AS3 has moderately shared a similar community composition compared to NS1 and AS2, whereas in the case of fungal communities the sites NS2, AS1, AS2, and AS3 were observed to share similar community composition compared to the site AS3. Further, among the anthropogenic site, AS2 and AS3 were found to share more similar fungal community composition.

Overall inference of the microbial community diversity dynamics shows that the bacterial communities have exhibited an uneven distribution over all the sites studied explaining the anthropogenic influence over the bacterial bioaerosols dispersed from the sites. Further, Gini–Simpson's diversity index (1-*D*) and Shannon diversity index (*H*) have inferred that the site AS3 has exhibited highest bacterial diversity and AS1a was found to exhibit a highest

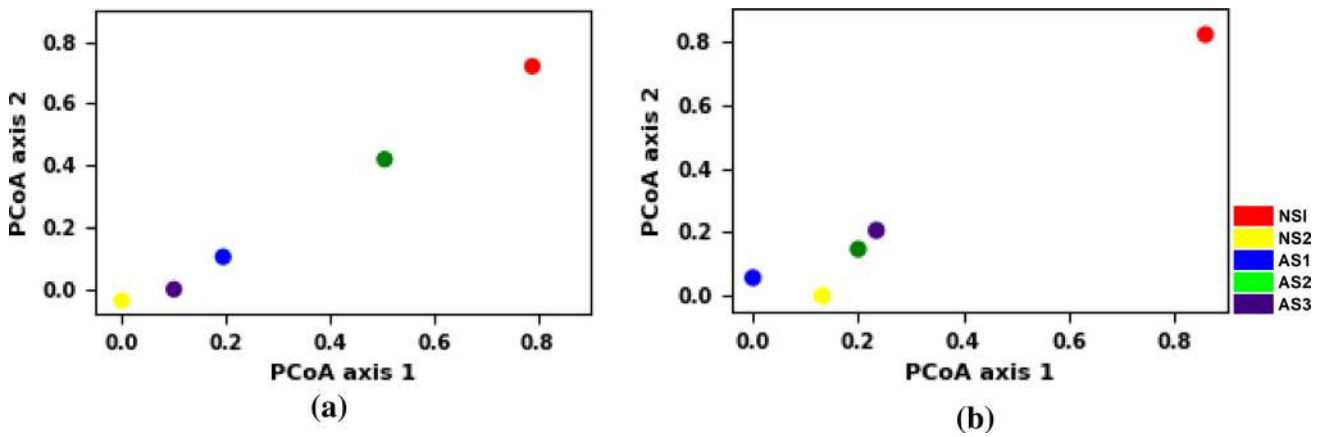


Fig. 4 PCoA plot based on the Bray–Curtis dissimilarity indices of the bioaerosols collected from the study region: **a** bacterial communities; **b** fungal communities

fungal abundance and AS2 with highest fungal diversity. Moreover, beta diversity analysis based on PCoA clearly inferred that the site NS2, AS1, and AS3 were found to share similar bacterial and fungal community composition that overlapped with each other indicating the influence of the most similar activities compared to NS1 and AS2 with site-specific community composition.

4 Discussion

Bioaerosols released play an important role in the spread of diseases to plants, humans, and animals (Fig. 5). Such dispersal of the pathogenic bioaerosols in the atmosphere due to the relatively natural and anthropogenic activities mentioned in this study may have negative implications on the ecosystem health [17, 23, 26, 27, 90]. Much attention has been given by many researchers [65, 74] on investigating the abundance, diversity, and types of naturally released bioaerosols and studies investigating the potential source, type, and diversity of anthropogenic bioaerosols are limited especially in a marine urban setup.

Size-dependent estimation of the atmospheric bioaerosols in the study has shown the presence of bacterial bioaerosols that exhibited an extreme variation in CFU concentrations (Fig. 2) similar to the reported observations of Hurtado et al. [41] and Chen et al. [11]. Furthermore, it was found that the meteorological conditions, microbial morphology, and source community composition have played a significant role in influencing the bacterial bioaerosols as stated by Ganthaler and Mayr [28], Kembel et al. [48], Sache [72], and Tang [78].

Concurrently, size-dependent measurements of fungal bioaerosols have shown the presence of high spore concentration at 2–5 µm similar to the observations of Valsan et al. [82] over the southern Indian region and many other researchers across the globe like Hjelmroos-Koski et al. [36], Huffman et al. [40], Schumacher et al. [75], and Healy et al. [34]. Analogously, the peak observed at 1–2 µm for fungal bioaerosols (Fig. 2) would have been contributed by the liberation of immature and desiccated conidia released from some *Aspergillus* sp. (Table S2) due to the uncontrolled anthropogenic activities and human influence observed in the site [87, 92]. Morphological observations (Fig. S1) of the bioaerosols collected on the filter papers have also shown the presence of tiny-large fungal spores [21, 31, 32] and bacterial rods complementing the results obtained in the size-dependent measurements of bioaerosols in the study region (Fig. 2). Further, the size of the bio-particles (fungi and bacteria) was of 100 nm to about 5 µm [87] with an elemental composition of 85–95% of carbon and 5–15% of oxygen (Fig. S4) augmenting the presence of organic materials in the collected aerosol particles,

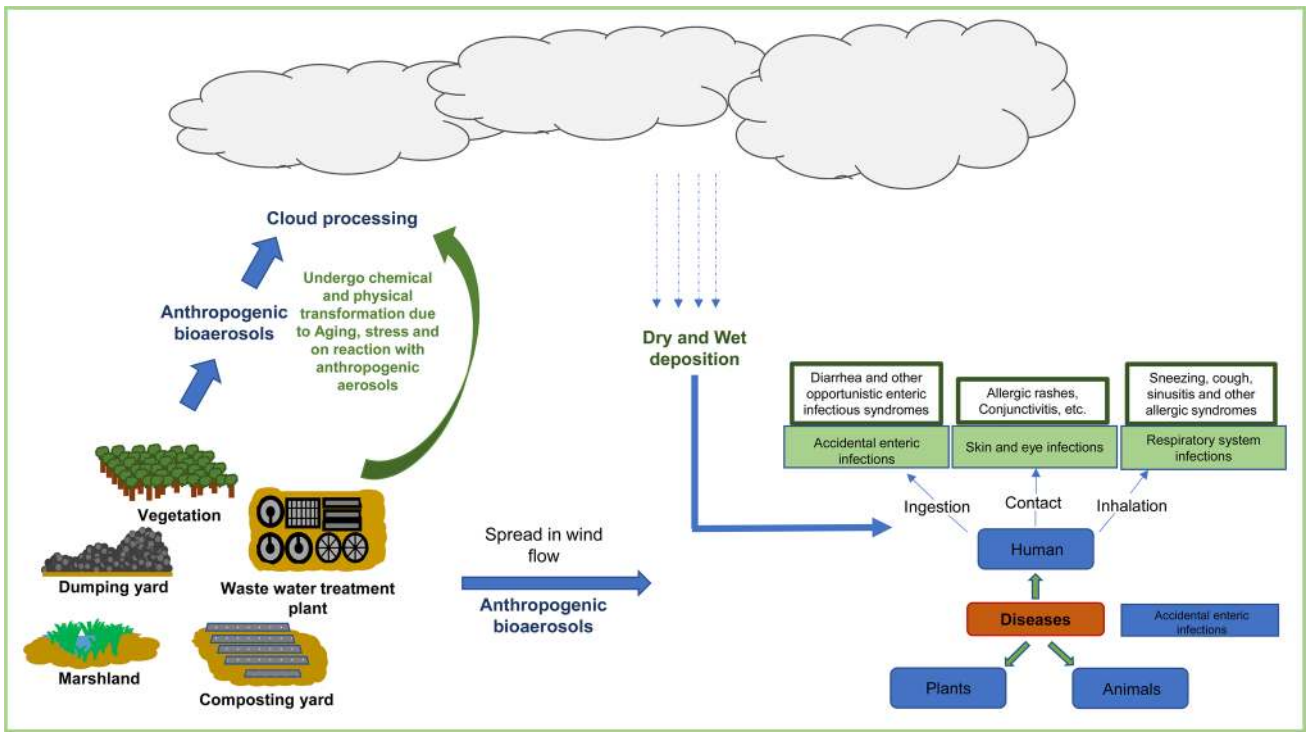


Fig. 5 Role of anthropogenic bioaerosols in the spread of disease, cloud processing, and precipitation

corroborating the fact that the investigated particles are of biological origin [14, 83, 84].

Du et al. [19] and Cao et al. [8] have stated that the studies on characterization of the bacterial and fungal bioaerosols in the atmospheric air play a vital role in understanding the atmospheric microbiota. Community taxonomic composition (Fig. 3a–c) of the sites studied deduced the presence of the phyla *Actinomycetales*, *Firmicutes*, *Alphaproteobacteria*, *Beta-proteobacteria*, *Gamma-proteobacteria*, *Bacteroidetes*, *Chloroflexi, Spirochetes*, other undefined phyla for bacterial bioaerosols and *Ascomycota*, *Basidiomycota*, *Mucormycota*, *Zygomycota*, *Zoopagomycota*, other undefined phyla for the fungal bioaerosols with the characteristic site-specific concentration (Figs. S2 and S3). Similar observations were made by researchers like Wery [89], Albrecht et al. [2], Le Goff et al. [53], Karra and Katsivela [46], and Dehghani et al. [16] for composting and wastewater treatment plant. Overall study inferred that the *Firmicutes* were the predominant phyla observed for bacterial bioaerosols [12, 38, 47, 68] and *Ascomycetes* for fungi as stated by Priyamvada et al. [65] in their study on occurrence of terrestrial macro-fungi in the tropical evergreen biome of southern India.

Anthropogenic bioaerosols of the study region demonstrated the presence of major families like *Bacillaceae*, *Planococcaceae*, *Staphylococcaceae*, *Pseudomonadaceae*, *Exiguobacterium*, *Oxalobacteraceae*, *Enterobacteriaceae*, *Micrococcaceae*, *Streptococcaceae*, *Cytophagaceae*, *Paenibacillaceae*, *Planococcaceae*, *Brucellaceae*, *Vibrionaceae*, *Xanthomonadaceae*, *Microbacteriaceae*, *Burkholderiaceae*, *Streptomycetaceae*, *Mycobacteriaceae*, *Corynebacteriaceae* for bacteria and *Pleosporaceae*, *Trichocomaceae*, *Mucoraceae*, *Cunninghamellaceae*, *Nectriaceae*, *Didymellaceae*, *Leptosphaeriaceae*, *Syncephalastraceae*, *Montagnulaceae*, *Bartaliniaceae*, *Amphisphaeriaceae*, *Davidellaceae*, *Ceratobasidiaceae*, *Saccharomycetaceae*, *Botryosphaeriaceae*, *Sordariaceae*, *Apiosporaceae*, *Didymosphaeriaceae*, *Mycosphaerellaceae* for fungi (Fig. 3a, b). Further, the bacterial and fungal bioaerosols have demonstrated the characteristic predominance of the pathogenic and environmental microbial families specific for each site (Fig. 3a–c) which are majorly influenced by various factors like the meteorological factors, activities observed, and the source characteristics of the site as discussed by researchers like Goudarzi et al. [29], Niazi et al. [61], Goudarzi et al. [30], Du et al. [19], and Grisoli et al. [33]. Furthermore, it is evident from Fig. 3c, Tables 1, S1, and S2 that the source has played an imperative role in contributing the microbes to the atmospheric air at the investigated site with anthropogenic activities as reported by researchers like Valsan et al. [82, 83] and Hjelmroose-Koski et al. [36]. A significant contribution from the source material (Fig. 3c) of about 53% for bacterial and ~ 34% for fungal bioaerosols was observed at NS1, about 30.9% for bacterial and 33.5% for fungal at NS2, 58.3% for bacterial, and 30.8% for fungal bioaerosols at AS1 has evidenced the role of source materials in contributing the bioaerosols to the atmospheric air. Furthermore, above results show that the source has contributed to almost half of the total bioaerosols concentration at the sites. Relatively, AS2 has exhibited a lower contribution rate from source materials of about 25% for bacteria and 27.8% for fungi, and AS3 with 42.9% and 23.1% for bacteria and fungi, respectively.

From the above observations, it is elucidated that the diverse nature of waste dumping yard (AS3) played a primary role in disseminating the anthropogenic microbes in the form of bioaerosols to the ambient atmosphere. This was followed by the marshland, wastewater treatment plant, composting site, and then the IITM site. Similarly, observations have been reported in many studies recognizing wastewater treatment plant as a potential source of bioaerosols that can cause human health implications [9, 24, 33, 63]. Moreover, it is also clearly evident from Fig. 3a–c and Table S2 that the source material present in the site has played a synergistic role in deciding the type and concentration of bioaerosols in the atmosphere. Further, anthropogenic activities in an urban setup can add up in the naturally pre-existing bioaerosols thus causing the elevated concentration, which could pose increased risk in acquiring respiratory system illness due to the sedimentation and deposition of these bioaerosols in the respiratory tract [18, 52, 85, 87].

Likewise, from Figs. 2, S2, and S3, it is conspicuous that the sites NS1, NS2, and AS1 have demonstrated a maximum risk to the whole respiratory system, AS2 posing possible risk to the tracheobronchial and alveolar region, and the site AS3 possibly risking the whole respiratory system as reported by Hofmann [37], Hussain et al. [42], and Nazaroff [59]. Interestingly, in accordance to the results obtained based on the size distribution of the bioaerosols (Figs. 2, S2, and S3), many literatures have evidenced the occurrence of hypersensitivity pneumonitis, reduction in lung function, severe asthma, organic dust toxic syndrome, airway inflammation, and respiratory disorders on prolonged human exposure to the fungal spores liberated from these sites [33, 55, 70]. Further, many other researchers have stated that the exposure to the anthropogenic activities including waste treatment facilities has resulted in the diseases like respiratory issues, skin disorders, gastrointestinal disorder, fever, eye irritation, headaches, nausea, and fatigue [20, 62, 81].

Furthermore, NCBI-BLAST analysis of the bioaerosols samples featured in Table S2 depicts the presence of plant pathogenic fungal species like *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium* sp., etc. and infectious bacterial species like *Bacillus*, *Pseudomonas*, *Staphylococcus*,

Streptococcus, *Streptomyces*, *Exiguobacterium*, *Klebsiella*, *Mycobacterium*, *Vibrio*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, etc. This clearly shows that the bioaerosols of the study region could pose a wide range of health effects like plant infections, animal infections, and human infections namely allergy, skin, eye, urinary, arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, cerebritis, and subcutaneous infections affecting the ecosystem health as evidenced from Rylander [71], Thorn and Kerekes [80], Orsini et al. [63], Smit et al. [76], Heinonen-Tanski et al. [35], Madsen et al. [55], Korzeniewska [49], Fazlzadeh et al. [22], Huang et al. [39], Kallawicha et al. [45], Allermann et al. [3], Brown et al. [7], Coccia et al. [12], Frankel et al. [25], Hospodsky et al. [38], and Soto et al. [77]. Especially, the presence of species like *Bacillus anthracis* (please refer Table S2 for the accession numbers of all the reported organism) is also demonstrated in the study, which are highly unlikely as such organisms can cause severe infectious syndrome to human and animal population residing near the study region as reported by other researchers like Langer et al. [51], Adams et al. [1], Bhangar et al. [5, 6], Meadow et al. [56, 57], Qian et al. [67, 68], and You [91].

Further, Du et al. [19] in their study have explained clearly the necessity and importance of the study related to diversity of microbial community in the bioaerosols as the load varies with season, meteorological conditions, geographical locations, and dispersal/release mechanisms adopted by the microorganisms present in the source materials. Hence, the alpha diversity (Table 1) analysis of the study region has revealed that the fungal communities exhibited highest diversity than the bacterial community which is in agreement with the findings made by Priyamvada et al. [66] and Valsan et al. [82] for the natural sites. Moreover, Gini–Simpson's diversity index (1-*D*) and Shannon diversity index (*H*) have inferred that the site AS3 (dumping yard) has exhibited highest bacterial diversity and AS1a (grit chamber of wastewater treatment plant) exhibited a highest fungal abundance with highest diversity at NS2 (marshland site) and AS2 (composting site) as described by Wei et al. [88] for the abundance and community structure of the airborne microbiota in an urban setup.

The beta diversity analysis using PCoA (Fig. 4) of the study region has shown that the bacterial community (Fig. 4a) has demonstrated different community compositions specific to the source materials, with NS2, AS1, and AS3 sharing some common species indicative of the presence of similar source materials in the site. Similarly, the fungal community composition at NS2, AS1, AS2, and AS3 was found to share similar species indicating the similar nature of source materials and the activity observed in these sites. Further, Fig. 4b infers the presence most similar fungal communities in AS2 and AS3, and overall presence of similar microbial community composition at NS2, AS1, and AS3 indicating the presence of similar source materials and activities in the sites.

5 Conclusion

The study provides comprehensive information about the size-dependent bacterial and fungal CFU concentrations at five different potential seminatural and anthropogenic bioaerosol sources of the marine urban southern tropical Indian city Chennai along with its community composition and diversity. The type and diversity reported here for various sites are the representative of very peculiar scenario corresponding to the very nature of the site under specific condition and season. Therefore, the type, diversity, and concentration observed should not be generalized over a larger spatiotemporal scale. For the further improved understanding of the mechanism, long-term sampling in the close vicinity of these potential sources is highly recommended. From the study, it is clearly evident that all the five sites located in the southern, central, and northern parts of the city have played an important role in the contribution of the pathogenic bioaerosols load to the ambient atmosphere of the study region. Further, the dumping yard located at the southern parts of the city has contributed considerably to the pathogenic bioaerosols which was further enhanced by the marshland site, and other sites. Studies on the size-dependent community composition revealed the presence of the different bacterial and the fungal phyla which pose a definite risk to respiratory diseases targeting the tracheobronchial, alveolar, and the air ways region. Further, studies on the microbiota of the study region inferred the presence of pathogens that can cause eye, skin, and enteric infections along with some plant, animal, and insect pathogens. Alpha and beta diversity indices conclude that the community of microbes present in each site has demonstrated a specificity to the source material and the activities pertinent to the site. Further, it has also inferred that the sites with similar activities and source materials have shared many species of both the bacterial and fungal communities.

However, the physical and chemical transformations of these bioaerosols along with the naturally occurring bioaerosols that take place due to their prolonged presence in the atmosphere are still poorly understood. Further studies, investigating the detailed source characteristics and properties of specific bacterial and/or fungal spores of the anthropogenic origin, are necessary to study their impact on ecosystem health and regional climate. Such information could be of great interest to climatologists and environmentalists to conserve the ecosystem and ecosystem health, and to better understand the role of bioaerosols on regional climate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Adams RI, Bhangar S, Pasut W, Arens EA, Taylor JW, Lindow SE, Nazaroff WW, Bruns TD (2015) Chamber bioaerosol study: outdoor air and human occupants as sources of indoor airborne microbes. PLoS ONE 10:0128022. https://doi.org/10.1371/journal.pone.0128022
2. Albrecht A, Witzenberger R, Bernzen U, Jackel U (2007) Detection of airborne microbes in a composting facility by cultivation based and cultivation independent methods. Ann Agric Environ Med 14:81–85
3. Allermann L, Wilkins CK, Madsen AM (2006) Inflammatory potency of dust from the indoor environment and correlation to content of NAGase and fungi. Toxicol In Vitro 20:1522–1531. https://doi.org/10.1016/j.tiv.2006.06.004
4. Arzt J, Juleff N, Zhang Z, Rodriguez LL (2011) The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound Emerg Dis 58:291–304. https://doi.org/10.1111/j.1865-1682.2011.01204.x
5. Bhangar S, Adams RI, Pasut W, Huffman JA, Arens EA, Taylor JW, Bruns TD, Nazaroff WW (2015) Chamber bioaerosol study: human emissions of size-resolved fluorescent biological aerosol particles. Indoor Air 23:193–206. https://doi.org/10.1111/ina.12195
6. Bhangar S, Huffman JA, Nazaroff WW (2014) Size-resolved fluorescent biological aerosol particle concentrations and occupant emissions in a university classroom. Indoor Air 24:604–617. https://doi.org/10.1111/ina.12111
7. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. Sci Transl Med 4:165–213. https://doi.org/10.1126/scitranslmed.3004404
8. Cao C, Jiang W, Wang B, Fang J, Lang J, Tian G, Jiang J, Zhu TF (2014) Inhalable microorganisms in Beijing's PM2.5 and PM10 pollutants during a severe smog event. Environ Sci Technol 48(3):1499–1507
9. Carducii A, Tozzi E, Rubulotta E, Casini B, Cantiani L, Rovini E, Muscillo M, Pacini R (2000) Assessing airborne biological hazard from urban wastewater treatment. Water Res 34(4):1173–1178
10. Castillo JA, Staton SJR, Taylor TJ, Herckes P, Hayes MA (2012) Exploring the feasibility of bioaerosol analysis as a novel fingerprinting technique. Anal Bioanal Chem 403:15–26. https://doi.org/10.1007/s00216-012-5725-0
11. Chen XR, Pixin H, Kin F, Lu W, Li B, Gu Z, Song C, Wang J (2012) Concentrations and size distributions of airborne microorganisms in Guangzhou during summer. Aerosol Air Qual Res 12(6):1336–1344
12. Coccia AM, Gucci PM, Lacchetti I, Paradiso R, Scaini F (2010) Airborne microorganisms associated with waste management and recovery: biomonitoring methodologies. Ann Ist Super Sanita 46(3):288–292
13. Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce-N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci 102:5074–5078. https://doi.org/10.1073/pnas.0501526102
14. Coz E, Artinano B, Clark LM, Hernandez M, Robinson AL, Casuccio GS, Lersch TL, Pandis SN (2010) Characterization of fine primary biogenic organic aerosol in an urban area in the northeastern United States. Atmos Environ 44:3952–3962. https://doi.org/10.1016/j.atmosenv.2010.07.007
15. Crist TO, Veech JA, Gering JC, Summerville KS (2003) Partitioning species diversity across landscapes and regions: a hierarchical analysis of α, β, and γ-diversity. Am Nat 162:734–743
16. Dehghani M, Sorooshian A, Ghorbani M, Fazlzadeh M, Miri M, Badiee P, Parvizi A, Ansari M, Baghani AN, Delikhoon M (2018) Seasonal variation in culturable bioaerosols in a wastewater treatment plant. Aerosol Air Qual Res 18:2826–2839
17. Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Fröhlich-Nowoisky J, Elbert W, Andreae MO, Pöschl U, Jaenicke R (2012) Primary biological aerosol particles in the atmosphere: a review. Tellus B. https://doi.org/10.3402/tellusb.v64i0.15598
18. Douwes J, Thorne P, Pearce N, Heederik D (2003) Bioaerosols: health effects and exposure assessment: progress and prospects. Ann Occup Hyg 47(3):187–200
19. Du P, Du R, Ren W, Lu Z, Zhang Y, Fu P (2018) Variation of bacteria and fungi in PM2.5 in Beijing, China. Atmos Environ 172:55–64
20. Dutkiewicz J, Cholewa G, Sitkowska J, Krysinska Traczyk E, Skorska C, Prazmo Z (2003) Exposure to bioaerosols in a municipal sewage treatment plant. Ann Agric Environ Med 10:241–248
21. Ertz D, Lawrey JD, Sikaroodi M, Gillevet PM, Fischer E, Killmann D, Serusiaux E (2008) A new lineage of lichenized *Basidiomycetes* inferred from a two-gene phylogeny: the *Lepidostromataceae* with three species from the tropics. Am J Bot 95:1548–1556
22. Fazlzadeh M, Sadeghi H, Bagheri P, Poureshg Y, Rostami R (2016) Microbial quality and physical chemical characteristics of thermal springs. Environ Geochem Health 38:413–422
23. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194. https://doi.org/10.1038/nature10947
24. Fracchia L, Pietronave S, Rinaldi M, Martinetti MG (2006) The assessment of airborne bacterial contamination in three composting plants revealed site-related biological hazard and seasonal variations. J Appl Microbiol 100(5):973–984
25. Frankel M, Beko G, Timm M, Gustavsen S, Hansen EW, Madsen AM (2012) Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. Appl Environ Microbiol 78(23):8289–8297. https://doi.org/10.1128/AEM.02069-12
26. Frohlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pohlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, Su H, Hoor P, Thines E, Hoffmann T, Despres VR, Poschl U (2016) Bioaerosols in Earth system: climate, health, and ecosystem

interactions. Atmos Res 182:346–376. https://doi.org/10.1016/j.atmosres.2016.07.018
27. Fröhlich-Nowoisky J, Pickersgill DA, Despres VR, Pöschl U (2009) High diversity of fungi in air particulate matter. Proc Natl Acad Sci 106:12814–12819. https://doi.org/10.1073/pnas.0811003106
28. Ganthaler A, Mayr S (2015) Temporal variation in airborne spore concentration of *Chrysomyxa rhododendri*: correlation with weather conditions and consequences for Norway spruce infection. For Pathol 45(6):347
29. Goudarzi G, Shirmardi M, Khodarahmi F, Hashemi Shahraki A, Alavi N, Ankali KA, Babaei AA, Soleimani Z, Marzouni MB (2014) Particulate matter and bacteria characteristics of the Middle East Dust (MED) storms over Ahvaz, Iran. Aerobiologia 30:345–356
30. Goudarzi G, Soleimani Z, Sadeghinejad B, Alighardashi M, Latifi SM, Moradi M (2016) The impact of visiting hours on indoor to outdoor ratio of fungi concentration at university hospitals in Ahvaz, Iran. J Adv Environ Health Res 4:1–8
31. Grand LF, Moore RT (1970) Ultracyto taxonomy of *Basidiomycetes*: scanning electron microscopy of spores. J Elisha Mitchell Sci Soc 86:106–117
32. Grand LF, Vandyke CG (1976) Scanning electron-microscopy of basidiospores of species of *Hydnellum*, *Hydnum*, *Phellodon*, and *Bankera* (*Hydnaceae*). J Elisha Mitchell Sci Soc 92:114–123
33. Grisoli P, Rodolfi M, Villani S, Grignani E, Cottica D, Berri A, Picco AM, Dacarro C (2009) Assessment of airborne microorganism contamination in an industrial area characterized by an open composting facility and a wastewater treatment plant. Environ Res 109:135–142
34. Healy DA, Huffman JA, O'Connor DJ, Pöhlker C, Pöschl U, Sodeau JR (2014) Ambient measurements of biological aerosol particles near Killarney, Ireland: a comparison between real-time fluorescence and microscopy techniques. Atmos Chem Phys 14:8055–8069. https://doi.org/10.5194/acp-14-8055-2014
35. Heinonen-Tanski H, Reponen T, Koivunen J (2009) Airborne enteric coliphages and bacteria in sewage treatment plants. Water Res 43:2558–2566
36. Hjelmroos-Koski MK, Macher JM, Hammond SK, Tager I (2006) Considerations in the grouping of plant and fungal taxa for an epidemiologic study. Grana 45:261–287
37. Hofmann W (2011) Modelling inhaled particle deposition in the human lung-a review. J Aerosol Sci 42:693–724. https://doi.org/10.1016/j.jaerosci.2011.05.007
38. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, Peccia J (2012) Human occupancy as a source of indoor airborne bacteria. PLoS ONE. https://doi.org/10.1371/journal.pone.0034867
39. Huang HL, Lee MK, Shih HW (2017) Assessment of indoor bioaerosols in public spaces by real-time measured airborne particles. Aerosol Air Qual Res 17:2276–2288
40. Huffman JA, Sinha B, Garland RM, Snee-Pollmann A, Gunthe SS, Artaxo P, Martin ST, Andreae MO, Pöschl U (2012) Size distributions and temporal variations of biological aerosol particles in the Amazon rainforest characterized by microscopy and real-time UV-APS fluorescence techniques during AMAZE-08. Atmos Chem Phys 12:11997–12019. https://doi.org/10.5194/acp-12-11997-2012
41. Hurtado L, Rodriguez G, Lopez J, Castillo JE, Molina L, Zavala M, Quintana PJE (2014) Characterization of atmospheric bioaerosols at 9 sites in Tijuana, Mexico. Atmos Environ 96:430–436. https://doi.org/10.1016/j.atmosenv.2014.07.018
42. Hussain M, Madl P, Khan A (2011) Lung deposition predictions of airborne particles and the emergence of contemporary diseases, part-I. Health 2:51–59
43. Jaenicke R (2005) Abundance of cellular material and proteins in the atmosphere. Science 308(5718):73–73
44. Kaasalainen U, Fewer DP, Jokela J, Wahlsten M, Sivonen K, Rikkinen J (2012) Cyanobacteria produce a high variety of hepatotoxic peptides in lichen symbiosis. Proc Natl Acad Sci 109:5886–5891. https://doi.org/10.1073/pnas.1200279109
45. Kallawicha K, Chen YC, Chao HJ, Shen WC, Chen BY, Chuan YC, Guo YL (2017) Ambient fungal spore concentration in a subtropical metropolis: temporal distributions and meteorological determinants. Aerosol Air Qual Res 17:2051–2063
46. Karra S, Katsivella E (2007) Microorganisms in bioaerosols emissions from wastewater treatment plants during summer at a Mediterranean site. Water Res 41:1355–1365. https://doi.org/10.1016/j.watres.2006.12.014
47. Kaushik R, Balasubramanian R (2013) Discrimination of viable from non-viable Gram-negative bacterial pathogens in airborne particles using propidium mono azide-assisted qPCR. Sci Total Environ 449:237–243
48. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, Bohannan BJ, Brown GZ, Green JL (2012) Architectural design influences the diversity and structure of the built environment microbiome. ISME J 6(8):1469
49. Korzeniewska E (2011) Emission of bacteria and fungi in the air from wastewater treatment plants—a Review. Front Biosci 3:393–407
50. Legendre P, Legendre L (1998) Numerical ecology, 2nd edn. Elsevier, Amsterdam
51. Langer V, Hartmann G, Niessner R, Seidel M (2012) Rapid quantification of bioaerosols containing *L. pneumophila* by Coriolis® µ air sampler and chemiluminescence antibody microarrays. J Aerosol Sci 48:46–55. https://doi.org/10.1016/j.jaerosci.2012.02.001
52. Laumbach RJ, Kipen HM (2005) Bioaerosols and sick building syndrome: particles, inflammation, and allergy. Curr Opin Allergy Clin Immunol 5:135–139
53. Le Goff O, Bru-Adan V, Bacheley H, Godon JJ, Wery N (2010) The microbial signature of aerosols produced during the thermophilic phase of composting. J Appl Microbiol 108:325–340. https://doi.org/10.1111/j.13652672.2009.04427.x
54. Lee J-H, Jo W-K (2006) Characteristics of indoor and outdoor bioaerosols at Korean High-rise apartment buildings. Environ Res 101:11–17
55. Madsen AM, Schlünssen V, Olsen T, Sigsgaard T, Avci H (2009) Airborne fungal and bacterial components in PM1 dust from biofuel plants. Ann Occup Hyg 53:749–757
56. Meadow JF, Altrichter AE, Bateman AC, Stenson J, Brown G, Green JL, Bohannan BJM (2015) Humans differ in their personal microbial cloud. Peer J 3:1258. https://doi.org/10.7717/peerj.1258
57. Meadow JF, Altrichter AE, Kembel SW, Kline J, Mhuireach G, Moriyama M, Northcutt D, O'Connor TK, Womack AM, Brown GZ, Green JL, Bohannan BJM (2014) Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor Air 24:41–48. https://doi.org/10.1111/ina.12047
58. Morris CE, Conen F, Huffman AJ, Phillips V, Poschl U, Sands DC (2014) Bioprecipitation: a feedback cycle linking earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. Glob Change Biol 20:341–351. https://doi.org/10.1111/gcb.12447
59. Nazaroff WW (2015) Indoor bioaerosol dynamics. Indoor Air. https://doi.org/10.1111/ina.12174
60. Nevalainen A, Pastuszka J, Liebhaber F, Willeke K (1992) Performance of bioaerosol samplers: collection characteristics and sampler design considerations. Atmos Environ 26A:531–540
61. Niazi S, Hassanvand MS, Mahvi AH, Nabizadeh R, Alimohammadi M, Nabavi S, Faridi S, Dehghani A, Hoseini M, Moradi-Joo M (2015) Assessment of bioaerosol contamination (bacteria and

fungi) in the largest urban wastewater treatment plant in the Middle East. Environ Sci Pollut Res 22:16014–16021
62. Oppliger A, Hilfiker S, Vu Duc T (2005) Influence of seasons and sampling strategy on assessment of bioaerosols in sewage treatment plants in Switzerland. Ann Occup Hyg 49:393–400
63. Orsini M, Laurenti P, Boninti F, Arzani D, Lanni A, Romano-Spica V (2002) A molecular typing approach for evaluating bioaerosol exposure in wastewater treatment plant workers. Water Res 36(5):1375–1378
64. Pankhurst LJ, Deacon LJ, Liu J, Drew GH, Hayes ET, Jackson S, Longhurst PJ, Longhurst JWS, Pollard SJT, Tyrrel SF (2011) Spatial variations in airborne microorganism and endotoxin concentrations at green waste composting facilities. Int J Hyg Environ Health 214:376–383. https://doi.org/10.1016/j.ijheh.2011.06.001
65. Priyamvada H, Akila M, Singh RK, Ravikrishna R, Verma RS, Philip L, Marathe RR, Sahu LK, Sudheer KP, Gunthe SS (2017) Terrestrial macrofungal diversity from the tropical dry evergreen biome of southern India and its potential role in aerobiology. PLoS ONE 12(1):1–21. https://doi.org/10.1371/journal.pone.0169333
66. Priyamvada H, Singh RK, Akila M, Ravikrishna R, Verma RS, Gunthe SS (2017) Seasonal variation of the dominant allergenic fungal aerosols—one-year study from southern Indian region. Sci Rep 7:1–12. https://doi.org/10.1038/s41598-017-11727-7
67. Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J (2012) Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. Indoor Air 22:339–351. https://doi.org/10.1111/j.1600-0668.2012.00769.x
68. Qian J, Peccia J, Ferro AR (2014) Walking-induced particle resuspension in indoor environments. Atmos Environ 89:464–481. https://doi.org/10.1016/j.atmosenv.2014.02.035
69. Rohde RA, Muller RA (2015) Air pollution in China: mapping of concentrations and sources. PLoS ONE 10(8):135749
70. Rostami R, Naddafi K, Aghamohamadi A, Saleh HN, Davil MF (2009) Survey of peanut fungal contamination and its relationship with ambient conditions in the Bazar of Zanjan. Iran J Environ Health Sci Eng 6:295–300
71. Rylander R (1999) Health effects among workers in sewage treatment plants. Occup Environ Med 56:354–357
72. Sache I (2000) Short-distance dispersal of wheat rust spores by wind and rain. Agronomie 20(7):757–767
73. Schloss PD, Westcott SL (2011) Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. Appl Environ Microbiol 77(10):3219–3226
74. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Horn DJV, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537–7541. https://doi.org/10.1128/AEM.01541-09
75. Schumacher CJ, Pöhlker C, Aalto P, Hiltunen V, Petäjä T, Kulmala M, Pöschl U, Huffman JA (2013) Seasonal cycles of fluorescent biological aerosol particles in boreal and semiarid forests of Finland and Colorado. Atmos Chem Phys 13:11987–12001. https://doi.org/10.5194/acp-13-11987-2013
76. Smit LA, Spaan S, Heederik D (2005) Endotoxin exposure and symptoms in wastewater treatment workers. Am J Ind Med 48:30–39
77. Soto T, Lozano M, Vicente-Soler J, Cansado J, Gacto M (2009) Microbiological survey of the aerial contamination in urban areas of the city of Murcia, Spain. Ann Biol 31:7–13
78. Tang JW (2009) The effect of environmental parameters on the survival of airborne infectious agents. J R Soc Interface 6(Suppl. 6):S737–S746
79. Tesson SVM, Skjøth CA, Šantl-Temkiv T, Löndahl J (2016) Airborne microalgae: insights, opportunities, and challenges. Appl Environ Microbiol 82:1978–1991. https://doi.org/10.1128/AEM.03333-15
80. Thorn J, Kerekes E (2001) Health effects among employees in sewage treatment plants: a literature survey. Am J Ind Med 40:170–179
81. Uhrbrand K, Schultz AC, Madsen AM (2011) Exposure to airborne noroviruses and other bioaerosol components at a wastewater treatment plant in Denmark. Food Environ Virol 3:130–137
82. Valsan AE, Priyamvada H, Ravikrishna R, Despres VR, Biju CV, Sahu LK, Kumar A, Verma RS, Philip L, Gunthe SS (2015) Morphological characteristics of bioaerosols from contrasting locations in southern tropical India—a case study. Atmos Environ 122:321–331. https://doi.org/10.1016/j.atmosenv.2015.09.071
83. Valsan AE, Ravikrishna R, Biju CV, Pohlker C, Despres VR, Huffman JA, Poschl U, Gunthe SS (2016) Fluorescent biological aerosol particle measurement at a tropical high-altitude site in southern India during southwest monsoon season. Atmos Chem Phys 16:9805–9830. https://doi.org/10.5194/acp-16-9805-2016
84. Vestlund TA, Al-Ashaab R, Tyrrel FS, Longhurst JP, Pollard SJT, Drew GH (2014) Morphological classification of bioaerosols from composting using scanning electron microscopy. Waste Manag 34:1101–1108
85. Walinder R, Norback D, Wessen B, Venge P (2001) Nasal lavage biomarkers: effects of water damage and microbial growth in an office building. Arch Environ Occup Health 56:30–36
86. Warfel JM, Beren J, Merkel TJ (2012) Airborne transmission of *Bordetella pertussis*. J Infect Dis 206:902–906. https://doi.org/10.1093/infdis/jis443
87. Wei K, Zou Z, Yao M (2014) Charge levels and Gram (±) fractions of environmental bacterial aerosols. J Aerosol Sci 74:52–62. https://doi.org/10.1016/j.jaerosci.2014.04.002
88. Wei K, Zou Z, Zheng Y, Li J, Shen F, Wu CY et al (2016) Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing. Sci Total Environ 550:751–759. https://doi.org/10.1016/j.scitotenv.2016.01.137
89. Wery N (2014) Bioaerosols from composting facilities—a review. Front Cell Infect Microbiol 4:1–9
90. Womack AM, Bohannan BJM, Green JL (2010) Biodiversity and biogeography of the atmosphere. Philos Trans R Soc B 365:3645–3653. https://doi.org/10.1098/rstb.2010.0283
91. You R (2013) Measuring the short-term emission rates of particles in the "personal cloud" with different clothes and activity intensities in a sealed chamber. Aerosol Air Qual Res 13:911–921. https://doi.org/10.4209/aaqr.2012.03.0061
92. Yousef AD, Joanne F (1984) Mould allergy. Domson, Lea, Febiger, Philadelphia. https://trove.nla.gov.au/version/25790935
93. Yu OHY, Keet AWW, Sheppard DC, Brewer T (2010) Articular aspergillosis: case report and review of the literature. Int J Infect Dis 14:433–435. https://doi.org/10.1016/j.ijid.2009.05.012
94. Zhou Y, Khu S-T, Xi B, Su J, Hao F, Wu J, Huo S (2014) Status and challenges of water pollution problems in China: learning from the European experience. Environ Earth Sci 72(4):1243–1254