

Assessment of airborne microbial community in Indian cities during the Middle East dust storm

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Globally dust storms have greatly affected air quality, and nearly 5.0 billion tonnes of dust undergoes migration every year. A plethora of microorganisms spread far and wide along with the dust particles during dust storms. In April 2015, a dust storm originated from the Middle East and travelled to many Indian cities. We analysed the impact of the dust storm on the diversity and composition of aerial microorganisms using cultivation-based methods. Sampling was done in three cities (Mumbai, Lonavala and Pune) during a dust storm day (DSD) and a post-dust storm day (PSD). A total of 580 morphologically different bacteria and then ten mycelial fungi were isolated during the study. Identification based on MALDI-TOF MS biotyping, sequencing of 16S rRNA gene and ITS region revealed that the bacterial isolates belonged to 32 different genera and fungi to four different genera. Principal coordinate analysis exhibited separate grouping of DSD and PSD samples, indicating the shift in microbial communities. Osmotolerant and halotolerant bacterial genera, viz. *Psychrobacter* and *Exiguobacterium* were recorded specifically during DSD. The proportion of opportunistic pathogens, including *Staphylococcus* and *Enterobacter* was high during DSD in comparison to PSD. Overall, the study reveals the influence of dust storms on the aerial microbial composition and indicates the possible spread of specific microbial species during a dust storm event.

Keywords: Airborne, dust storm, high-throughput cultivation, microorganisms, mass spectroscopy.

THE study on migration and spread of microorganisms has lured researchers since long, because of its likely impacts on the environment, human and animal health¹. The primary modes of microbial dispersion involve currents of water and wind and can survive through harsh conditions efficiently, thus establishing their presence and growth in new lands². Dust storms originate when high-velocity winds blow-off the topsoil from the dry

grounds and deserts³. These strong wind currents are known to blow-off the topsoil particles, dust and soil-inhabiting microbes being carried by the wind. A minimum of 1 million tonnes of airborne soil is known to circumvent the earth in a year, carrying approximately 10^{16} dust-borne bacteria⁴. Microbiological analyses of dust storm aerosol samples have proven the presence of marine bacteria, plant pathogens and animal pathogens which cause outbreaks of infection and disease⁵.

Dust Storms are frequent in the Sahara desert located in the Northern Africa, Taklamakan and Badain Jaran deserts of China, and the Arabian desert stretching from Yemen and Oman to Jordan and Iraq⁶. Phenomena such as suspension and saltation are responsible for the drifting of fine dust particles and microbes over extremely long distances⁷. These processes affect coral reefs, impact human health, fertilize oceans, influence photochemistry and cloud formation and also carry microorganisms⁸. Previously, African dust storms have transported pathogenic *Aspergillus* species that caused aspergillosis in the sea fans of Virgin Islands⁸. Air samples over the Arctic and Atlantic waters were found to carry microorganisms that were similar to the microorganisms recovered from the Caribbean atmosphere⁹. These studies indicate that dust storms are a major factor in the transport of microbes, helpful or harmful, making it important to characterize the microbial diversity of a dust storm to ensure its impact on environmental health.

On 2 April 2015, an intense dust storm was seen spanning over the Middle East and by 4 April, it was visibly moving towards the Indian peninsula. The storm hit the Arabian coast on 7 April 2015, affecting parts of Maharashtra and significantly reduced visibility of the affected areas. This was further confirmed by satellite images (Figure 1 i). The present study was undertaken to enhance our understanding of the status of dust-borne microorganisms reaching the Indian peninsula travelling from the Gulf countries and also to determine if culturable populations could be detected after travelling more than 2000 km across the Arabian Sea. Moreover, we also evaluated airborne microbial community on a dust storm day (DSD) and on a post-dust storm day (PSD) in three Indian cities.

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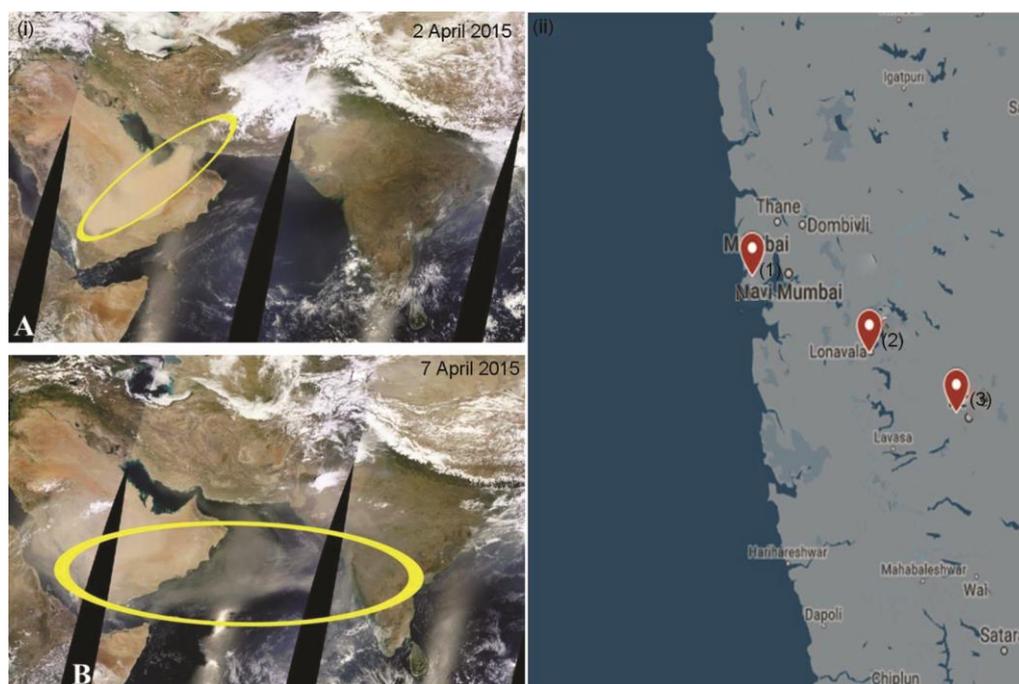


Figure 1. Satellite images of the dust storm, (A) its origin at the Arabian Peninsula on 2 April 2015 and (B) reaching the Indian peninsula on 7 April 2015, Source: earthdata.nasa.gov. Sample collection sites marked in the state of Maharashtra, India, (site 1) Mumbai (19.07°N, 72.87°E, elevation 14 m amsl); (site 2) Lonavala (18.75°N, 73.4°E, elevation 624 m amsl); and (site 3) Pune (18.5°N, 73.85°E, elevation 562 m amsl).

Materials and methods

Sample sites

With the dust storm moving towards the Indian peninsula from the Arabian coast, samples were collected at three sites in Maharashtra, India. All locations were chosen with varying distances from the coast – Mumbai (19.07°N, 72.87°E, elevation 14 m amsl) which is along the coast of Maharashtra, Lonavala (18.75°N, 73.4°E, elevation 624 m amsl) at a distance of 95 km from the coast and Pune city (18.5°N, 73.85°E, elevation 562 m amsl) around 203 km away from the seashore (Figure 1 ii). Mumbai city is situated along the coast while Lonavala is located on Sahyadri mountain slopes and Pune on the Deccan Plateau.

Humidity, velocity and particulate matter

Data on humidity, velocity and particulate matter (PM_{2.5} and PM₁₀) were shared by the Indian Institute of Tropical Meteorology (IITM) Pune, which were collected under the System of Air Quality and Weather Forecasting and Research (SAFAR) project.

Collection of samples

Sampling was performed by exposing pre-sterilized culture plates of different media to air at the three locations. Two batches of sterile culture media plates were exposed

for 20 and 60 min respectively. The media used were Reasoner's 2A agar, nutrient agar, dilute nutrient agar (1 : 10), actinomycete isolation agar, rose bengal agar and water agar. The plates were incubated at 27°C for 24, 48 and 72 h. A pure culture of each isolate was stored in 20% glycerol as frozen stock at –80°C.

Identification using mass spectrometry

Different morphotypes were chosen from each plate and pure bacterial cultures were subjected to Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF-MS). A thin film of bacterial cells was coated with 1 µl of α -cyano-4-hydroxycinnamic acid (HCCA) (10 mg/ml) matrix, dried and subjected to further analysis. Ethanol/formic acid extraction method was used for the samples that could not generate MALDI-TOF mass spectra by direct sample method¹⁰. Principal component analysis (PCA) dendrograms were generated using MALDI Biotyper software for isolates from each sampling location.

16S rRNA gene sequencing

DNA isolation was done with the Invitrogen PureLink Pro96-Genomic DNA isolation kit using the manufacturer's protocol. The DNA was quantified by NanoDrop™. The 16S region of the genomic DNA was then amplified using 16S specific amplification primers 27F and 1525R

Table 1. Concentration of particulate matter during dust storm and normal days

Location	Dust storm day				Normal day			
	PM 2.5 ($\mu\text{g}/\text{m}^3$)	PM 10 ($\mu\text{g}/\text{m}^3$)	Relative humidity (%)	Velocity (km/h)	PM 2.5 ($\mu\text{g}/\text{m}^3$)	PM 10 ($\mu\text{g}/\text{m}^3$)	Relative humidity (%)	Velocity (km/H)
Mumbai	170.6	102.5	67	34	68.07	153.6	60	8.5
Lonavala	111.7	80.12	44	32	50.12	130.1	40	9
Pune	52.88	57.9	20	32	31.78	98.28	22	9.3

*Data collected by System of Air Quality and Weather Forecasting and Research (SAFAR) systems, IITM, Pune.

Table 2. Colony forming units on the respective media at different exposure times

Location	Exposure time (min)	R2A	NA	DNA	AIA	WA
Pune	DS20	201 ^d	44 ^{bc}	74 ^b	13 ^{bcd}	10 ^{abc}
	DS60	280 ^{bc}	70 ^{ab}	85 ^{ab}	12 ^{bcd}	9 ^{abc}
	CN20	103 ^e	35 ^c	25 ^{cd}	8 ^{cde}	7 ^c
	CN60	111 ^e	55 ^{abc}	32 ^{cd}	8 ^{cde}	11 ^{abc}
Lonavala	DS20	256 ^c	77 ^{ab}	96 ^a	15 ^{bc}	8 ^{bc}
	DS60	280 ^{bc}	60 ^{abc}	88 ^{ab}	16 ^b	8 ^{bc}
	CN20	85 ^{ef}	52 ^{abc}	26 ^{cd}	4 ^e	4 ^c
	CN60	97 ^{ef}	40 ^c	22 ^d	5 ^e	16 ^a
Mumbai	DS20	300 ^{ab}	78 ^{ab}	44 ^c	39 ^a	15 ^{ab}
	DS60	327 ^a	83 ^a	69 ^b	46 ^a	17 ^a
	CN20	70 ^f	42 ^{bc}	20 ^d	2 ^e	5 ^c
	CN60	97 ^{ef}	40 ^c	40 ^{cd}	3 ^e	16 ^a

Values are the mean of three replicates. Values with common letters in a column do not differ statistically according to Tukey's HSD test at $P \leq 0.01$. Here $a > b > c > d > e > f$. R2A, Reasoner's 2 agar; NA, Nutrient agar, DNA, Diluted nutrient agar; AIA, Actinomycete isolation agar; WA, Normal water agar.

(ref. 11). The amplified PCR product was purified by polyethylene glycol (Peg)-NaCl precipitation¹². The DNA was then sequenced using a Sanger sequencer. The generated sequence was further edited using ChromasPro software and searched for identity in the 16S rRNA gene sequence database and taxonomy search tool of EzBiocloud¹³. Newly generated sequences were submitted to NCBI Genbank and the accession numbers are listed in [Supplementary Table 1](#).

Statistical analysis

Data on total microbial count were checked for normality and subjected to one-way analysis of variance (ANOVA) using the STATISTICA data analysis software system version 7 (StatSoft Inc., Tulsa, USA, 2004). We applied Good's coverage for a better understanding of the isolations performed¹⁴. We also applied Welch's *t*-test with Benjamini-Hochberg FDR correction to examine the significantly different bacterial families between normal and dust storm. Kruskal-Wallis test was applied to examine the location specific microbial species.

Results

Particulate matter analysis

The aerial particulate matter PM_{2.5} during the DSD (7 April 2015) ranged from 58.88 to 170.56 and showed

significant ($P < 0.05$) increase in concentration in comparison to PSD (18 May 2015) from 31.78 to 68.07 (Table 1). In contrast, PM₁₀ was more on PSD (range 98.28–153.62) in comparison to DSD (range 57.9–102.53). The aerial particulate matter and humidity were higher in Mumbai during both sampling days in comparison to Pune and Lonavala.

Total microbial count

The microbial count recorded on plates exposed during DSD was significantly high compared to that recorded during PSD (Table 2). Among different growth media, the highest microbial count was observed on R2A medium. The count on R2A plates during DSD was almost double in comparison to colony count on the same medium plates during PSD. Microbial colonies obtained on nutrient agar, dilute nutrient agar and actinomycete isolation agar showed significantly higher number in comparison to water agar and rose Bengal medium. An increase was observed in the number of colonies in plates with 60 min exposure in comparison to those exposed for 20 min; however, this increase was not statistically significant.

Identification of microorganisms

A total of 283 isolates were selected from DSD samples based on their diverse morphotypes, while 286 isolates

were selected in case of PSD samples. MALDI-TOF MS-based identification resulted in 310 (53.44%) isolates. In addition, good quality spectra were generated for 270 isolates. The PCA dendrograms constructed based on MALDI-TOF mass spectra of microbial isolates (163 and 152 isolates for PSD and DSD respectively) led to the grouping of isolates under 13 and 15 major clusters at similarity levels 6 and 5 for PSD and DSD samples respectively.

Next, 16S rRNA gene sequencing was carried out for 50 bacterial isolates including 37 isolates with no reliable identity found during comparison with the Biotyper database and 13 isolates randomly selected among the identified isolates by Biotyper. These 50 isolates belonged to various genera, viz. *Bacillus*, *Micromonospora*, *Nocardiosis*, *Lysinibacillus*, *Jeotgalicoccus*, *Curtobacterium*, *Arthrobacter*, *Exiguobacterium*, *Noviherbaspirillum*, *Gracilibacillus*, *Micromonospora*, *Streptomyces*, *Staphylococcus*, *Paracoccus*, *Psychrobacter*, *Ruegeria*, *Microbacterium*, *Brachybacterium*, *Stenotrophomonas*, *Providencia*, *Enterobacteria* and *Curvibacter* ([Supplementary Table 1](#)). The identification results by MALDI-TOF MS and sequencing were comparable as there was no misidentification using both methods. The results of ITS gene sequencing of ten fungal isolates showed the presence of four genera, viz. *Aspergillus*, *Alternaria*, *Curvularia* and *Cladosporium* ([Supplementary Table 2](#)).

Table 3. Biodiversity indices calculated for normal and dust storm days

Biodiversity index	Normal day	Dust storm day
Number of genera	17	21
Shannon index	4.88	5.07
Simpson's index	0.05	0.08
Chao I	36.39	36.61
ACE	39.39	41.12
Jackknife	40.89	41.81
Good's coverage	0.929	0.966

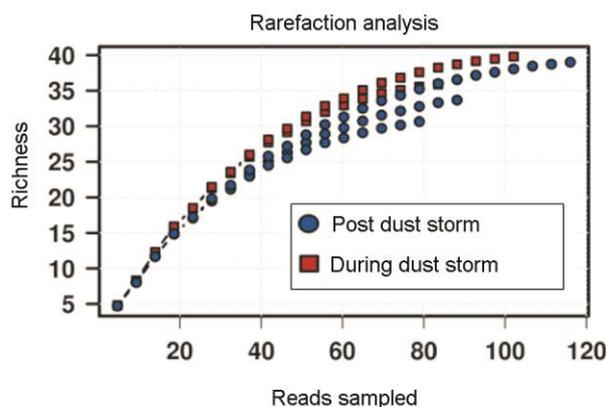


Figure 2. Rarefaction curves generated based on data number and identity isolates from post-dust storm day (PSD) and dust storm day (DSD) samples and analyses performed in CALYPSO using the furthest neighbour assignment algorithm.

Biodiversity analyses

Good's coverage indicated that a sufficient number of colonies was picked to represent bacterial diversity recovered in the growth media for both PSD and DSD samples (Table 3). Alpha diversity indices for Shannon, Simpson, Chao1, ACE and Jackknife were almost similar for both PSD and DSD. The rarefaction curve generated based on the presence, absence and abundance of unique microbial species recorded from each sample indicated that all the samples grew rapidly at first and then reached a plateau (Figure 2).

Bacterial community composition

The overall analysis of DSD and PSD samples revealed the presence of 92 different microbial species phylogenetically distributed across 32 genera which belong to four phyla, viz. *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroides* (Figure 3a and b). Members of *Bacillus*, *Arthrobacter*, *Staphylococcus*, *Kocuria*, *Streptomyces* and *Lysinibacillus* were the most dominant during both PSD and DSD, representing 51% of the total observed microbial species. The Venn diagram showed that members of the most dominating genera were present during both days, while 11 genera were uniquely present during PSD and 15 genera during DSD (Figure 4a and b). Species-level comparisons exhibited a complete shift in microbial composition during both days at all sites ([Supplementary Figure 1](#)).

In the UniFracPCoA plot, all samples collected during PSD were placed on the negative side of PCoA1 while samples collected during DSD were placed separately on the positive side of PCoA1 (Figure 5). The mean abundance of bacterial genera was significantly different for PSD and DSD samples at $P < 0.01$ (Figure 6). The differential genera during PSD were *Arthrobacter*, *Masilla*, *Noviherbaspirillum* and *Ochrobactrium*, and during DSD *Arthrobacter*, *Brachybacterium*, *Curtobacterium*, *Curvibacter*, *Dietiza*, *Enterobacter*, *Ensifer*, *Exigobacterium*, *Jeotgalicoccus*, *Providencia*, *Regureia* and *Serratia*.

Discussion

Dust deposition during dust storms has been considered relevant for nutrient deposition and soil development¹⁵⁻¹⁷. Recent studies on the shift in aerial microbial communities suggested that dust deposition can also introduce new soil-derived microorganisms, which may include pathogens, and consequently might lead to diverse effects on the environment and public health¹⁸⁻²⁰. In the present study we analysed the cultivable microbial communities during PSD and DSD in Mumbai, Lonavala and Pune.

The PM_{2.5}/PM₁₀ ratios during PSD were below the threshold (0.5–0.6 µg/m³) in all the three sites indicating

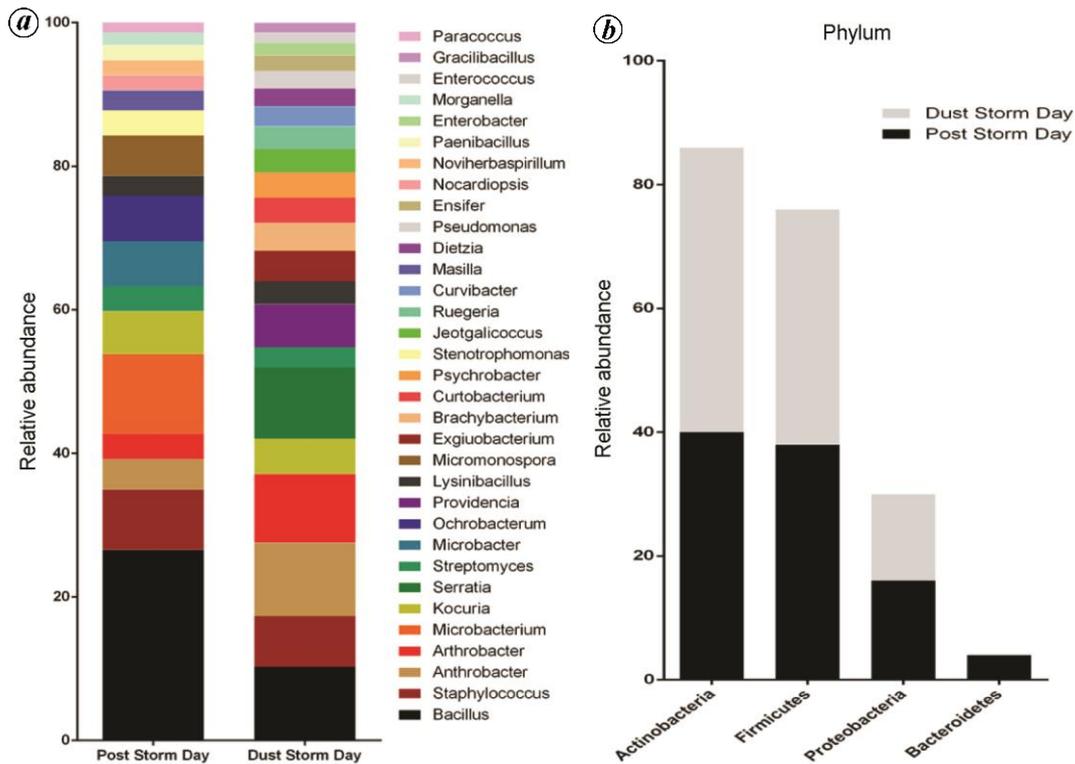


Figure 3. *a*, Distribution and abundance of genera. *b*, Relative abundance of four major phyla in PSD and DSD samples.

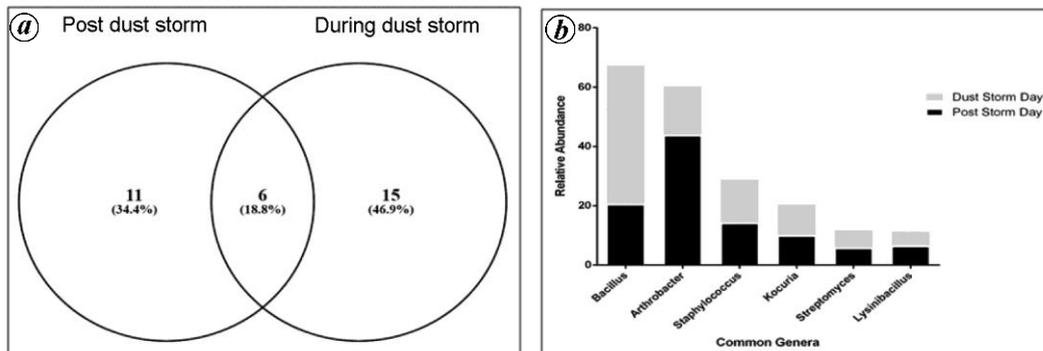


Figure 4. *a*, Venn diagram showing shared genera between PSD and DSD samples. *b*, Relative abundance of six genera shared by PSD and DSD samples.

a good air quality (Table 1). A comparison of data on GIS topography, land use and vegetation indicated that dust deposition and PM concentration in these sites are generally low^{21,22}. A significant ($P < 0.05$) increase was observed in PM_{2.5}/PM₁₀ ratios during DSD, suggesting depletion of air quality in the sites due to dust storm (Table 1). A similar impact on air quality (particle matter concentration) has also been observed during dust storm events in Cairo, Egypt and Ahvaz, Iran^{9,22}.

The different climatic conditions, sampling conditions, sampling time, particle concentrations, anthropogenic activities, types of region and population densities influence diversity of aerial microbial communities⁶. In the present study, microbial count recorded during DSD was significantly high compared to that recorded during PSD, thus

revealing the impact of dust storms on aerial microbial build-up (Table 2). Our findings are consistent with other similar studies conducted across Iran, Egypt and the Mediterranean region²¹⁻²⁴. The higher microbial count during DSD was recorded at all sampling sites (Table 2). However, a steady decrease in microbial count was observed for sampling sites. The highest count was recorded in Mumbai, followed by Lonavala and Pune. Among the growth media, R2A supported the growth of maximum microbial colonies, suggesting the presence of oligotrophic microorganisms in the air. Zhang considered R2A medium suitable for isolation of oligotrophic microorganisms from environmental habitats.

Identification of microorganisms by MALDI-TOF MS is rapid, cost-effective and becoming the technique of

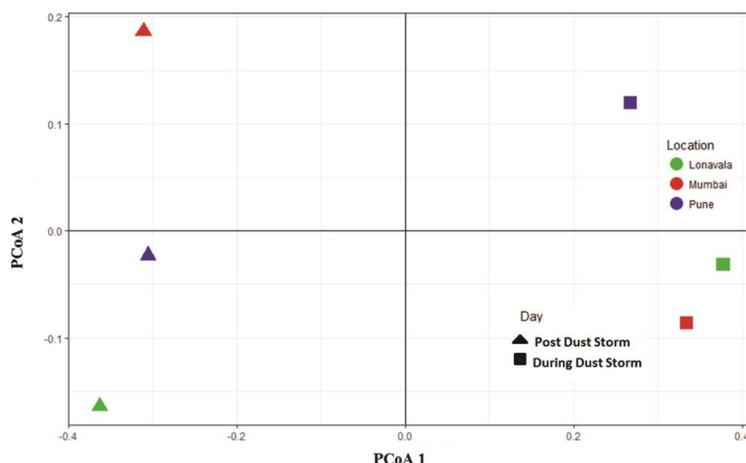


Figure 5. Principal coordinate analysis bi-plot constructed based on the microbial community composition of PSD and DSD samples.

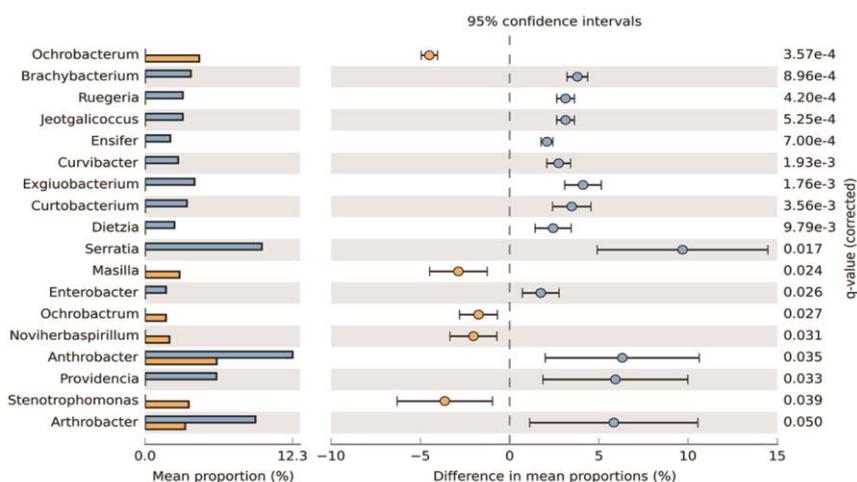


Figure 6. Extended bar plot representing the differential abundant genera present in PSD and DSD samples.

choice for high-throughput microbial identification¹⁰. MALDI-TOF MS allowed species-level identification of 310 isolates and dereplication of 580 isolates. The identified genera members are well represented in the Biotyper database. The isolates with ‘no reliable identification’ in MALDI-TOF-MS were further identified by sequencing of 16S rRNA gene, and belonged to the genera *Micromonospora*, *Nocardopsis*, *Curtobacterium*, *Exgiubacterium*, *Noviherbaspirillum* and *Psychrobacter*, which might be because of their underrepresentation in the Biotyper database¹⁰. Identification of the members of *Bacillus*, *Staphylococcus*, *Arthrobacter*, *Microbacterium* and *Enterobacter* by MALDI-TOF MS has remained inconsistent in various studies^{25–27}. Identification of few selected isolates by both techniques provided in the same results confirm the accuracy of MALDI-TOF MS-based identifications.

The high Good’s coverage for PSD (0.929) and DSD (0.966) indicated good sampling depth, suggesting that the isolates picked for identification are representing the total cultivated members, and the chances to get any new

unique species are low (Table 3). However, the values for Good’s coverage in this study are relatively lower to those observed by uncultivable methods for dust storms in Syria, Saudi Arabia and North Africa²⁰. Similar values for all diversity indices were recorded during PSD and DSD, signifying minimal impact of the dust storm on species richness (Table 3).

A total of 32 bacterial genera and four fungus genera were identified, indicating high diversity of microorganisms in the air samples (Table 3). A similar study during an African dust storm showed the presence of 25 bacterial and 10 fungal genera²⁴. *Bacillus*, *Arthrobacter*, *Staphylococcus*, *Kocuria*, *Streptomyces* and *Lysinibacillus* were common genera recorded during both sampling days, representing 50% of the total isolates (Figures 4 and 6). Bacteria belonging to these genera have been reported frequently from air and many other habitats^{4,5,20–22,28,29}. Though there were few common genera, the overall microbial composition isolated from air differed significantly during DSD and PSD (Figure 6). Members of 15 genera were specifically present during DSD and 11 genera

during PSD, indicating a shift in microbial communities in response to the dust storm. Among the specific genera, isolates belonging to *Serratia* were present in high proportions were present during DSD while isolates belonging to *Microbacterium* during PSD. The abundance of *Psychrobacter adeliensis* (3.53%) and *Exiguobacterium aurantiacum* (4.24%) during DSD, which are well-known osmotolerants and halotolerants, indicated their survival and possible migration along with dust storms^{30–35}. Species-level comparison at all three sites showed a dynamic change in microbial composition during PSD and DSD ([Supplementary Table 3 a, b](#) and [Supplementary Figure 1](#)). The fungal isolates belonged to four genera, *Aspergillus*, *Alternaria*, *Curvularia* and *Cladosporium*, of which *Cladosporium* was uniquely present in DSD ([Supplementary Table 3](#)). Members of genus *Aspergillus* were recorded during both days, but *Aspergillus niger* was specifically recorded during PSD and *Aspergillus dowii* during DSD. Various reports have shown the frequent occurrence of *A. dowii* in sea fan corals in the Caribbean Sea, Australian coast of the Indian Ocean and Pacific Ocean^{36–38}. On the contrary, *A. niger* has been considered as normal flora in the air, especially in indoor environments³⁹. The species-level identification of microbial isolates recovered during dust storm and post-dust storm sampling, lead us to the identification of marker microbial species, which are specifically present during a dust storm event.

The shift in microbial composition could be attributed to the fact that several microorganisms were transported from the origin of the dust storm and introduced into the aerial microbial communities of the sampling sites. The spread of microorganisms along with dust storms has been demonstrated previously in the case of an African desert storm reaching the Caribbean Sea²⁴. Our study corroborates well with previous observations revealing that the major portion of aerial microbial communities comprised microbes of terrestrial origin^{21,40}.

The separate grouping of samples from DSD and PSD in the PCoA bi-plot demonstrated the heterogeneity in microbial composition (Figure 5). The abundance of 13 genera was significantly higher in DSD samples and five genera in PSD samples which contributed to the specific grouping of these samples in PCoA plot (Figures 5 and 6). A similar shift in microbial abundance of *Acinetobacter* and *Pseudomonas* was seen in studies conducted by Griffin *et al.*^{28,41}. Detailed literature survey on the possible pathogenicity of microorganisms recorded during this study revealed that members of *Lysinibacillus* (1.1%) were opportunistic human pathogens, those of *Ochrobacterium*, *Bacillus* and *Microbacterium* (9.1%) were human pathogens and few members of *Bacillus* (4.5%) were plant pathogens in PSD samples ([Supplementary Tables 4 and 5](#)). An increase in the proportion of microorganisms was observed during DSD, viz. for members of *Arthrobacter*, *Bacillus*, *Exiguobacterium* and *Providencia* (8.11%) which were opportunistic human pathogens,

while few members of *Arthrobacter*, *Bacillus*, *Dietzia*, *Enterococcus*, *Pseudomonas* and *Serratia* (20.88%) were human pathogens and some members of *Arthrobacter* and *Curvibacter* (6.66%) were animal pathogens^{40,42,43}. The presence of stress-tolerant, opportunistic and potential pathogens as marker microbial species in the samples collected during DSD, indicated the plausible spread of these organisms along with dust particles. This study has generated preliminary but essential information on the microbial population structure during and post dust storm events. However, we recognize that inclusion of more sampling points and analysis of microbial communities using next-generation sequencing approaches followed by high-throughput cultivation will generate more specific information.

In conclusion, this study provides a comprehensive comparison between bacterial communities in aerial dust that originated in the Middle East and travelled across Indian cities. Considering the expected changes in the severity, frequency and origin of dust storms, it suggests that dust storm-induced fluctuations in microbial community composition and presence of specific microbial species during a dust storm event will be helpful in generating information for microbial surveillance.

Conflict of interest: The authors declare no conflict of interest.

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