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ORIGINAL PAPER

Metagenomic characterisation of bioaerosols during the dry season in Mexico City

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Abstract Air pollution in urban areas is one of the main problems because of its effects on human health and the environment. The levels of critical pollutants such as ozone and airborne particles and their impacts on human health have been widely studied, neglecting the microbiological communities present in the air, which alone or in combination with chemical contaminants can have detrimental effects on human health. In this study, we employed a metagenomic approach to characterise the bacterial and fungal communities, using 16S rRNA and the internal transcribed spacer region of the nuclear ribosomal RNA. The study took place in Mexico City during the dry season, at days with high levels of ozone and suspended particles (March 14 to 18, 2016). We found a total of 147 bacterial genera, of which the most abundant ones were *Microbispora* (9%), *Paracoccus* (6%), *Exiguobacterium* (6%), *Kocuria* (3.0%), *Friedmanniella* (3%), *Rubellimicrobium* (2%), *Sphingomonas* (2%) and *Methylobacterium* (2%). We also found a total of 211 fungal genera, mainly *Cladosporium* (26%), *Phoma* (15%), *Aureobasidium* (11%) and *Cryptococcus* (3%). Some bacterial and fungal genera reported in this study have been reported as a cause of allergic, respiratory or infectious diseases. Our findings may serve as a reference for further monitoring of pathogens present in the air during periods with high levels of ozone and airborne particles, studying their distribution patterns and evaluating the possible combined effects of those particles and pollutants as a risk factor for the health of the general population.

Dr. René Drucker Colin for his contribution and diffusion of science and for believing in the work and capacity of Mexican scientists.

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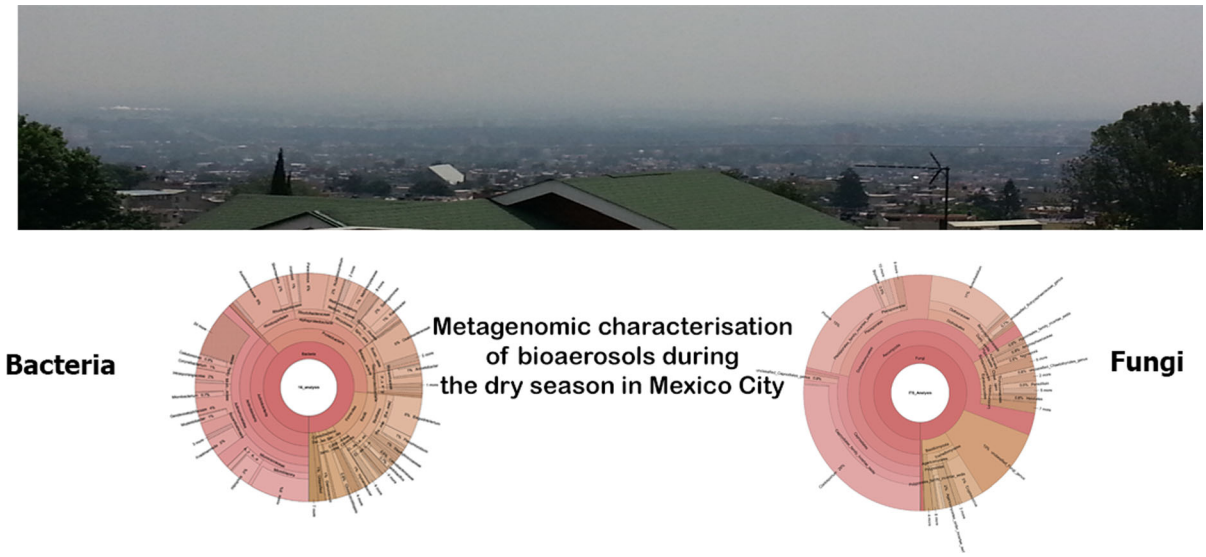
Graphic abstract



Keywords Fungi · Bacteria · Ozone · Airborne particles · Air pollution · Bioaerosols

1 Introduction

According to the World Health Organization (WHO 2019), about 7 million premature deaths each year can be attributed to outdoor and household air pollution. Mexico City is one of the most densely populated cities in the world, with 8,985,339 inhabitants occupying an area of 1495 km^2 (INEGI 2015). Several pollutants emitted by the large urban agglomeration alter the air quality in the city and in the metropolitan area.

Primarily during the dry season, certain physiographic and meteorological conditions trigger the accumulation, transformation and dispersion of pollutants in the atmosphere (SEDEMA 2016). The dry season occurs due to the dominance of the westerly winds, which are generally dry in the mountains and rarely cause rain. This season includes two periods: one from November to February, when the temperature drops directly towards the minimum, and one from March to April, when the temperature increases until reaching its maximum in April and May.

In 2016, air pollution in Mexico City and surrounding areas increased due to weather conditions on a global and regional scale. In the southern region of Mexico City, on March 14, the levels of O_3 reached 150 ppb at 15 h (Fig. 1), exceeding the permissible limits (WHO 2005). On the other hand, the concentration of particles smaller than 10 µm (PM10) reached 103 µg/m^3 of air in a maximum of 24 h and with an annual average of 44.3 µg/m^3 (SEDEMA 2016). In the southern zone of Mexico City, on March 14, the maximum concentrations of PM10 were reached at 11 and 12 h with 95 and 40 µg/m^3, respectively (Fig. 1).

Previous studies investigating the presence of particles in Mexico City air have shown that these particles are probably the leading environmental risk factor for mortality in Mexico (Amador-Muñoz et al. 2013; Chirino et al. 2015; Rosales-Castillo et al. 2001). Furthermore, chemical pollutants and bioaerosols (particles of biological origin) can form airborne agglomerates. The impact on public health is high since particles of various sizes reach various regions of the respiratory tract; the larger particles (> 2.5 mm) are deposited in the upper respiratory tract, while the smallest particles reach the trachea and alveoli (Riojas-Rodríguez et al. 2014).

Some studies have evaluated the chemical composition of the airborne compounds of Mexico City, identifying several polycyclic aromatic hydrocarbons (PAHs) and metals, among other compounds (Chirino et al. 2015; Manzano-León et al. 2013). Likewise,

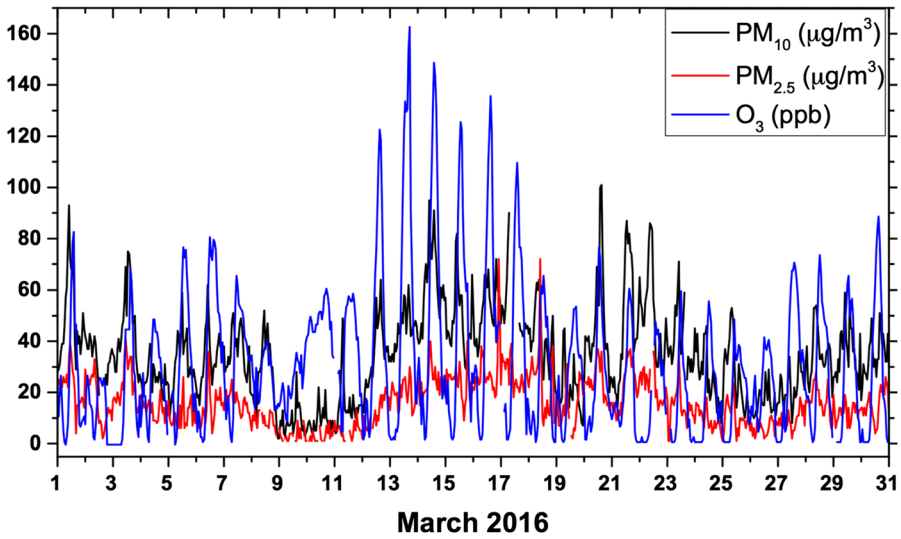


Fig. 1 Monthly variation of ozone, PM10 and PM2.5 concentrations in the southern area of Mexico City. The x-axis represents the days and the y-axis the concentrations in ppb and µg/m^3

various studies have determined the mutagenic activity of soluble organic matter, as well as the elemental composition and the solubility of oligo-elements associated with airborne particles (Mugica-Álvarez et al. 2012; Villalobos-Pietrini et al. 1995, 2006). The mutagenicity and PAH associated with the organic matter of airborne PM10 particles from Mexico City have also been evaluated (Villalobos-Pietrini et al. 2011). However, there is a lack of information on the composition of the atmospheric bioaerosols of the City of Mexico, both of microorganisms and their derivatives (endotoxin, mycotoxins), mainly in the cold dry season (January to March), when the levels of air pollutants are high (Chirino et al. 2015; Calderón-Ezquerro et al. 2016; García-Mena et al. 2016; Serrano-Silva and Calderón-Ezquerro 2018).

There is even less information on the possible health effects of the association of complex mixtures of chemical and biological pollutants suspended in the air on the inhabitants of urban areas.

The worldwide prevalence of chemical and biological pollutants is associated with human diseases such as pneumonia, influenza, measles, asthma, gastrointestinal disorders, infectious diseases, acute toxic effects, allergies and cancer (Kim et al. 2016; Srikanth et al. 2008). Therefore, it is essential to evaluate and determine the type of atmospheric bioaerosols to which the population is exposed.

The metagenomic approach allows the detection of airborne microorganisms with greater depth and breadth and without the bias represented by the use of specific culture media. With this technique, it is possible to determine a great diversity of microbial communities in the air and to focus on pathogenic and infectious microbial agents (Serrano-Silva and Calderón-Ezquerro 2018; Behzad et al. 2015; Cao et al. 2014).

Therefore, this study aimed to determine the composition of the aerosolised microbial community during the dry season, when high concentrations of ozone and airborne particles are present. Our findings allow us to understand the role of air quality in the composition of bioaerosols, the interactions between microbiological agents and chemical contaminants, and how this association could be a risk factor to the health of the population.

2 Materials and methods

2.1 Sampling area

The sampling site was located on the rooftop (15 m) of the "Centro de Ciencias de la Atmósfera" building (19°19′35″ N, 99°10′34″ W) at the Universidad Nacional Autónoma de México (UNAM) in Mexico City. The monitoring site is one of seven air quality stations of the Mexican Aerobiology Network (REMA). Sampling occurred from March 14 to March 18, 2016, during a severe period of atmospheric pollution by ozone and PM10 in Mexico City.

2.2 Sample collection

Three different samplers were used: a total suspended particles (TSP) sampler (Graseby Andersen), a volumetric Hirst-type spore trap (HST) (Burkard Manufacturing Co Ltd, Rickmansworth, UK) and a high-throughput "Jet" spore and particle sampler (Jet sampler) (Burkard Manufacturing Co Ltd, Rickmansworth, UK). All samplers were continuously and simultaneously operated during the 5 days of sampling. The conditions for the performance of each sampler are described below.

2.3 Total suspended particles (TSP) sampler

The TSP sampler operated with an airflow of 1.13 m^3/min, using a sterile nitrocellulose filter previously exposed to ultraviolet light (UV) for 20 min. After particle collection, the filter was carefully carried to the Atmospheric Bioaerosols Laboratory (ABL) in a sterile plastic box and was handled under sterile conditions inside a UV light laminar flow hood previously cleaned with ethanol (70%). The particles were removed from the filter with a sterile scalpel by swabbing, weighed and deposited in a sterile glass jar.

2.4 Hirst-type spore trap (HST)

The HST sampler operated with an airflow of 10 L/min and by impaction of bioaerosols onto cellophane tape (Melinex DuPont®, USA), which was adhered over the surface of the drum and covered with a thin layer of a mixture of petroleum jelly and hexane (1:5) (Calderón-Ezquerro et al. 2016). After sampling, the drum was carried to the laboratory and handled under sterile conditions inside a UV light laminar flow hood previously cleaned with ethanol (70%). The Melinex tape was removed and cut into pieces of 4.8 × 1.9 cm (each one corresponding to 1 day of sampling). The pieces were placed in 2.0-mL screw-capped tubes containing 0.2 g acid-washed, sterile Ballotini beads.

2.5 High-throughput "Jet" sampler (Jet sampler)

The Jet sampler was operated as described by Serrano-Silva and Calderón-Ezquerro (2018), with an air volume of 600 L/min. Prior to assembly, each part of the equipment was washed and sprayed with benzalkonium chloride (0.1%). The Eppendorf tube and plastic funnel were also sterilised in an autoclave.

Negative controls were included for each sampler. For the TSP sampler, a sterile nitrocellulose filter was placed in the sampler 2 h prior to the operation of the equipment. For the HST, a drum was placed inside the sampler with the vacuum pumps turned off for 2 h, and for the Jet sampler, the Eppendorf tube used for dust collection was placed inside the sampler with the vacuum pumps turned off for 2 h.

Immediately after the sampling period, the bioaerosol samples collected by each sampler and the negative controls were preserved at − 80 °C until the extraction of metagenomic DNA.

2.6 Extraction, purification and quality of DNA

The samples collected with the TSP and Jet samplers were processed under a laminar flow hood sterilised with UV light and benzalkonium chloride (0.1%). The DNA was extracted following the method described by Serrano-Silva and Calderón-Ezquerro (2018). The particles collected were placed in 2.0-mL screw-capped tubes containing 0.2 g of acid-washed, sterile Ballotini beads and 200 µL of sterilised extraction buffer. Subsequently, 4 µL of proteinase K (20 mg/µL) (Thermo Fisher Scientific, Inc) was added and mixed by vortexing. The samples were processed in a FastPrep® (Thermo Electron, Corp) for two periods of 40 s at 4 m/s, with cooling on ice between each cycle. After this, 4 µL of RNase A (100 mg/µL) (Thermo Fisher Scientific, Inc) was added and mixed thoroughly by vortexing, followed by incubation at 65 °C for 15 min with occasional vortexing. Subsequently, 200 µL of phenol/chloroform/isoamyl alcohol (25:24:1) (Thermo Fisher Scientific, Inc) was added, and the samples were centrifuged for 15 min at 20,000×*g*. The aqueous phase (150 µL) was set in a new Eppendorf tube and purified with 270 µL of Agencourt AMPure® XP beads (Beckman Coulter, Inc) according to the manufacturer´s instructions. The DNA was eluted in 50 µL of DNase-free water.

Lysis of the samples collected with the HST was done as described before, but with the addition of 150 µL of phenol: chloroform: isoamyl alcohol (25:24:1) to remove proteins and cellular debris. Subsequently, 100 µL of the aqueous phase were set in a new Eppendorf tube. The DNA was obtained and purified with 180 µL of Agencourt AMPure® XP beads

(Beckman Coulter, Inc) according to the manufacturer´s instructions and eluted in 15 µL of DNase-free water (Serrano-Silva and Calderón-Ezquerro (2018)).

The DNA extraction of the negative controls was carried out using nitrocellulose filters (TSP sampler), cellophane tap-Melinex (HST sampler) and Eppendorf tubes (Jet sampler). No PCR products were obtained from the negative controls.

Three DNA samples were obtained for this study (one by each sampler); DNA purification and quantification were performed in a NanoDrop Lite Spectrophotometer (Thermo Scientific) and via Qubit fluorometric quantitation (Thermo Fisher Scientific), measuring the absorbance ratio at 260 and 280 nm. The DNA was verified by electrophoresis in agarose gel (1%) and stored at − 80 °C until subsequent analysis.

2.7 DNA amplification, library preparation and sequencing

The DNA extracted from the airborne samples was amplified using the Nextera XT DNA Library Preparation Kit (Illumina, Inc). Adapter sequences for compatibility with the Illumina index were appended to the primer pair to amplify the variable regions V3 and V4 of the bacterial 16S rRNA gene and the fungal ITS2 region. Partial 16S and ITS regions were amplified by polymerase chain reactions (PCRs) with 12.5 ng DNA and primers 341F/805R and ITS3_KY02/ITS4, respectively (Table 1). The amplified regions were purified using Ampure XP beads (Beckman Coulter). In a second PCR reaction, dual-index barcodes were added to the amplicons to be sequenced after a second PCR clean up with Ampure XP beads (Beckman Coulter). The prepared libraries were sequenced in an Illumina MiSeq system (Illumina Inc). The detailed protocol for library preparation and sequencing can be revised in the manufacturer's instruction manual available on https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.

2.8 Bioinformatic analysis

Reads with Phred scores lower than Q30 in the outer sections were discarded using Trimmomatic PE (Bolger et al. 2014). A Qiime v. 1.9 metagenomic pipeline was used to analyse 16S and ITS sequences to taxonomic units for bacteria and fungi, respectively. Operational taxonomic unit (OTU) assignment was done via the open reference approach with USEARCH v. 6.1 clustering at 99% identity for bacteria and fungi (Edgar et al. 2010). The minimum OTU size was 10 sequences for bacterial assignment and four sequences for fungi. The representative phylogenetic OTUs (phylotypes) were assigned using the UCLUST consensus taxonomy assigner. The Greengenes v. 13.8 database was used for bacteria and a combined UNITE v. 7.0 plus the Findley data set for fungi. Chimeric sequences were removed, given the percent similarity to the reference database (< 80%), either using Greengenes or UNITE + Findley. Microbial diversity was determined by calculating the Shannon and Simpson indices, Chao1 and observed species from rarefaction curves. The relative abundances and microbial diversity of bacteria and fungi obtained of the three libraries from the different sampling devices were integrated into a single analysis to construct rarefaction curves and Krona graphics.

Table 1 Primers used in the amplification of 16S rRNA region of bacteria and ITS2 region of fungi

Name	Primer sequence (5′ to 3′)	Alignment temperature (°C)	References
341F	CCTACGGGNGGCWGCAG	55	Herlemann et al. (2011)
805R	GACTACHVGGGTATCTAATCC		Herlemann et al. (2011)
ITS3_KYO2	GATGAAGAACGYAGYRAA	52	White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC		White et al. (1990)

3 Results

3.1 Sequencing data

The DNA concentration and purity obtained from the samples were suitable for the sequencing process (on average, 89.7 ng/μL and an absorbance ratio A260/280 = 1.65). A total of 734,910 reads (raw sequences) were obtained from the sequenced samples, of which 53% were valid after quality filtering and removal of chimeras (post-QC sequences) for subsequent analysis (Table 2). The retrieved sequences were clustered based on 99% (for bacteria) and 97% (for fungi) sequence identity, resulting in 993 bacterial OTUs and 1413 fungal OTUs (Table 2). A high number of bacterial and fungi genera could not be assigned to known genera (Table 2).

3.2 Nucleotide sequence accession number

All relevant data are available in the NCBI Sequence Read Archive (SRA) under accession number SRX5279113.

3.3 Airborne bacteria

According to the rarefaction curves and diversity indices, the bacterial diversity during the study was adequately characterised (Fig. 2), with a specific diversity of Chao1 = 979, Shannon index = 8.47 and Simpson index = 0.99.

Overall, 14 phyla represented the airborne bacterial community during the evaluated period (March 2016) in Mexico City. The most abundant bacterial phyla were Actinobacteria (38%), Proteobacteria (38%) and Firmicutes (15%). Bacteroidetes (3%), Cyanobacteria (3%), Chloroflexi (1%) and Thermi (1%) were also identified (Fig. 3; Fig. S1 Supplementary material). Planctomycetes, Acidobacteria, Armatimonadetes, FBP (a bacterial lineage that is known only by molecular sequence data from environmental surveys) and Gemmatimonadetes were present in low proportions (< 1.0%), as well as Fusobacteria and TM7 (a bacterial lineage that is known only by molecular sequence data from environmental surveys).

Based on analysis at the genus level, a high number of sequences (37.2%) could not be assigned. We found a total of 147 bacterial genera (Table 2); the dominant genera were *Microbispora* (9%), *Paracoccus* (6%), *Exiguobacterium* (6%), *Kocuria* (3.0%), *Friedmanniella* (3%), *Rubellimicrobium* (2%), *Sphingomonas* (2%) and *Methylobacterium* (2%) (Table S1). However, *Skermanella*, *Planomicrobium*, *Acinetobacter*, *Kaistobacter*, *Hymenobacter*, *Corynebacterium*, *Deinococcus* and *Modestobacter* were present in proportions from 1.0 to 2.0%. Other genera, such as *Alkanindiges, Leuconostoc, Enterococcus, Gordonia, Lautropia, Ruminococcus, Faecalibacterium, Bacteroides, Prevotella, Flavobacterium, Bifidobacterium, Comamonas, Collinsella, Sarcina, Arcobacter, Propionibacterium, Fusobacterium* and *Eubacterium*, were also present with relative abundance values < 0.1% (Table S1).

Around 30% of unique bacterial genera found in this study were pathogens; those with proportions higher than 0.1% are shown in Table S2.

Table 2 Descriptive characteristics of the metagenomic analysis (16S and ITS clone libraries)

Characteristic	16S	ITS
Raw sequences	734,910	
Post-QC sequences	389,086	
Total assigned OTUs	993	1413
Unclassified OTUs at genus level	418	657
Classified OTUs at genus level	575	756
Unique genera	147	211
OTUs with minimal size representatives	73	202

3.4 Airborne fungi

Fungal diversity during the period studied was reliably characterised as the rarefaction curve became nearly asymptotic (Fig. 4), with a specific diversity of Chao1 = 1776, Shannon index = 6.75 and Simpson index = 0.95. Based on the analysis, we found a total of 211 fungal genera (Table 2); the dominant fungal genera belonged to the divisions Ascomycota (81%) and Basidiomycota (8%), with 10% unclassified fungi (Fig. 5; Fig. S2 Supplementary material). Among the Ascomycota, the most abundant genera were *Cladosporium* (26%), *Phoma* (15%) and *Aureobasidium* (11%) (Fig. 5). Other genera, such as *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria*, were also identified, albeit with a relative abundance ≤ 1% (Table S3). On the other hand, the most abundant

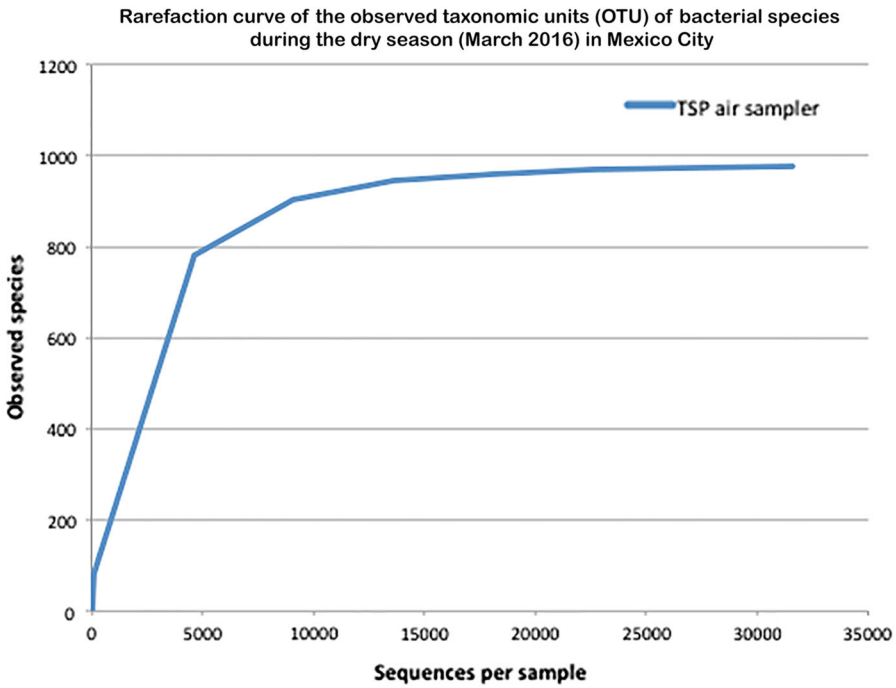


Fig. 2 Rarefaction curve of the observed taxonomic units (OTU) of bacterial species during the dry season (March 2016) in Mexico City

Basidiomycota was *Cryptococcus* (3%), and other genera, such as *Ustilago*, *Heterobasidion*, *Hyphodontia* and *Peniophora with a relative abundance* $\leq$ 1%, as well as Zygomycota, were also identified (*Rhizopus* and *Mucor*) (Table S3). The main allergenic/pathogenic fungal genera found during this study are shown in Table S4.

4 Discussion

The airborne microbiota is involved in the function and diversity of ecosystems, either aquatic, terrestrial or directly linked to human, animal and plant health through dispersion in the atmosphere (Polymenakou 2012). Bioaerosols can be transported in the air either individually, in aggregates or attached to particles, exposing the inhabitants to the inhalation of viruses, bacteria, fungi and their spores, lichen fragments, protists, spores and fragments of plants, pollen, small seeds and invertebrates, as well as their fragments (Lacey and West 2006).

The chemical composition of gasses and particulate matter in the air has been extensively studied (Shen et al. 2011; Valavanidis et al. 2008). However, few studies have investigated the microbial community composition and its role in the atmosphere, as well as its potential impact on ecosystems and human health (Behzad et al. 2015). There are even less studies that have evaluated the structure and composition of the communities of airborne microorganisms on days with poor air quality (but see Cao et al. 2014; Du et al. 2018).

The atmosphere of Mexico City contains a complex mixture of solid and liquid aerosols of organic and inorganic substances that constitute the PM suspended in the air, comprising TSP, $\leq$ PM10 and $\leq$ PM2.5, which can penetrate and lodge in the lungs or cross the pulmonary barrier and enter the blood system (WHO 2003). Such PM and bioaerosols could participate in the development, exacerbation and spread of infectious and non-infectious diseases. Atmospheric bioaerosols may be responsible for transmitting human diseases such as staphylococcal and streptococcal respiratory infections, pneumococcal pneumonia, influenza, tuberculosis, anthrax, aspergillosis, nocardiosis, histoplasmosis, sporotrichosis, cryptococcosis, coccidioidomycosis, among many others.

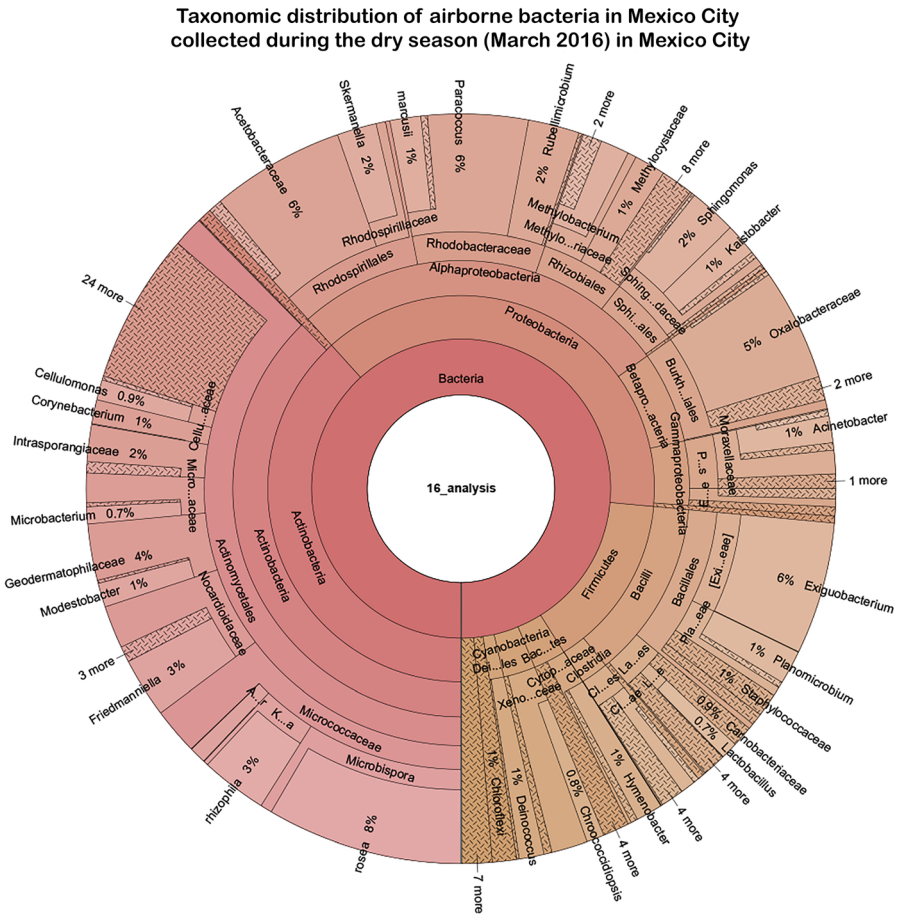


Fig. 3 Taxonomic distribution of airborne bacteria in Mexico City collected during the dry season (March 2016) in Mexico City

Other airborne biological particles are toxins or proteins with an allergenic potential (Yan et al. 2016; Calderón-Ezquerro et al. 2018; Humbal et al. 2018; Oh et al. 2014), as well as pollen grains and fungal spores, which can cause allergies or asthma (Babich and Lightart 1974).

Airborne bacteria are one of the main components of atmospheric bioaerosols (Gangamma 2014) and are also contained in PM (Jaenicke 2005), affecting the upper and lower respiratory tracts through immunological reactions or by causing infections. In addition to the diseases caused by most of the bacteria found in this study (Table S2), there are endotoxins and peptidoglycans (components of the cell walls of Gram-negative and Gram-positive bacteria, respectively), which are pro-inflammatory agents that can induce respiratory symptoms such as fever, headache, cough and respiratory distress (Douwes et al. 2003; Fröhlich-Nowoisky et al. 2016; Bauer et al. 2012; Earl et al. 2015).

In the present study, the most abundant airborne bacterial phyla were Gram-negative proteobacteria (38.2%), Gram-positive actinobacteria (38.3%) and firmicutes (14.6%). Some studies have suggested that endotoxins and peptidoglycans in airborne bacteria contribute significantly to the proinflammatory response (Brook et al. 2010; Seaton et al. 1999; Monn and Becker 1999; Ning et al. 2000; Rosales-Castillo et al. 2001).

Furthermore, several studies based on experimental and epidemiological data have shown that environmental exposure to bacterial endotoxins, particulate air pollution and ozone has the potential to induce neutrophilic airway inflammation, airway obstruction and symptoms of asthma by non-allergic mechanisms (Douwes et al. 2003; Bauer et al. 2012). These findings

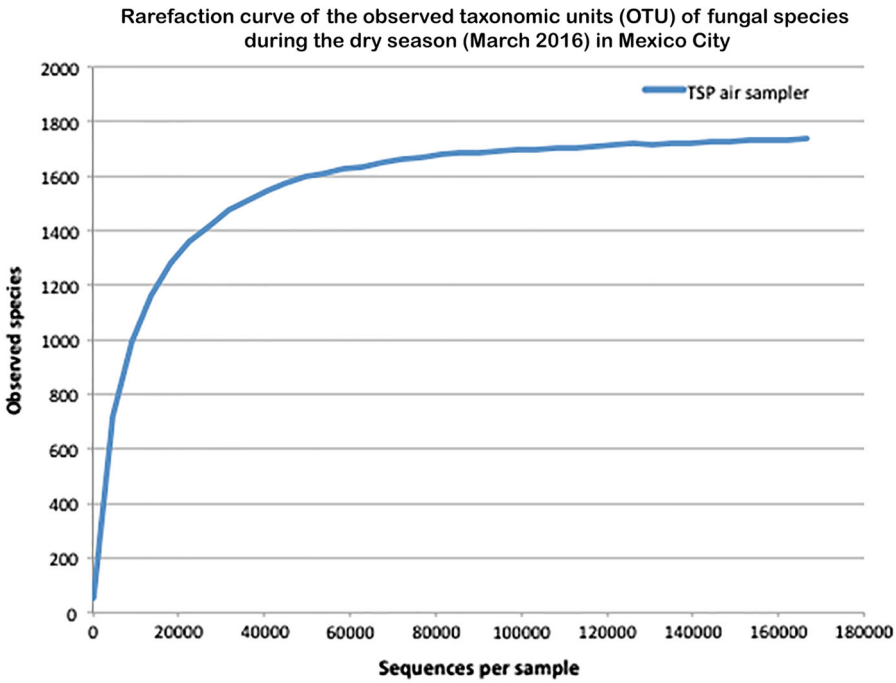


Fig. 4 Rarefaction curve of the observed taxonomic units (OTU) of fungal species during the dry season (March 2016) in Mexico City

indicate the high health risk to which the inhabitants of polluted urban areas, such as Mexico City, are exposed, mainly on days of high pollution when the airborne bacterial and fungal communities form conglomerates of particles of the atmosphere (Serrano-Silva and Calderón-Ezquerro 2018).

On the other hand, even though fungi represent one of the largest groups of eukaryotic organisms (Hawksworth and Lücking 2017), studies on airborne fungi identification with a metagenomic approach during pollution events in urban spaces are scarce (Behzad et al. 2015; Cao et al. 2014; Yan et al. 2016). Metagenomics has shown many advantages because it does not require culture-dependent methods. However, there are still obstacles in the analysis because of the low representativeness of fungi in the primary metagenomics databases. The nuclear ribosomal internal transcribed spacer (ITS) region has been widely used as a DNA metabarcode to characterise the diversity and composition of fungal communities (Blaalid et al. 2013). However, the resolution at level species in several groups is limited, but it is a first step in the characterisation.

The results of this study provide new findings of what is present in outdoor air and allow us to deepen our knowledge of infection sources and pathogens that can affect human health.

In this study, 10% of the total sequences were assigned as "unclassified fungi" (Fig. 5), which may be due to the available taxa in the databases (UNITE and Findley) (Nilsson et al. 2009). Fungal genera frequently identified in outdoor airborne environments, such as *Cladosporium*, *Phoma*, *Aureobasidium*, *Aspergillus* and *Penicillium* (Rosas et al. 1998), were also identified here.

These fungi are allergens and can trigger or exacerbate allergic diseases, such as allergic rhinitis, asthma and allergic conjunctivitis, and can cause infectious diseases and respiratory pathologies (Zukiewicz-Sobczak 2013).

It is worth mentioning that the genus *Cryptococcus* has not previously been reported in urban spaces in Mexico City. In this study, *Cryptococcus* was identified, albeit at low levels (3%). However, it is necessary to carry out studies with specific molecular tools and specific sampling methods to determine the presence, quantity and viability of *Cryptococcus* human-pathogen species (*C. neoformans* and *C. gattii*). This stands out because human cryptococcosis has become recognised as a significant health threat with the emergence

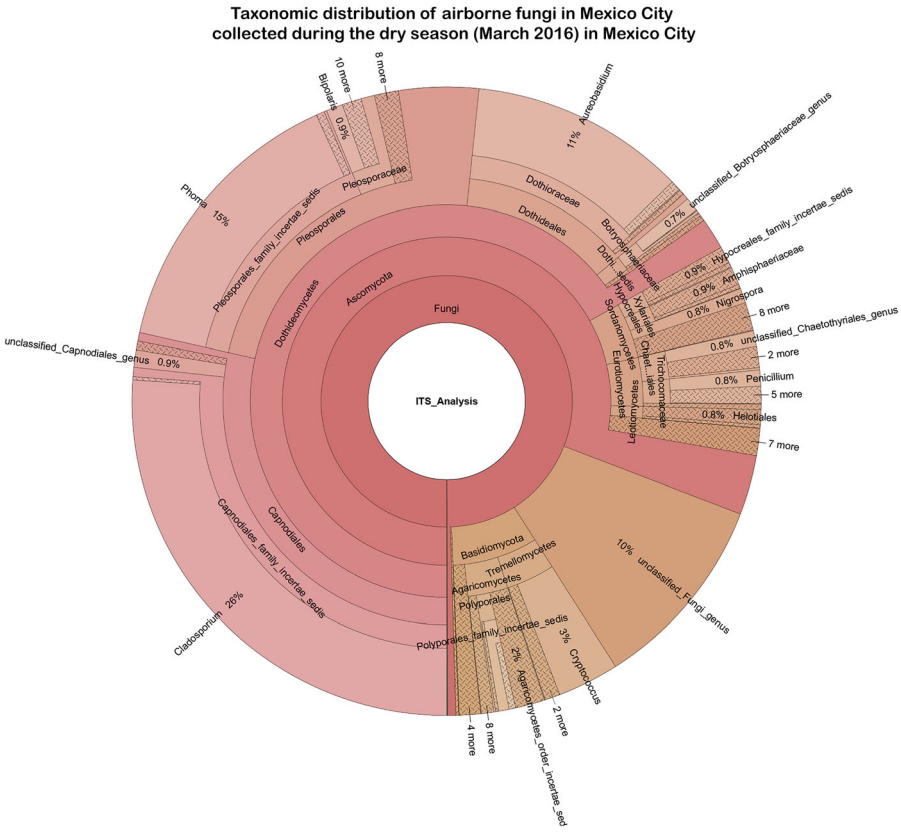


Fig. 5 Taxonomic distribution of airborne fungi in Mexico City collected during the dry season (March 2016) in Mexico City

of AIDS (May et al. 2015), organ transplantation and cancer chemotherapy. It is essential to mention that this genus has 356 records according to de Index Fungorum and that most of the species are phytopathogens.

This study revealed, via the Shannon, Simpson and Chao1 indices, a great richness of species, as well as the presence of "uncommon" or low-abundance species in the Mexico City atmosphere, compared to those species reported in previous studies using traditional culture techniques and biochemical tests (Santos-Burgoa et al. 1994; Rosas et al. 1996, 1997,1998; Calderon et al. 1997).

It is essential to consider that both bacterial and fungal composition and abundance in the air can vary. The microbial community composition can be influenced by sampling methods, anthropogenic activity and environmental conditions, mainly on days with high contamination levels.

4.1 Bioaerosols, chemical pollutants and health effects

According to the WHO (2005), ozone (O_3), particulate matter (PM), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) are the primary outdoor air pollutants linked to harmful health effects (https://www.who.int/air-pollution/news-and-events/how-air-pollution-is-destroying-our-health).

The microbial community identified in bioaerosols in this study was associated with PM, which coincides with what has been reported in air quality studies conducted in Europe (Baldacci et al. 2015; Guerreiro et al. 2013), Bejing, China (Cao et al. 2014) and in previous studies carried out in Mexico City (Serrano-Silva and Calderón-Ezquerro 2018). The studies performed in Mexico City reported that higher levels of outdoor pollen or fungi were associated with higher rates of hospital asthma admissions (Calderón-Ezquerro et al. 2018; Rosas et al. 1998).

Exposure to low levels of ozone, diesel and other chemical air pollutants causes a synergistic reaction with airborne pollen and fungi, resulting in a significant decrease in lung function compared to that caused by exposure to chemical contaminants, pollen grains or fungal spores alone (Molfino et al. 1991; Janssen et al. 2003). Therefore, the risk to health increases mainly on days with high air pollution, which, in Mexico City, frequently happens during the dry season. The population most vulnerable to high O_3 levels are mainly children, older adults, outdoor workers and individuals with asthma.

The microbiota involved in air pollution can be both a source of gaseous pollutants and a sink for pollutants. Likewise, the airborne microbiota can be considered a pollutant if it is involved in the spread of infectious and allergic diseases or can transform pollutants into more or less toxic forms.

Chemical contaminants in the air can be toxic, inhibitors or stimulants for microorganisms and can influence their reproductive potential, alter their morphology and produce other effects (Babich and Lightart 1974).

5 Conclusions

Aerobiological characterisation during the dry season, with high levels of ozone and particles, allows to deepen the recognition of the microbial composition of the air and to determine the risk to which the inhabitants are exposed. This combination can potentiate the hazard for the health of the inhabitants, mainly the most vulnerable ones such as children and the elderly.

Further studies are necessary to expand our knowledge on the variation in time of the composition of bioaerosols and the interaction between bioaerosols and chemical pollutants, as well as their effects on health, in particular with high levels of air pollution. For this purpose, long-term monitoring should be established.

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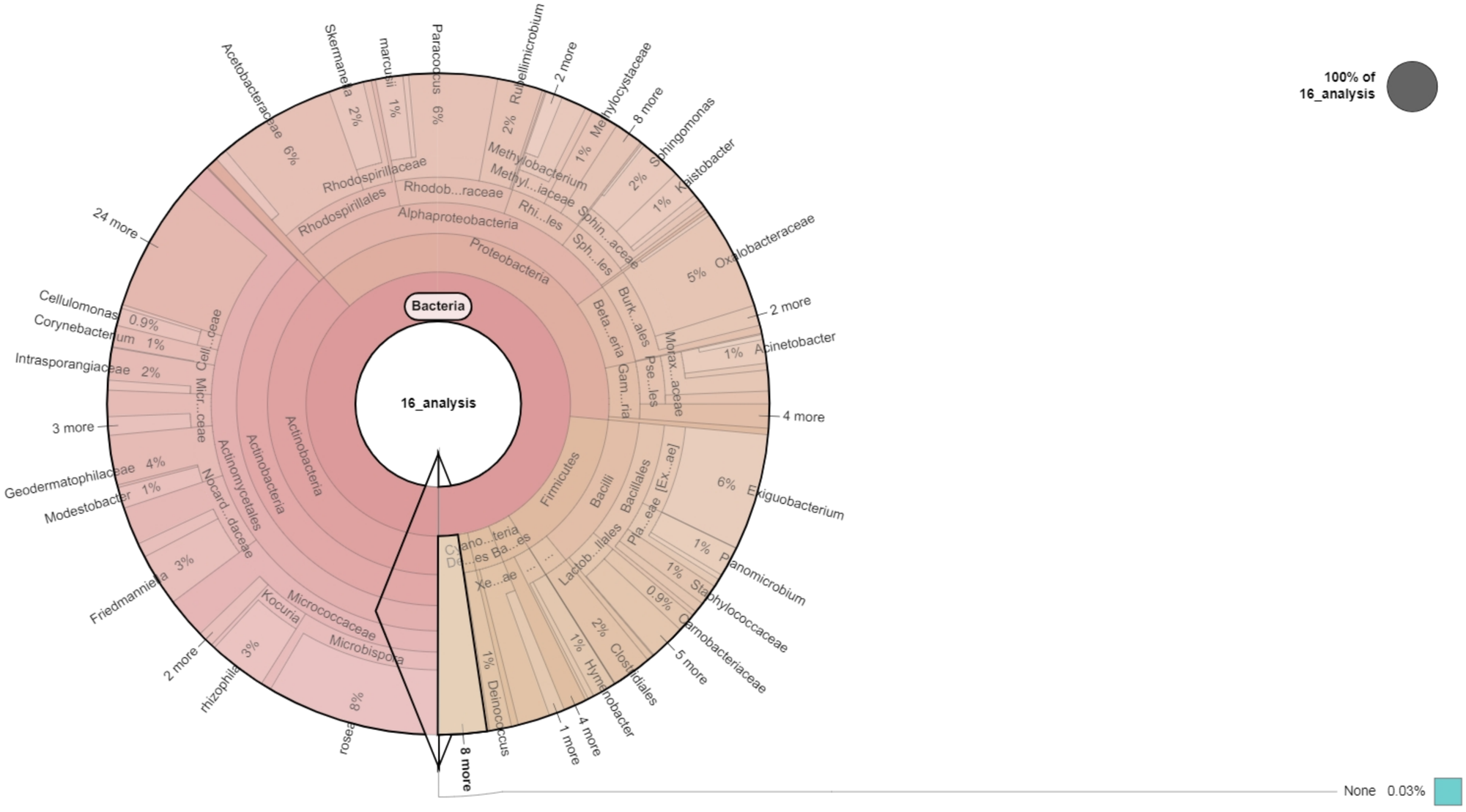

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Rubellimicrobium
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Oxalobacteraceae
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Pla...eae [Ex...ae]
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Cyano...teria
De...es Ba...es
Xe...ae
Clostridiales
Hymenobacter
Deinococcus
Actinobacteria
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Nocard...daceae
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Microbispora
Micr...ceae
Cell...ceae
Acetobacteraceae
Skermanella
marcusii
Paracoccus
Rhodospirillales
Cellulomonas
Corynebacterium
Intrasporangiaceae
Geodermatophilaceae
Modestobacter
Friedmanniella
rhizophila
rosea
Exiguobacterium
Planomicrobium
Staphylococcaceae
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24 more
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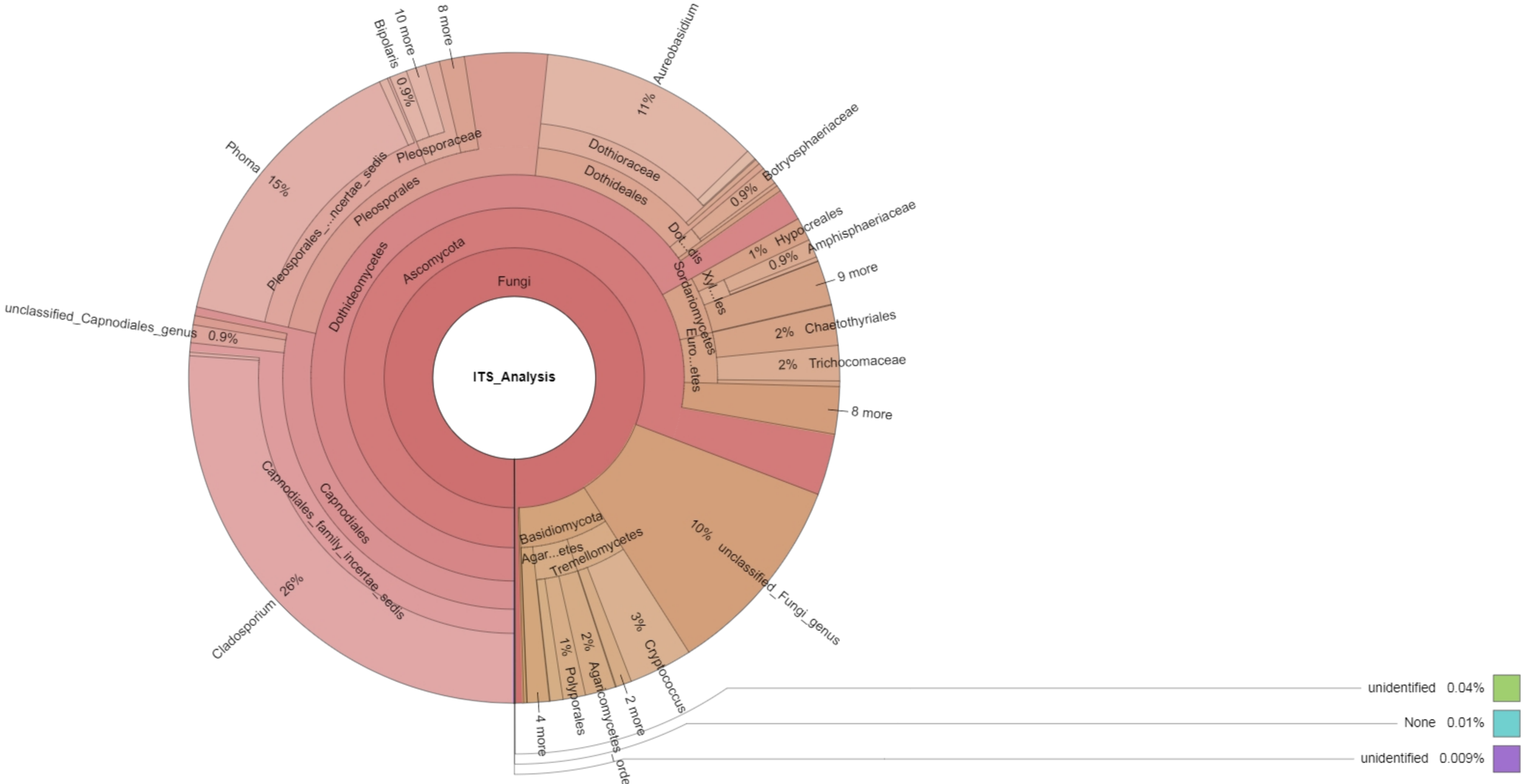
None 0.03%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Phoma 15%
Bipolaris 0.9%
10 more
8 more
Dothideales
Dothioraceae
Aureobasidium 11%
Botryosphaeriaceae 0.9%
Sordariomycetes
Hypocreales 1%
Amphisphaeriaceae 0.9%
9 more
Chaetothyriales 2%
Trichocomaceae 2%
8 more
unclassified_Capnodiales_genus 0.9%
Capnodiales
Capnodiales_family_incertae_sedis
Cladosporium 26%
Basidiomycota
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Tremellomycetes
Polyporales 1%
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2 more
Cryptococcus 3%
unclassified_Fungi_genus 10%
4 more
unidentified 0.04%
None 0.01%
unidentified 0.009%

Table S1. Bacterial genera present in the atmosphere during the dry season (March 2016) in Mexico City.

Phyla	**Genera**	**Relative abundance >0.1%**
Several Phyla	Unasigned	37.178%
Actinobacteria	Microbispora	9.000%
Proteobacteria	Paracoccus	6.000%
Firmicutes	Exiguobacterium	6.000%
Actinobacteria	Kocuria	3.000%
	Friedmanniella	3.000%
Proteobacteria	Rubellimicrobium	2.000%
	Sphingomonas	2.000%
	Methylobacterium	2.000%
	Skermanella	1.705%
Firmicutes	Planomicrobium	1.464%
Proteobacteria	Acinetobacter	1.400%
	Kaistobacter	1.365%
Bacteroidetes	Hymenobacter	1.149%
Actinobacteria	Corynebacterium	1.087%
[Thermi]	Deinococcus	1.074%
Actinobacteria	Modestobacter	1.032%
	Cellulomonas	0.858%
Cyanobacteria	Chroococcidiopsis	0.766%
Actinobacteria	Arthrobacter	0.754%
	Microbacterium	0.741%
Firmicutes	Lactobacillus	0.724%
Actinobacteria	Brachybacterium	0.701%
Proteobacteria	Roseomonas	0.575%
Actinobacteria	Dietzia	0.515%
Proteobacteria	Pseudomonas	0.500%
Firmicutes	Staphylococcus	0.478%
Actinobacteria	Pseudonocardia	0.455%
Proteobacteria	Erwinia	0.445%
Firmicutes	Bacillus	0.439%
Actinobacteria	Propionicimonas	0.422%
	Arsenicicoccus	0.388%
	Rhodococcus	0.344%
Firmicutes	Jeotgalicoccus	0.338%
	Desemzia	0.330%
	Turicibacter	0.324%

Phyla	**Genera**	**Relative abundance <0.1%**
Proteobacteria	Amaricoccus	0.097%
Bacteroidetes	Pontibacter	0.095%
Proteobacteria	Beijerinckia	0.093%
Bacteroidetes	Adhaeribacter	0.089%
Proteobacteria	Alkanindiges	0.087%
Firmicutes	Leuconostoc	0.083%
Actinobacteria	Georgenia	0.076%
Firmicutes	Enterococcus	0.075%
Proteobacteria	Lautropia	0.075%
Actinobacteria	Gordonia	0.075%
Firmicutes	[Ruminococcus]	0.069%
	Blautia	0.067%
Bacteroidetes	Dyadobacter	0.059%
Firmicutes	Faecalibacterium	0.059%
Actinobacteria	Terracoccus	0.058%
	Curtobacterium	0.054%
Bacteroidetes	Bacteroides	0.054%
Firmicutes	Rummeliibacillus	0.053%
	Planococcus	0.051%
Proteobacteria	Sphingobium	0.048%
Bacteroidetes	Prevotella	0.047%
Firmicutes	Megamonas	0.045%
Cyanobacteria	Scytonema	0.044%
Bacteroidetes	Flavobacterium	0.042%
Proteobacteria	Ramlibacter	0.042%
Firmicutes	Solibacillus	0.040%
Actinobacteria	Phycicoccus	0.039%
Chloroflexi	Ardenscatena	0.039%
Actinobacteria	Bifidobacterium	0.037%
Proteobacteria	Comamonas	0.036%
Actinobacteria	Collinsella	0.034%
Proteobacteria	Rhodobacter	0.034%
Actinobacteria	Sanguibacter	0.033%
Bacteroidetes	Spirosoma	0.033%
Firmicutes	Macrococcus	0.033%
	Sarcina	0.033%

Proteobacteria	Rhodoferax	0.324%
Firmicutes	Aerococcus	0.319%
Actinobacteria	Mycobacterium	0.311%
	Streptomyces	0.304%
Firmicutes	Trichococcus	0.286%
Bacteroidetes	Chryseobacterium	0.271%
Proteobacteria	Burkholderia	0.266%
Actinobacteria	Brevibacterium	0.254%
Proteobacteria	Novosphingobium	0.251%
Actinobacteria	Kineococcus	0.223%
Proteobacteria	Devosia	0.215%
Actinobacteria	Williamsia	0.204%
	Carnobacterium	0.199%
Firmicutes	Clostridium	0.192%
Proteobacteria	Janthinobacterium	0.187%
Actinobacteria	Actinotalea	0.179%
	Actinomycetospora	0.176%
	Aeromicrobium	0.170%
Firmicutes	Streptococcus	0.168%
Firmicutes	Salinicoccus	0.167%
Proteobacteria	Balneimonas	0.165%
	Agrobacterium	0.156%
Bacteroidetes	Pedobacter	0.153%
Actinobacteria	Microlunatus	0.151%
Bacteroidetes	Flavisolibacter	0.135%
Acidobacteria	Terriglobus	0.131%
	Luteococcus	0.131%
	Geodermatophilus	0.128%
Proteobacteria	Psychrobacter	0.121%
[Thermi]	Truepera	0.120%
Firmicutes	Facklamia	0.118%
Proteobacteria	Enhydrobacter	0.118%
Actinobacteria	Rathayibacter	0.112%
Firmicutes	Sporosarcina	0.112%
Actinobacteria	Salana	0.106%
	Nocardioides	0.104%
Proteobacteria	Wolbachia	0.104%
Actinobacteria	Tessaracoccus	0.103%
Cyanobacteria	Calothrix	0.103%

Proteobacteria	Lysobacter	0.033%
	Luteimonas	0.033%
Acidobacteria	Edaphobacter	0.028%
Proteobacteria	Arcobacter	0.026%
Actinobacteria	Propionibacterium	0.025%
Bacteroidetes	[Prevotella]	0.025%
Firmicutes	Proteiniclasticum	0.025%
	Marinilactibacillus	0.025%
Proteobacteria	Steroidobacter	0.025%
Actinobacteria	Nonomuraea	0.023%
Chloroflexi	Caldilinea	0.023%
Fusobacteria	Fusobacterium	0.023%
Proteobacteria	Marinomonas	0.023%
	Sorangium	0.022%
Actinobacteria	Dactylosporangium	0.020%
Firmicutes	[Eubacterium]	0.020%
	Alkalibacterium	0.020%
Actinobacteria	Virgisporangium	0.019%
Bacteroidetes	Rhodocytophaga	0.019%
Cyanobacteria	Nostoc	0.019%
Firmicutes	Phascolarctobacterium	0.019%
Proteobacteria	Labrys	0.019%
	Roseococcus	0.019%
Actinobacteria	Lentzea	0.017%
	Yaniella	0.017%
Bacteroidetes	Segetibacter	0.017%
	Cloacibacterium	0.017%
Firmicutes	Oscillospira	0.017%
Actinobacteria	Nesterenkonia	0.016%
	Catenuloplanes	0.016%
	Patulibacter	0.016%
Bacteroidetes	Gillisia	0.016%
	Gelidibacter	0.016%
Bacteroidetes	CF231	0.016%
Cyanobacteria	Leptolyngbya	0.016%
Proteobacteria	Variovorax	0.016%
	Afifella	0.016%

Table S2. Airborne bacterial pathogenic genera present in the atmosphere during the dry season (March 2016) in Mexico City.

Genera*	**Relative abundance (%)**	**Main pathogenic species**	**Clinical infections**
Paracoccus $^{(-)}$	6%	*P. yeei*	Opportunistic infections, arthritis
Exiguobacterium $^{(+)}$	6%	*Exiguobacterium spp.*	Bacteremia, cutaneous infection
Kocuria $^{(+)}$	3%	*K. rosea, K. kristinae*	Sepsis, peritonitis, bacteremia
Sphingomonas $^{(-)}$	2%	*S. paucimobilis, S. mucosissima, S. adhesiva*	Bacteremia, pseudobacteraemia, septic arthritis, osteomyelitis.
Methylobacterium $^{(-)}$	2%	*Methylobacterium spp., M. radiotolerans, M. thiocyanatum, M. aminovorans, M. lusitanum*	Bloodstream infections, peritonitis, pneumonia, hospital-acquired bacteremia
Acinetobacter $^{(-)}$	1.4%	*Acinetobacter spp., A. baumannii*	Septicemia, pneumonia, endocarditis, meningitis, skin and wound infection, urinary tract infection, peritonitis, ophthalmic infections
Corynebacterium $^{(+)}$	1.0%	*C. diphtheriae, Corynebacterium spp C. minutissimum, C. jeikeium, C. urealyticum*	Diphtheria, Respiratory and cutaneous disease, ocular infections, Trichomycosis erythrasma, infections in immunocompromised individuals and those with medical devices, urinary tract infecitons
Cellulomonas $^{(+)}$	0.8%	*C. hominis, C. denverensis*	Bacteremia, sepsis, endocarditis
Arthrobacter $^{(+/-)}$	0.7%	*Arthrobacter sp., A. scleromae, A. woluwensis, A. cumminsii*	Dematosis, bacteremia, skin infection, endophthalmitis, infective endocarditis
Microbacterium $^{(+)}$	0.7%	*M. oxydans, M. paraoxydans, M. foliorum, M. hydrocarbonoxydans, M. esteraromaticum, M. oleivorans, M. phyllosphaerae, M. thalassium*	Human clinical specimens
Lactobacillus (+)	0.7%	*Lactobacillus spp.*	Dental caries
Roseomonas (-)	0.5%	*R. mucosa, R. gilardii*	Bacteremia, catheter-related infection, peritonitis
Dietzia (+)	0.5%	*D. maris, D. cinnamea, D. papillomatosis*	Septic shock, prosthetic hip infection, aortitis confluent and reticulated papillomatosis, inflamed skin
Pseudomonas (-)	0.5%	*Pseudomonas spp, P. aeruginosa, P. pseudomallei*	Nosocomial pneumonia, opportunistic infections, ocular infections, urinary tract infection

Staphylococcus (+)	0.4%	*S. aureus, S. epidermidis, S. warneri, S. lugdunensis, S. saprophyticus*	Pneumonia, ocular infections, atopic dermatitis device-associated infections, bacterial endocarditis, urinary tract infections
Erwinia (-)	0.4%	*E. billingiae, E. persicinus*	Cutaneous infection, bacteremia, urinary tract infection
Bacillus (+)	0.4%	*B. anthracis, B. cereus, B. licheniformis, B subtilis.*	Anthrax, meningitis, food poisoning, bacteremia, abscesses, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis, respiratory and urinary tract infections.
Rhodococcus (+)	0.3%	*R. equi, R. erythropolis*	Lung diseases (pneumonia, lung abscesses); disseminated diseases (meningitis, pericarditis); opportunistic infections (wound infection, peritonitis, traumatic endophthalmitis), septicemia
Aerococcus (+)	0.3%	*A. urinae, A. sanguinicola*	Urosepsis, infective endocarditis, bacteremia, postoperative infection
Mycobacterium (+/-)	0.3%	*M. tuberculosis* complex, *M. leprae, M. ulcerans, M. avium, M. intracellulare*	Bacteremia, tuberculosis, leprosy, Buruli ulcers, Crohn's disease
Streptomyces (+)	0.3%	*Streptomyces spp., S. somaliensis, S. griseus, S. paraguayensis, S. albus, S. coelicolor, S. pelletieri, S. bikiniensis*	Mycetoma; bacteremia, pneumonia, lung nodule, brain abscess, peritonitis, chronic pericarditis, lymphadenitis, endocarditis
Chryseobacterium (-)	0.2%	*C. meningosepticum, C. indologenes*	Meningitis, sepsis, pneumonia, endocarditis
Burkholderia (-)	0.2%	*B. cepacia, B. cenocepacia, B. multivorans*	Opportunistic pathogens, chronic granulomatous disease
Brevibacterium (+)	0.2%	*B. casei*	Bacteremia, pericardial infection osteomyelitis, peritonitis
Williamsia (+)	0.2%	*W. deligens, W. muralis, W. serinedens*	Pulmonary infection, bacteraemia, endophthalmitis, perinatal sepsis
Clostridium (+)	0.1%	*C. botulinum, C. tetani, Clostridium spp, C. perfringens, C. bifermentans, C. tertium, C. Sporogenes, C. difficile*	Food poisoning, tetanus, Intra-abdominal infections, gas gangrene, myonecrosis, polymicrobial infections (peritonitis, intra-abdominal abscesses, septicaemia), food poisoning, pseudo-membranous colitis

Streptococcus (+)	0.1%	*S. pyogenes, S. pneumonia, S. oralis, S. sanguis, S. mitis biovar 1, S. mutans, S. sobrinus*	Ocular infections, pneumonia, meningitis, otitis media, sinusitis, pharyngitis, bacterial endocarditis, periodontal diseases, dental caries
Agrobacterium (-)	0.1%	*A. radiobacter*	Bacteremia, peritonitis
Psychrobacter (-)	0.1%	*Psychrobacter sp, P. faecalis, P. pulmonis, P. sanguinis, P. phenylpyruvicus, P. immobilis*	Meningitis, opportunistic infections as chronic peritonitis, bacteremia, endocarditis, infective arthritis, ocular infections, wound infection
Facklamia (+)	0.1%	*F. hominis, Facklamia spp, F. languida*	Bacteremia, genitourinary infections, wound infections, bacterial meningitis

* The cell wall structure for each genus is given in parentheses.

Table S3. Fungal genera present in the atmosphere during the dry season (March 2016) in Mexico City with a relative abundance ≤ 1%.

Genera	Relative abundance
Acremonium	0.05%
Amphobotrys	0.00%
Coniosporium	0.30%
Leptodontidium	0.00%
Peltaster	0.03%
Phaeomoniella	0.03%
Phialemonium	0.01%
Scolecobasidium	0.00%
Capnobotryella	0.02%
Devriesia	0.11%
Cercospora	0.16%
Mycocentrospora	0.02%
Mycosphaerella	0.03%
Dothidea	0.29%
Hormonema	0.21%
Botryosphaeria	0.04%
Epicoccum	0.20%
Stomiopeltis	0.00%
Venturia	0.01%
Elsinoe	0.02%
Myriangium	0.00%
Rhytidhysteron	0.05%

Curreya	0.02%
Leptosphaeria	0.13%
Lophiostoma	0.04%
Massarina	0.07%
Letendraea	0.00%
Paraconiothyrium	0.05%
Paraphaeosphaeria	0.09%
Phaeosphaeria	0.03%
Alternaria	0.49%
Bipolaris	1.15%
Cochliobolus	0.17%
Curvularia	0.09%
Dendryphiella	0.01%
Embellisia	0.00%
Exserohilum	0.05%
Lewia	0.05%
Pyrenochaeta	0.02%
Stagonospora	0.04%
Corynespora	0.03%
Leptosphaerulina	0.29%
Preussia	0.07%
Sarcinomyces	0.45%
Capronia	0.01%
Cladophialophora	0.03%
Cyphellophora	0.01%
Exophiala	0.08%
Phaeococcomyces	0.34%
Aspergillus	0.25%
Emericella	0.03%
Eurotium	1.10%
Neosartorya	0.04%
Penicillium	1.51%
Thysanophora	0.01%
Mycocalicium	0.01%
Auxarthron	0.00%
Chrysosporium	0.01%
Verrucaria	0.32%
Lecanora	0.00%
Bryoria	0.00%
Punctelia	0.01%
Phaeophyscia	0.17%
Physcia	0.03%
Ramalina	0.01%

Caloplaca	0.04%
Acephala	0.02%
Cadophora	0.00%
Naemacyclus	0.01%
Phialocephala	0.00%
Botryotinia	0.01%
Erysiphe	0.03%
Golovinomyces	0.06%
Neoerysiphe	0.01%
Podosphaera	0.03%
Sawadaea	0.00%
Geomyces	0.00%
Pseudeurotium	0.00%
Lophodermium	0.01%
Arthrobotrys	0.01%
Dactylaria	0.02%
Ascobolus	0.01%
Morchella	0.02%
Pseudaleuria	0.01%
Kazachstania	0.01%
Saccharomyces	0.01%
Candida	0.00%
Debaryomyces	0.02%
Hanseniaspora	0.01%
Phaeoacremonium	0.00%
Harknessia	0.01%
Pilidiella	0.00%
Phomopsis	0.03%
Valsa	0.02%
Colletotrichum	0.01%
Glomerella	0.01%
Paecilomyces	0.01%
Hypocrea	0.03%
Beauveria	0.03%
Cordyceps	0.08%
Fusarium	0.73%
Isaria	0.04%
Myrothecium	0.02%
Sarocladium	0.05%
Stachybotrys	0.10%
Gibberella	0.19%
Nectria	0.04%
Periconia	0.12%

Kernia	0.01%
Scedosporium	0.02%
Scopulariopsis	0.06%
Chaetomium	0.20%
Corynascus	0.00%
Podospora	0.01%
Neurospora	0.30%
Arthrinium	0.34%
Magnaporthe	0.02%
Phialophora	0.02%
Nigrospora	0.89%
Pestalotiopsis	0.12%
Seiridium	0.04%
Truncatella	0.44%
Diatrype	0.00%
Diatrypella	0.00%
Eutypella	0.03%
Annulohypoxylon	0.00%
Hypoxylon	0.02%
Nodulisporium	0.02%
Dicyma	0.02%
Microdochium	0.01%
Taphrina	0.02%
Agaricus	0.02%
Marasmius	0.00%
Moniliophthora	0.01%
Oudemansiella	0.00%
Strobilurus	0.06%
Pleurotus	0.16%
Coprinellus	0.27%
Coprinopsis	0.05%
Psathyrella	0.02%
Hypholoma	0.01%
Omphalina	0.01%
Xeromphalina	0.02%
Amylocorticium	0.01%
Gloeophyllum	0.08%
Trechispora	0.00%
Athelia	0.05%
Heterobasidion	0.59%
Cylindrobasidium	0.01%
Hyphodontia	0.37%
Limonomyces	0.00%

Phanerochaete	0.06%
Phlebia	0.04%
Sistotrema	0.01%
Tubulicrinis	0.02%
Bjerkandera	0.05%
Peniophora	0.32%
Amylostereum	0.18%
Stereum	0.10%
Auricularia	0.01%
Exidia	0.05%
Myxarium	0.02%
Leucogyrophana	0.02%
Serpula	0.01%
Coniophora	0.01%
Botryobasidium	0.01%
Ceratobasidium	0.03%
Geastrum	0.00%
Fuscoporia	0.00%
Inocutis	0.01%
Resinicium	0.01%
Ganoderma	0.14%
Polyporus	0.03%
Trichaptum	0.00%
Antrodia	0.05%
Antrodiella	0.00%
Coriolopsis	0.04%
Phlebiopsis	0.05%
Steccherinum	0.01%
Trametes	0.24%
Tomentellopsis	0.01%
Bensingtonia	0.00%
Curvibasidium	0.07%
Erythrobasidium	0.00%
Rhodotorula	0.24%
Sporobolomyces	0.07%
Wallemia	0.08%
Microbotryum	0.00%
Cronartium	0.01%
Melampsora	0.01%
Puccinia	0.04%
Melampsoridium	0.00%
Septobasidium	0.01%
Rhodotorula	0.11%

Sporobolomyces	0.01%
Cystofilobasidium	0.05%
Udeniomyces	0.06%
Cryptococcus	0.47%
Bulleromyces	0.00%
Tremella	0.02%
Dioszegia	0.03%
Trichosporon	0.01%
Thecaphora	0.01%
Pseudozyma	0.14%
Sporisorium	0.01%
Tranzscheliella	0.01%
Ustilago	0.30%
Rhizopus	0.02%

Table S4. Main allergenic/pathogenic fungal genera found in the atmosphere during the dry season (March 2016) in Mexico City.

Division	Allergens	Clinical cases published	Anamorph / Teleomorph	Synonym
Ascomycota				
Alternaria[a]	*	*		
Arthrinium[a]		*	*Apiospora*	
Aspergillus[a]	*	*	*Eurotium* (more 70 names)	DH
Aureobasidium	*	*		Columnosphaeria, DH
Chaetomium	*	*		
Cladosporium[a]	*	*	*Davidiella*	DH
Cochliobolus[t]	*	*	*Bipolaris, Curvularia*	
Epicoccum[a]	*	*		DH
Fusarium[a]	*	*	*Gibberella, Nectria*	DH
Leptosphaeria[t]	*		*Phoma*	
Neurospora[t]	*		*Chrysonilia*	
Nigrospora		*		*Khuskia*
Penicillium[a]	*	*		DH
Phaeococcomyces		*		*Exophiala*
Phoma[a]		*		Westerdykella
Sarocladium		*		DH
Truncatella[a]		*		

Basidiomycota				
Coprinellus	*			
Cryptococcus	*	*		
Ganoderma	*			
Pseudozyma		*		
Rhodotorula		*		
Ustilago	*	*		
Others				
Mucor		*		
Rhizopus		*		

Superscripts indicate sexual state (a = anamorph and t = teleomorph); each genus is marked with an asterisk when clinical cases have been reported for allergy or other pathology; DH = Dematiaceous hyphomycetes.