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RESEARCH ARTICLE

Airborne bacteria associated with particulate matter from a highly urbanised metropolis: A potential risk to the population's health

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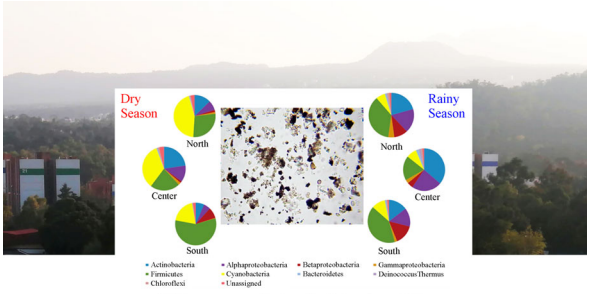
HIGHLIGHTS

- The airborne bacteria of Mexico City are representative of urban environments.
- Particle material < 10 μm influenced the type and quantity of airborne bacteria.
- The diversity and richness of bacteria were higher in the rainy season.
- The emission & transport of airborne bacteria determine the atmosphere's microbiome.
- Bacterias as *Kocuria*, *Paracoccus,* and *Staphylococcus* were in the air of Mexico City.

GRAPHIC ABSTRACT



Airborne particles in Mexico City

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ABSTRACT

Bacteria in the air present patterns in space and time produced by different sources and environmental factors. Few studies have focused on the link between airborne pathogenic bacteria in densely populated cities, and the risk to the population's health. Bacteria associated with particulate matter (PM) were monitored from the air of Mexico City (Mexico). We employed a metagenomic approach to characterise bacteria using the 16S rRNA gene. Airborne bacteria sampling was carried out in the north, centre, and south of Mexico City, with different urbanisation rates, during 2017. Bacteria added to the particles were sampled using high-volume PM_{10} samplers. To ascertain significant differences in bacterial diversity between zones and seasons, the Kruskal-Wallis, Wilcoxon tests were done on alpha diversity parameters. Sixty-three air samples were collected, and DNA was sequenced using next-generation sequencing. The results indicated that the bacterial phyla in the north and south of the city were Firmicutes, Cyanobacteria, Proteobacteria, and Actinobacteria, while in the central zone there were more Actinobacteria. There were no differences in the alpha diversity indices between the sampled areas. According to the OTUs, the richness of bacteria was higher in the central zone. Alpha diversity was higher in the rainy season than in the dry season; the Shannon index and the OTUs observed were higher in the central zone in the dry season. Pathogenic bacteria such as *Kocuria*, *Paracoccus*, and *Micrococcus* predominated in both seasonal times, while *Staphylococcus*, *Corynebacterium*, and *Nocardioides* were found during the rainy season, with a presence in the central zone.

1 Introduction

The biosphere integrates all living beings and their interactions. Among them, the microbiome constitutes a fundamental component of this system. The alteration of the quality of the biosphere caused by anthropogenic activities, the emission of greenhouse gases, and other environmental pollutants generate severe impacts on human health and ecosystems and have economic, social, cultural, and political repercussions.

The airborne microbiome comprises bacteria, viruses, fungi, and aerosolised toxins, among others. Their presence in the air is considered merely transitory and not as a place or biotope in which they carry out

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interspecific interactions. Several studies have demonstrated specific microbial community structures, either in intramural, extramural, urban, rural, or agricultural areas or shifts in the composition related to seasonality (Gandolfi et al., 2013; Du et al., 2018).

The urbanisation process has a significant impact on health since accelerated population growth has led to new concerns, such as air, water, and soil contamination; urban heat islands; and non-communicable diseases, such as heart disease, stroke, asthma, and other respiratory illnesses. Over 55% of the world's population lives in urban areas, a proportion that is expected to increase to 68% by 2050 (Joint Research Centre, 2019).

Urbanisation implies changes in the ecosystem. Therefore, it is necessary to know the composition of the microbial community, mainly the one that favours the development of potential human pathogenic bacteria and their relative abundance in the environment, including the air. As levels of urbanisation increase, the health risk appears to increase due to potentially pathogenic bacteria (Li et al., 2019). It has been reported that the inherent characteristics of an urban area, such as its air quality, vegetation, buildings, vehicular traffic, and population density, alter the structure of the composition of microbiomes, understanding that these are formed by both the microbiota or by its "activity", that is, by metabolites, mobile genetic elements (such as transposons, phages, and viruses), and DNA, embedded in or coupled to airborne particulate matter in each zone (Berg et al., 2020) and to which we are exposed. However, despite the transcendent relationship between microbial exposure in urban areas and its impact on human health, much research is still needed on the subject (Flies et al., 2017, 2019, 2020).

It has been reported that on the earth's surface in natural settings almost 25% of all particles in the air of undisturbed habitats may consist of biological materials (Matthias-Maser and Jaenicke, 1994; Matthias-Maser and Jaenicke, 2000; Jones and Harrison, 2004; Clauß, 2015), while in urban and agricultural environments the percentage is usually higher. Because meteorological conditions regulate the communities of airborne bacteria in extramural environments, the various types of bacteria can be used as indicators of specific sources of bioaerosols, observing differences in the microbiome of urban and rural environments (Ruiz-Gil et al., 2020). Some studies have indicated that the environmental microbiomes of rural areas are more diverse than their urban peers (Flies et al., 2020). For example, a greater diversity of genera of Proteobacteria and Bacteroidetes has been reported in rural areas than in urban areas, as well as the presence of some pathogens, such as Salmonella and Legionella which are characteristics of urban areas (Ruiz-Gil et al., 2020).

Among the main atmospheric bioaerosols (a complex mixture of viable and non-viable microorganisms and other biomass) (Burrows et al., 2009a; 2009b; Després et al., 2012; Polymenakou, 2012; Gandolfi et al., 2013; Fröhlich-Nowoisky et al., 2016; Innocente et al., 2017) are the communities of bacteria that are commonly found in aggregates (Hesse, 1884,1888; Clauß, 2015) or adhered to particulate/dust material that is less than 10 μm in diameter, which could act as a carrier of bacteria (Smets et al., 2016; Zhen et al., 2017). People living in urban environments can be exposed to the inhalation of up to 100 million bacteria every day (Després et al., 2007). The adhesion of various types of bioaerosols, such as bacteria, to coarse particles has been determined; although, they are also found in the finest fractions and on other biological particles, such as whole or fragmented pollen (Meklin et al., 2002; Alghamdi et al., 2014). Likewise, some studies have shown that it is possible to detect airborne bacteria not associated with particles and impacted (in aggregates) on surfaces, such as nitrocellulose membranes (Chatterjee and Sigler, 2015) and cellophane tapes used in air sampling during consecutive days (Serrano-Silva and Calderón-Ezquerro, 2018).

The emission of bacteria from various sources (excretions of organisms, plant debris, leaf litter, agricultural activities, water reservoirs, waste treatment plants, sea spray, transport vehicles, and dust, among others) has been described as principal emission sources of bioaerosols into the atmosphere (Després et al., 2012; Tomasi et al., 2017), and their transport in the air of urban areas determines the atmospheric microbiome, thus marking the species' diversity and richness. Bacteria attached to particulate matter are carried by the wind from long distances and locally, which significantly determines the type of bacterial populations present, depending on the sources and environmental conditions. Moreover, airborne bacteria have an essential role in the ecological balance (Fang et al., 2007), and some bacteria are involved in atmospheric processes (Gandolfi et al., 2013, 2015).

The importance and influence of meteorological factors on the development, survival, and transport of bacteria in the atmosphere have been demonstrated in various studies. Among the reported findings, it has been indicated, for example, that in the spring season the ambient temperature, relative humidity, and magnitude of the wind are the determining factors in the type of bacterial communities present in the urban area (Du et al., 2018). Regarding temperature, the increase in temperature favours bacterial development and promotes the dispersion of bacteria in the air (Smets et al., 2016). On the other hand, it has been found that relative humidity is negatively correlated with the abundance of bacteria in the air (Zhen et al., 2017), while the magnitude of the wind has a strong impact on airborne bacteria because the force of the wind raises dust from various types of surfaces, aerosolising bacteria from local sources or carrying them from more distant places (Acosta-Martinez et al., 2015). The impact of meteorological factors on bacterial communities in the air is more significant than the presence of environmental pollutants, such as O_3, NO_2, SO_2, CO, and $PM_{2.5}$ (particulate matter

less than 2.5 μm in size) (Zhen et al., 2017). However, some authors have shown that the survival of microorganisms in the air can be affected by hydrocarbons, NO_2 (Ho et al., 2005), and trace elements (Jackson, 1978).

Studies on atmospheric bioaerosols have made it possible to evaluate the effectiveness of detecting biological agents that can cause diseases or even epidemics, mainly in densely populated urban areas (Be et al., 2015). Investigating potential bacterial pathogens and environmental factors influencing their community is critical to understanding the impact of bioaerosols on human health. Therefore, the objective of this study was to determine the structure of the airborne bacterial community composition associated with PM_{10} (particulate matter less than 10 μm) in three areas with different rates of urbanisation in Mexico City (Mexico) (north, centre, and south) during the dry and rainy season of 2017, with an emphasis on pathogenic bacteria, in order to detect possible biological agents that impact the health of the population.

2 Materials and methods

2.1 Sampling area

The study was carried out in Mexico City at 19°36′–19°02′ N and 98°56′–99°22'W, at an altitude of 2240 meters during three months of the dry season (January to April) and three months of the rainy season (August to October) of 2017. Mexico City has a cool dry season from November to February, followed by a warm dry season until April and a rainy season from May to October. Temperatures are moderate with low humidity. Mexico City is surrounded by mountains, with weak winds within the valley (Molina et al., 2009; Calderón-Ezquerro et al., 2016).

Aerobiological monitoring stations were installed in three different territorial demarcations of the city (Alcaldías Coyoacán, Cuauhtémoc, and Gustavo A. Madero). An urbanisation rate of each of the territorial demarcations selected was assigned considering the total area of each territorial demarcation and the number of inhabitants. Additionally, the percentages of some land-use types, such as parks, wildlife areas, and water bodies, were considered to characterise each area (Table 1).

The first aerobiological station was located at 19°32′52″N, 99°17′51″W in the south of the city [S] in the "Alcaldía Coyoacán" on the rooftop of the "Instituto de Ciencias de la Atmósfera y Cambio Climático" building, within the campus of the "Universidad Nacional Autónoma de México" (UNAM) (Table 1). The second station was located at 19°26′08″N, 99°08′22″W in the centre of the city (Historical) [C] in the "Alcaldía Cuauhtemoc" on the rooftop of the "Palacio de Minería" building, with scarce green areas, and the third station was located at 19° 30'43"N, 99°8'16"W in the north of the city [N] in the "Alcaldía Gustavo A. Madero" on the rooftop of the "Escuela de Medicina y Homeopatía" building within the campus of the "Instituto Politécnico Nacional" (IPN) (Table 1).

2.2 Sampling of bioaerosols attached to airborne particles

Particulate matter with an aerodynamic size of 10 μm was sampled using high volume samplers (PM_{10}) (GMW Model 1200, VFC $HVPM_{10}$; Sierra Andersen, Smyrna, GA, USA), which were cleaned with alcohol (70%) before and after each sampling. Samples were collected 3 times a week for 24 h periods. The sampler had an airflow rate of 1.13 m^3/min, and the particles impacted on nitrocellulose membranes (11302-131, Sartorius, Goettingen, Germany) were previously sterilised with ultraviolet light for 30 min. Each week, after 72 h of sampling, the membranes were collected from the sampler using sterile nitrile gloves and N95 masks and stored in sterile aluminium paper and then stored in sterile envelopes. The membranes with the impacted particles were stored at –70 °C until use. The particles impacted on the membranes were collected by placing each membrane independently in a glass beaker. The particles were mechanically detached by sweeping with a fine brush or scalpel that was previously sterilised with ultraviolet light for 30 min. Subsequently, the particles were collected in sterile glass vials, covered with aluminium foil, and stored in a plastic container at –70 °C until use. All glass bottles used to collect and store the particles were previously washed with Extran® 10% liquid soap, kept in the soap for 24 h, and rinsed with

Table 1 Characteristics of the sampling areas

Territorial demarcation	Urban land*	Green area*	Other (waterbody)*	Total	Number of inhabitants**	Urbanisation rate& (9209944 Inhabitants of Mexico City***)	Area (km^2)*
Coyoacán (S)	93.05%	6.95%	0.00%	100%	620416	6.74	53.62
Gustavo A. Madero (N)	83.53%	16.31%	0.16%	100%	1185772	12.9	87.38
Cuauhtémoc (C)	100.00%	0.00%	0.00%	100%	521348	5.66	32.34

Notes: *INEGI (2017); **INEGI (2020); ***UNIATMOS (2020) (Computer Science Unit for Atmospheric and Environmental Sciences. Institutional Repository Institute of Atmospheric Sciences and Climate Change, UNAM (2020)). & Urbanization rate (%) (number of inhabitants of each demarcation concerning the number of inhabitants of Mexico City (INEGI, 2020)).

running water and distilled water. The glass bottles were dried at 250 °C for 1 h. The glass and caps were sterilised in an autoclave.

2.3 Extraction of DNA

The samples were processed in a laminar flow cabinet that was previously cleaned with a benzalkonium chloride solution (0.1%) and sterilised with ultraviolet radiation (UV) light. To obtain metagenomic DNA, 10 mg of particles was resuspended in 2 mL tubes with a screw cap and semi-conical bottom with 400 μL of extraction buffer (0.1 M Tris-HCl, pH 7.5; 0.05 M EDTA (ethylenediaminetetraacetic acid), pH 8.0; 1 M KCl; and 0.1% Nonidet P40). For the extraction of genomic DNA, the Fast DNA Spin Kit for Soil (MP BIOMEDICALS, USA) was used following the manufacturer's recommendations. Three replicas of each sample (250 μL) were processed in 2 mL tubes with a screw cap. Two elutions with free nuclease water were made, each with a volume of 50 μL, to obtain a higher yield of DNA. The elutions were concentrated in a volume of approximately 25 μL with a SpeedVac Concentrator (DNA120 Savant equipment, Thermo Scientific, USA).

Metagenomic DNA was quantified in a Qubit fluorometer (Thermo Fisher Scientific, USA), which was then submitted to Macrogen (Macrogen Inc., Seoul, Republic of Korea) for 16S rRNA library construction. Briefly, the V3-V4 hypervariable region of the 16S rRNA gene was amplified using the primers S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013), according to Illumina protocol. The prepared libraries were sequenced in an Illumina MiSeq system (Illumina Inc., USA).

All sequencing data supporting the findings of this study can be found in the National Center for Biotechnology Information (NCBI) (Mexico) using accession number PRJNA771096 and BioSample accessions numbers SAMN22252569 to SAMN22252640.

2.4 Bioinformatic analysis and data processing

Paired-end reads were joined with FLASH (v.1.2.11) (Magoč and Salzberg, 2011). CD-HIT is a comprehensive clustering package. De-duplication was done using CD-HIT-DUP (identifies duplicates from single or paired Illumina reads) at 100% identity (Li et al., 2012). Chimera filtering and denoising were performed with CD-HIT-OTU (multi-step pipeline to generate operational taxonomic unit (OTU) clusters for ribosomal ribonucleic acid (rRNA) tags from 454 and Illumina platforms) (Li et al., 2012). Sequences with a minimum quality score of 33 were retained for downstream analysis. High-quality sequences were clustered into OTUs at 97% similarity using the CD-HIT-EST module (cluster deoxyribonucleic acid (DNA) data sets) (Li et al., 2012). The taxonomy assignment was done using UCLUST from QIIME (ver. 1.9.1) (Caporaso et al., 2010) against the Ribosomal Database Project (RDP). Statistical analysis was done in R (Team, 2020). Alpha diversity measures (observed OTUs, Shannon, and Simpson) were calculated from the observed OTU table rarefied to 2161 sequences per sample in QIIME (ver. 1.9.1) (Caporaso et al., 2010). The Kruskal-Wallis and Wilcoxon tests from the package stats (Team, 2020) were used to detect significant OTU richness differences between zones and seasons, respectively. Heat maps were done in the pheatmap package (Kolde, 2015).

Beta diversity analysis was done at the class level with Bray-Curtis distances matrices using non-metric dimensional analysis (NMDS) performed in the vegan package (Oksanen et al., 2017). Differences between zones and seasons at a class level were explored through permutational multivariate analysis of variance using the distance matrices test (adonis) done in the vegan package (Oksanen et al., 2017). The Kruskal-Wallis test and post hoc Dunn's test were done with all samples at class and genus levels to determine differences between zone and seasonal samples using stats (Team, 2020) and Fisheries Analyses with R (FSA packages) (Ogle, 2018).

2.5 Indicator taxa analysis

Indicator species analysis can be used as an ecological indicator of the presence of an organism in a specific environment (Dufrêne and Legendre, 1997; De Cáceres et al., 2010). To assess the specificity of some genus from each area or season, the data of relative abundance at the genus level was used to calculate the indicator species value (IndVal) (Dufrêne and Legendre, 1997) (in the indicspecies package ver. 1.7.1 (De Cáceres et al., 2010)) from R (Team, 2020). The IndVal was used to identify the prevalence of a specific genus for each zone and season. We considered the values > 0.95 and $p \leqslant 0.001$ as indicator taxa.

3 Results

3.1 Bacterial community structure

A total of 473058 sequences after quality filtering and 2161 observed OTUs were obtained from 63 air samples. The rarefaction curves showed enough sequencing depth to ascertain the full bacterial diversity. The number of species was calculated based on the total OTUs obtained in all samples, thus determining the species richness through observed species. The asymptote showed adequate sampling according to the total number of identified species (Fig. 1). A total of 18 phyla, 35 classes, 75 orders, 179 families, and 485 genera were identified. According to the diversity alpha indices, there were no significant

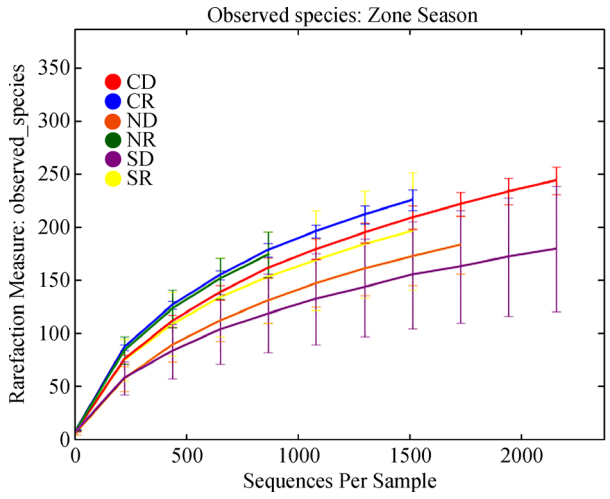

Fig. 1 Rarefaction curve for observed OTU metrics according to zone and season. The lines indicate the airborne samples from the centre zone (blue line), north zone (green line), and south zone (yellow line) in the rainy season and the airborne samples of the centre zone (red line), north zone (orange line), and south zone (lilac line) in the dry season. The error bars indicate the standard deviation (sd).

differences among the three sampling areas (Kruskal-Wallis test), but the richness was higher (according to the observed OTUs) in the centre zone than in the north and south areas ($p < 0.007$) (Table 2A, Fig. 2(a)).

The alpha diversity was higher in the rainy season than in the dry season ($p < 0.001$) (Table 2B). In addition, during the rainy season there were no significant differences in alpha diversity between the studied zones; however, during the dry season, the Shannon index and observed OTUs were significantly higher in the centre zone than in the other zones (Table 3).

3.2 Bacterial community structure by site

The bacterial phyla composition in the north and south zone were similar; although, their relative abundance to each site varied, with Firmicutes (N = 33%; S = 50%), Cyanobacteria (N = 26%; S = 15%), Proteobacteria (N = 22%; S = 23%), and Actinobacteria (N = 17%; S = 11%) being the dominant phyla. In the centre zone, Actinobacteria (30%) predominated, followed by Proteobacteria (24%), Cyanobacteria (21%), and Firmicutes (21%) (Fig. S1).

A heatmap of the relative abundances of the most abundant bacterial taxa in each site is represented in Fig. 3. For example, in the northern zone the Oscillatoriales (Cyanobacteria), Clostridiales, and Bacilliales (Firmicutes) had the highest values. In the central zone, Oscillatoriales, *Kocuria* (*Actinobacteria*), and *Paracoccus* (*Proteobacteria*) had the highest values, while in the southern zone *Bacillus*, *Clostridium* (Firmicutes), and Oscillatoriales had the highest values (Cyanobacteria) (Figs. 3(a) and S1).

The main genera in the north zone were *Clostridium* (8%), *Kocuria* (6%), *Turicibacter* (6%), *Bacillus* (5%),

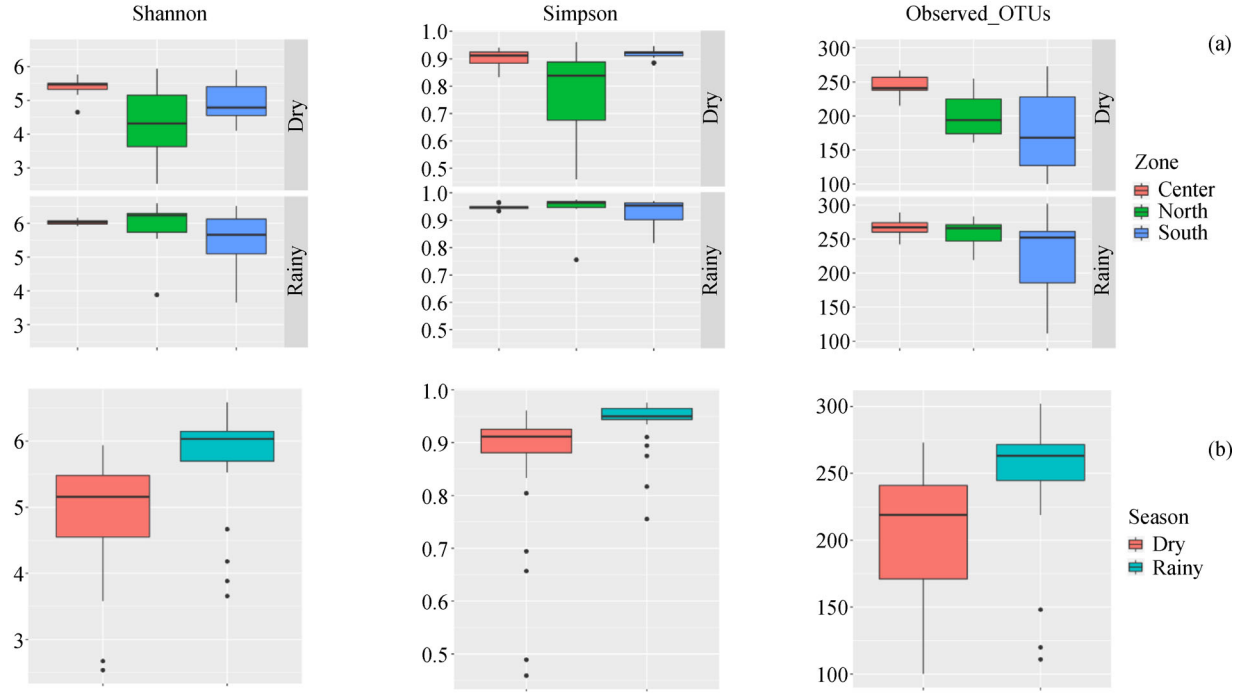

Fig. 2 (a) Alpha diversity indices and observed OTUs of the three sampling areas. (b) Alpha diversity indices and observed OTUs between the dry and rainy seasons.

Table 2A Alpha diversity indices in the three different sampling areas

Alpha diversity parameter	Zone			Kruskal-Wallis test
	Mean North	Mean Centre	Mean South	*p* value
Simpson	0.846	0.924	0.923	0.789
Shannon	5.004	5.689	5.199	0.134
Observed OTUs	226.000	253.000	199.000	0.007*

Table 2B Alpha diversity indices by season

Alpha diversity parameter	Season		Wilcoxon test
	Mean Dry	Mean Rainy	*p* value
Simpson	0.868	0.938	< 0.001
Shannon	4.908	5.800	< 0.001
Observed OTUs	208.000	248.000	< 0.001

Notes: *Post hoc Dunn test, Centre vs. South, p value = 0.005.

Paracoccus (4%), and *Bordetella* (3%). In the central zone, *Kocuria* (14%), *Paracoccus* (6%), *Clostridium* (4%), *Bacillus*, and *Turicibacter* (3%) were the main genera, and in the southern zone *Bacillus* (14%), *Clostridium* (9%), *Raistonia* (10%), *Turicibacter* (7%), and *Kocuria* (3%) were the main genera (a list of genera with relative abundances of >1% is shown in Fig. S1).

3.3 Bacterial community structure by season

According to the Wilcoxon test, the diversity indices showed a highly significant difference between dry and rainy seasons ($p < 0.005$). Higher richness and diversity values were recorded during the rainy season than during the dry season (Table 2B, Fig. 2(b)).

The main bacterial phyla collected from the air of Mexico City, in general, showed differences in relative abundance between the dry and rainy seasons, registering higher species richness during the rainy season. Firmicutes (36%) and Cyanobacteria (32%) predominated during the dry season, while the phyla Firmicutes (32%), Proteobacteria (31%), and Actinobacteria (24%) were more abundant during the rainy season (Figs. S2 and S3).

3.4 Spatio-temporal analysis

3.4.1 Dry season

Firmicutes predominated (58%) in the south of the city, while Cyanobacteria showed greater abundance in the northern zone (46%), followed by the central zone (35%) and south (20%). The highest relative abundances within this phylum were represented by the Oscillatoriales, showing the highest values in the north zone (45%) (in central and south areas, the values were 33% and 19%, respectively). Actinobacteria (24%) and Proteobacteria (16%) (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) were recorded with the highest relative abundances in the central area (Fig. S4).

At the genera level, *Bacillus* recorded 20%, *Turicibacter* 10% and *Clostridium* 9% all them in the south area) (Figs. 3(b), 3(c) and S4).

3.4.2 Rainy season

In the rainy season, the highest abundance of Firmicutes (41%) was found in the southern zone, followed by the north (37%) and centre (19%) areas. The phyla Actinobacteria had the second greatest abundance and was higher in the centre area (37%), followed by the northern (21%) and southern (15%) areas. The phyla Proteobacteria was found in the three areas of the city and with similar abundance (32%–31%). Simultaneously, the phylum Cyanobacteria (10%) registered its highest abundance in the southern zone, although with much lower percentages in north (7%) and central areas (7% and 8%, respectively) than those registered during the dry season (Figs. 4 and S4).

At the genera level, *Kocuria* was the most abundant airborne bacteria in all samples, presenting differences between the rainy and dry seasons in both centre ($p < 0.01$) and south zones ($p < 0.05$). It was followed mainly by *Clostridium* with 10% and 8% (north and south areas, respectively) and by *Bacillus* and *Paracoccus*, both

Table 3 Alpha diversity indices corresponding to the three different sampling areas during the dry and rainy seasons

Season	Alpha diversity parameter	Zone			Kruskal-Wallis test
		North	Centre	South	*p* value
Dry	Simpson	0.769	0.903	0.918	0.011*
	Shannon	4.277	5.400	4.948	0.007**
	Observed OTUs	201.000	242.000	179.000	0.002***
Rainy	Simpson	0.939	0.948	0.929	0.268
	Shannon	5.893	6.027	5.498	0.424
	Observed OTUs	257.000	266.000	223.000	0.183

Notes: * Post hoc Dunn test, South vs. North, p value = 0.002; ** Post hoc Dunn test, Centre vs. North, p value = 0.002; *** Post hoc Dunn test, Centre vs. South, p value = 0.001.

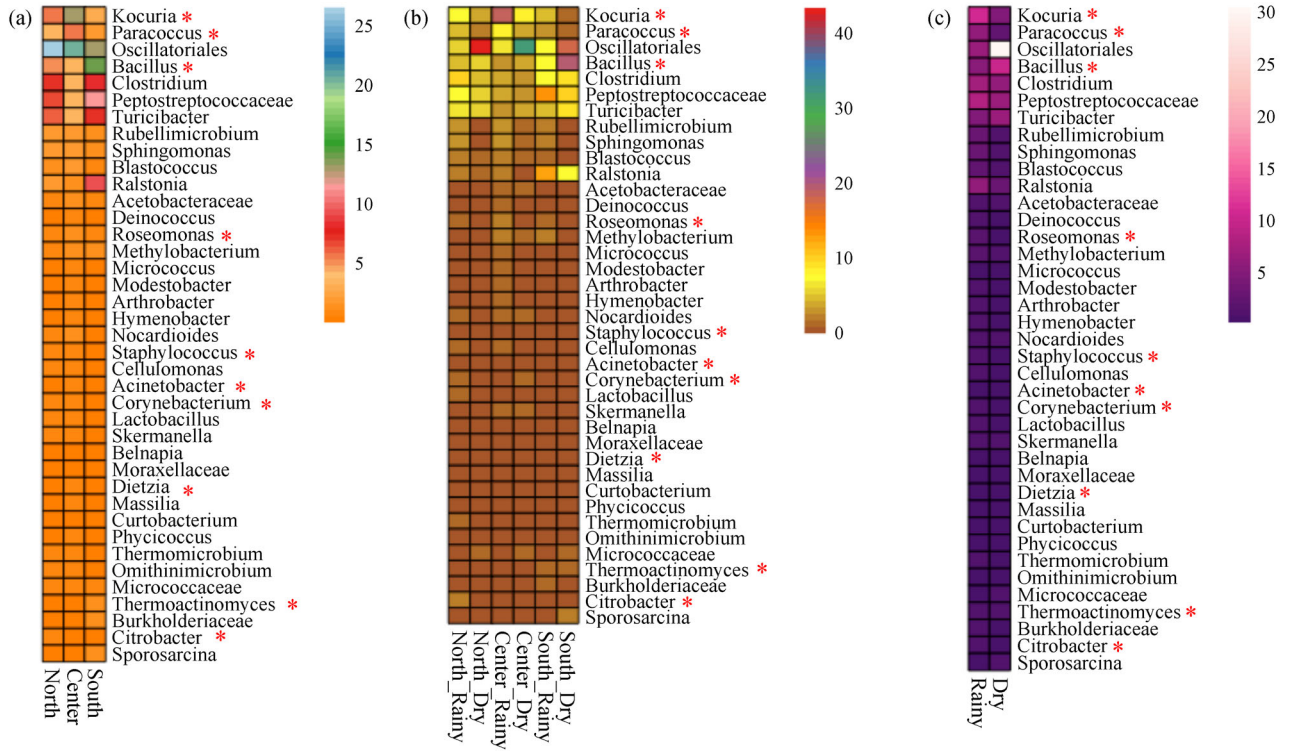

Fig. 3 Heat map of the relative abundance of the most abundant taxonomic groups of airborne bacteria (a) by zone, (b) by zone and time of year, and (c) by season. *Genera of bacteria reported as pathogens.

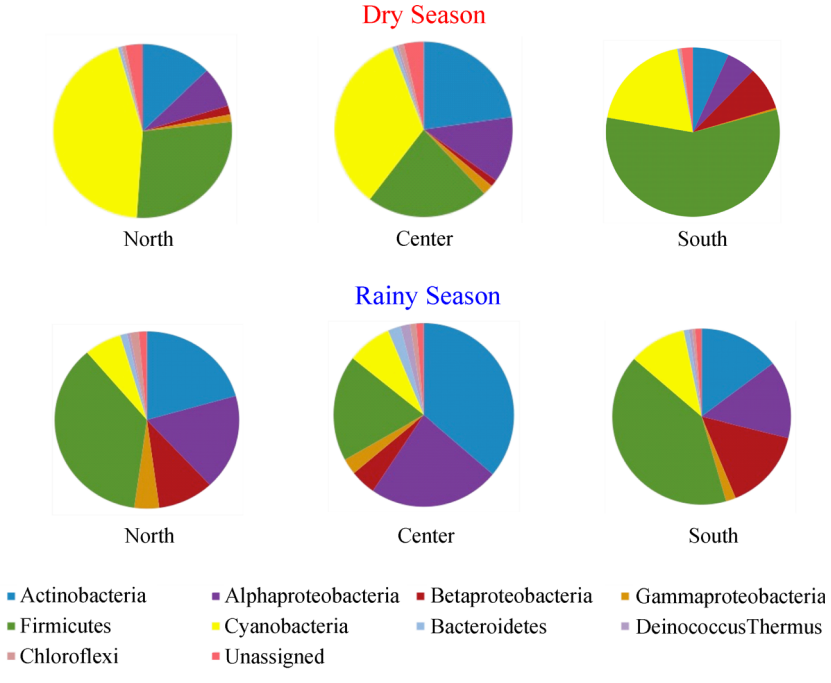

Fig. 4 Main taxa of airborne bacteria registered in the northern, central, and southern zones of Mexico City during the dry and rainy seasons.

with 8% (south and central areas, respectively) (Figs. 3(b), 3(c) and S4). The relative abundance of *Kocuria*, *Paracoccus*, *Roseomonas*, *Belpania*, and *Massilia* was higher and significantly different in the rainy season than in the dry season in the centre zone (Table S1).

Within the Enterobacteriaceae, the *Citrobacter* genus was recorded in greater abundance during the rainy season north of the city, followed, to a lesser extent, by the south. Likewise, *Escherichia* was collected mainly in the northern zone during the rainy season. *Lactobacillus* was found mainly in the northern and central areas in both seasons, although with a higher percentage in the rainy season. Similarly, *Streptococcus* was found with a lower abundance in the southern zone in dry and rainy seasons. The rest of the determined genera showed low abundance, mainly in the dry season. The relative abundance of the main genera of airborne bacteria found by zone and season, related to the faecal microbiome reported in the literature, is shown in Table S2.

The bacterial community structure at the class level was significantly different between the dry and rainy seasons, according to the Bray-Curtis and adonis test ($p < 0.001$), shown in Fig. S5.

3.5 Indicator bacteria

Ricketssia and *Hymenobacter* were genera that were frequently present during the rainy season, while *Cylindrospermum* was present during the dry season. The presence of specific microorganisms was mainly influenced by seasonal conditions (Fig. S6).

The taxa that showed significant differences (post hoc Dunn test) between zones and stations in the rainy season were Actinobacteria, *Kocuria*, *Paracoccus*, *Cytophagia*, *Deinococcus*, and *Micrococcus*, among others, while during the dry season they were Cyanobacteria, Oscillatorialles, Actinobacterias, Clostridia, *Kocuria*, *Bacillus*, *Ralstonia*, *Paracoccus*, and Nocardioides (Table S3). From the abovementioned, the central zone showed the highest relative abundance, both in the rainy season and the dry season, of the taxa mentioned in Table S3. Genera that may contain pathogenic species are indicated in red and with an asterisk, observing that the presence of some pathogenic genera, such as *Kocuria, Paracoccus*, and *Micrococcus*, were found both times of the year; although, during the rainy season they were more abundant. Likewise, the highest relative abundance percentage of these genera was found in the central zone, followed by the north and south zones.

In the dry season, some bacteria containing pathogenic species, such as *Staphylococcus*, *Corynebacterium*, and *Nocardioides*, were recorded, among other bacteria, with more significant presence in the central zone.

4 Discussion

The general analysis of annual airborne bacteria among the sampling areas showed that the highest relative abundances corresponded mainly to the phyla Firmicutes, Proteobacteria, Actinobacteria, and Cyanobacteria. The above agrees with studies in urban areas where Firmicutes and Proteobacteria were the dominant phyla (Be et al., 2015; Li et al., 2019). In the central zone, the most abundant phyla were Actinobacteria (30%), followed by Firmicutes and Cyanobacteria in the same proportion (21%) and by Proteobacteria. Likewise, this coincides with that recorded in studies on atmospheric bioaerosols in urban areas, which showed that bacterial communities in the air, such as in Milan, Italy, and New York, USA, were dominated by Actinobacteridae (Bowers et al., 2011; Bertolini et al., 2013). This study also agrees with a study of the atmosphere in Seoul, Republic of Korea, where bacteria attached to dust transported by the wind from arid and semi-arid areas were predominated by Proteobacteria, followed by Actinobacteria and Firmicutes in meagre abundances, while in the urban area of Seoul the community composition was similar but with variations in the abundances since Actinobacteria and Firmicutes increased (Cha et al., 2017).

Some authors have mentioned Proteobacteria as the most abundant airborne bacterial phylum; the most representative orders are Pseudomonadales, Burkholderiales, Rhizobiales, Rhodospirillales, and Sphingomonadales (Bowers et al., 2013; Gandolfi et al., 2013; Ruiz-Gil et al., 2020). In the present study, Proteobacteria showed similar relative abundances in the three monitored areas; however, this phylum was surpassed by Actinobacteria in the centre zone and Firmicutes in the north and south zones. These changes in the structure of the airborne bacterial community have been associated with atmospheric sources and other factors. Smets et al. (2016) mentioned that these shifts might have a pattern for each season of the year according to specific zones. Although the airborne bacterial community composition occurs seasonally, the interaction between atmospheric factors and bacterial diversity has been indicated (Gao et al., 2015; Ruiz-Gil et al., 2020).

Another important group identified was the phylum Cyanobacteria (Oscillatoriales). Thanks to the metagenomic approach, it has been possible to determine with greater precision the airborne spatio-temporal variation of this group, which allowed for greater knowledge about their presence in the air, compared to the information that was previously available when conventional sampling and identification techniques were used.

Cyanobacteria were recorded mainly in the northern and central areas and with lower values in the southern area.

Previous studies carried out in Mexico City have shown the presence of Cyanobacteria in the aerial microbiome (Roy-Ocotla and Carrera, 1993; Serrano-Silva and Calderón-Ezquerro, 2018; Calderón-Ezquerro et al., 2020; Calderón-Ezquerro et al., 2021). Some studies have confirmed that these phyla are found in the air as a consequence of their emission from the ground, vegetation, buildings, trees and roofs, rain, snow, lakes, sea and freshwater reservoirs, and untreated water (Antoinette van Overeem, 1937; Carson and Brown, 1976; Sharma et al., 2007; Genitsaris et al., 2011; May et al., 2018; Wiśniewska et al., 2019). Likewise, Cyanobacteria emissions from water bodies can be produced by human activity (Benson et al., 2005; Backer et al., 2010; Wiśniewska et al., 2019). They can also be released into the atmosphere as particle fragments (Fröhlich-Nowoisky et al., 2016; Wiśniewska et al., 2019).

It has been reported that particulate material is carried in the atmosphere of Mexico City through the prevailing winds at different times of the day (Edgerton et al., 1999; Molina et al., 2009; Calderón-Ezquerro et al., 2018; Wiśniewska et al., 2019) so that the airborne microbiota, including the Cyanobacteria attached to the particles, are transported in the air from their sources towards more distant areas. The north zone showed the highest relative abundance of Cyanobacteria. This zone was the largest of the areas studied and the one with the highest urbanisation rate and green areas and a high percentage of urban land use and water bodies, constituting sources of emission of Cyanobacteria, such as "Río de los Remedios", which is located at the north of the city near the sample site. This riverbed is known for its contamination since it is an open-air drainage of wastewater from Mexico City (Gonzáles, 2007).

Although the central zone was recorded with meagre green areas, it was possible to detect Cyanobacteria in the air. It must be considered that 5 km away from the sampling station is the Chapultepec Forest (678 ha), with 4 artificial lakes. Therefore, Cyanobacteria can be emitted from various sources, including the water bodies of Chapultepec, and transported from one place to another depending on the prevailing wind and its direction (Molina et al., 2009). The transport of bioaerosols in the atmosphere of Mexico City has been studied, indicating that the wind is a determining factor for the dispersion of aerosols in the atmosphere (Calderón-Ezquerro et al., 2018). Likewise, it has been reported that the wind carries phytoplankton and cyanobacteria such as *Microcystits aeruginosa* (very toxic) from one area to another (Chrisostomou et al., 2009).

The southern zone showed a lower relative abundance of Cyanobacteria in the air, similar to that reported in other studies in the same city (Serrano-Silva and Calderón Ezquerro, 2018; Calderón-Ezquerro et al., 2020, 2021). This zone is also characterised as an urban area; however, at approximately 15 km to the SE, it borders the Xochimilco demarcation, which has 79.9% of conservation areas with the "Parque Ecológico Xochimilco" that includes polluted water channels being sources of bioaerosols (Calderón-Ezquerro et al., 2021) transported by the wind and dispersed throughout the city (Bravo-Alvarez and Torres-Jardón, 2002).

Regarding the green areas, it has been shown that plants are a more potent source of bacteria than bare soil (Lindemann and Upper, 1985; Jones and Harrison, 2004). Therefore, the type of land use determines the microbiomes present in the atmosphere (Mhuireach et al., 2016; Flies et al., 2017, 2018). It has been found that the biodiversity of green areas that delimit cities may contain more diverse aerobiomes than those that occur inside the urban area (Flies et al., 2020).

It is interesting to note that in the study carried out by Serrano-Silva and Calderón-Ezquerro (2018), three methods for sampling bioaerosols were tested, indicating that the structure of airborne bacterial communities was affected by the device used. A great number of Cyanobacteria in southern Mexico City were collected with the Durham passive spore trap. In the present work, a similar type of high volume PM_{10} air sampler was used in the same zone during the dry season, identifying Cyanobacteria, although with lower relative abundance.

4.1 Genera of bacteria and PM_{10}

In this study, we found that the bacteria collected from PM_{10} filters, such as *Kocuria*, *Paracoccus*, and *Sphingomonas*, coincide with the same airborne bacteria associated with particulate matter that has been found in the Beijing atmosphere. These findings allow us to expand our knowledge about the structure of the bacterial community in various atmospheric conditions and geographic regions (Yan et al., 2018).

Regarding Actinobacteria, *Kocuria* was the most abundant genus; Firmicutes was represented by *Bacillus*, *Clostridium*, and *Turicibacter*, and Proteobacteria was formed mainly by Rhodobacterales, *Paracoccus*, and *Raistonia*. The influence of environmental pollution on the microbial communities present in the atmosphere of urban areas has been previously demonstrated. Several studies related to airborne bacteria made evaluations on days with low levels of air pollution, finding Firmicutes, Proteobacteria, and Actinobacteria as the predominant phyla. Unlike highly contaminated areas, it has been found that in terms of abundance, the predominance of the phyla changes, with the Actinobacteria phyla being more abundant, followed by Proteobacteria and Firmicutes (Bowers et al., 2011, 2013; Lymperopoulou et al., 2016; Mhuireach et al., 2016; Calderón-Ezquerro et al., 2020; Flies et al., 2020). The phyla predominance registered in our study agrees with that registered in urban areas with

high levels of environmental pollution, mainly for the central and northern areas of Mexico City. We can state that the presence of the diversity of bacteria emitted into the atmosphere comes from different local sources and those carried by the wind. Likewise, when evaluating the structures of the bacterial communities of the different regions of the city with marked differences in climatic conditions and land uses (Estrada et al., 2009; Calderón-Ezquerro et al., 2018), it was possible to determine a greater consistency at the phyla level than at the genus level. This agrees with that reported by Mu et al. (2020) when studying different regions of China. Likewise, with what was indicated in a review study on the possible influence of urbanisation on aerial microbiota (Flies et al., 2020), the authors pointed out that environmental variation is due to urban growth (changes in land use, number of inhabitants, and dwellings; altered populations of wild animals and plants). In the analysis of the reviewed studies, they concluded that urbanisation reduces the abundance and diversity of the aerial microbiota, concerning those registered in rural areas. More studies are needed to determine how these changes affect human health.

4.2 Bacterial community structure by season

In this study, the airborne bacterial diversity varied with the seasons and among the evaluated areas. The seasonal differences of bacterial communities were more significant than the spatial differences, which is consistent with what has been reported by Mu et al. (2020).

The alpha diversity parameters indicated significant differences ($p < 0.001$) concerning the diversity and richness of the phyla of bacteria collected between the dry and rainy seasons. Firmicutes and Cyanobacteria predominated in the dry season, while during the rainy season Firmicutes, Proteobacteria, Actinobacteria, and Cyanobacteria predominated. Although similarity was observed in the community composition at the phylum level, there was higher species richness during the rainy season.

At the genera level, *Kocuria* and *Paracoccus* predominated during the dry and rainy seasons. Also, during the dry season, bacteria such as *Nocardioides* and some genera of the Clostridia class (*Micrococcus*) and the Bacilli class (*Bacillus*) were detected.

The genus *Paracoccus* was found in the three study areas, showing significant differences between rainy and dry seasons, with greater abundance during the rainy season. In urban areas, bacteria such as *Kocuria*, *Clostridium*, *Bacillus*, and *Paracoccus* have been found adhering to particulate matter that is > 2.5 μm in size (Ruiz-Gil et al., 2020).

In this study, it was possible to detect bacteria from the particles impacted on the PM_{10} filters used for their collection, despite the large amount of other inorganic and organic particles present in an urban area with high atmospheric pollutants such as Mexico City.

The taxa of airborne bacteria collected in this study broadly coincide with those reported for other urban and suburban areas of the world (Franzetti et al., 2011; Bertolini et al., 2013; Gandolfi et al., 2015; Xu et al., 2017; Zhen et al., 2017; Liu et al., 2018; Li et al., 2019; Tanaka et al., 2019; Calderón-Ezquerro et al., 2020; Ruiz-Gil et al., 2020; Calderón-Ezquerro et al., 2021).

Cyanobacteria showed a marked seasonal pattern, showing greater abundance during the dry season when temperatures in Mexico City are high (25 to 30°C) and relative humidity decreases (40% and 60%), which coincides with what has been reported in some studies regarding the increase in Cyanobacteria during the warm seasons during the Spring (Wiśniewska et al., 2019). This could be explained by the fact that Cyanobacteria have excellent tolerance to variations in humidity. The bioaerosols of soil origin are more acclimatised to dryness, and they have a broad spectrum of moisture tolerance (Ehresmann and Hatch, 1975).

Cyanobacteria have also been considered the most resistant group to unfavourable conditions of the atmosphere, such as desiccation, atmospheric pollutants, and UV radiation (Sharma and Singh, 2010). Therefore, it is possible to find these organisms in hot and dry climates. Various studies in India have indicated that Cyanobacteria dominate during the warm season, while green algae are in greater abundance during colder months (Sharma et al., 2006a, 2006b). In addition, Lewandowska et al. (2017) conducted airborne microalgae and Cyanobacteria analyses in the southern Baltic Sea Region (Poland), finding that the highest number of Cyanobacteria taxa occurred in April during the warm spring, and later the diversity decreased. The authors suggested that the observed variability is due to the vegetation period of the algae and Cyanobacteria in the Baltic Sea, which act as a source of bioaerosols.

4.3 Bacteria and human health

Exposure and inhalation to bioaerosols, such as pathogenic bacteria present in the environment, represent a risk of developing infectious diseases, allergic reactions, inflammation, and respiratory diseases. Once bioaerosols enter the respiratory system, some bacteria can access other systems (such as gastrointestinal and cardiovascular systems) (Stetzenbach, 2009), producing adverse effects on human health (Stetzenbach, 2009; Camatini et al., 2012). In urban areas, exposure to bioaerosols can substantially impact human health since they contain particulate matter in the air. The association between air pollution in these areas and bioaerosols have provided evidence of a more significant impact on human health (Gouveia and Maisonet, 2006). Also, bioaerosols in ecosystems can spread plant and animal pathogens (Hirano and Upper, 1983; Pillai and Ricke, 2002).

Air is considered a carrier medium for bacterial pathogens, such as *Streptococcus pneumoniae*, *S. pyogenes*, *Mycoplasma pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonasaeruginosa*, and *Mycobacterium tuberculosis*, among many others (Smets et al., 2016). Microbes in the air have been found to adhere to airborne particles (Chen et al., 2020), playing a fundamental role in the transport of the microbiota in the atmosphere (Griffin, 2007). In our study, among the main phylum of bacteria collected together with the PM_{10} from the atmosphere of Mexico City were those of the phylum Firmicutes, which includes genera that cause diseases such as pharyngitis, conjunctivitis, meningitis, pneumonia, endocarditis, erysipelas, necrotising fasciitis, food poisoning, herpetic and exfoliative dermatitis, sepsis, bacteraemia, abscesses, meningitis, nosocomial infections, and infections of implanted prostheses (for example, heart valves and catheters). Likewise, we found bacteria of the phylum Proteobacteria, which contain genera that have been reported to be common and abundant in urban areas (Genitsaris et al., 2011). For example, although it has been reported that most *Paracoccus* species are environmental, some, such as *P. yeei*, have been associated with opportunistic human infections. These infections are mainly related to immunosuppressed people.

Actinobacteria have a wide distribution in various environments and trigger severe respiratory diseases (Paściak et al., 2014). In our study, Actinobacteria were among the main recorded phyla of urban air. The genus *Kocuria* was one of the genera collected from the air with the highest relative abundance, mainly in the central area (historical centre) of the city, where there is a large concentration of people due to the economic and tourist activities of the site. The previous agrees with what has been published by Calderón-Ezquerro et al. (2021) in a study also carried out in the urban area of Mexico City, as well as with studies of bacteria in the air reported by Zhou et al. (2008) and Yan et al. (2018) in the atmosphere of the cities of Xinjiang and Beijing, China, respectively. The genus *Kocuria* includes 11 species, of which only 3 have been determined to be pathogenic to humans. These are *K. rosea*, *K. varians*, and *K. kristinae*, which have been identified, although infrequently, as a cause of disease in immunosuppressed patients, such as those with HIV, causing severe bacteraemia (Stackebrandt et al., 1995).

We only found three taxa that were considered bioaerosol indicators: *Rickettsia*, *Hymenobacter*, and *Cylindrospermum* (Cyanobacteria). *Rickettsia* is a collection of obligate intracellular bacteria found in ticks, lice, fleas, mites, chiggers, and mammals. These bacteria cause infectious diseases transmitted in aerosols. In our study, these bacteria collected on PM_{10} filters during the rainy season and probably came from those transported in aerosols, raindrops, or attached to particulate matter in the air.

Likewise, in our study, we collected *Hymenobacter* (Bacterioidetes) as an indicator species, also with low relative abundance in the rainy season, which contrasts with what was reported in a study carried out in New York (USA) by Yooseph et al. (2013) on a metagenomic framework for the study of airborne microbial communities. The authors of this study reported *Hymenobacter* as one of the bacterial genera with the highest relative abundance (17.6%). Some other studies have reported that *Hymenobacter* species can survive in unfavourable conditions, such as desiccation or high radiation levels (Zhang et al., 2007; Krieg et al., 2010). We consider that it is necessary to continue monitoring aerial microbiota using various sampling equipment since it has been determined that using different airborne particle samplers increases the possibility of collecting a greater diversity of airborne bacteria during an entire annual cycle (Serrano-Silva and Calderón-Ezquerro, 2018).

The last type of indicator bacteria collected was the genus *Cylindrospermum* during the dry season. This is a genus of filamentous Cyanobacteria found in terrestrial (soil) and aquatic environments. It is a nitrogen-fixing cyanobacterium, and it is known to produce neurotoxins, which act in the transmission of the nerve impulse and cause death due to respiratory paralysis (González, 2015). Cyanobacteria such as *Microcystits aeruginosa* and *Anabaena* produce toxins such as microcystins and anabasine. Cyanotoxins belong to different groups of chemical substances, each of which has specific toxicity mechanisms in vertebrates. Some are potent neurotoxins and others have toxic activity on the liver (Microsistins nodularin). They have a *B-N* substance, methylamino-L-alanine, a neurotoxic amino acid associated with Alzheimer's-like dementia disease, with signs of parkinsonism and atypical motor neuron disease (Chorus and Welker, 2021).

Among the diversity of bacteria that we identified in the atmosphere of Mexico City, we detected genera of the Enterobacteriaceae *Citrobacter*, together with *Enterobacter*, *Klebsiella*, and *Escherichia*, forming the coliform group of enteric bacteria that can be found in the air. *Citrobacter* and *Escherichia* were detected with greater abundance in the northern and central areas of the city, which are areas characterised by being densely populated and with very high urban activity, in addition to the presence of "stray dogs" and "stray cats" that are not under the care of anyone, which increases faecalism in the open air. The detections made in our study coincide with that reported by Santos-Burgoa et al. (1994), who found the presence of *E. coli*, among other enterobacteria, in the air of Mexico City. Likewise, it has been reported in urban areas that it is common to find the faecal microbiome (Chen et al., 2020). *Escherichia* causes intestinal tract infections (diarrhoea), urinary tract infections, haemorrhagic colitis, and haemolytic-uremic syndrome in humans (Ye and Zhang, 2011). Also, in our study, we identified other pathogenic species that make up the faecal micro-

biome, such as *Lactobacillus* and *Streptococcus*, the latter with species that cause pharyngitis, conjunctivitis, meningitis, pneumonia, endocarditis, erysipelas, and necrotising fasciitis.

Despite the presence of such diverse bioaerosols in the atmosphere and, therefore, the risk of exposure to pathogenic bacteria and fungi, some studies provide growing evidence that some type of aerobiome exposure can have significant protective effects. Early exposure to bioaerosols can cause systemic changes in immune function that suppress allergies, asthma, and inflammation (Flies et al., 2020). On this basis, early exposure to abundant aerial fungi (Iossifova et al., 2007) or bacteria (Stein et al., 2016) is associated with a reduced risk of developing allergies/asthma.

It is essential to continue conducting aerobiological studies that allow us to monitor and understand the biological quality of the air, even within the same urban area, and to look for potential pathogenic taxa, their concentrations in the air, and their spatio-temporal variation.

These data will make it possible to evaluate the risk to the health of the inhabitants exposed both to pathogenic bacteria and to the particulate material present in the atmosphere of highly polluted urban areas.

5 Conclusions

Airborne phyla bacteria associated with particulate matter are representative of those found in the atmosphere of urban areas and present local and seasonal differences. The presence of airborne pathogenic bacteria registered in the study areas is a potential risk to the health of the population.

In Memory of
Dr. René Drucker Colin, for his love and dedication to science and especially for his support of Environmental Sciences, focused on biological and chemical pollution of the atmosphere and promoting Mexican scientists.

Declaration of Competing Interest
The authors have no conflicts of interest to declare.

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