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Microbial Contamination of Airborne Particulate Matter ($PM_{2.5}$ and PM_{10}) In Kuala Lumpur's Ambient Air

Nurul Tasha Zulkifle[1], Nurmizani Hashim[1], Hui-min Neoh[2], Nur Faizah Abu Bakar[3], Siti Shahara Zulfakar[1,♥]

[1] Environmental Health and Industrial Safety Program, Centre for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz,50300 Kuala Lumpur, Malaysia
[2] UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, Jalan Ya'acob Latiff, Cheras, Kuala Lumpur 56000, Malaysia
[3] Centre for Diagnostic, Therapeutic & Investigative Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300, Kuala Lumpur, Malaysia

ABSTRACT

Regional haze episodes frequently affect developing countries, causing severe air pollution and potential health risks. However, conventional air quality monitoring often overlooks the biological components of airborne particulate matter (PM). This study investigates microbial contamination associated with fine ($PM_{2.5}$) and coarse (PM_{10}) particulates in Kuala Lumpur during the Southwest Monsoon and Inter-monsoon II periods. Samples were collected using High Volume (HVS) and Low Volume Samplers (LVS) equipped with glass microfibre filters. Culture-based enumeration on Tryptone Soy Agar (TSA) and shotgun metagenomic sequencing were performed to quantify bacterial loads and characterize community composition. The highest bacterial counts in PM_{10} were recorded during the Southwest Monsoon and Inter-monsoon II, at 5.42 ± 0.49 and 5.40 ± 0.73 log CFU/g, respectively. No significant differences were observed between monsoon periods ($p > 0.05$), and bacterial abundance was not correlated with PM concentration. Metagenomic analysis revealed greater bacterial diversity during the Southwest Monsoon, dominated by *Firmicutes* and *Pseudomonadota*, including potentially pathogenic genera such as *Staphylococcus, Bacillus* and *Burkholderia*. In contrast, *Bacillus* and *Clostridium* predominated during Inter-monsoon II. These findings highlight seasonal variations in airborne bacterial communities and emphasize the need to incorporate microbial assessment into air quality monitoring frameworks.

Keywords: *Airborne bacteria; Particulate Matter ($PM_{2.5}$ and PM_{10}); Monsoon Seasons (SW and IM II)*

1. Introduction

Airborne particulate matter (PM), particularly fine ($PM_{2.5}$) and coarse (PM_{10}) fractions, is a key urban pollutant with serious environmental and health risks. These particles are not merely chemical and physical entities, but also serve as carriers for bioaerosols, comprising the bacteria, fungi and viruses, collectively known as the airborne microbiome. The biological fraction of PM has gained increasing attention due to its potential influence on atmospheric processes, human health and disease transmission. The coexistence of these bacterial fractions reflects the complex and dynamic nature of the airborne microbiome, influenced by environmental conditions and anthropogenic activities. Bacteria associated with PM can exist in both culturable and non-culturable states. Culturable bacteria can be isolated and quantified using traditional microbiological techniques, while

♥ *Corresponding author.*
E-mail address: sitishahara.zulfakar@ukm.edu.my

non-culturable bacteria, which may remain viable but undetectable by conventional methods, require molecular-based approaches, such as metagenomic sequencing for identification.

Seasonal variations, particularly the Southwest Monsoon and Inter-monsoon II periods, critically influence the airborne microbiome in tropical cities like Kuala Lumpur. The Southwest Monsoon's dry, windy weather increases PM levels and disperses bacteria over long distances [1]. In contrast, the Inter-monsoon II period reported by Zulkifle *et al.,* [2], washes the air with heavy rain, despositing particles and altering microbial communities through wet scavenging effects. Since key meteorological factors like temperature, humidity, wind speed and rainfall, directly control the concentration and viability of these airborne microbes, it is essential to understand their specific effects on both culturable and non-culturable bacteria associated with $PM_{2.5}$ and PM_{10}. This understanding, in turn, is key to assessing microbial air quality and protecting public health in urban tropical environments.

2. Methodology

2.1 Sample Collection

Particulate matter (PM) sampling was conducted on the rooftop of the Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur (3°8'20.4108"N, 101°41'12.678"E), during the Southwest Monsoon (May–September) and Inter-monsoon II (October–November). Glass microfibre filters (Whatman QMA, UK) were prepared according to Li *et al.,* [3], including sterilised at 450°C for 4 h, stored in a desiccator for 24 h and weighed before use. A high-volume sampler (HVS) (Tisch Environmental HVS 300–3X) was used for $PM_{2.5}$ and a low-volume sampler (LVS) (MiniVol TAS–5.0) for PM_{10} collection. $PM_{2.5}$ was collected for 24 hours at 1.13 m^3/min, while PM_{10} was collected for 120 hours at 0.010 m^3/min. All procedures were conducted aseptically [4]. Twelve samples were collected each season and blank filters were processed as controls. Meteorological parameters were recorded in situ and validated with data from the Malaysian Meteorological Department.

2.2 Cultivation of Airborne Bacteria

Half of each $PM_{2.5}$ and PM_{10} filter were used based on their particulate loads. Filters were cleaned with 75% ethanol, cut into 1 × 1 cm pieces, and placed in 50 mL tubes with phosphate buffer saline (PBS) and 0.1% Tween 20 to aid microbial release. Following the procedure outline by Luhung *et al.,* [5], samples were sonicated at 65°C for 30 minutes, vortexed for 15 minutes, and centrifuged at 2500 rpm for 10 minutes to separate cells and free DNA. Approximately 1 mL aliquot of the homogenised mixture was transferred into a microcentrifuge tube for analysis. Tenfold serial dilutions were also conducted by using 0.85% NaCl and 0.1 mL from each dilution was plated on tryptone soya agar (TSA) in duplicates [6]. Plates were incubated at 37°C for 24 hours to determine the total bacterial plate count.

2.3 Molecular Identification of Airborne Bacteria

Genomic DNA was extracted from particulate matter retained on the sampling filters using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's protocol and optimised for low biomass environmental samples as reported by Pan *et al.,* [7]. Following Ma *et al.,* [8], DNA concentration and purity were determined using a NanoDrop spectrophotometer by measuring absorbance at 260 nm, while integrity was verified by agarose gel electrophoresis, ensuring suitability for downstream sequencing. Approximately 200 ng of high-quality genomic DNA per sample was

used for next-generation sequencing (NGS) library preparation. DNA was randomly fragmented to an average size of 300-350 bp using a Covaris system, followed by end-repair, adapter ligation, and PCR amplification (8 cycles). The resulting libraries were purified and evaluated for fragment size distribution and quality using the Agilent 2100 Bioanalyser. As described by Alcock *et al.,* [9], only libraries meeting the required quality thresholds were subjected to paired-end sequencing (2 x 150 bp) on the Illumina NovaSeq platform.

2.4 Statistical and Bioinformatic Analysis

The concentrations of $PM_{2.5}$ and PM_{10} in Kuala Lumpur's ambient air during the Southwest Monsoon and Inter-monsoon II were analysed to assess microbial contamination and characterise bacteria. Descriptive analysis, one-way ANOVA, and Pearson's correlation were conducted using SPSS version 29.0 to compare seasonal variations and examine the relationship between particulate concentration and microbial load. The raw sequences from the shotgun metagenomic sequencing were assessed with FastQC v0.12.1 and filtered using Trimmomatic v0.39 to remove adapters and low-quality bases (Q<20) [10]. Clean reads were assembled using SPAdes v4.1.0 (metaSPAdes), and assembly quality was verified with QUAST v5.3.0 (MetaQUAST) [11].

3. Results

The concentration of particulate matter ($PM_{2.5}$ and PM_{10}) and associated bacterial loads showed temporal variation between the Southwest Monsoon and Inter-monsoon II periods in Kuala Lumpur (Table 1). The mean concentration of $PM_{2.5}$ (10.3 ± 3.1 μg/m³) and PM_{10} (15.9 ± 4.1 μg/m³) was higher during the Southwest Monsoon compared to Inter-monsoon II ($PM_{2.5}$: 6.7 ± 0.8 μg/m³; PM_{10}: 9.9 ± 1.0 μg/m³). The increase in particulate matter during the monsoon season could be attributed to drier atmospheric conditions and stronger surface winds, which promote the resuspension of soil dust, vehicular emissions and secondary aerosols [2]. In contrast, the higher humidity (78.8 %) and rainfall intensity (14.7 mm) during Inter-monsoon II likely enhanced wet deposition, reducing airborne particle concentrations [12].

Table 1
Meteorological parameters and sampling information for particulate matter samples collected during Southwest monsoon and Inter-monsoon II

Variables	SW Monsoon 6 July – 19 Sept 2025	Inter-monsoon II 1 Oct – 19 Nov 2025
Bacterial Count in $PM_{2.5}$ (log CFU/g)	3.40 ± 0.4	3.04 ± 0.6
Bacterial Count in PM_{10} (log CFU/g)	5.42 ± 0.49	5.40 ± 0.73
$PM_{2.5}$ Concentration (μg/m^3)	10.3 ± 3.1	6.7 ± 0.8
PM_{10} Concentration (μg/m^3)	15.9 ± 4.1	9.9 ± 1.0
Temperature (°C)	30.2 ± 1.3	28.0 ± 0.7
Wind Speed (m/s)	0.6 ± 0.0	0.6 ± 0.0
Relative Humidity (%)	65.9 ± 0.0	78.8 ± 2.7
Rainfall Density (mm)	3.9 ± 1.8	14.7 ± 1.3

Bacterial abundance followed a similar seasonal trend. The highest bacterial counts were recorded in PM_{10} samples during both the Southwest Monsoon (5.42 ± 0.49 log CFU/g) and Inter-monsoon II (5.40 ± 0.73 log CFU/g) while $PM_{2.5}$ samples exhibited comparatively lower bacterial concentrations (3.40 ± 0.4 log CFU/g and 3.04 ± 0.6 log CFU/g, respectively). Statistical analysis

indicated no significant difference ($p > 0.05$) between seasons, suggesting that bacterial load was relatively stable despite meteorological variation. This aligns with previous study reported by Wang *et al.,* [13] that airborne microbial concentrations are influenced more by local resuspension sources than by short-term climatic fluctuations. Meteorological parameters strongly shape microbial behaviour in the atmosphere. During the Southwest Monsoon, elevated temperature (30.2°C) and lower humidity (65.9%) may have enhanced bacterial aerosolisation from the soil and vegetative surfaces [2]. Conversely, the low temperature (28.0°C) and more humid (78.8%) conditions of Inter-monsoon II favoured microbial deposition through wet scavenging, leading to lower viable bacterial counts as reported by Yan *et al.,* [12]. The average wind speed remained stable at 0.6 m/s during both monsoon periods, implying a limited effect on local dispersion. However, long range air mass movement could still contribute to bacterial transport [14].

Metagenomic sequencing revealed distinct shifts in bacterial community composition between seasons (Figure 1). The Southwest Monsoon exhibited higher bacterial diversity, dominated by *Firmicutes* and *Pseudomonadota*, particularly genera, such as *Staphylococcus, Bacillus, Burkholderia*, and *Ralstonia*. These taxa are commonly associated with soil, vegetation and anthropogenic sources, such as vehicular exhaust and human activity, consistent with increased resuspension during dry periods [1]. The Inter-monsoon II samples, in contrast, were largely dominated by *Bacillus* and *Clostridium*, genera known for their spore-forming capabilities and tolerance to environmental stress [2]. This aligned with previous study conducted by Rahim *et al.,* [14] who suggested that the prevalence of these taxa under humid conditions influenced their resilience to moisture-driven deposition and limited dispersal. The presence of potentially pathogenic genera, such as *Staphylococcus* and *Burkholderia* highlights possible public health implications [15]. As noted by Long *et al.,* [16], urban populations may be exposed to opportunistic pathogens via inhalation, especially during dry monsoon periods when PM carrying bacteria remain suspended longer. These findings underscore the importance of incorporating bioaerosol monitoring into conventional air quality frameworks, as physical PM measurements alone may underestimate biological exposure risks.

Overall, the study demonstrates that both meteorological conditions and particle size influence bacterial abundance and diversity in urban air. The Southwest Monsoon favoured higher PM and bacterial concentrations, while the Inter-monsoon II period exhibited compositional shifts toward stress-resistant genera. Continuous monitoring combining culture-based and molecular approaches is therefore essential for understanding seasonal microbial dynamics in tropical urban atmospheres, such as Kuala Lumpur.

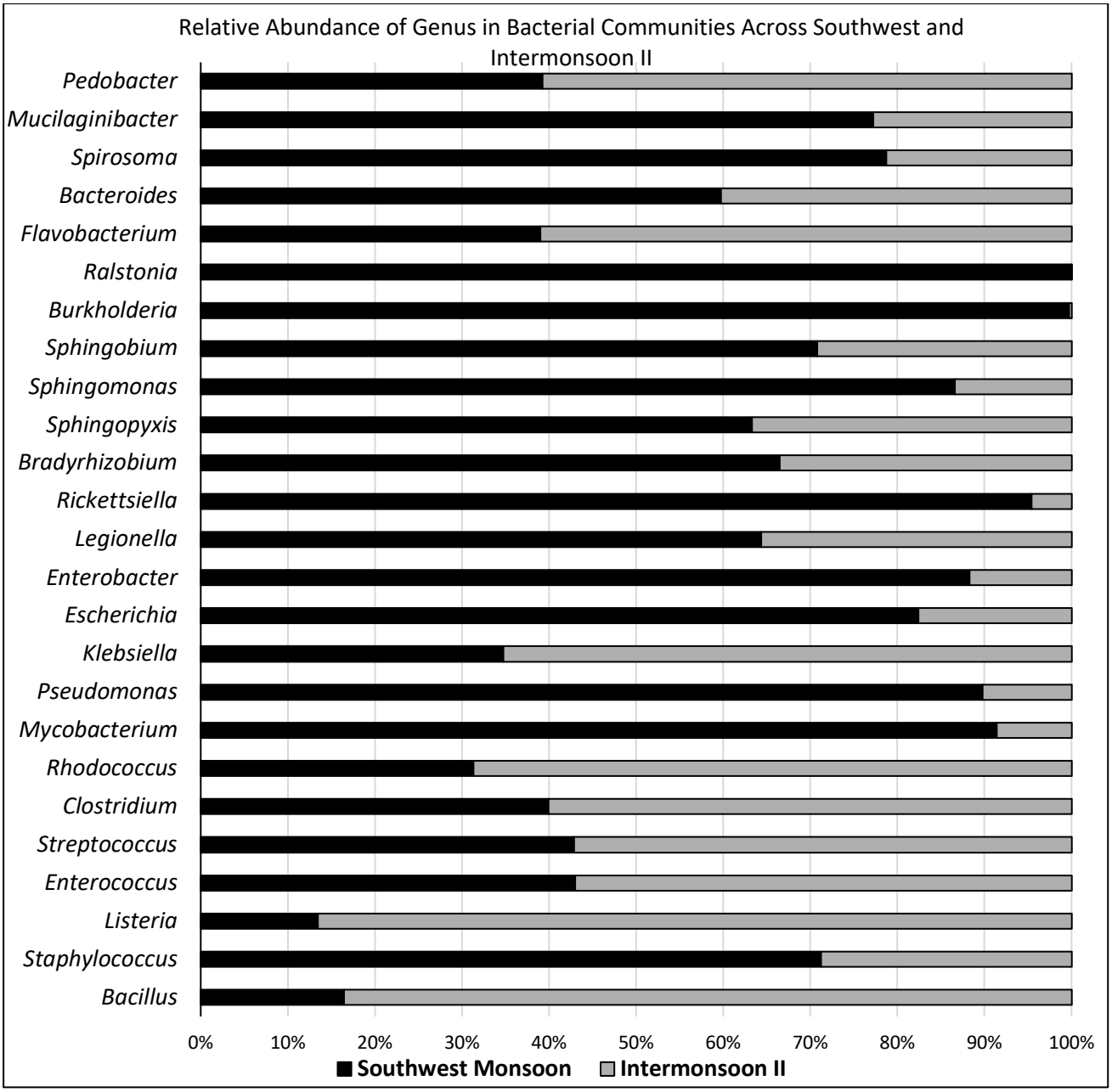

Fig. 1. Relative abundance of genus in bacterial communities across Southwest monsoon and Inter-monsoon II

4. Conclusions

This study documented distinct seasonal shifts in the bacterial communities associated with $PM_{2.5}$ and PM_{10} in Kuala Lumpur. While overall bacterial concentrations did not differ significantly between the Southwest Monsoon and Inter-monsoon II periods, the taxonomic composition changed significantly. The Southwest Monsoon featured higher bacterial diversity, dominated by *Firmicutes* and *Pseudomonadota*, while the Inter-monsoon II was characterised by a predominance of *Bacillus* and *Clostridium*. The compositional shifts are likely driven by meteorological factors, such as temperature, humidity, rainfall and wind speed that influence microbial dispersion and survival. The findings highlight that particulate matter acts as a key vector for bacterial transport and underscore the need for continuous bioaerosol monitoring to assess public health risks in urban environments.

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