Seasonal variations of microbes in particulate matter obtained from Dhaka City in Bangladesh

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ABSTRACT

The present study, for the first time, evaluated the seasonal variation of PM10-associated bacterial and fungal concentrations at four locations (CARS premises, Doyel Chattar, Postogola, and Chittagong Road) in Dhaka, Bangladesh. In this study, PM10 samples were collected four times on 20.3 × 25.4 cm irradiated glass fibre filter from November 2018 to August 2019. The concentrations of total airborne bacteria (14,073 ± 8,897 CFU/m3) were found to be significantly higher (one-way ANOVA; p < 0.05) in Chittagong Road, which is known for traffic congestion, than that of the other locations. The total airborne bacterial concentrations occurred in the following descending order: winter > spring > summer > rainy. Bacillus spp. and Staphylococcus spp. were found to be the dominant species present in PM10 particles. Based on Pearson correlation analysis and stepwise multiple-regression analysis, relative humidity was found to be the most important variable controlling the concentrations of total airborne bacteria. Common fungi such as Aspergillus, Penicillium, Cladosporium and Fusarium genera were identified in the PM10 samples. The highest fungal concentration (1,974 ± 1,173 CFU/m3) was found at Chittagong Road. The total fungal spore concentrations occurred in the following descending order: summer > spring > winter > rainy. From correlation analysis and stepwise regression analysis, the temperature was found to be the most important variable influencing the concentrations of fungi in PM10 samples. A dose-rate estimation study revealed that the children were more vulnerable compared to adults with respect to exposure to bacterial and fungal dose rates. The present study has enormous implications considering the health hazards the bacterial and fungal communities pose to humans.

1. Introduction

Over the last two decades, south Asian countries have seen brisk economic growth. The concomitant developmental activities in these countries have given rise to excessive air pollutants that are posing hazardous conditions for the population residing in the region [1,2]. Particulate matters (PMs) have been identified as a major air pollutant in densely populated cities or urban areas [3]. In the recent past, PMs have been given serious attention because of their potential detrimental impacts on public health. In humans, PMs can induce severe sickness or, in the worst case, even death [3]. Depending on their size, different PMs may travel varied distances and affect different parts of the human body. For instance, PM2.5 (<2.5 μm) can remain suspended in the air for several weeks and be transported over great distances. These particles can penetrate and deposit deeper in the tracheobronchial and alveolar regions [4]. Through the respiratory system, PM2.5 may eventually find its way to the blood circulatory system [5]. The coarse fraction (PM2.5–10) is usually comprised of crystal materials and fugitive dust stemming from the resuspension of road dust and construction sites. Because of their larger sizes, they can easily deposit and thus travel for short distances. PM2.5–10 (<10 μm) deposit mainly in the head airways. The still coarser fraction, i.e., total suspended particles (TSP, <100 μm) trigger skin allergies.

Particulate matter is composed of solid particles and liquid droplets containing acids, organic chemicals, metals, soil, or dust [6], and compounds of biological origin known as bioaerosol [7]. In this decade, the occurrence of diseases due to airborne microorganisms or bioaerosols has substantially increased. Microorganisms, particularly bacteria and fungi, are common in soil, plants, and on surface water. They can be easily released into the air from these sources. In addition to bacteria and fungi (<10 μm), bioaerosols contain pollens (<100 μm), viruses (<0.1 μm), and any fragments of plant and animal origin. Bioaerosol has been found to be linked with adverse health effects, including infectious diseases, acute toxic effects, allergies, and cancers [8–14]. There are ~1.5 million fungal species on earth [15,16]. Only 80 individual genera are
documented for their allergenic properties and accompanying respiratory tract disorders. The most observed fungi in ambient air are Cladosporium, Alternaria, Penicillium, Aspergillus, Mucor, Fusarium, Epicoccum, a variety of yeasts, smuts and rusts, and other basidiomycetes. Cladosporium, for example, persists for extended durations in the environment and is directly connected to human respiratory diseases [17,18]. Bacteria are also prevalent in the atmosphere. Common genera observed in outdoor air using culture methods include, e.g. Bacillus, Clavibacter, Corynebacterium, Curtobacterium, Micrococcus, Pseudomonas and Staphylococcus [19–21].

To the best of our knowledge, no study has been conducted here in Bangladesh to assess the seasonal variation in bacterial and fungal concentrations. In the present study, particles with an aerodynamic diameter of less than <10 µm were investigated. Particles >5 µm exhibit significantly higher concentrations of microorganisms compared to the smaller ones [22]. Given the high levels of air pollution in Dhaka, Bangladesh, the current study was aimed at investigating the occurrence of airborne bacteria and fungi attached to PM. The broader goal was to provide baseline information regarding the composition of PM-bound bioaerosols in Dhaka city, Bangladesh. The specific objectives were as follows: (1) to estimate the bacterial and fungal concentrations associated with PM10 particles present in the air at different locations in Dhaka city, Bangladesh, (2) to observe the seasonal variation in bacterial and fungal communities in the selected locations, and (3) to assess the vulnerability of different age-group vis-à-vis exposure to bacterial and fungal dose rates.

2. Materials and methods

2.1 Description of sampling site

The samples for the present study were all collected from the capital city of Dhaka, Bangladesh. Dhaka is one of the most populated megacities in the world with about 20.2 million people residing within a 300-km² area. The city is currently facing twofold challenges of high population and uncontrolled vehicular growth. The relentless pressure of urbanization has negatively affected the ambient air quality of Dhaka city. For many years, Dhaka [23] and other cities in Bangladesh have been experiencing some of the highest PM concentrations among the world’s cities [23]. In the present study, four different locations (Figure 1), including the Center for Advanced Research in Sciences (CARS) premises, Doyel Chattar, Postogola, and Chittagong Road were selected for the measurement of the PM10 concentration. The locations selected include two roadside areas, one industrial area, and one university campus area with some vegetation coverage (Figure 1 and Table 1).

2.2 Sample collection

Sampling was carried out four times at each monitoring site: winter of 2018 and 2019 (December – January), spring of 2019 (February – March), summer of 2019 (April – June), and the rainy season of 2019 (July – August). The duration of sampling was 24 h. A respirable dust sampler (TEI-RDSBL 108, Thermo Environmental Instruments) was used for the collection of ambient dust samples. The samples were collected at a constant flow rate of 1 m³/min. The concentration of PM10 was determined with the help of embedded cyclone separator technology. PM10 was collected on a 20.3 × 25.4 cm irradiated glass fibre filter. The filter was sterilized and stored in a sterile environment prior to sample collection. The outdoor sampler was kept on the curbside or ~1 m above the ground. The collected PM10 samples were then used for biological characterizations.

2.3 Identification of airborne microorganisms

2.3.1 Determination of pH

A pH meter (BOECO, BT-600, Germany) was used to determine the pH of the collected particulate matter.

Figure 1. Map of Dhaka city with the monitoring sites.
samples. The dust samples were dissolved in distilled water (1:9) and the pH values were then measured with a calibrated pH meter.

### 2.3.2 Microbiological analysis

After the samples were collected, the presence of airborne microorganisms was confirmed by various cultural, and biochemical analyses. Approximately 5 g of particles-laden filter paper was taken in a Stomacher® bag and dissolved with 45 mL of distilled water and then put in a stomacher machine (STOMACHER 400 CIRCULATOR). The stomached sample was then surface plated (0.1 mL) on different non-selective and selective microbiological culture media. The following day, the colonies which appeared were counted using a colony counter (BOECO, Germany COLONY COUNTER CC-1) and the number of total airborne bacteria (TAB) and total airborne fungi (TAF) were determined. For isolation and identification of coliforms and other Gram-positive and Gram-negative bacteria, the presumptive colonies were streaked on different media such as Chromocult agar for *E. coli* (*Escherichia coli*), Mannitol salt agar (MSA) for *Staphylococcus* spp., NaCl glycine Kim, and Goepfert (NGKG) agar for *Bacillus* spp., cetramide agar for *Pseudomonas* spp., Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* spp. The inoculated culture media were then incubated at 25/30/37°C for 18–24 h. Sabouraud Dextrose Agar (SDA) media was used for the maintenance and total counting of fungi.

For the isolation and identification of coliforms and other bacteria, cultural and biochemical properties, especially colony characteristics were investigated. Colony characteristics play a vital role in PM samples. On the basis of presumptive colony characteristics and Gram reaction, the bacterial species were presumptively confirmed. The presumptively identified *Bacillus* spp. and *Staphylococcus* spp. bacterial isolates were confirmed by API 50 CHB and API Staph (bioMérieux, USA), respectively (Figures S1 and S2). For the isolation and identification of coliform bacteria, API 20E (bioMérieux, USA) was employed. On the other hand, the identification of fungal spores was performed by examining their microscopic features.

### 2.4 Dose rate estimation of airborne microbes

The United States Environmental Protection Agency’s model was used for the estimation of the dose rate of the bacterial and fungal components associated with PM$_{10}$ (Eq. 2). The model was developed to assess the risks of environmental exposure for susceptible populations [24,25].

\[
\text{Dose rate (CFU/kg)} = \frac{C \times \text{InhR} \times \text{ET}}{\text{BW}}
\]  

where $C$ is the bacterial and fungal aerosol concentration (CFU/m$^3$), InhR is the inhalation rate (m$^3$/day), ET is the exposure time (h/day), and BW is the body weight (kg) (Table S1).

### 2.5 Statistical analyses

Data was analyzed by Microsoft Excel and Minitab Statistical software (version 19). The raw data were checked for normality and homoscedasticity prior to a parametric test. A one-way ANOVA followed by Tukey’s post-hoc test was performed to determine if there were any significant differences between the locations and seasons in terms of different tested parameters. Pearson correlation coefficients were determined between different parameters of interest. Additionally, stepwise multiple-regression analysis was performed to decipher the combined effects of different parameters. Significance was determined based on whether the p-values are <0.05 or not.
3. Results and discussions

3.1 Environmental parameters

Environmental parameters such as temperature (T) and relative humidity (RH) were recorded during PM$_{10}$ sampling. The summary of environmental parameters recorded is given in Table 2. Significant differences were observed in temperature among the seasons (one-way ANOVA; p < 0.001). The highest temperature was observed in summer followed by rainy, spring, and winter seasons. A statistically higher value was recorded in summer compared to that of other seasons. The lowest temperature was observed in winter which was significantly different than the values of other seasons (p < 0.05). There were no statistically significant differences between the spring and rainy seasons (p > 0.05). The one-way ANOVA indicated that there were significant differences among the values of relative humidity recorded in different seasons (p < 0.001). The highest value was recorded in the rainy season which was significantly different than that of the spring and winter seasons (p < 0.05). On the other hand, the lowest value was recorded in winter.

3.2 PM$_{10}$ concentrations

The PM$_{10}$ concentrations varied among the locations. However, no statistically significant differences were observed among the sampling locations (one-way ANOVA; p > 0.05). The average PM$_{10}$ concentrations were 266.20 ± 115.74 μg/m$^3$, 248.84 ± 52.85 μg/m$^3$, 341.44 ± 77.51 μg/m$^3$ and 295.14 ± 64.77 μg/m$^3$ for CARS, Doyel Chatter, Postogola, and Chittagong Road, respectively (Table 3). In all the sampling sites, the PM$_{10}$ levels were found to be higher than the limit set by the World Health Organization (WHO) guideline which is 50 μg/m$^3$ for a 24-h period and the Environmental Protection Agency (EPA) limit value which is 150 μg/m$^3$ for a 24-h period. The high particulate concentrations at the studied locations in Dhaka city may be attributed to biomass burning, brick kilns, diesel-powered and gasoline vehicles, industrial fossil fuel burning, and construction activities in and around the city [26,27]. The highest value recorded in Postogola was presumably due to the presence of higher industrial activities in the area which had already been declared as the metal area of Dhaka city. On the other hand, the lowest level of PM$_{10}$ in Doyel Chatter area may possibly be due to the washout effects of heavy rainfall during the sampling times.

The seasonal variation of PM$_{10}$ concentrations in the sampling locations of Dhaka city is shown in Figure 2. The seasonal average concentrations of PM$_{10}$ were 376.16 ± 52.10 μg/m$^3$ in winter, 308.64 ± 62.37 μg/m$^3$ in spring, 271.99 ± 44.27 μg/m$^3$ in summer, and 194.83 ± 39.03 μg/m$^3$ in rainy seasons. The average PM$_{10}$ level was found to be significantly higher in the winter season than in the rainy season (one-way ANOVA; p < 0.05). The highest average PM$_{10}$ concentration observed in the winter season could be ascribed to the temperature inversion phenomenon and the shallow mixing depth, which affects the accumulation of a considerable number of particles in the lower layer of the atmosphere [28–30]. In summer, the PM$_{10}$ concentrations were relatively lower than in winter, which may be because of the stronger winds and the deeper mixing height in the season leading to better dispersion conditions for the PM$_{10}$ particles [31]. The minimum PM$_{10}$ concentrations were recorded in the monsoon season which is characterized by heavy rainfall; heavy rainfall plays an important role in washing out the PM particles from the atmosphere [32]. The results of the present study corroborate the findings of a previous study by 29.

3.3 pH of PM$_{10}$ samples

pH values of the PM$_{10}$ samples at different sampling sites are shown in Table 3. There is a strong relationship between microbial count and pH. For example, if the sample pH is acidic or alkaline, the microbiological study cannot detect microbial flora. The pH values for all the samples were found to be around 7.0 indicating a conducive condition for most of the bacteria; most bacteria are neutrophiles and grow optimally at pH between 5 and 9 [33].

3.3.1 Concentrations and diversity of bacterial species

After 24 h of sampling, the average concentration of culturable total aerobic bacterial colonies in PM$_{10}$ was recorded as 7,580 ± 7,510 CFU/m$^3$. The total concentration range of bacterial colonies across the sites varied from 185 CFU/m$^3$ to 23,856 CFU/m$^3$. The mean concentration of culturable bacteria was 1,193 ± 707 CFU/m$^3$ in CARS, 11,312 ± 6,911 CFU/m$^3$ in Doyel Chatter, 3,743 ± 2,407 CFU/m$^3$ in Postogola, and 14,073 ± 8,897 CFU/m$^3$ in Chittagong Road (Table 3).

In previous studies, it was found that the bioaerosol concentrations in the clean area are significantly lower than those in the heavily trafficked area or the densely populated area [34]. The findings of the previous studies are corroborated by the findings of the present study. The concentrations of total airborne bacteria were found to be significantly higher in the Chittagong Road and Doyel Chatter areas than those of the other sampling sites. These two sites are considered roadside areas and are highly traffic-congested. There were statistically significant differences among the sampling sites (one-way ANOVA; p < 0.05) with the highest concentration in
Table 2. Temperature (T) and relative humidity (RH) in winter, spring, summer, and rainy seasons.

| Season | Temperature, T (°C) | Relative humidity (RH) (%) |
|--------|---------------------|---------------------------|
| Winter | 28.8 ± 1.0 c        | 34.5 ± 7.6 c              |
| Spring | 34.3 ± 1.7b         | 42.8 ± 9.3bc              |
| Summer | 39.3 ± 1.7a         | 57.5 ± 5.9ab              |
| Rainy  | 35.3 ± 1.7b         | 65.5 ± 7.4a               |

A one-way ANOVA followed by Tukey’s HSD post-hoc test was performed to determine if there were any significant differences among the seasons with respect to temperature and relative humidity. The values are given as mean ± Standard Deviation. Different letters in the same column indicate significant differences at p <0.05.
Table 3. Concentrations of PM$_{10}$ (µg/m$^3$), total airborne bacteria (CFU/m$^3$), and total airborne fungi (CFU/m$^3$) at the sampling sites. pH values of PM$_{10}$ samples are also included in the table.

| Sampling sites   | PM$_{10}$ (µg/m$^3$) | Total airborne bacteria, TAB (CFU/m$^3$) | Total airborne fungi, TAF (CFU/m$^3$) | pH values of PM$_{10}$ samples |
|------------------|-----------------------|------------------------------------------|--------------------------------------|-------------------------------|
| CARS             | 266.20 ± 113.74a      | 1,193 ± 707b                             | 489 ± 378b                           | 6.93 ± 0.10a                  |
| Doyel Chatter    | 248.84 ± 52.85a       | 11,312 ± 6,911ab                         | 594 ± 326b                           | 6.96 ± 0.05a                  |
| Postogola        | 341.44 ± 77.51a       | 3,743 ± 2,407ab                          | 812 ± 690b                           | 6.95 ± 0.10a                  |
| Chittagong Road  | 295.14 ± 64.67a       | 14,073 ± 8,897a                          | 1,974 ± 1,173a                       | 7.00 ± 0.08a                  |

A one-way ANOVA followed by Tukey’s HSD post-hoc test was performed to determine if there were any differences among the sampling sites with respect to the studied parameters. The values are given as mean ± Standard Deviation. Different letters in the same column indicate significant differences at p < 0.05.

**Figure 2.** Concentrations of PM$_{10}$ mass in different seasons. A one-way ANOVA followed by Tukey’s HSD post-hoc test was performed to determine if there were any differences among the locations with respect to PM$_{10}$ concentrations. Different letters above the bars indicate significant differences between seasons at p < 0.05.

Chittagong Road and the lowest concentration in CARS.

In the present study, two individual bacterial genera recognized as gram-positive bacteria were identified as dominant species in PM samples. The average concentrations of Bacillus spp. and Staphylococcus spp. were 2,058 ± 1,751 CFU/m$^3$ and 1,466 ± 1,152 CFU/m$^3$, respectively. From a study in North China, which is known for heavy traffic and human activities like Dhaka city, the concentrations of airborne bacteria were found to range from 92 to 3,836 CFU/m$^3$ [22,35], whereas lower concentrations (4 to 911 CFU/m$^3$) were observed in relatively pollution-free Eastern European Cities. Other bacteria, in the present study, included Escherichia coli belonging to total coliforms, and Salmonella spp. and Pseudomonas spp.; their concentrations, however, were found to be below the detection limit. Of all these, Staphylococcus and Bacillus are the dominant bacteria that infect human skin. It is assumed that the major sources of these bacterial aerosols might be contaminated soil, sewage treatment plant, and traffic-congested areas in and around Dhaka city. The dispersion of bioaerosol at the source is driven by climatic conditions and geographical location.

3.3.2 Seasonal variability of bacterial aerosols

Meteorological parameters such as temperature, relative humidity, rainfall, wind speed, and wind direction mainly control the seasonal variations of the biological aerosols [35,36]. Therefore, depending on the time of the day, weather conditions, season, geographical location, and the presence of sources, the population and genus of bacterial aerosols differ. In this study, it was found that the average concentrations of total airborne bacteria varied with seasons and can be arranged in the following descending order: winter (12,705 ± 10,281 CFUs/m$^3$) > spring (8,870 ± 6,851 CFUs/m$^3$) > summer (7,361 ± 6,320 CFUs/m$^3$) > rainy (1,385 ± 985 CFUs/m$^3$). In the case of Bacillus spp., the average concentrations were 3,806 ± 2,021 in winter, 2,521 ± 1,512 in spring, 1,435 ± 732 in summer, and 469 ± 429 in the rainy season. For Staphylococcus spp., the concentrations were 2,415 ± 1,567 in winter, 1,832 ± 868 in spring, 1,245 ± 618 in summer, and 375 ± 134 in rainy seasons. Figure 3 shows the types and concentrations of bacteria associated with PM$_{10}$ based on seasons. There were no significant variations among the seasons in terms of total airborne bacterial concentrations (one-way ANOVA; p > 0.05). However, the isolates of bacterial species, i.e. Bacillus spp. and Staphylococcus spp. varied significantly across the
Figure 3. Concentrations of PM$_{10}$-bound total airborne bacteria (TAB) and *Staphylococcus* and *Bacillus* bacteria in different seasons. A one-way ANOVA followed by Tukey’s HSD post-hoc test was performed to determine if there were any differences among the locations with respect to bacterial concentrations. Different letters within the same species indicate significant differences at p < 0.05.

Table 4. Correlation between different types of bacteria and meteorological parameters.

| Types of bacteria | Relative humidity (RH) | Temperature |
|-------------------|------------------------|-------------|
| Total airborne bacteria | r = −0.742 | r = −0.219 |
| p = 0.001 | p = 0.416 (NS) |
| *Staphylococcus* spp. | r = −0.345 | r = −0.568 |
| p = 0.000 | p = 0.022 |
| *Bacillus* spp. | r = −0.493 | p = 0.052 (NS) |

Note: NS = not significant

higher bacterial aerosol concentrations in the months of winter and spring could be ascribed to the higher concentration of dust particles in the air samples during that period (Figure 2). The higher values in winter could also be attributed to the low wind velocity and the foggy and hazy days in winter [40]. On the other hand, the lower bacterial aerosol concentrations in summer and rainy seasons may presumably be due
to the washout effects of rainfall events in those seasons [41]. The high temperature in combination with a harsher atmospheric environment (e.g., strong UV intensity, a higher ozone level, etc.) was also reported to reduce the outdoor bacterial levels [19,24,35,40].

3.3.3. Correlations between bacterial concentrations and meteorological parameters

Relationships between the concentration of bacterial bioaerosols and temperature and between the concentration of bacterial bioaerosols and relative humidity (RH) were determined using Pearson correlation analysis. The correlation coefficient values are tabulated in Table 4. Significant correlations with \( r > 0.5 \) (therefore explaining 25% of total variation) were only considered for further discussion [42]. Figure 4 shows the correlation between bacterial concentration and relative humidity. There are no significant correlations between total airborne bacteria, *Staphylococcus* spp., *Bacillus* spp. and temperature. On the other hand, strong significant negative correlations were found between total airborne bacteria, *Bacillus* spp. and *Staphylococcus* spp. and relative humidity, meaning the higher the RH, the lower the bacterial count and vice versa. Negative correlations between airborne microbes and RH were observed in some previous studies [43–45]. However, the effects of RH on microbial release are varied. Different microbes exhibit different reactions towards RH and sometimes they show no reaction at all [46].

3.3.4. Stepwise regression analyses to understand the effects of meteorological parameters on bacterial concentrations

From the stepwise regression analyses in Table 5, relative humidity (RH), \( \text{PM}^{10} \) concentrations, and pH of \( \text{PM}^{10} \) particles were found to be important factors controlling the concentrations of bacteria in \( \text{PM}^{10} \) particles. Relative humidity and \( \text{PM}^{10} \) particles had a negative correlation with the concentrations of total airborne bacteria. On the other hand, pH had a positive correlation with the concentrations of total airborne bacteria. The developed regression model explained about 76% of the variation in bacterial concentration in the studied areas. The temperature was not found to be significant (\( p > 0.05 \)) for the bacterial concentration and was not included in the developed regression model. Our regression model is in slight disagreement with some of the previous studies. Li et al. (2011) employed a multiple linear regression model and obtained positive correlations between temperature and culturability and the total number of bioaerosol microbes collected from a coastal region. The researchers, however, found negative correlations between relative humidity and culturable and the total number of bioaerosol microbes, which is in line with our findings. Mouli et al. (2005) also developed a regression model, where meteorological factors such as temperature, RH, and wind speed accounted for about 50% variation in the bacterial concentrations. In their study, wind speed was found to be the most important parameter controlling bacterial concentration. In our study, the wind speed parameter was not recorded and consequently not used for the stepwise regression analyses. In the future studies, this parameter should be considered to determine its importance in controlling the concentrations of airborne bacteria and fungi in Dhaka city.

3.3.5. Total fungal concentrations and diversity

The culturable airborne fungal concentration was also estimated from the samples that were collected from different parts of Dhaka city. The concentration of airborne fungi was found to vary greatly across the different sampling sites (one-way ANOVA; \( p < 0.05 \)). The range of culturable total fungal concentrations was 174 to 3,588 CFU/m\(^3\) with a mean concentration of 967 ± 892 CFU/m\(^3\). The mean concentrations of total fungi were 489 ± 378 CFU/m\(^3\) in CARS, 594 ± 326 CFU/m\(^3\) in Doyel Chattar, 812 ± 690 CFU/m\(^3\) in Postogola, and 1,974 ± 1,173 CFU/m\(^3\) in Chittagong Road (Table 3).

From Table 3, the highest fungal concentration was found at Chittagong road, which is a highly trafficked area [34], and the second highest in Postogola, which is a well-known industrial area with a dense population. High heat produced from different industries could be one of the primary reasons for enhanced fungal growth in Postogola.

Fungal colonies identified based on their morphological characteristics exhibited significant variation among the PM samples. In the present study, different types of individual genera and some

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Table 5. Stepwise regression analysis of the concentrations of total airborne bacteria and \( \text{PM}^{10} \) concentrations, pH of \( \text{PM}^{10} \) particles, temperature (T), and relative humidity (RH). Temperature was not included in the model because of non-significance.

| Term                  | Coefficient | SE* of Coefficient | T-Value | P-Value | VIF** |
|-----------------------|-------------|--------------------|---------|---------|-------|
| Constant              | -158,410    | 82,816             | -1.91   | 0.080   |       |
| \( \text{PM}^{10} \)  | -57.8       | 18.2               | -3.19   | 0.008   | 2.37  |
| pH of \( \text{PM}^{10} \) | 30,800     | 11,902             | 2.59    | 0.024   | 1.01  |
| Relative Humidity (RH)| -633        | 103                | -6.15   | 0.000   | 2.36  |

Regression model

Total airborne bacteria = -158,410 - 57.8 \( \text{PM}^{10} \) + 30,800 pH - 633 RH; \( R^2 \) (adj) = 75.62%; \( F = 16.51 \); \( p < 0.001 \)

SE* = Standard error; VIF** = Variance Inflation Factor
species of fungi were identified in the PM samples. The genera and species identified include Aspergillus (A. flavus, A. niger, A. fumigatus), Penicillium, Cladosporium, Rhizopus (R. stolonifer), Fusarium, Monilia, Syncephalastrum, Bipolaris, Alternaria, Trichoderma, Neurospora, etc. and several non-sporing isolates. Non-Sporing Isolates are those that do not produce spores even after 30 days of incubation. Among all these fungal types, previous researchers identified Aspergillus, Penicillium, Cladosporium and Fusarium as the most common allergens for their high allergic properties [47,48].

3.3.6 Seasonal variability of fungal aerosols

Bioaerosol concentrations may change seasonally due to the variations in local sources and air diffusion conditions. These concentrations are also affected by meteorological factors and the degree of air pollution. Fungal conidia exhibited considerable variability with the change in seasons. The total fungal concentrations were found to be 394 ± 207 CFU/m³, 777 ± 225 CFU/m³, 1843 ± 1224 CFU/m³ and 379 ± 320 CFU/m³ in winter, spring, summer, and rainy seasons, respectively (Figure 5). Different fungi species found at different locations are presented in Table 6.

Aspergillus spp. was found to be the most prevalent genus of fungi in all the sampling sites, as determined by culture. The genera Penicillium/Fusarium/Monilia was the second most common fungal genera, followed by Rhizopus stolonifer, Syncephalastrum spp., Trichoderma spp., Penicillium spp., Streptomyces spp., Penicillium variabile, and Eurotium stelodami. Alternaria alternata and Cladosporium sphaerospermum were not found in any of the samples at all.

The concentrations of total fungal conidia were the maximum during the summer season, the minimum in

![Figure 5. Concentrations of PM_{10}-bound total airborne fungi (TAF) in different seasons. A one-way ANOVA followed by Tukey’s HSD post-hoc test was performed to determine if there were any differences among the locations with respect to fungal concentrations. Different letters above the bars indicate significant differences between seasons at p <0.05.]

| Fungal species/genera | Doyel Chatter | CARS | Postogola | Chittagong Road |
|-----------------------|--------------|------|-----------|-----------------|
| Alternaria spp.       | R*           | -    | -         | -               |
| Aspergillus flavus     | W, SU        | S, SU| W         | W               |
| Aspergillus fumigatus  | W, S         | W    | W         | W               |
| Aspergillus niger      | W, S, SU, R  | W, S, SU, R | SU, R | W, S, SU, R |
| Aspergillus nidulans   | -            | -    | -         | S               |
| Aspergillus oryzae     | -            | -    | -         | S               |
| Aspergillus terreus    | -            | -    | -         | S               |
| Aspergillus spp.       | S            | R    | R         | R               |
| Bipolaris spp.         | -            | -    | -         | -               |
| Cladosporium spp.      | W, SU        | W    | W, S      | W, SU, S       |
| Curvularia spp.        | W            | -    | W, SU     | SU, S          |
| Eurotium stelodami    | S            | -    | R         | -               |
| Fusarium spp.          | W            | W    | W         | W               |
| Monilia spp.           | S, R         | W    | -         | -               |
| Neurospora spp.        | R            | -    | -         | R               |
| Penicillium brevicaespactum | -             | -    | -         | SU               |
| Penicillium spp.       | S, R         | R    | W, S, SU, R | W, S           |
| Rhizopus stolonifer    | -            | SU, R| SU, R    | R               |
| Syncephalastrum spp.   | -            | R    | SU, R     | R               |
| Trichoderma spp.       | W            | -    | -         | -               |
winter and rainy seasons, and moderate during spring months. Significant variations between summer and winter and between summer and rainy seasons were observed (one-way ANOVA; p < 0.05). The total fungal conidia concentrations occurred in the following descending order: summer > spring > winter > rainy. In a previous study, Li et al. (2011) observed the lowest concentration of airborne microbes in the winter season, which was attributed to the inhibitory effect of low temperature in the atmosphere. During summer, the high atmospheric temperatures and moistures increase cell membrane fluidity and promote microbial activity that is conducive for the germination, growth, and release of airborne fungi [36]. In another study, 48, reported the highest concentrations of fungal conidia in summer, which was attributed to the maximum wind speed in summer; the minimum wind speed resulted in the lowest concentrations in winter and monsoon months. The findings in the present study are inconsistent with the findings of the study carried out by Chakrabarti [47] who found higher fungal concentration in winter than other seasons in Kolkata, India, and ascribed it to the geographic characteristics of the sampling area. For instance, the fungal concentration peak in autumn in Ohio, USA, whereas the concentrations were found to peak in the summer in Cracow, Poland [39].

3.3.7 Correlations between fungal concentrations and meteorological parameters

The correlation between temperature and total fungal conidia was statistically significant and positive, i.e. the higher the temperature, the higher the fungal count and vice versa (r = 0.666; p < 0.01). The findings could be substantiated by the findings of earlier researchers, who all reported a positive correlation between temperature and fungal conidia [36,39,49,50]. Warm and rainy seasons favor sporulation which accounts for the higher fungal count in summer [51]. The phenomenon of convective upliftment of surface deposits was also ascribed to the higher fungal count in summer [48]. Wind-assisted transported material might add to the fungal spore fraction of ambient PM load. On the other hand, fungal count did not show any statistically significant correlation with relative humidity (RH) (r = 0.065, p > 0.05). Similar result was reported in a previous study [37]. However, some studies reported positive correlations between the concentrations of culturable bioaerosol and RH (Li et al., 2017). Figure 6 represents correlation between total fungal concentrations and atmospheric temperature.

3.3.8. Stepwise regression analyses to understand the effects of meteorological parameters on fungal concentrations

Table 7 represents the stepwise regression analyses performed for total fungal concentrations and parameters such as PM$_{10}$ concentrations, pH of PM$_{10}$ particles, temperature (T), and relative humidity (RH). Fungal concentrations were found to be influenced by ambient temperature and relative humidity. There was a positive correlation between temperature and the concentrations of total airborne fungi present in the PM$_{10}$ samples. On the other hand, relative humidity exhibited a negative correlation with the concentrations of total airborne fungi. The developed regression model explained about 60% of the variation in fungal concentration in the studied areas. PM$_{10}$ concentrations and pH of PM$_{10}$ particles were not found to be significant and consequently not included in the developed regression model. 39, studied the relationships between the concentrations of ambient inhalable airborne fungi and different environmental parameters. They found statistically significant positive correlations between total fungi and temperature and between total fungi and ozone. They also obtained significant positive correlations for Aspergillus/Penicillium, Alternaria, and Ascospores with both temperature

Figure 6. Correlation between temperature (°C) and total airborne fungi (CFU/m$^3$).

![Figure 6](image-url)
and ozone. However, from stepwise regression analyses, the temperature was found to be the main governing factor for the concentrations of total airborne fungi and the individual genera or groups of fungi.

### 3.4 Dose-rate estimation of biological contents

Table 8 represents the dose rates due to inhalation of bioaerosols such as bacteria and fungi for both children and adults in different seasons. It was observed that children were exposed to worse conditions vis-à-vis the bacterial and fungal dose rates. The average values of all four seasons were calculated and the children were found to be exposed to higher dose rates (2655.6 and 297.2 CFU/kg/day for bacteria and fungi, respectively) compared to the adults (295.1 and 32.4 CFU/kg/day for bacteria and fungi, respectively). Bacterial dose rates were higher for both children and adults than the fungal dose rates because higher concentrations of bacteria were found in the studied areas. In some previous studies, similar results were observed; the fact that children were more exposed to biological pollutants than the adults was attributed to their vulnerability, low weight, higher respiration rates per unit body weight, and high susceptibility [53]. From Figure 7, it can be observed that in each season the children were exposed to higher dose rates of both bacteria and fungi compared to the adults.

### 4. Conclusions

Bioaerosols, which account for ~5-10% of the total suspended particulate matter, have not been given due importance and have woefully been neglected in the past. The present study is the first of its kind in Bangladesh where the particulate matter was investigated for its bacterial and fungal concentrations and distributions. The current study assessed the location-wise and seasonal variations in bacterial and fungal concentrations. The stepwise regression model exhibited associations between bacterial concentrations and relative humidity and PM$_{10}$ concentrations. The regression model also revealed the combined role of temperature and relative humidity

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**Table 7.** Stepwise regression analysis of the concentrations of total airborne fungi and PM$_{10}$ concentrations, pH of PM$_{10}$ particles, temperature (T), and relative humidity (RH). PM$_{10}$ concentrations, pH of PM$_{10}$ particles were not included in the model due to non-significance.

| Term                        | Coefficient | SE of Coefficient | T-Value | P-Value | VIF** |
|-----------------------------|-------------|-------------------|---------|---------|-------|
| Constant                    | -4.268      | 12.54             | -3.40   | 0.005   |       |
| Temperature (T)             | 220.0       | 44.6              | 4.94    | 0.000   | 1.60  |
| Relative Humidity (RH)      | -46.5       | 12.8              | -3.62   | 0.003   | 1.60  |

Regression model:

Total airborne fungi = -4.268 + 220.0 T - 46.5 RH; Rs (adj) = 60.45%; F = 12.46; p < 0.01

SE* = Standard error; VIF** = Variance Inflation Factor

**Table 8.** Age-specific dose rates (CFU/kg/day) of PM$_{10}$-bound biological contents.

| Season | Dose-rate of Bioaerosols | Bacteria | Fungi |
|--------|--------------------------|----------|-------|
| Winter | Adults                   | 494.6    | 15.1  |
|        | Children                 | 4450.8   | 138.0 |
| Spring | Adults                   | 345.3    | 29.7  |
|        | Children                 | 3107.5   | 272.3 |
| Summer | Adults                   | 286.6    | 70.4  |
|        | Children                 | 2578.8   | 645.8 |
| Rainy  | Adults                   | 53.9     | 14.5  |
|        | Children                 | 485.2    | 132.7 |

**Figure 7.** Dose-rate of bacteria and fungi to adults and children.
on fungal concentrations. Bangladesh is one of the countries in the world, who is at the receiving end of the consequences of the global warming phenomenon. If previous years’ trend is taken into account, Dhaka city is expected to experience spells of the heat wave in the coming decades. Unceasing population growth, urbanization, and industrialization are likely to aggravate the scenario in Dhaka city and other cities, for that matter. As indicated by the study, the increasing temperature could promote the growth of fungi which will, in turn, exacerbate an already bad air pollution condition in Dhaka city. Diseases associated with fungi will pose serious health hazards to children who are the most vulnerable group. The incidence of respiratory allergic diseases will likely increase which will create new vulnerable groups. The findings of the present study will provide a strong basis for the policymakers in Bangladesh to regulate different industrial operations and other activities that are known to enhance the number of viable microorganisms in the air. The study will also pave the way for other scientists to delve into this aspect of air pollution. Further studies on the source apportionment of bioaerosols are warranted.

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