



Identification and Characterization of Size-Segregated Bioaerosols at Different Sites in Delhi

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ABSTRACT

Ambient levels of culturable bioaerosol were measured at four different sites of Delhi, India in six size ranges ($> 7.0 \mu\text{m}$, $7.0\text{--}4.7 \mu\text{m}$, $4.7\text{--}3.3 \mu\text{m}$, $3.3\text{--}2.1 \mu\text{m}$, $2.1\text{--}1.1 \mu\text{m}$, $< 1.1 \mu\text{m}$). The study also accounted the seasonal variation (monsoon, post monsoon, winter and pre-monsoon) of the air microbes. The sampling was carried out for three different fractions of bioaerosols viz. fungi, gram positive and negative bacteria during August 2010 to April 2011 using a six-stage viable cascade impactor sampler. Unlike gram positive and negative bacteria, the concentration of fungal bioaerosol found in different stages at each site seems to follow a typical pattern in all four season. The typical pattern of concentration depicts that majority of the fungal species found in the diameter range of $3.3\text{--}2.1 \mu\text{m}$, which coincides with the penetration range in the secondary bronchi of the lungs in the human body. This reveals that majority of the immunotoxic and allergic fungi found at this stage are mostly prone to affect the secondary bronchi in human lungs when inhaled. At all four sites maximum fungal concentration ($1740.5\text{--}3224.7 \text{CFU m}^{-3}$), gram-positive bacterial concentration ($2790.6\text{--}9428.3 \text{CFU m}^{-3}$) and gram-negative bacterial concentration ($1990.3\text{--}7609 \text{CFU m}^{-3}$) were found in post monsoon season. In the majority of the sites, minimum concentrations were found in monsoon period which probably may be due to rain wash during the sampling. For all the three bioaerosol fractions no particular relationship pattern was found to exist between their respective concentrations with temperature and relative humidity (RH). However, higher range of variation was observed at higher concentration levels and lower range of variation at low concentration levels for all the three bioaerosol fractions. Most of the fungal bioaerosol identified such as *Penicillium sp.*, *Alternaria sp.* and *Aspergillus sp.* are associated with immunotoxic and allergic diseases.

Keywords: Bioaerosol; Air Pollution; Fungi; Bacteria; Delhi.

INTRODUCTION

Bioaerosol is the term that is often used for airborne particles that mostly originate from different biological materials. Regardless of their viability bioaerosol comprises both fractions and whole microorganisms along with biopolymers and other products released from various living things (ACGIH, 1989). Along with living or dead fungi and bacteria (both pathogenic and non-pathogenic), bioaerosol may also consist of peptidoglycans, endotoxins, mycotoxins, pollens etc. (Douwes *et al.*, 2003; Stetzenbach *et al.*, 2004).

Both natural (soil, vegetations, etc.) and man-made (building materials, organic wastes, etc.) sources act as the major reason for the entirely unique assemblage of bioaerosols. Animal housings, water damped buildings,

food processing plants, dumping sites etc. are various indoor and outdoor environments wherein higher concentration of bioaerosol have been associated with several health impacts indicating poor air quality (Eduard *et al.*, 1993; Fischer *et al.*, 2003; Horner *et al.*, 2004; Shantha *et al.*, 2009; Ghosh *et al.*, 2015). Since airborne microorganisms mostly fall into respirable size range (with diameter $< 10 \mu\text{m}$), they usually have the capability to penetrate deep down into human lungs causing several health hazards (Reponen *et al.*, 2001; Gorny and Dutkiewicz, 2002). Hence, their presence in indoor environments is often associated with sick building syndrome (SBS) (Norback *et al.*, 2016).

Apart from indoor sources, various outdoor habitats such as sewage treatment plant, municipal waste dumping site etc., also emit various airborne microbes into the atmosphere depending upon the type and location of the facility (Maharia and Srivastava, 2015). The other factors on which the concentration of microorganisms and their range depend are weather condition and time of the year. Apart from temperature and relative humidity air movement is also an important factor as it acts as a mechanism for biological dispersal. Hence, dumping grounds can act as a

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potential source of several diseases (Lis *et al.*, 2004; Srivastava *et al.*, 2011). The various routes through which human are exposed to bioaerosol are inhalation, ingestion, and dermal contact, out of which inhalation is the predominant one. In general, bioaerosols range between 0.3 to 100 μm in diameter out of which the respirable size fraction which is of primary concern is 1 to 10 μm (Cox and Wathes, 1995). However, only particles ranging between 1.0 to 5.0 μm generally remain in the air, while the rest larger particles are shortly deposited on surfaces (Mohr, 2001).

The aim of the study was to estimate the concentration of different fractions of bioaerosols in different size ranges at various locations in Delhi, India. The study also accounted the seasonal variation viz. monsoon, post- monsoon, winter, and pre-monsoon.

MATERIAL AND METHODS

Samplings of bioaerosol were carried out in the capital of India, Delhi (28°25' latitude and 76° 50' longitude), which is situated in the Northern part of India (details of study area has been given in the supplementary material).

Sampling Sites

Sampling was carried out at four different sites (that includes one indoor and three outdoor sites) in Delhi namely Garbage site (S1) [outdoor], Jhelum mess (S2) [indoor],

Munirka (S3) [outdoor] and Kaushambi (S4) [outdoor]. Their locations in Delhi have been provided in Fig. 1. Sampling sites were selected on the basis of a previous onsite survey. Among the four sites, S1 and S2 were inside the Jawaharlal Nehru University (JNU). JNU, which is situated in the bush forest of Aravali hill ridges covering an area of 1,000 acres (4 km²) and is in the southwest of New Delhi. The third sampling site Munirka (S3) was in the close vicinity to JNU. It is a heavily populated residential area with enormous numbers of buildings and houses. Sampling was done at the balcony of a three-story building which is around 200m away from the main road. The fourth site Kaushambi (S4) was far-off (≈ 24 km) from JNU. This is a scarcely, populated (5000) residential area of Ghaziabad of Delhi-NCR. The locality is experienced by a large dumping area and butcher houses in the vicinity of the area.

Bioaerosol Sampling

The sampling was carried out for three different fractions of bioaerosols viz. fungi, gram-positive bacteria (GPB) and gram-negative bacteria (GNB) during August 2010 to April 2011. According to Indian Meteorological Department (IMD), the meteorological seasons over India are winter (January–February), pre-monsoon (March–May), monsoon (June–September) and post-monsoon (October–December). Sampling carried out in these nine months (August–April) cover more or less all the four seasons. Sampling was carried

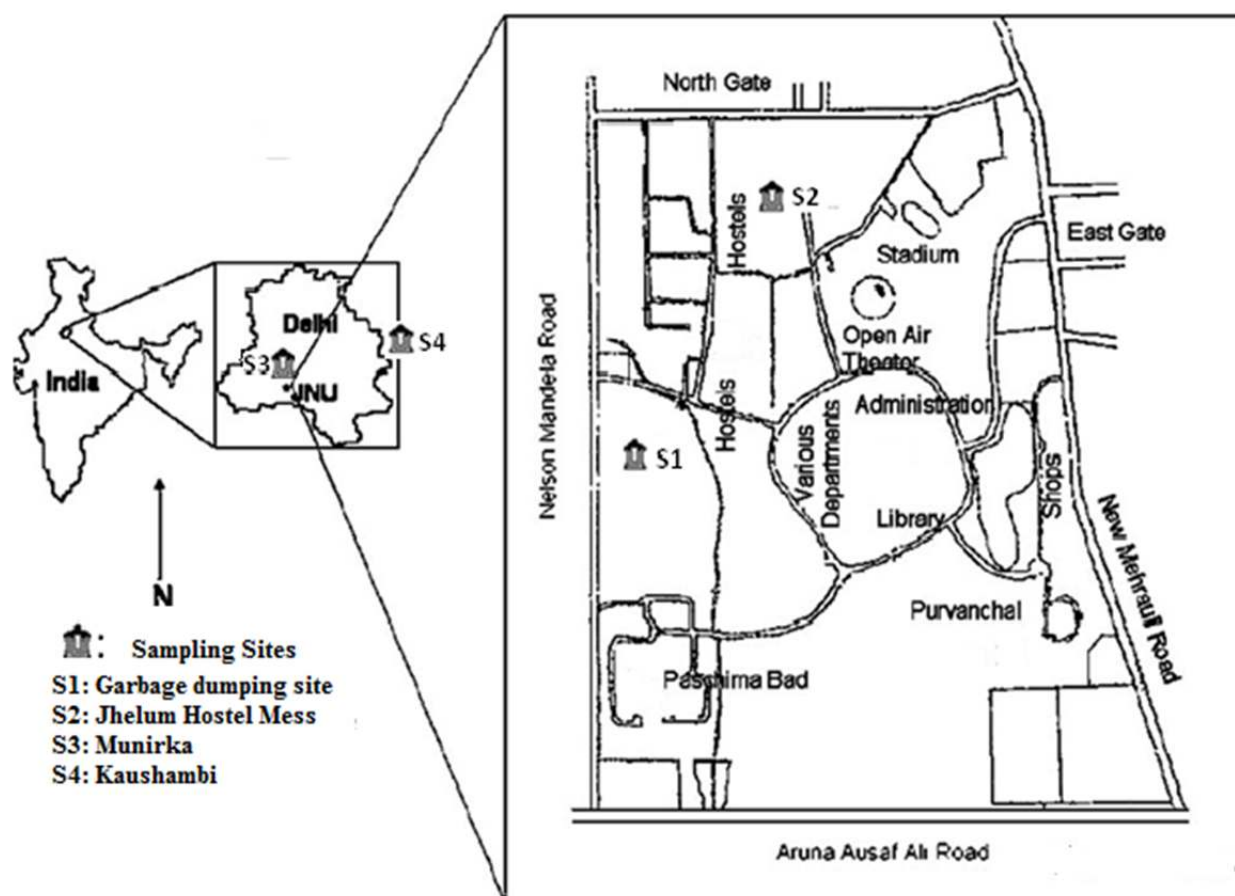


Fig. 1. Location of sampling sites (not to scale).

out using a six-stage viable cascade impactor sampler (Tisch Environmental, South Miami, OH, USA). This sampler collects aerosols in six different size ranges. Size range for each stage and its possible association with the human respiratory system is given in Table 1. The Anderson sampler was kept at a height of 1.4 m above the floor of the building when indoor and above the ground when outdoor to simulate exposure in the human breathing zone. Sampling was carried out for 20 minutes at a flow rate of 28.3 L min⁻¹ twice a month.

Culture Media

For detection and enumeration of fungi and bacteria, suitable media were used on the collection plates. The fungal fraction was sampled over Potato dextrose agar media (Titan Biotech Ltd. India). Potato dextrose agar is common microbiological media made from potato infusion, and dextrose (corn sugar). Potato infusion and carbohydrate promote the growth of fungi while low pH and antibiotic present inhibit the growth of bacteria (Eddleman, 2005; Mandal *et al.*, 2008).

GNB fractions of bioaerosols were collected on Eosin Methylene Blue (EMB) agar media (Titan Biotech Ltd. India). This is selective-differential plating medium for the detection and isolation of gram-negative bacteria. GPB were collected on blood agar. Blood Agar Media (Titan Biotech Ltd. India) is used with blood for both isolations as well as cultivation of a wide variety of fastidious microorganisms (FDA, 1998).

Preparation, Enumeration and Identification of Culturable Bioaerosols

Sample Preparation

Inoculated agar plates were incubated at the appropriate temperature for a time ranging from hours for a fast-growing bacterium to days for a fungus to develop into a visible colony. In this study, the bacterial plates were incubated at 30 to 37°C for 48 h for both GNB and GPB. Fungal plates were incubated at 28°C for 3 days. The resultant colonies were reported as colony-forming units (CFU m⁻³). Fungal isolates were mainly identified by the direct observation on the basis of spore and colony morphological features.

Enumeration

The concentration (in terms of CFU m⁻³) of culturable microorganisms is calculated by dividing the volume of air sampled from the total number of colonies observed on the plate. A colony is a macroscopically visible growth of

microorganisms on a solid culture medium. Concentrations of culturable bioaerosols normally are reported as colony forming units (CFU) per unit volume of air sampled. CFU is the number of microorganisms that can replicate to form colonies, as determined by the number of colonies that develop.

$$\text{Bioaerosol Concentration (CFU m}^{-3}\text{)} = \frac{\text{No. of colonies}}{\text{(Flow rate} \times \text{ sampling duration (minutes))}} \quad (1)$$

$$\text{Flow rate} = 28.3 \text{ L min}^{-1} = 0.0283 \text{ m}^3 \text{ min}^{-1}.$$

$$\text{Bioaerosol Concentration (CFU m}^{-3}\text{)} = \frac{\text{No. of colonies}}{(0.0283 \times \text{ sampling duration (minutes))}} \quad (2)$$

In many cases it is difficult to identify multiple colonies at one location on a plate because of several reasons, firstly lack of differential colony morphology and secondly chemicals secreted by one microorganism might inhibit the growth of other microorganisms at that same location (Burge, 1977). In addition, some organisms produce large, spreading colonies, while others produce microcolonies. Moreover, the morphology of the colony of a specific microorganism may completely obscure that of another, and a slow-grower might get obscured by the faster one. In these cases, a statistical adjustment of the observed number of colonies is needed to account for the probability that more than one particle impacted the same site (Anderson 1958; Leopold, 1988; Macher, 1989). The adjusted concentration of culturable microorganisms is calculated by following the reference http://www.skinc.com/catalog/pdf/Multiple_Jet_Impactors.pdf.

Identification

A small portion of the fungal colony is taken with the help of inoculum loop and placed to a slide containing 4% of NaCl. A drop of lactophenol cotton blue stain is added to it immediately and left for about 1–2 minutes. The area is then covered with a coverslip and it is ready for microscopic examination and identification. Identification was done comparing the fungal spore of the samples with the existing result viz. published papers, available literature and images available on the internet.

Statistical Analysis

Statistical analyses (regression and correlation analyses) were carried out with the help of Microsoft Office Excel 2007 and SPSS 16.

Table 1. Cutoff size for each stage and the extent to which particles can penetrate human respiratory system.

Stages	Range of particle sizes (µm)	Parts of human respiratory system where particles can be lodged
1	7.0 and above	Pre-separator
2	4.7–7.0	Pharynx
3	3.3–4.7	Trachea and primary bronchi
4	2.1–3.3	Secondary bronchi
5	1.1–2.1	Terminal bronchi
6	0.65–1.1	Alveoli

Source: <https://tisch-env.com/wp-content/uploads/2015/06/TE-10-800-Viable-Cascade-Impactor.pdf>.

RESULTS AND DISCUSSION

Size-Segregated Distribution of Bioaerosols

The size-segregated concentration of fungal bioaerosol at each site seems to follow a typical pattern in all the four seasons. It can be inferred from Fig. 2 that, there is an increasing trend in concentration from stages 1–4 with a further decreasing order from 4–6 which was found to be synonymous to the fungal bioaerosol study carried out at a university campus and sewage treatment plant in Delhi by Srivastava *et al.* (2012) and Maharia and Srivastava (2015) respectively. In all three figures the highest concentration of fungus is found in stage 4 (size range 2.1 to 3.3 μm) and lowest concentration in stage 6 (size range 0.6 to 1.1 μm) except in winter season which has a higher concentration in stage 3. The reason for the high concentration of fungal bioaerosol at stage 3 in winter season is due to the climatic conditions of Delhi, winter is characterized by low mixing height for which higher agglomeration/accumulation of airborne particles are observed (Hazarika *et al.*, 2015; Hazarika and Srivastava, 2017; Hazarika *et al.*, 2017). This might be the cause of the higher amount of fungi in winter. The typical pattern of concentration depicts that majority of the fungal species have a similar diameter as that of stage 4 which is a synonym to the secondary bronchi of the lungs in the human body. This reveals that majority of the immunotoxic and allergic fungi found at this stage are mostly prone to affect the secondary bronchi in human lungs when inhaled. The almost similar result was found to be depicted in the size segregated fungal study carried out in a landfill site in Delhi by Agarwal *et al.* (2016) wherein concentration peak was observed in the size range of 2.1 to 5.8 μm (that is synonymous to stages 2 to 4).

Unlike fungus, in all the four seasons gram-positive bacteria seem to follow no pattern at all which was similar to

the gram positive bacterial result depicted by Srivastava *et al.* (2012). It is clear from Fig. 3 that, maximum concentration is found in stage 4 at most of the sites with an exception at Munirka where maximum concentration is found to be in stage 6 (0.65–1.1 μm). Minimum concentration is different at different sites. In the case of Jhelum mess and Munirka the minimum concentration is in stage 6 and 1 while for Garbage site and Kaushambi it is in stage 5 and 6 respectively. High degree of concentration found in stage 4 reveals the fact that primary and secondary bronchi are mostly affected. Again Fig. 3 reveals a different pattern of distribution at various sites in post monsoon season. The maximum concentration of GPB in Jhelum mess and Munirka is found to be associated with stage 3 (3.3–4.7 μm), while in the case of Garbage site and Kaushambi it is stages 2 and 4 respectively. At most of the sites, the minimum concentration is observed in stage 6 with few exceptions. Maximum concentration found in stages 2, 3 and 4 reveals the fact that in winter period pharynx; trachea, primary and secondary bronchi seems to have high probability to be affected by gram-positive bacteria.

Gram-negative bacterial concentration does not seem to follow any typical pattern in all the four seasons as seen in fungus concentration in different stages. As seen in Fig. 4, the maximum concentration of GNB is found in stage 3 (3.3–4.7 μm) and stage 4 (2.1–3.3 μm) which is a synonym to the tracheal region with primary bronchi and secondary bronchi respectively. The high concentration at these stages reveals that the above-mentioned parts of lungs are more prone to infection. High concentration found in stage 5 (1.1–2.1 μm) reveals that terminal bronchi of lungs are at risk to be infected due to its diameter range being a synonym to stage 5. The pre-monsoon season for GNB shows a totally different pattern. The maximum concentration of gram-negative bacteria is found in stage 3 (3.3–4.7 μm) and minimum in stage 2 (4.7–7.0 μm). Stage 3 being a

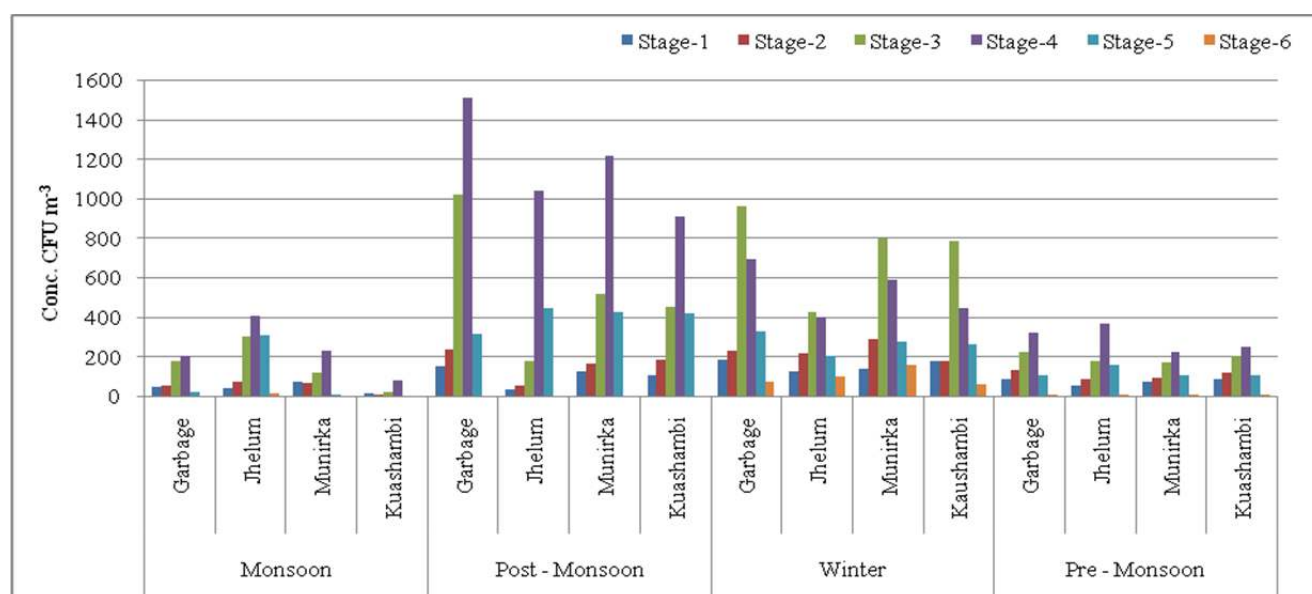


Fig. 2. Average concentration of size segregated fungal bioaerosols at four sites in four different seasons. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

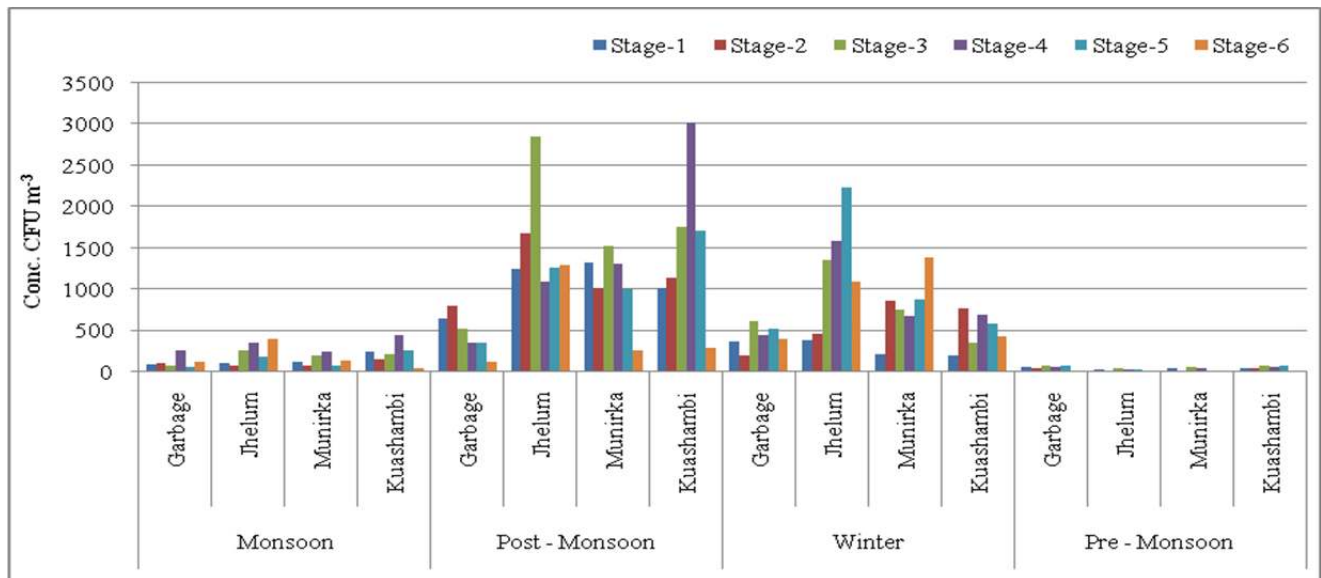


Fig. 3. Average concentration of size segregated GPB bioaerosols at four sites in four different seasons. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

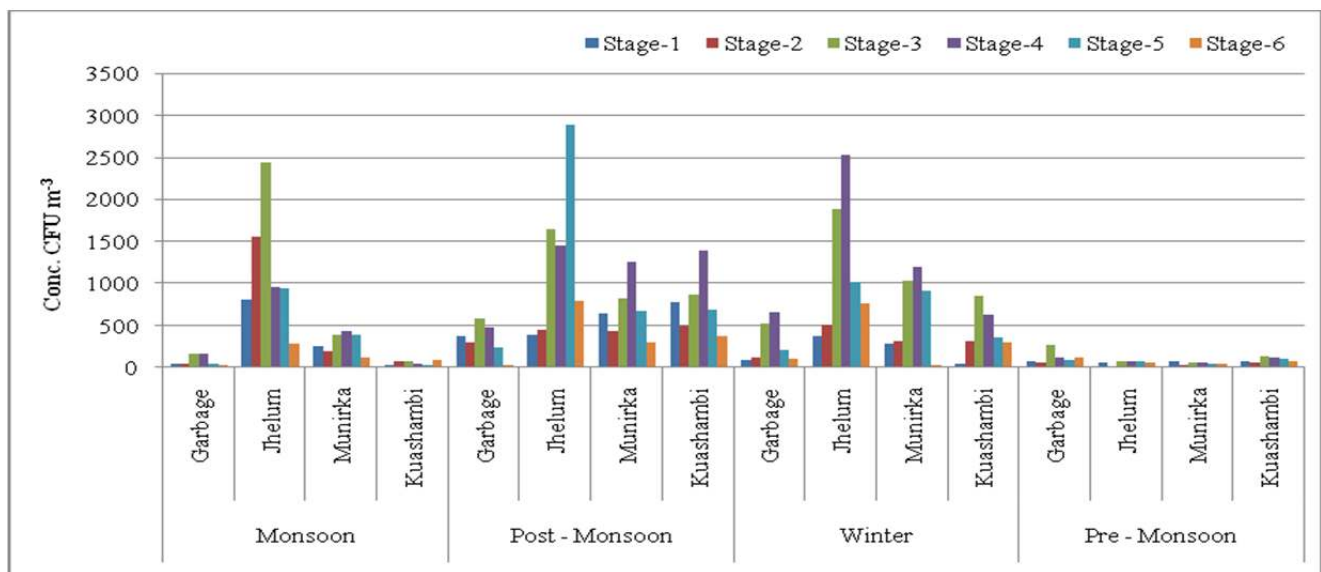


Fig. 4. Average concentration of size segregated GNB bioaerosols at four sites in four different seasons. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

synonym to the trachea and primary bronchi, these parts seem to have high probability to be affected by gram-negative bacteria during the winter period.

Unlike the similarity found with size segregated fungal distribution, the size segregated bacterial distribution shown in this study (with few exceptions) totally differs from the result reported by Agarwal *et al.* (2016) wherein bacterial concentration was higher at the size range 0.65 to 2.1 μm (that is synonymous to stages 4 to 6).

Seasonal Variation of Bioaerosol Concentration with Site

Fig. 5 and Table S1 (supplementary data) shows the

seasonal variation of fungal concentration at each site in the varied temperature range. At all four sites, maximum fungal concentration (1740.5–3224.7 CFU m⁻³) is found at a temperature range (19.2–32.2°C) in post monsoon season. At most of the sites, minimum concentration (113.7–499.4 CFU m⁻³) is found at a temperature range of 28.3–30°C in monsoon period with an exception at Jhelum mess where the much higher concentration of 1140 CFU m⁻³ is observed. In Monsoon due to rain, most of the fungal species get washed out hence low fungal concentration is found in outdoor sites such as Garbage site and Kaushambi with respect to the corresponding indoor sites such as Jhelum

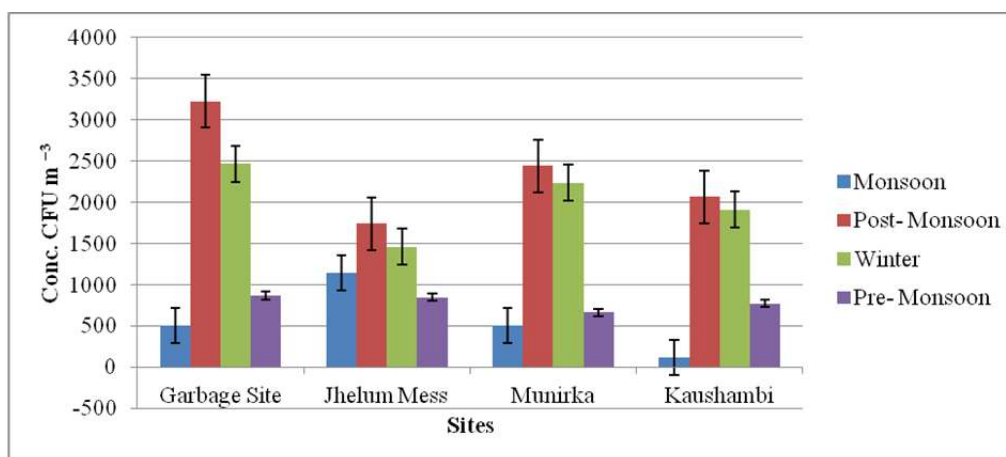


Fig. 5. Average fungal concentration at different sites in different season. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

mess and Munirka. Around $662.5\text{--}865.7\text{ CFU m}^{-3}$ of fungal concentration is found at a temperature range of $30.1\text{--}35^\circ\text{C}$ during pre-monsoon season. High temperature being associated with low humidity is one of the major reasons of less fungal concentration in pre-monsoon season in comparison to post monsoon season. However, absences of rain wash out gears up the growth of fungi in post monsoon, winter and pre-monsoon season in comparison to monsoon. These findings are totally in contrast to the study carried out by Agarwal *et al.* (2016) wherein maximum and minimum fungal concentration has been reported in winter and pre monsoon season. However, higher concentration in post monsoon and lower in monsoon and winter has been reported in other few studies carried out in Delhi itself (Lal *et al.*, 2013; Maharia and Srivastava, 2015).

Fig. 6 shows the seasonal variation of GPB concentration in the varied temperature range. The maximum concentration of gram-positive bacteria ($2790.6\text{--}9428.3\text{ CFU m}^{-3}$) is found to exist at a temperature range of $29\text{ to }32^\circ\text{C}$ in post

monsoon season similar to fungi. Although the temperature range is nearly favorable for bacterial growth yet absence of rain washout is the primary reason of high concentration in post monsoon season. Minimum bacterial concentration ($164.3\text{--}325.1\text{ CFU m}^{-3}$) is found at a temperature range of $30.6\text{--}36.1^\circ\text{C}$ during pre-monsoon period. It is a well-known fact that with increasing temperature, humidity decreases. So, in the pre-monsoon period even though the temperature is optimum for bacterial growth yet low humidity is the reason for low concentration. In monsoon gram, positive bacterial concentration of $711.9\text{--}1371.4\text{ CFU m}^{-3}$ range is found at a temperature range of $26\text{--}30.8^\circ\text{C}$.

Fig. 7 shows the seasonal variation of GNB concentration in the varied temperature range. Like fungi and GPB maximum concentration of GNB ($1990.3\text{--}7609\text{ CFU m}^{-3}$) is found at a temperature range between $28\text{--}3^\circ\text{C}$ in the post monsoon season. The presence of favorable conditions of temperature and relative humidity may be the major cause of higher concentration. In monsoon period quite low

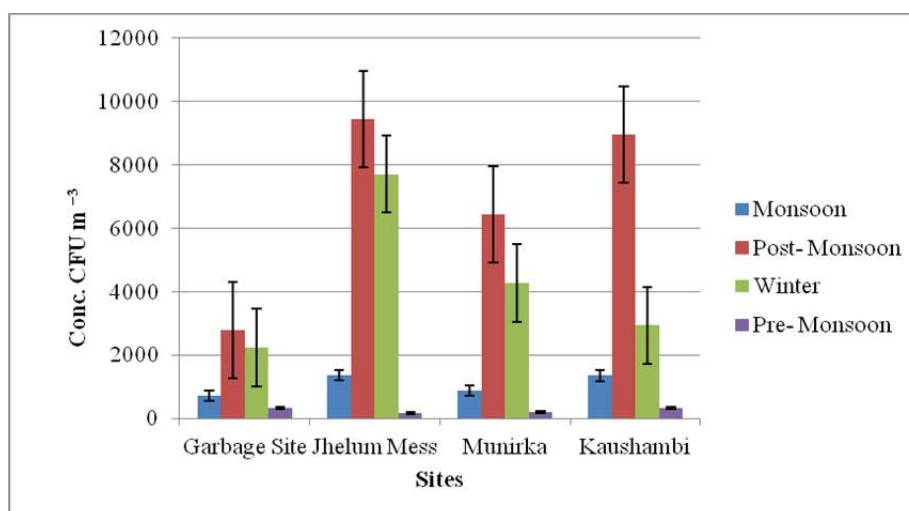


Fig. 6. Average Bacterial (gram positive) concentration at different sites in different season. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

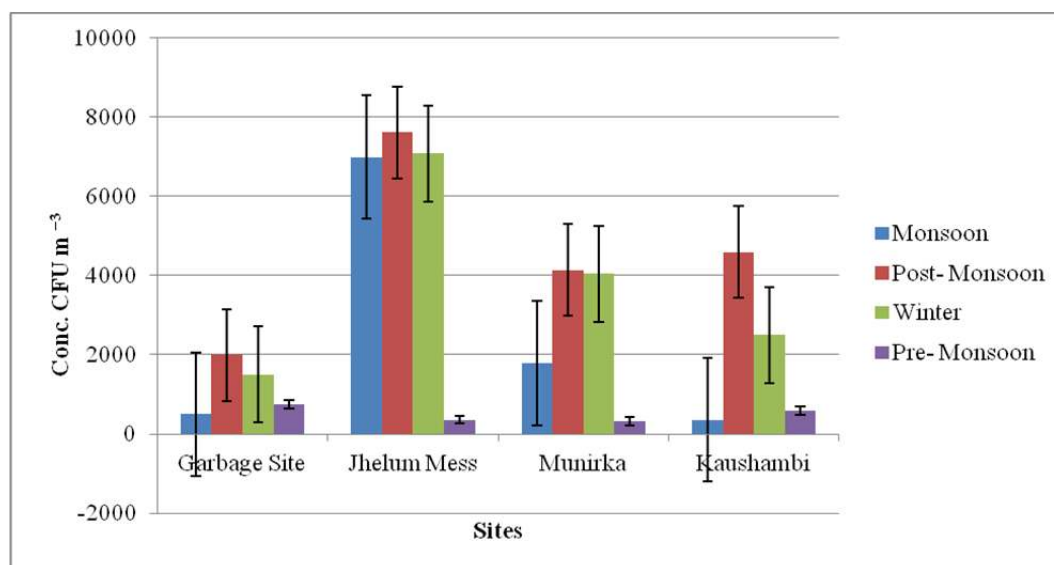


Fig. 7. Average Bacterial (gram negative) concentration at different sites in different season. The bars refer to the variation in average concentration due to no. of samples viz. Monsoon ($n = 4$); Post-Monsoon ($n = 6$); Winter ($n = 4$); Pre-Monsoon ($n = 4$).

concentration around 356.0–498.4 CFU m⁻³ of GNB was found in outdoor sites such as at Garbage site and at Kaushambi in comparison to the high concentration of 6990.5 CFU m⁻³ in Jhelum mess an indoor site. Even though temperature ranges i.e., 27–32°C is near to the optimum temperature of bacterial growth yet rain washout is the main reason for lesser concentration in the outdoor environment. A low range of 322.7–750.3 CFU m⁻³ is found at a temperature range of 30.6–36°C in the pre-monsoon period. Though the temperature is within the optimum range yet low humidity content of the atmosphere is the main reason for the low concentration. In case of the maximum bacterial concentration, the findings in this study are in contrast to the study carried out by Agarwal *et al.* (2016) wherein maximum bacterial concentration has been reported in winter but has similarity in terms of minimum concentration in pre monsoon season. The results also differ from the data reported by Kumar *et al.* (2013) where in maximum bacterial concentration was found in monsoon with the lowest in winter. However, higher concentration in post monsoon and lower in monsoon and pre monsoon has been reported by Lal *et al.* (2013) in a study carried out in Delhi.

Statistically it was also observed (as shown in Table 2) that the mean bioaerosol concentration for all the three fractions namely fungus, GPB and GNB was much higher in the post monsoon season (2368.1 ± 638.6 ; 6900 ± 3036.2 and 4581.2 ± 2315.2 respectively) while it was lowest in the monsoon season for fungal bioaerosol (562.7 ± 426.6) and pre-monsoon season for GPB and GNB (255.3 ± 85.3 and 505 ± 201.7 respectively).

Regression Analysis

Regression analyses were carried out between coarse and fine fractions of bioaerosols. The sum of stages 1–3 was considered as coarse fraction while the sum of stages 4–6 was considered as a fine fraction. Various sites and different

seasons were presumed as the repetition of data. This was done keeping in mind the less number of the data set.

The result of Regression analyses is shown in Figs. 8, 9 and 10. It may infer from these figures that there is a good regression ($R^2 = 0.7196$) of fine over coarse for a fungal fraction of bioaerosol. Similarly in case of GPB and GNB excellent regression of fine over coarse was observed ($R^2 = 0.7912$ and 0.8920 respectively). Maharia and Srivastava (2015) carried out similar regression analysis among fine and coarse fungal bioaerosol which is relevant to this study.

Correlation Analysis

Correlation analyses were carried out between the concentration of each stage and total as well as among each stage. As seen in Table 3 in the case of fungi, a very good correlation exists between each stage except stage 6 which has no correlation with any of the stages at all. Almost similar results have been found for size segregated fungal bioaerosol in Delhi region by Maharia and Srivastava, 2015.

As seen in Table 3 in the case of GPB, a very good correlation exists between all the stages. Unlike GPB, in the case of GNB stage 1 and 2 has no correlation with stage 5 and 6. However, a good correlation exists between all the remaining stages.

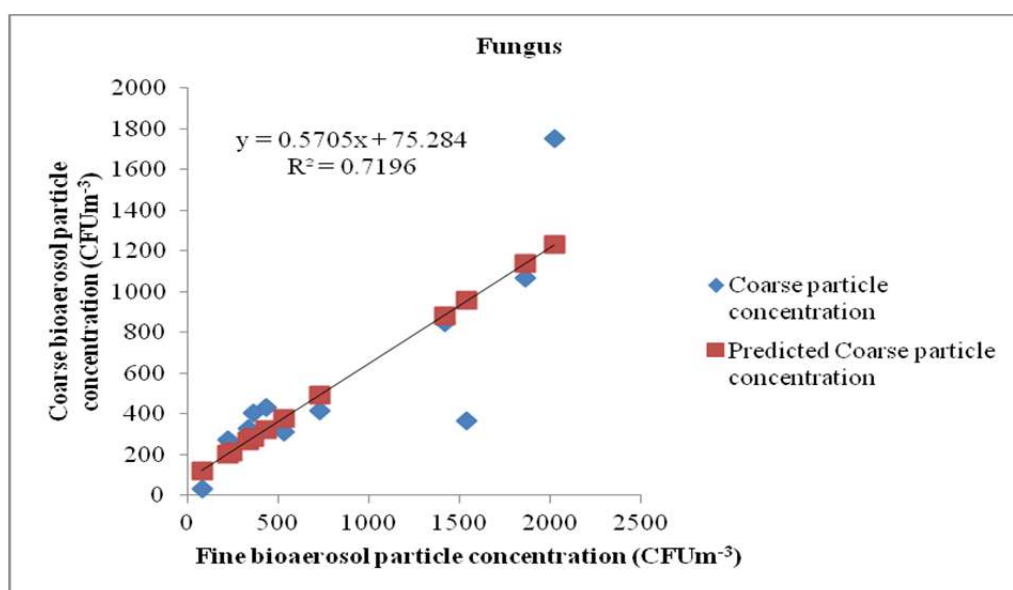
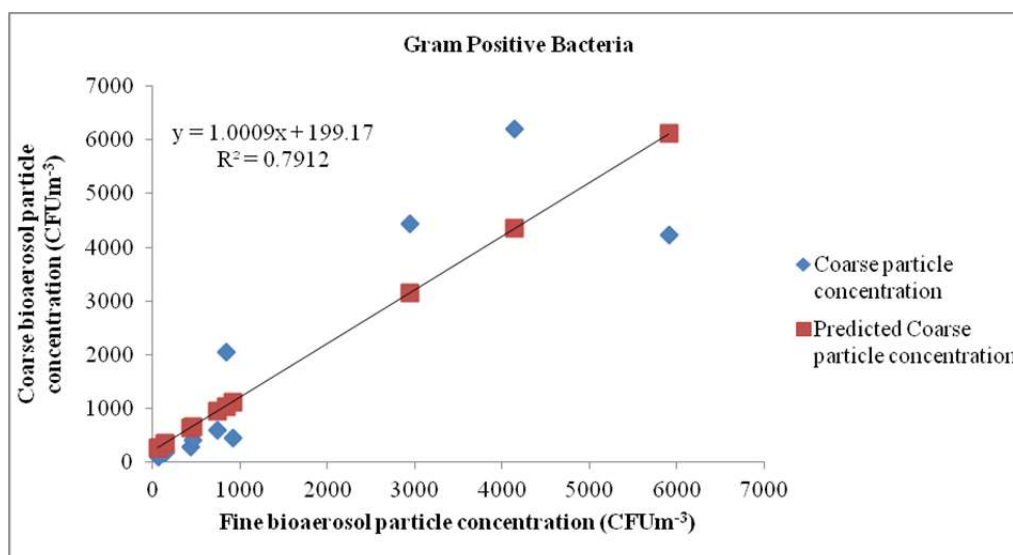
Relationship between Bioaerosol Concentration with Temperature and RH

In the case of fungus (given in supplementary data), we can see from Figs. S1 and S2 that no particular relationship pattern exists between fungal bioaerosol concentration with temperature and relative humidity. Similarly, in the case of gram-negative bacteria (Figs. S3 and S4) and gram-positive bacteria (Figs. S5 and S6) too, no relationship pattern was observed between their respective bioaerosol concentration with temperature and relative humidity.

However, it could be observed that for all the three

Table 2. Bioaerosol concentration (CFU m⁻³) in different four seasons.

Seasons	Fungus			Gram Positive Bacteria			Gram Negative Bacteria		
	Mean	Standard deviation	Standard error	Mean	Standard deviation	Standard error	Mean	Standard deviation	Standard error
Monsoon	562.7	425.6	212.8	1076.5	334.3	167.1	2408.5	3121.9	1561.0
Post monsoon	2368.1	638.6	319.3	6900.0	3036.2	1518.1	4581.2	2315.2	1157.6
Winter	2016.7	434.6	217.3	4282.8	2433.2	1216.6	3779.75	2435.2	1217.6
Pre monsoon	785.4	91.9	45.9	255.3	85.3	42.7	505.0	201.7	100.8

**Fig. 8.** Relationship plot between coarse and fine bioaerosol particle concentration (fungus) at Delhi.**Fig. 9.** Relationship plot between coarse and fine bioaerosol particle concentration (gram positive bacteria) at Delhi.

bioaerosol fractions higher variation existed at their higher concentration level while a very low or nominal variation existed at lower concentration level.

Identification of Fungal Bioaerosol at Different Sites

Nine genera of fungal bioaerosol were identified in

different seasons are given in Table 4. Among the nine genera identified, three genera which are found in maximum number in all the seasons are *Rhizopus*, *Aspergillus*, and *Penicillium*. Remaining six fungal genera were found in very less concentration. In monsoon period major finding was *Aspergillus*, *Rhizopus*, *Mucor*, and *Penicillium*. During post

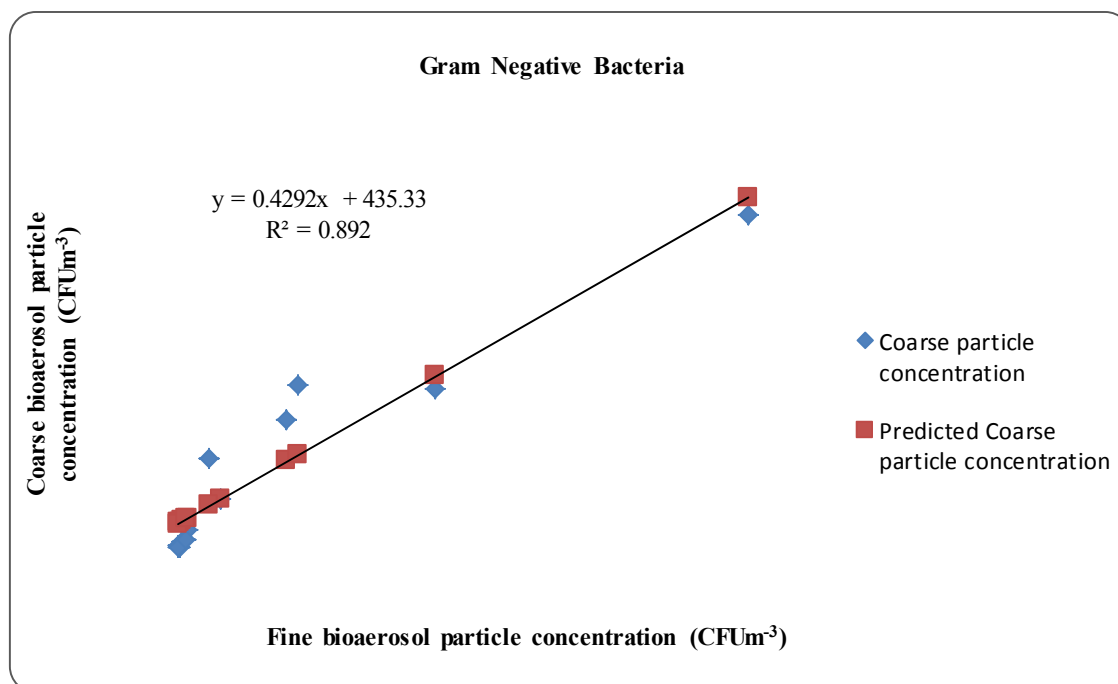


Fig. 10. Relationship plot between coarse and fine bioaerosol particle concentration (gram negative bacteria) at Delhi.

Table 3. Correlation matrix between various size fractions of different bioaerosols.

		Stage1	stage 2	stage 3	stage 4	stage 5	stage 6	Total
Fungi	Stage1	1						
	Stage 2	0.8962	1					
	Stage 3	0.7958	0.9526	1				
	Stage 4	0.7689	0.8171	0.8892	1			
	Stage 5	0.5867	0.5863	0.6909	0.8899	1		
	Stage 6	-0.3132	-0.2136	-0.3121	-0.4732	-0.2369	1	
	Total	0.8187	0.8911	0.9463	0.9851	0.8646	-0.3837	1
Gram Positive Bacteria	Stage 1	1						
	Stage 2	0.9178	1					
	Stage 3	0.9239	0.9737	1				
	Stage 4	0.6945	0.6857	0.7132	1			
	Stage 5	0.8548	0.9174	0.9365	0.9017	1		
	Stage 6	0.6804	0.8366	0.8703	0.3799	0.7159	1	
	Total 7	0.9206	0.9592	0.9765	0.8428	0.9853	0.7762	1
Gram Negative Bacteria	Stage 1	1						
	Stage 2	0.7998	1					
	Stage 3	0.7434	0.9293	1				
	Stage 4	0.8289	0.6151	0.7573	1			
	Stage 5	0.3706	0.4271	0.7191	0.7699	1		
	Stage 6	0.4389	0.4149	0.6876	0.8355	0.9695	1	
	Total 7	0.6559	0.6985	0.8994	0.8932	0.9345	0.9258	1

Bold digits: Good correlation.

monsoon, *Aspergillus*, *Penicillium*, and *Rhizopus* were majorly found in most of the sites while in winter major concentration of *Fusarium*, *Rhizopus*, *Aspergillus*, *Alternaria*, and *Penicillium* were found at each site. During pre-monsoon period *Alternaria* was found in bulk.

Among above mentioned *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* are immunotoxic; *Aspergillus*, *Drechslera*, *Curvularia*, and *Penicillium* are allergic while

Ulocladium and *Mucor* are harmless. *Aspergillus* and *Penicillium* are also known to cause respiratory infections along with allergic reactions (Kanaani *et al.*, 2008). In fact infections such as Aspergillosis may occur specifically in immune compromised patients due to inhalation of spores as well as toxins released by certain *Aspergillus* species (Swan *et al.*, 2002). *Penicillium* species have also been reported to cause numerous hypersensitivity pneumonitis

Table 4. Fungal Genus characterized at different sites.

Sampling Sites Season Fungi ▼	▶ Garbage Site				Jhelum Mess				Munirka				Kaushambi			
	▶ M	Po	W	Pr	M	Po	W	Pr	M	Po	W	Pr	M	Po	W	Pr
	M	M		M	M	M		M	M	M		M	M	M		M
Aspergillus≠×	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Fusarium ×			Y				Y				Y				Y	
Drechslera≠	Y	Y			Y					Y			Y			
Ulocladium+				Y				Y				Y				Y
Curvularia≠	Y			Y	Y								Y			
Penicillium≠×	Y	Y	Y	Y	Y		Y	Y	Y		Y	Y	Y	Y	Y	Y
Alternaria×			Y	Y			Y	Y			Y	Y			Y	Y
Mucor+	Y				Y				Y				Y			
Rhizopus×	Y	Y	Y	Y	Y		Y	Y	Y		Y	Y	Y	Y	Y	Y

× IMMUNOTOXIC, +HARMLESS, ≠ ALLERGIC, M = Monsoon, PoM = Post-Monsoon, W = Winter, PrM = Pre-Monsoon.

epidemics (Kreiss and Hodgson, 1984). *Curvularia* species has been found to cause allergic fungal sinusitis mainly due to the growth of fungus in the area of abnormal tissue drainage (Luong and Marple, 2004; Bush et al., 2006). Among the various toxins produced by different *Fusarium* species vomiting and nausea have been reported to be caused by deoxynivalenol while Fumonisin B1 has been found with the ability to cause oesophagus cancer in humans (Bucci et al., 1996; Rotter et al., 1996; Bennett and Klich, 2003).

CONCLUSIONS

A seasonal and size- segregated characterization of atmospheric bioaerosol was carried out at 4 different sites in Delhi, India. The concentration of fungal bioaerosol found in different stages at each site seems to follow a typical pattern in all the four seasons. The highest concentration observed to be associated with size range 3.3 to 2.1 μm (synonymous to the secondary bronchi of the lungs in the human body) and lowest concentration with size range 0.6 to 1.1 μm . Hence, the majority of the immunotoxic and allergic fungi found to be a potential threat to the secondary bronchi in human lungs when inhaled. Unlike fungi, gram positive and gram negative bacteria seems to follow no pattern at all.

Detailed study of bioaerosol distribution in different seasons revealed a pattern in which all the three fractions of airborne microorganisms are found to be maximum in post monsoon season in comparison to pre-monsoon, monsoon and winter period in both the indoor as well as the outdoor environment. In monsoon season even though temperature and relative humidity are found to be at optimum range for bioaerosol growth yet rain wash out is the major reason for less microorganism concentration in comparison to the winter season. Due to the similar reason lesser concentration of bioaerosols are found at outdoor sites than indoor sites. In the case of a pre-monsoon season, high temperature and correspondingly low relative humidity justify the reason of less bioaerosol concentration.

Out of nine fungal genera identified *Rhizopus*, *Aspergillus* and *Penicillium* were found in abundance at all the four

sites in all the seasons. Out of these three, *Penicillium* is harmless while *Aspergillus* is allergic (with the potential to produce carcinogenic Ochratoxin A and Aflatoxins) and *Rhizopus* is immunotoxic in nature. *Fusarium* and *Alternaria* are found in abundance in winter and pre-monsoon season respectively.

The good regression coefficient of fine over coarse in the case of all three different type of bioaerosol suggests that variation in the concentration of fine fraction of bioaerosol affects the variation of concentration of coarse fraction of bioaerosol. A good correlation exists between different fractions of fungal bioaerosol with each other and with the total. The only exception is size range 1.1–0.65 μm , which is not at all correlated with any of the stages. Similarly, more or less good correlation exists between the fraction of gram negative and gram positive bacteria with each other and total. No relationship pattern was found to exist between the concentration of different bioaerosol fractions with temperature and relative humidity. However, higher variation was observed for all the three bioaerosol fractions at higher concentration levels and very low variation at low concentration levels.

ACKNOWLEDGEMENT

The author takes the opportunity to thank Mr. Mahendra Kumar and Mrs. Neelam for facilitating sampling in Kaushambi. Thanks to Rajesh and Manish for helping during sampling and analysis.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at <http://www.aaqr.org>.

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Received for review, June 5, 2015

Revised, February 15, 2017

Accepted, February 17, 2017