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# Spatial Variation of Biogenic Aerosols at Different Land Use Configurations in Urban Delhi

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**Abstract**

Air particulates associated with biological origins are termed as “bioaerosol”, consist of different species of bacteria, fungi, pollen, spores etc. and can be allergenic and infectious in nature. The present study tries to understand the variability in bioaerosol concentration at three different land use configuration-commercial complex, dumping site and vegetated ridge with respective microclimatic variation. The result showed that dumping site had maximum bacterial count (207.38±10.66 CFU/plate/hr. to 383.47±57.67 CFU/plate/hr.) whereas vegetated ridge recorded highest fungal count (133.69±36.41CFU/plate/hr. to 287.04±40.06 CFU/plate/hr.). Although spatio-temporal variation was quite distinct but meteorological parameter independently failed to show a uniform and conclusive relationship. The land use pattern and human activity seems to be more important factor in determining aerosolized microbial diversity than meteorological variables.

**Keywords:** Bioaerosol, Bacteria, Fungi, Temperature, Relative Humidity, Colony Forming Unit (CFU)

## INTRODUCTION

The biogenic materials or bioaerosols present in the atmosphere in the form of pollens, fungal spores, bacteria, viruses and fragments of plants and animals [1-2]. They are the suspension of air-borne particles containing living organisms or their fragments or products released from them ranging between one micrometer to one hundred micrometers[3-5]. The predominant fraction of these biological pollutants are attributed as a cause of various toxic effects, infectious diseases, respiratory allergies and also responsible for sick building syndrome[6-9]. They act as an irritant to the respiratory system and time to time exacerbating various respiratory morbidity[10-15]. The competence of aerosolization and colonization of bio-aerosol are dependent on its physical properties (like size, density & shape) and it encounters with influencing meteorological variability, which include magnitude and direction of air currents, relative humidity and temperature.[9-10,16-17]

India has yet to ascertain the national standards explicitly mentioning the ambient and threshold limits of the bioaerosols in outdoor as well as in the indoor environment. High Spatio-temporal variability in count and composition of bioaerosols along with difficulty in their identification and complexities in quantifying human exposure makes the studies more challenging to exhibit any plausible association between bioaerosol, other cofactors and their consequential effects. Various studies have been conducted in India to ascertain levels of bioaerosols at different land use sites[18-24] but most of these studies are limited to a single site. As a result, till now the literature in relation to bioaerosols exposure and associated regulatory limits is very scanty. A conceptual framework for bioaerosol study is a dire need to understand their variability at least at a wide level.

The intention of this study is to provide a concept about seasonal growth pattern of ambient biogenic aerosols (i.e. bacteria and fungi) at various land use configuration of urban Delhi and to establish its relation with meteorological variability like temperature and relative humidity.

## MATERIALS AND METHODS

### Study area

Delhi is situated in the northern part of India having geographical location 28.70ºN,77.10ºE and about 211 meter above mean sea level. It has an area of 1,483 sq.kms. The city experiences a sub-tropical climate pattern, with well delineated dry and wet seasons, the arrival of latter being heralded by the monsoons. The city also experiences extremes of temperatures, with temperature in summer reached up to around 48ºC and that in Winter cools down to around 2 ºC[25].

## Sampling Site

Three Sampling sites were selected to typecast different land use pattern with diverse anthropogenic involvement which create different micro-environment for the survival of diverse pathogenic or non-pathogenic organisms and their capacity to get aerosolized. One of the sampling site was a commercial complex, dominated (70%) by printing and photocopy shops, second was an open dumping site, located near mixed cluster zones like traffic intersection, hospital and commercial market area and the third one was a vegetated ridge area which was a northern extension of the ancient Aravalli hill range dominated by indigenous and native plant species like *Acacia nilotica* of family Leguminosae, Capparisdecidua of family Capparaceae, etc. (fig. 1).

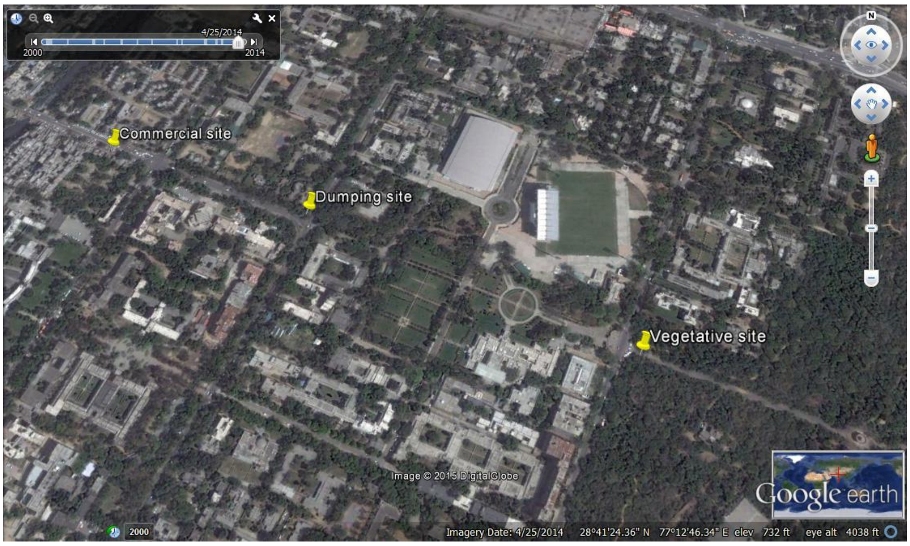



**Figure 1.** Map of Delhi University showing location of the sampling sites

## Sampling and analysis of Bioaerosols

The bioaerosol level was estimated using standard passive non-volumetric settle plate (Gravitational) method. The level was measured at each site weekly for a duration of five months starting from April to mid November, 2013. The Petri plate using blood agar media, prepared in the sterile condition was exposed (face upwards) for one hour at a vertical height of approx. 1m to collect air borne particles at pre identified location following the 1-1-1 principle such that no major wall or obstacle was present within a radius of one meter[26-27]. The plate was incubated at 35°C for 48 hours, identified and then resultant colonies were reported as Colony-forming units (CFU/plate/hr.).

## Bacterial and fungal characterization

The bacterial and fungal colonies were isolated on the basis of morphology and further sub-cultured on their respective media for isolation and identification. Mac Conkey and

Blood agar media were used for bacteria and Sabraouds dextrose agar (SDA) was used for fungal culture. The culture was incubated at 37ºC for 24 hours for bacteria and at 25ºC for 3-4 days for fungus respectively. Bioaerosols population were identified according to their phenotypic characteristics i.e. color, shape, size and nature of the colony. *Gram staining* was done for bacterial identification and *lacto-phenol cotton blue* (*LPCB*) stain mount was used for the identification of fungal growth. The microscopic examination was done to identify different morphological characteristics of fungi based on the presence of spores, types, shape of spores and septal presence[28]. The bacterial and fungal agents were confirmed as per conventional laboratory methods[29-30].

## Meteorological parameter

The meteorological parameters (Relative humidity and Temperature) were monitored regularly at each sampling site using hand held weather monitor (kestrel A33, USA).

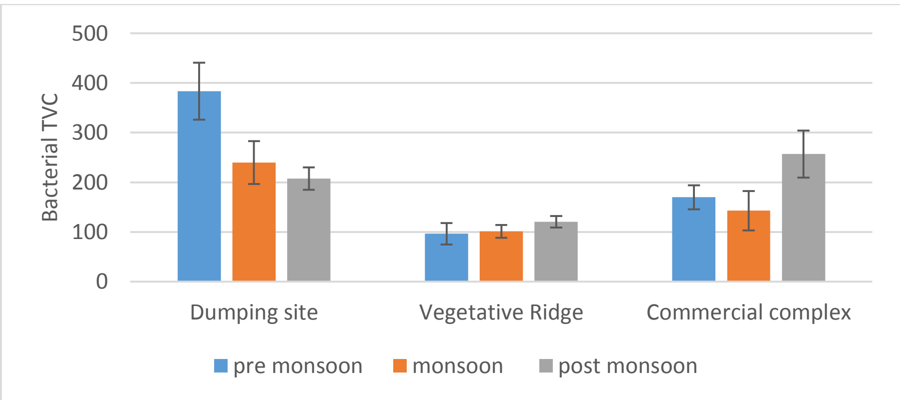
## Statistical Analysis

The data was checked for normality and homogeneity of variance using Shapiro-wilks test and levenes test respectively. Mixed model was used to analyse the fixed effect of land use pattern and season on total viable count (TVC) of aerosolized microbes (bacteria and fungi). Pearson Correlation Coefficient was carried out to establish relationship between microbial pollutant and meteorological parameters such as relative humidity and temperature.

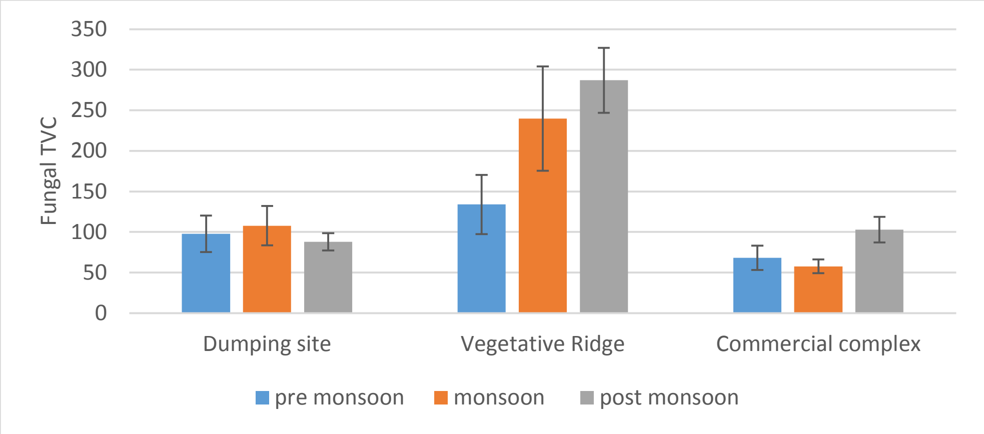
# RESULTS

## Spatial Enumeration of Biogenic Aerosols

The seasonal variation in total viable count (TVC) of bioaerosols from three land use sites are presented in figure 2(a) & (b).




**(a)**




**(b)**

**Figure 2:** Average bacterial (a) and fungal (b) TVC during different seasons (unit= CFU/plate/hr.)

A considerable difference was observed in microbial TVC among the three land use sites. The highest bacterial TVC was noted at the dumping site followed by commercial complex whereas maximum fungal TVC was noted at the vegetative ridge. A variation in the seasonal trend was also observed amongst the three land use site. The bacterial TVC was maximum during pre monsoon season (383.47±57.67 CFU/plate/hr.) which was followed by a fall during monsoon (239.89±43.25 CFU/plate/hr.) and post monsoon season (207.38±10.66 CFU/plate/hr.) specifically at dumping site. The commercial complex observed lowest TVC during monsoon season. The influence of season on the bacterial TVC has been reported statistically significant on fixed effect analysis (p= 0.03).

The fungi had a consistent TVC at the dumping site where as a rising trend was observed at vegetative ridge between pre monsoon season to post monsoon (pre monsoon = 133.69±36.41CFU/plate/hr., monsoon = 239.91±64.26 CFU/plate/hr. & post monsoon =287.04±40.06 CFU/plate/hr.). The trend in fungal TVC is similar to that shown by bacterial TVC at commercial complex. The season was found to had a statistically significant effect on bacterial TVC (p= 0.27).

In term of comparing the difference in the microbial TVC among all the land use site, the Emmean command of mixed modelling was applied by entering land use site as a fixed effect. The pairwise comparison for TVC of bacteria and fungi at diverse land-use configuration are presented in **Table 1 & 2** respectively.

**Table 1:** Pairwise comparison of mean bacterial total viable count at different land use sites

| Site A | Site B | Mean Difference (A-B) (CFU/plate/hr.) | p-value | 95% confidence interval for difference | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| **Dumping Site** | **Vegetative Ridge** | 171.173 | **<0.01** | 111.235 | 231.111 |
| | **Commercial Complex** | 74.009 | **0.01** | 14.071 | 133.947 |
| **Vegetative Ridge** | **Commercial complex** | --97.164 | **0.001** | -157.102 | -37.226 |

**Table 2:** Pairwise comparison of mean fungal total viable count at different land use sites

| Site A | Site B | Mean Difference (A-B) (CFU/plate/hr.) | p-value | 95% confidence interval for difference | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| **Dumping Site** | **Vegetative Ridge** | -122.459 | **<0.01** | -162.281 | -82.636 |
| | **Commercial Complex** | 21.547 | 0.459 | -18.276 | 61.370 |
| **Vegetative Ridge** | **Commercial complex** | 144.005 | **<0.01** | -183.828 | -104.183 |

The bacterial viable count at the dumping site differed significantly from both commercial complex (p=0.011) and the vegetative ridge (p<0.01). The mean bacterial TVC was also significantly (p=0.001) higher at the commercial complex than the vegetative ridge.

In case of fungi, a statistically significant higher concentration was observed at the vegetative ridge in comparison to dumping site (p<0.01) and commercial complex (p<0.01) whereas very low and statistically non-significant (p=0.459) difference of mean was observed between dumping site and commercial complex.

**Effect of meteorological variables on biogenic aerosols:**

In order to understand the relation between microbial TVC with meteorological parameters, Pearson Correlation Coefficient was calculated (**Table 3**). Temperature was reported to had a moderately negative correlation with bacterial and fungal TVC at commercial and vegetative ridge respectively. The correlation at the dumping site, however, remained non conclusive. A positive correlation was observed between fungal TVC and relative humidity at the commercial complex (r = 0.331, p = 0.048) and also at the vegetative ridge (r = 0.758, p<0.01).

**Table 3:** Correlation matrix of bacterial and fungal TVC with temperature and relative humidity

| | | **Bacteria** | **Fungi** |
|---|---|---|---|
| **Temperature** | **Dumping Site** | r = 0.31<br>p = 0.095 | r = 0.197<br>p = 0.296 |
| | **Vegetative Ridge** | r = -0.369<br>p = 0.051 | r = -0.4977<br>**p = 0.005*** |
| | **Commercial Complex** | r = -0.570<br>**p = 0.001*** | r = 0.179<br>p = 0.514 |
| **Relative Humidity** | **Dumping Site** | r = -0.2375<br>p = 0.207 | r = -0.035<br>p = 0.854 |
| | **Vegetative Ridge** | r = 0.2788<br>p = 0.135 | r = 0.758<br>**p <0.01*** |
| | **Commercial Complex** | r = 0.214<br>p = 0.112 | r = 0.331<br>**p = 0.048*** |

*p-value significant for a value less than 0.05

## DISCUSSION

### Spatial Enumeration of Biogenic Aerosols

The highest bacterial viable count was recorded at the dumping site because it had a constant input of waste within various stages of degradation which provided a good substratum for microbial growth[28]. Unhindered access to rag pickers and animals increased process of aerosolisation at dumping site. However, the highest fungal TVC was noted at vegetative ridge. The high evapo-transpiration and regular watering of ridge and moderation in temperature provided optimum conditions for fungal growth[31]. Commercial complex, on the other hand, had highest human activities among all the sites. Human releases microbes in air by talking, sneezing, coughing or by shedding skin cells. Constant human activity and persistent vehicular traffic prevents settling down of aerosolize microbes and causes re-suspension and recirculation of bioaerosols. Various studies pertaining to bioaerosol emission in different micro-climatic environment reported human occupancy and different human activities as an important contributor (Ghosh et al[23], Sharma et al[32] and Mehta et al[24]).

**Seasonal variation of Biogenic Aerosols**

The bacterial and fungal viable count followed different trend at the three land use sites. The dumping sites had highest bacterial TVC during summer season due to faster degradation of waste[33]. The bacterial and fungal TVC during monsoon remained low or comparable to post monsoon season despite presence of favorable condition for microbial growth, could be due to rain wash effect and can be supported with observations by other scientists (Weber et al[34], Chakrabarti et al[35], Lal et al[36] and Chao et al[37]).

**Effect of meteorological variables on biogenic aerosols**

In diverse land-use sites the information on the correlation between the microbial TVC and meteorological parameters was not very uniform. Earlier studies conducted by various scientist (Giorgio et al at Marseilles[38], Wang et al at China[39], Niazi et al at the middle east[40], Oppliger et al at Switzerland[41], Peternel et al at Croatia[42], Sabariego et al at Spain[43], Burch & Levetin[44], Stennett and Beggs at Australia[45]) observed a significant correlation with meteorological parameters whereas other studies (Carducci et al at Italy[46], Sudharsanam et al[47] & Pathak et al in Jabalpur[48]) reported negatively. These studies were, however, limited to a single site. The present study which was conducted at three different land use site located with in or near university complex failed to provide a conclusive relation between microbial TVC and meteorological parameters which suggests that microclimatic conditions of diverse land use configuration plays more determining role than meteorological parameters in influencing population and composition of bioaerosol, although the study was conducted for a small timeframe.

**Morphological Characterization of isolated Biogenic Allergens at Selected sites**

**Bacteria**

*Streptococcus* genera was found to be most prevalent at all the sites (72%) followed by *Staphylococcus* and both genus were characterized on basis of catalase test. When cultured on blood agar media, different species of *Streptococcus* were identified based on their colony characteristics and morphology on gram staining. *Streptococcus pneumonia* cells were lancet-shaped cocci whereas the *Streptococcus pyogenes* as spherical, glossy, grayish-white, translucent and typically produces large zones of beta hemolysis when cultured on blood agar plates[49]. The bacteria having large, round, golden-yellow colonies and appeared as gram-positive coccus in grape-like clusters, was identified as *Staphylococcus* aureus. It is frequently found in the human respiratory tract and also on the skin. Other identified genera observed were *Bacillus, Klebsiella, Escherichia coli, lactobacillus* etc.

### Fungus

Among others, *Aspergillus* were the most abundant genus. Three species of aspergillus genus (89%) were prevalent namely *Aspergillus flavus*; *Aspergillus niger* and *Aspergillus fumigatus* at all experiment sites. The fungus appeared as powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface and with rapid growth has labeled as *Aspergillus flavus*[50]. Whereas *Aspergillus niger* species were with large black or brown conidia by phialides that are arranged in a globose head radiating from a vesicle or spherical conidiophore. The texture was lanose, margin was white with yellowish center. Conidial heads were blackish brown but reverse was bright yellow with no distinct odor[51]. The third species *Aspergillus fumigatus* had typical columnar, uniseriate conidial heads with short, smooth-walled Conidiophores and conical shaped terminal vesicles[52]. Other observed fungus species were *Fusarium, Mucor,* etc.

The gram positive cocci were most predominant bacteria found in all three sites. World wide similar observation were reported in various study[51-52], it may be due that cocci are more stress- resistant and can withstand stress weather condition[17&53]. The major fungi observed were *Aspergillus, Fusarium, Mucur.* Similar genera of fungi were observed in other studies conducted in India[54-56].

## CONCLUSION

This study reveal that viable count of bacteria and fungi varies with the land use sites and season. Aerosolisation of microbes follow a different spatio-temporal diversity. The spatial diversity occurs may be due to anthropogenic and micro-climatic variation along with changes in other influencing factors. The microclimate and human activity seems to play a more important role in determining microbial load in the air than meteorological parameter. The present study was, however, conducted for small time frame. Hence, a study spanning for a larger duration across multiple sites is recommended to ascertain a definite and conclusive relationship between aerosolized microbial count and meteorological parameters.

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