PROJECT REPORT

(UGC Major Research Project)

Characterization of Bioaerosol in different size ranges over Delhi Region.

Submitted to

University Grants Commission (UGC)
Bahadur Shah Zafar Marg, New Delhi
Pin: 110 002

by

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Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project……**Characterization of Size-segregated Bioaerosol of different size ranges over Delhi Region**…….

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR……**Dr. Arun Srivastava, School of Environmental Science, Jawaharlal Nehru University, New Delhi**……..

3. NAME AND ADDRESS OF THE INSTITUTION …… **School of Environmental Science, Jawaharlal Nehru University, New Delhi** …...

4. UGC APPROVAL LETTER NO. AND DATE……**42-420/2013(SR), 12th March 2013**…

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10. TITLE OF THE PROJECT …… **Characterization of Size-segregated Bioaerosol of different size ranges over Delhi Region** ……

11. OBJECTIVES OF THE PROJECT ……**Attached as Annexure 1**……

12. WHETHER OBJECTIVES WERE ACHIEVED …**Yes**……

(GIVE DETAILS)

13. ACHIEVEMENTS FROM THE PROJECT….**One M. Sc. Project completed and one M. Phil. Dissertations were done. One paper presented in a conference and two papers communicated**

14. SUMMARY OF THE FINDINGS ……**Attached as Annexure 2**……..

(IN 500 WORDS)

15. CONTRIBUTION TO THE SOCIETY ……

Papers have been published and proper standard regarding bioaerosol concentration has to be set in order to identifying major human health issues ….

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT ….**two**…

17. NO. OF PUBLICATIONS OUT OF THE PROJECT….**Two**… (and two communicated).

(PLEASE ATTACH)

(PRINCIPAL INVESTIGATOR) **(REGISTRAR/PRINCIPAL)**

(Seal)

CERTIFICATE

The research Project embodied in the project report entitled **"Characterization of Size-segregated Bioaerosol of different size ranges over Delhi Region"**, was sponsored by the University Grants Commission (UGC), New Delhi, under the major research project scheme **(Ref. No.: 42-420/2013(SR).** This work has been carried out at the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi.

Dr. Arun Srivastava

(Principal Investigator)

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CONTENTS

Chapter 1	Introduction	1
Chapter 2	Material and Method	4
	2.1. Study Area	4
	2.2. Sampling Sites	4
	2.3. Sampling Instrument	6
	2.3.1.Anderson sixth stage viable cascade impactor	6
	2.3.2. Aerodynamic particle sizing	6
	2.3.3. Six-Stage Viable Particle Sampler Description	7
	2.4. Sampling procedure	9
	2.5. Preparation, identification, and enumeration of culturable bio aerosols	9
	2.5.1. Sample Preparation	9
	2.5.2. Enumeration	9
	2.5.3. Identification	10
Chapter 3	Results and Discussion	12
	3.1. The concentration of bioaerosol at different seasons at different sites	12
	3.1.1. Fungi	12
	3.1.2. Gram-Negative Bacteria	13
	3.1.3. Gram-Positive Bacteria	14
	3.2. The concentration of total bioaerosol at different seasons at different sites	16
	3.3. Concentrations of fractions of bioaerosol at different seasons	18
	3.4. Size Segregations of Fungi over two years	19
	3.5. Size Segregations of Gram-Positive Bacteria (GPB) over two years	24

3.6. Size Segregations of Gram-Negative Bacteria (GNB) over two years 29
3.7 Identification of Fungal bioaerosols 33
3.8 Linear and multiple regression for bioaerosol concentration 34
Chapter 4 Conclusion 35
Chapter 5 References 38

List of figures

Figure 1:	Location of Sampling Sites	5
Figure 2:	Simulation of Human Respiratory System with Anderson Cascade Impactor	7
Figure 3:	Tisch Six-Stage Viable Sampler	8
Figure 4:	Concentration of fungal bioaerosol at different seasons at different sites	13
Figure 5:	Concentration of GNB bioaerosol at different seasons at different sites	14
Figure 6:	Concentration of GPB bioaerosol at different seasons at different sites	15
Figure 7:	Concentration of total bioaerosol at different seasons at different sites	16
Figure 8:	Concentrations of fractions of bioaerosol at different seasons	18
Figure 9(a):	Size segregation of fungal bioaerosol in PM season of 1st year (2013-14)	20
Figure 9(b):	Size segregation of fungal bioaerosol in Win season of 1st year (2013-14)	20
Figure 9(c):	Size segregation of fungal bioaerosol in Sum season of 1st year (2013-14)	21
Figure 9(d):	Size segregation of fungal bioaerosol in Mon season of 1st year (2013-14)	21
Figure 9(e):	Size segregation of fungal bioaerosol in PM season of 2nd year (2014-15)	22
Figure 9(f):	Size segregation of fungal bioaerosol in Win season of 2nd year (2014-15)	22
Figure 9(g):	Size segregation of fungal bioaerosol in Sum season of 2nd year (2014-15)	23
Figure 9(h):	Size segregation of fungal bioaerosol in Mon season of 2nd year (2014-15)	23
Figure 10(a):	Size segregation of GPB bioaerosol in PM season of 1st year (2013-14)	25
Figure 10(b):	Size segregation of GPB bioaerosol in Win season of 1st year (2013-14)	25
Figure 10(c):	Size segregation of GPB bioaerosol in Sum season of 1st year (2013-14)	26
Figure 10(d):	Size segregation of GPB bioaerosol in Mon season of 1st year (2013-14)	26
Figure 10(e):	Size segregation of GPB bioaerosol in PM season of 2nd year (2014-15)	27
Figure 10(f):	Size segregation of GPB bioaerosol in Win season of 2nd year (2014-15)	27
Figure 10(g):	Size segregation of GPB bioaerosol in Sum season of 2nd year (2014-15)	28
Figure 10(h):	Size segregation of GPB bioaerosol in Mon season of 2nd year (2014-15)	28
Figure 11(a):	Size segregation of GNB bioaerosol in PM season of 1st year (2013-14)	29
Figure 11(b):	Size segregation of GNB bioaerosol in Win season of 1st year (2013-14)	30
Figure 11(c):	Size segregation of GNB bioaerosol in Sum season of 1st year (2013-14)	30
Figure 11(d):	Size segregation of GNB bioaerosol in Mon season of 1st year (2013-14)	31

Figure 11(e): Size segregation of GNB bioaerosol in PM season of 2nd year (2014-15) 31
Figure 11(f): Size segregation of GNB bioaerosol in Win season of 2nd year (2014-15) 32
Figure 11(g): Size segregation of GNB bioaerosol in sum season of 2nd year (2014-15) 32
Figure 11(h): Size segregation of GNB bioaerosol in Mon season of 2nd year (2014-15) 33

List of Tables

Table 1:	Description of Sampling Sites	4
Table 2:	Table of jet orifice dimensions and particle size ranges of each stage	8
Table 3:	Table of characterized fungal bioaerosol	33
Table 4:	Statistical results of linear regression for fungal and bacterial concentration	34

Abbreviations

PM	Post Monsoon
Mon	Monsoon
Sum	Summer
Win	Winter
PDA	Potato Dextrose Agar
EMB	Eosin Methylne Blue Agar
BA	Blood Agar
LPM	Litres Per Minute
GPB	Gram-Positive Bacteria
GNB	Gram-Negative Bacteria
CFU	Colony Forming Units
CFU M^{-3}	Colony Forming Units per Cubic Meter
R.H	Relative Humidity
JNU	Jawaharlal Nehru University
C.P	Connaught Place (C.P)
H.NZM	Hazrat Nizamuddin
U.N	Uttam Nagar (U.N)

Annexure 1

Objectives of the Project

1. Determination of size distribution of bioaerosol and respirable fractions(PM_{10}) over Delhi.
2. Determination of correlation between different size fractions over Delhi.
3. Determination of correlation between bioaerosol of different size with the pollutant of different size.
4. Characterization of different bioaerosol using SEM (Scanning Electron Microscope).
5. To determine the correlation between bioaerosol, pollutants and different meterological parameters (temperature, wind direction, wind speed and humidity).

Annexure 2

Chapter 1

INTRODUCTION

Rapid urbanization and industrialization lead to the production of many industries and migration of many people from rural areas to urban areas. This creates a load of heavy vehicular traffic in urban areas as people need transport to go to one area to another area. This urbanization also creates a construction of landfill for safe disposal of solid waste. This introduces different types of pollutants in the environment producing adverse changes in the water, soil, air and climate.

Air pollution is known to cause local problem including damage of materials, human health and sometimes creating a zero visibility among a particular region as well as on geographical and global scale such as ozone depletion and climate change. Air pollution (both outdoor and indoor air pollution) was responsible for the premature death of 3 million people each year **(Lave et al., 1979)** and reached to 7 million by 2012 that is double of the previous estimate **(WHO, 2014)**. Air pollution can cause health hazards by affecting people in different ways, having both short term and long term effects **(Chen, 2008)**. Young children and elderly people are more sensitive to pollutants than others. Air pollution adversely affects people with heart, asthma, and lung diseases. The pollutant which is responsible for air pollution are of two types: abiotic (particulate matter, various metals, and gaseous pollutant such as Sulphur oxides (SOx), especially sulphur dioxide(SO_2), Nitrogen oxides (NO_X), especially nitrogen dioxide(NO_2), Carbon monoxide (CO), Volatile organic compounds (VOC_S)) & biotic (mainly include bioaerosol). The aerosol present in the near surface atmosphere which is typical of biological origin include mainly of fungal spores, bacteria, viruses, different form of pollen grain, any fragment from plant, animals or any living organism as well as by-product or secondary metabolite of microbial metabolism present in the form of particulates, liquid and volatile organic compounds (VOCs) are commonly known as the bioaerosol **(Stetzenbach et al., 2004)**.

It is well known that biogenic aerosols have a significant effect on human health, the environment, climate, atmosphere and another phenomenon **(Poschl, 2005; Jaenicke et al., 2007; Ho and Duncan, 2005; Huffman et al., 2010)**. Fungi live everywhere and they originate from different environments such as soil, plants, and water. Air borne fungal spore depends on

geographical location, air pollution as well as meteorological parameters**.(D'Amato et al., 1984; Lin and Li., 2000).**

Majority of bioaerosol fall in the respirable size ranges, namely; 0.003 µm for viruses **(Taylor, 1988)**, 1 to 30 µm for fungi **(Gregory, 1973)**, 0.25 to 20 µm for bacteria **(Thompson, 1981)** and from 17 to 58 µm for plant pollens **(Stanley and Linskin, 1974)**. The size distributions of bioaerosol were studied and found that it varied with regions. Particles of different sizes have been distributed into four categories according to their diameter by the US Environmental Protection Agency (EPA) such as ultrafine <0.1 µm, fine 0.1 -2.5 µm, coarse 2.5 -10 µm and super coarse > 10 µm **(EPA 2004)**. According to studies carried out in different countries such as Sweden **(Bovallius et al., 1978)**, The United States **(Lighthat and Shaffer, 1995)** as well as in Asian country such as China **(Fang et al., 2004; Liu et al., 2008; Xu et al., 2011)** majority of bacteria exist in coarse particles (>2.5 µm). Both density and shape of the particle play a key role in its aerodynamic diameter. Thus along with filtration and separation, respiratory deposition is also characterized by the aerodynamic diameter of the particle **(Hassan & Lau, 2009)**. The American Conference of Governmental Industrial Hygienists (ACGIH), the International Organization for Standardization (ISO), and the European Standards Organization (CEN) have reached agreement on definitions of the inhalable, thoracic and respirable fractions of dust particle **(ACGIH, 1999; ISO, 1995; CEN, 1993; ICRP, 1994)**. So in occupational hygiene terms such as "inhalable", "thoracic" and "respiratory" particles are mostly used rather than using the terms "fine" and "coarse" particles. Particles have the tendency to deposit in various parts of the human respiratory system, wherein the principal factor that determines the deposition location is the particle size. In relative to deposition concerns, ISO (1995) uses the following particle size classifications:

(a) Inhalable fraction – mass fraction of total airborne particles that can be inhaled through the nose and mouth.

(b) Thoracic fraction – mass fraction of inhaled particles that can penetrate beyond the larynx.

(c) Respirable fraction – mass fraction of inhaled particles that can reach the gas exchange region of the lung.

In addition, these countries have their own national air quality standards for the bioaerosol concentration. Table 1.1 present recommended international standards of bioaerosol.

According to European Standardization Committee, the 50% cut-off diameter for both respirable fraction and thoracic fraction are 4 μm and 10 μm respectively (CEN 1993). Out of the total airborne mass fraction, the inhalable fraction is made up of particles that are easily inhaled via both mouth and nose. Out of the particles with diameter >50 μm that enter the nose and mouth, particles with diameter >10 μm are found to be deposited on the ventilation pathway surfaces just above the trachea. Fine particles gain entry to the alveolar region of the lungs. In the case of fungi, although the particle size was found to be different in various regions yet their size distribution exhibited a single peak distribution. In addition, the airborne microbes were found primarily in coarse particles with diameters larger than 2.5 μm **(Li et al., 2011)**.

Sources of bioaerosol in the indoor environment include floor cavities, ceiling, wall contaminated with fungus; building materials and furnishings as well as the movement of spores, cells and cell fragments through gaps in structural joints and wall openings **(Srikanth et al., 2008; Ghosh et al., 2015, Lal et al., 2013)**. Other factors like dwellers, transporters, services, domestic animals, water spray, and putrefactions were found the major contributors in microbial concentration in the ambient environment **(Pathak and Verma, 2009).**

Chapter 2

MATERIALS AND METHODS

2.1 Study Area:

Delhi, being the capital, is the chief metropolitan city of India. It is spread between the latitudes of 28°24′17″ to 28°53′ and the longitudes of 76°20′37″ to 77°20′37″ at an elevation of around 213.3 to 305.4 m above sea level. The atmosphere of Delhi is subtropical and is characterized by a semi-arid climate which consists of summer (April to June), Monsoon (July to September), Autumn (September to November), Winter (December to January), Spring (February to March). Delhi experiences a maximum temperature of approximately 45- 48 ºC in June during the summer season and a minimum of about 1-2ºC in January during the winter season. The average rainfall is around 611mm. The air over Delhi is dry during the greater part of the year. Humidity is high in the monsoon season while April and May are the driest months. Normally, north–westerly winds prevail during the year, while in the months of June and July, south–easterly winds predominate. According to the 2011 Census, Delhi's population was 16.75 million.

2.2 Sampling Sites:

To fulfill the objectives of the study, samples of bioaerosol were collected at six different locations in Delhi. The description of sampling sites is given in table 1. The samples were collected on different weekdays in all the seasons at these locations (figure 1). Further, meteorological conditions viz temperature, relative humidity, and wind speed were also recorded at each sampling site during the duration of sampling.

Table 1: Description of Sampling Sites

S.No.	Sampling Site	Site Type
1.	Jawaharlal Nehru University (JNU)	Residential cum Institutional
2.	Connaught Place (C.P)	Commercial
3.	ITO	Heavy Traffic
4.	Okhla	Industrial
5.	Hazrat Nizamuddin (H.NZM)	Residential
6.	Uttam Nagar (U.N)	Sub Urban

Annexure 2

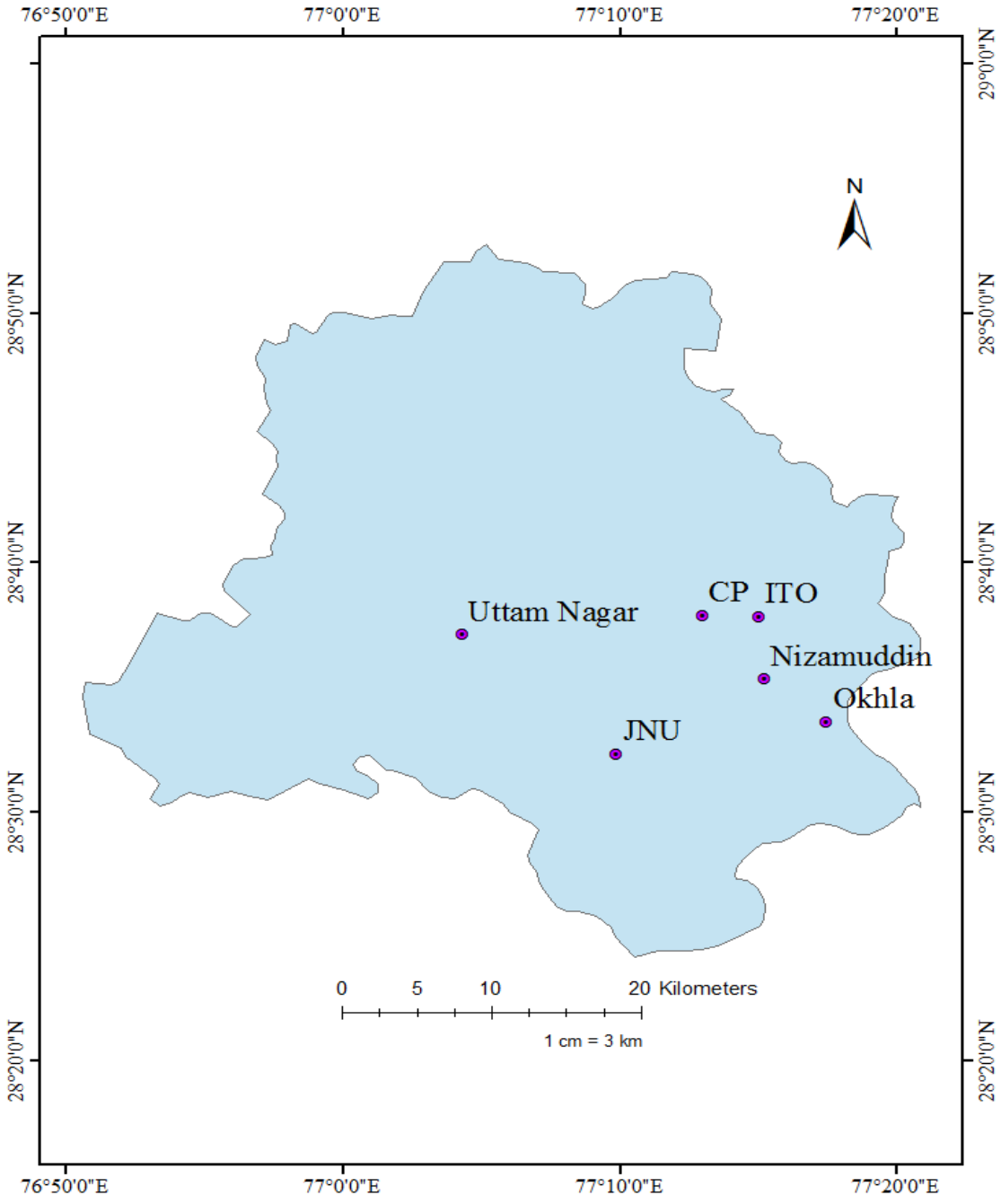

Figure 1: Location of Sampling Sites.

2.3 Sampling Instrument:

The bioaerosol sampling was done with the help of Anderson sixth stage viable cascade impactor (Tisch Environmental, Inc.)

2.3.1 Anderson sixth stage viable cascade impactor:

The six-stage Viable Particle Sampler is a multi-orifice, cascade impactor which is normally used to measure the concentration and particle size distribution of aerobic bacteria and fungi in ambient air. The instrument has been widely used as a standard for enumerating the viable particles in a microbial aerosol. Viable particles can be collected on a variety of bacteriological agar and incubated in situ for counting and identification. This sampler was calibrated so that all particles collected, regardless of physical size, shape, or density, are sized aerodynamically and can be directly related to human lung deposition.

2.3.2 Aerodynamic Particle Sizing:

The human respiratory system tract is an aerodynamic classifying system for airborne particles. A sampling device can be used as a substitute for the respiratory tract as a collector of viable airborne particles, and as such, it should reproduce to a reasonable degree the lung penetration by these particles. The fraction of inhaled particles retained in the respiratory system and the site of deposition vary with all the physical properties (size, shape, density) or the particles which make up the aerodynamic dimensions. Because the lung penetrability of unit density particles is known and since the particle sizes that are collected on each stage of the Tisch Viable Samplers have been determined, if a standard model of these samplers is used according to standard operating procedure, the stage distribution of the collected material will indicate that extent to which the sample would have penetrated the respiratory system.

Figure 2 shows the deposition efficiencies in the nasal-pharyngeal, tracheo-bronchial and pulmonary regions of the human respiratory tract as a function of particle size. Large particles deposit primarily in the nasal-pharyngeal area, whereas particles of sub-micrometer size particles deposit mainly in the pulmonary area Numerous small round jets improve collection (impaction) efficiency and provide a sharper cutoff of particle sizes on each stage of inertial impactors. Thus, the Six-Stage sampler with 400 small round jets per stage meet all the criteria for the efficient collection of airborne viable particles. Reports have discussed a reduced efficiency in cascade impactors when particles bounce off the impaction surface, are restrained and lost in the exhaust air. This effect is minimized when a sticky agar surface is used as the collection medium.

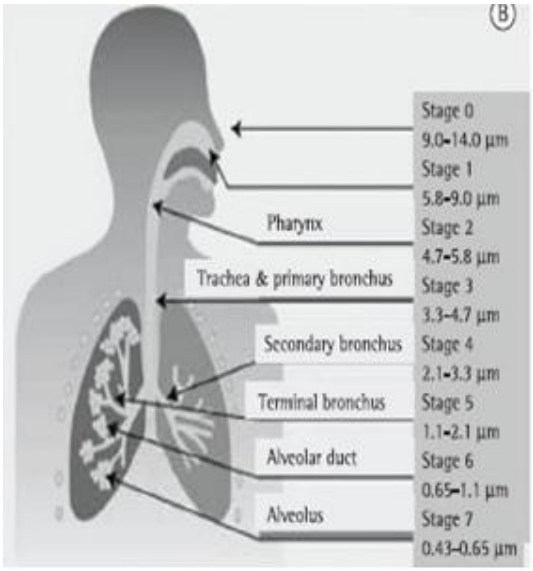

Figure 2: Simulation of Human Respiratory System with Anderson Cascade Impactor.

2.3.3 Six-Stage Viable Particle Sampler Description:

The Tisch Viable Particle sampler is constructed with six aluminum stages that are held together by three spring clamps and sealed with 0-ring gaskets (Figure 3). Each impactor stage contains multiple precision drilled orifices. When air is drawn through the sampler, multiple jets of air in each stage direct any airborne particles toward the surface of the agar collection surface for that stage. The size of the jet orifices is constant within each stage, but are smaller in each succeeding stage. The range of particle sizes collected in each stage depends on the jet velocity of the stage and the cutoff of the previous stage. Any particle not collected on the first stage follows the air stream around the edge of the Petri dish to the next stage.

Each stage contains 400 orifices with diameters ranging from 1.18 mm on the first stage to 0.25 mm on the sixth stage. Each stage has a removable glass Petri dish with a glass or metal cover. The exhaust section of each stage is approximately 19mm larger in diameter than the Petri dish, which allows unimpacted particles to go around the dish into the next stage.

Annexure 2

Figure 3: Tisch Six-Stage Viable Sampler

Table 2: Table of jet orifice dimensions and particle size ranges of each stage

The jet orifice dimensions and particle size ranges for each stage are:

Stage	Orifice Diameter (mm)	Range of Particle Sizes (Microns)
1	1.18	7.0 and above
2	0.91	4.7-7.0
3	0.71	3.3-4.7
4	0.53	2.1-3.3
5	0.34	1.1-2.1
6	0.25	0.65-1.1

2.4 Sampling procedure:

The air sample was collected on a Petri dish loaded with the selective media, namely Potato Dextrose Agar (PDA) for fungi, Blood Agar (B.A) for Gram-Positive Bacteria and Eosin Methylene Blue Agar (EMB Agar) for Gram Negative Bacteria with the help of Six Stage Anderson Viable Cascade Impactor. However, the selective media was used for culturing the bio aerosol but anti-fungal chemical (Cycloheximide) was added in the Blood Agar and EMB Agar. The air was drawn to the Petri dish at a flow rate of 28.3 LPM. Sampling time was 2 minutes for all the six sites throughout the sampling period. Sampling was done at a height of about 1.3meter to simulate the human respiratory tract. Apart from that the Blank Petri dish sample was prepared and was taken to the field at each time of sampling but the air was not withdrawn from them. The results were blank corrected.

2.5 Preparation, identification, and enumeration of culturable bio aerosols:

2.5.1 Sample Preparation:

After bio aerosol sampling, exposed agar plates were incubated at the appropriate temperature for times ranging from hours for a fast-growing bacterium to develop a micro colony to days for a fungus to develop into a visible colony. In this study, the bacterial colonies were incubated at 32^{o}C-37^{o}C for 3-5 days for both GNB and GPB. The fungal colonies were incubated at 25-27^{o}C for 3 days. After the incubation period, the growth of colony was observed and counted as Colony Forming Units (CFU m^{-3}). However in the case of Fungi, few colonies did not produce spore after 7 days of sampling, thus they called as non-sporulating colony. Fungi were identified by the direct observation of colony morphological feature basis.

2.5.2 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.

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

Flow rate = 28.3 lit/min = 0.0283 m^3/min

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

In many cases it is difficult to identify multiple colonies at one location on a plate because of several reasons, firstly the 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.skcinc.com/catalog/pdf/Multiple_Jet_Impactors.pdf.

2.5.3 Identification:

For identification of fungi, took the smaller portion of fungus colony with the help of inoculum loop and placed it on a slide containing 4% NaCl. A drop of Cotton Blue Stain is added over it for staining purpose and left for 1-2 min. The area is covered by a cover slip and now ready for microscopic examination and visual identification.

For Bacterial identification, on a clean slide, a thin film of bacterial culture was produced and stained with ammonium oxalate crystal violet solution for 1 min. Then the slide was washed with water to remove excess stain from it, immersed in an iodine solution for a

minute and again washed with water and allow to dry. It was then dipped in 95% ethanol for the 30s and safranin solution for 10s. After final water wash, glycerine was added and is covered with a cover slip for prior microscopic examination. In microscope GPB show purple in color and GNB show red in color. By microscopic examination, the only conformation of gram-positive and gram-negative bacteria has been done. No individual species could be determined using this method.

Chapter 3

Results and Discussion

The observed values of the concentrations of different bioaerosols at each site in all the four seasons, namely, summer, winter, post-monsoon, and monsoon, are presented in figures 4 to 6.

3.1 The concentration of bioaerosol at different seasons at different sites:

3.1.1 Fungi :

As seen in figure 4, in 2013-14, the fungal bioaerosol concentration ranged from (914.31- 130.30 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at Okhla site. In the Post Monsoon season, the highest fungal bioaerosol concentration (914.31 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest concentration (309.19 CFU M^{-3}) was found at Cannaught Place site. In winter season the highest fungal bioaerosol concentration (675.79 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest fungal bioaerosol concentration was at Cannaught Place site (494.79 CFU M^{-3}). In Summer season, the highest fungal bioaerosol concentration (456.42 CFU M^{-3}) was found at Uttam Nagar site while lowest fungal bioaerosol concentration was at Okhla site (188.45 CFU M^{-3}). In Monsoon Season, the highest fungal bioaerosol concentration (567.58 CFU M^{-3}) was found at ITO site while lowest fungal bioaerosol concentration was again at Okhla site (130.30 CFU M^{-3}).

In 2014-15, the fungal bioaerosol concentration ranged from (1174.91- 259.13 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at JNU site. In the Post Monsoon season, the highest fungal bioaerosol concentration (636.04 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest concentration (312.13 CFU M^{-3}) was found at ITO site. In Winter Season, the highest fungal bioaerosol concentration (1174.91 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest concentration (636.04 CFU M^{-3}) was found at ITO site. In Summer Season, the highest fungal bioaerosol concentration (412.25 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest concentration (259.13 CFU M^{-3}) was found at JNU site. In Monsoon Season, the highest fungal bioaerosol concentration (556.54 CFU M^{-3}) was found at ITO site while lowest concentration (295.94 CFU M^{-3}) was found at Okhla site. The reason of fungal bioaerosol concentration is due to factors like dwellers, transporters, services, domestic animals, water spray, and putrefactions were found the major contributors in microbial

concentration in the ambient environment **(Pathak and Verma, 2009).** Fungi live everywhere and they originate from different environments such as soil, plants, and water. Air borne fungal spore depends on geographical location, air pollution as well as meteorological parameters**.(D'Amato et al., 1984; Lin and Li., 2000).**

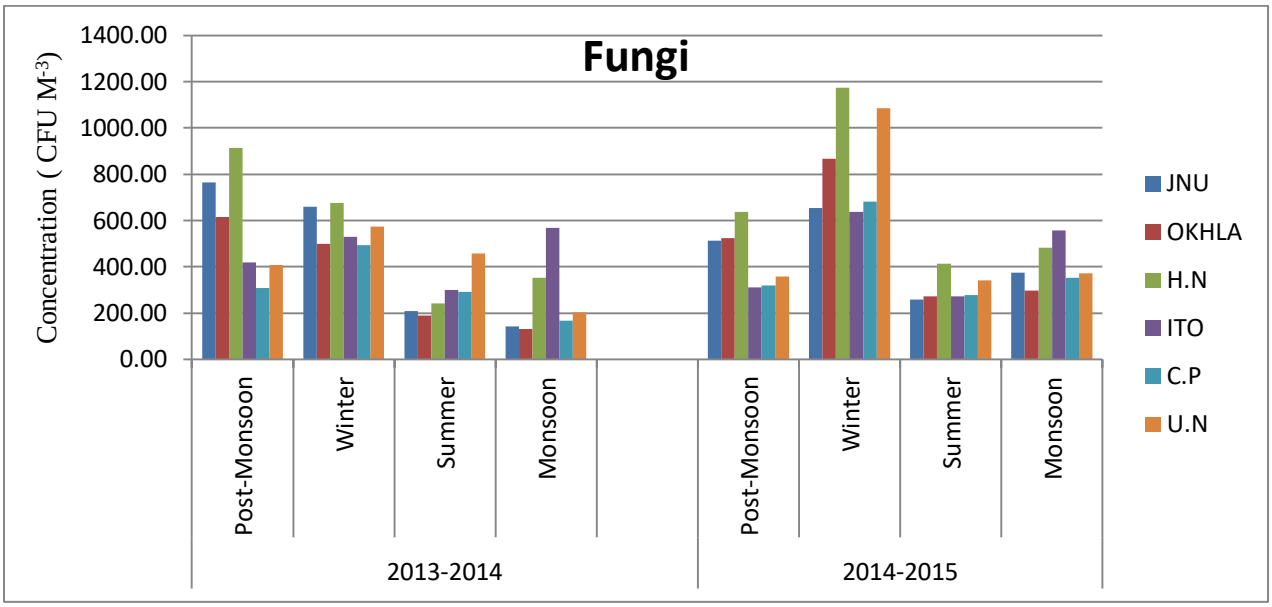

Figure 4: Concentration of fungal bioaerosol at different seasons at different sites

3.1.2 Gram-Negative Bacteria:

As seen in figure 5, in 2013-14, the Gram-Negative bacterial bioaerosol concentration ranged from (1104.24-132.49 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at ITO site. In the Post Monsoon season, the highest Gram-Negative bacterial bioaerosol concentration (856.89 CFU M^{-3}) was found at JNU site while lowest concentration (221.03 CFU M^{-3}) was found at Cannaught Place site. In the winter season, the highest Gram-Negative bacterial bioaerosol concentration (472.61 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Negative bacterial bioaerosol concentration was at Okhla site (225.26 CFU M^{-3}). In Summer season, the highest Gram-Negative bacterial bioaerosol concentration (1104.24 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Negative bacterial bioaerosol concentration was at JNU site (294.46 CFU M^{-3}). In Monsoon Season, the highest Gram-Negative bacterial bioaerosol concentration (280.45 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Negative bacterial bioaerosol concentration was again at ITO site (132.49 CFU M^{-3}).

In 2014-15, the Gram-Negative bacterial bioaerosol concentration ranged from (1107.18-70.67 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at Okhla and Connaught

Place site. In the Post Monsoon season, the highest Gram-Negative bacterial bioaerosol concentration (194.35 CFU M^{-3}) was found at JNU site while lowest concentration (70.67 CFU M^{-3}) was found at both sites (Cannaught Place and Okhla) respectively. In winter and summer season both, the highest Gram-Negative bacterial bioaerosol concentration(335.69CFU M^{-3}, 1107.18CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Negative bacterial bioaerosol concentration was at Cannaught Place and JNU site (114.84CFU M^{-3},541.81CFU M^{-3}). In Monsoon Season, the highest Gram-Negative bacterial bioaerosol concentration (591.87 CFU M^{-3}) was found at ITO site while lowest Gram-Negative bacterial bioaerosol concentration was at JNU site (340.11 CFU M^{-3}).

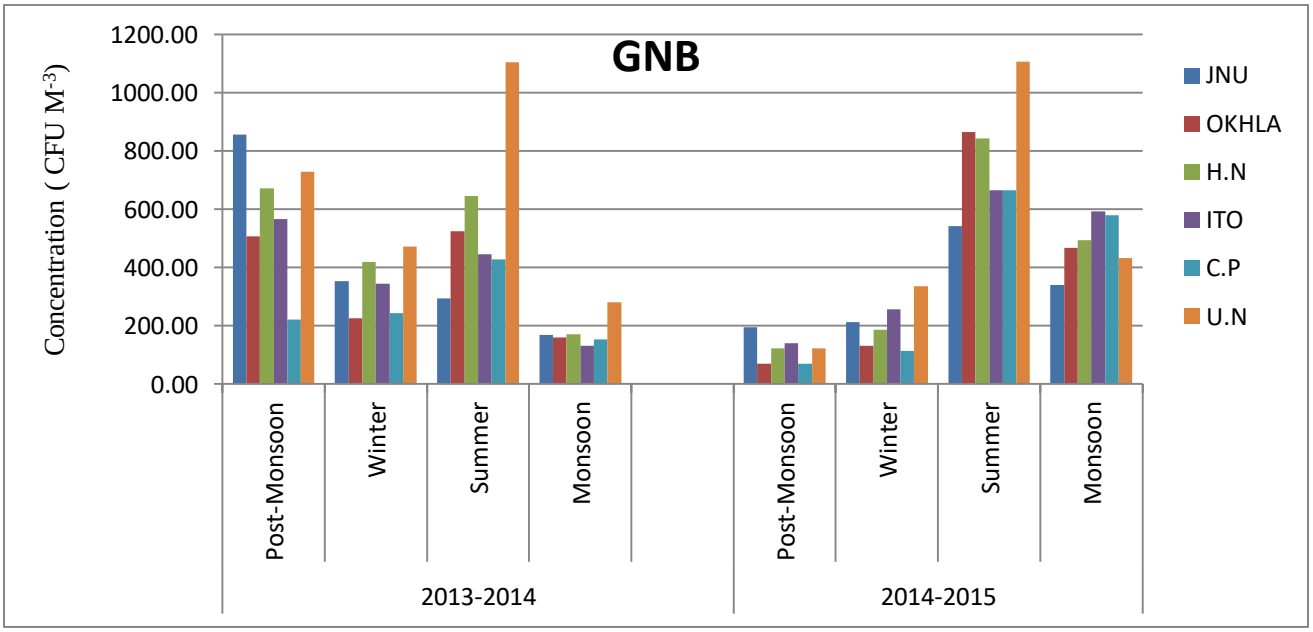

Figure 5: Concentration of GNB bioaerosol at different seasons at different sites

3.1.3 Gram-Positive Bacteria:

As seen in figure 6, in 2013-14, the Gram-Positive bacterial bioaerosol concentration ranged from (9667.26-320.24 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at JNU site. In the Post Monsoon season, the highest Gram-Positive bacterial bioaerosol concentration (1170.50 CFU M^{-3}) was found at JNU site while lowest concentration (552.08 CFU M^{-3}) was found at Cannaught Place site. In the winter season, the highest Gram-Positive bacterial bioaerosol concentration (2062.72 CFU M^{-3}) was found at Okhla site while lowest Gram-Positive bacterial bioaerosol concentration was at ITO site (513.24 CFU M^{-3}). In Summer

season, the highest Gram-Positive bacterial bioaerosol concentration (9667.26 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Positive bacterial bioaerosol concentration was at JNU site (971.73 CFU M^{-3}). In Monsoon Season, the highest Gram-Positive bacterial bioaerosol concentration (1199.19 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Positive bacterial bioaerosol concentration was again at JNU site (320.24 CFU M^{-3}). In 2014-15, the Gram-Positive bacterial bioaerosol concentration ranged from (1740.28-406.36 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at JNU site. In the Post Monsoon season, the highest Gram-Positive bacterial bioaerosol concentration (1154.30 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest concentration (583.04 CFU M^{-3}) was found at Cannaught Place site. In the winter season, the highest Gram-Positive bacterial bioaerosol concentration (1740.28 CFU M^{-3}) was found at Hazrat Nizamuddin site while lowest Gram-Positive bacterial bioaerosol concentration was at JNU site (406.36 CFU M^{-3}). In Summer season, the highest Gram-Positive bacterial bioaerosol concentration (1378.09 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Positive bacterial bioaerosol concentration was at Cannaught Place site (883.39 CFU M^{-3}). In Monsoon Season, the highest Gram-Positive bacterial bioaerosol concentration (971.73 CFU M^{-3}) was found at Uttam Nagar site while lowest Gram-Positive bacterial bioaerosol concentration was again at JNU site (538.87CFU M^{-3}).

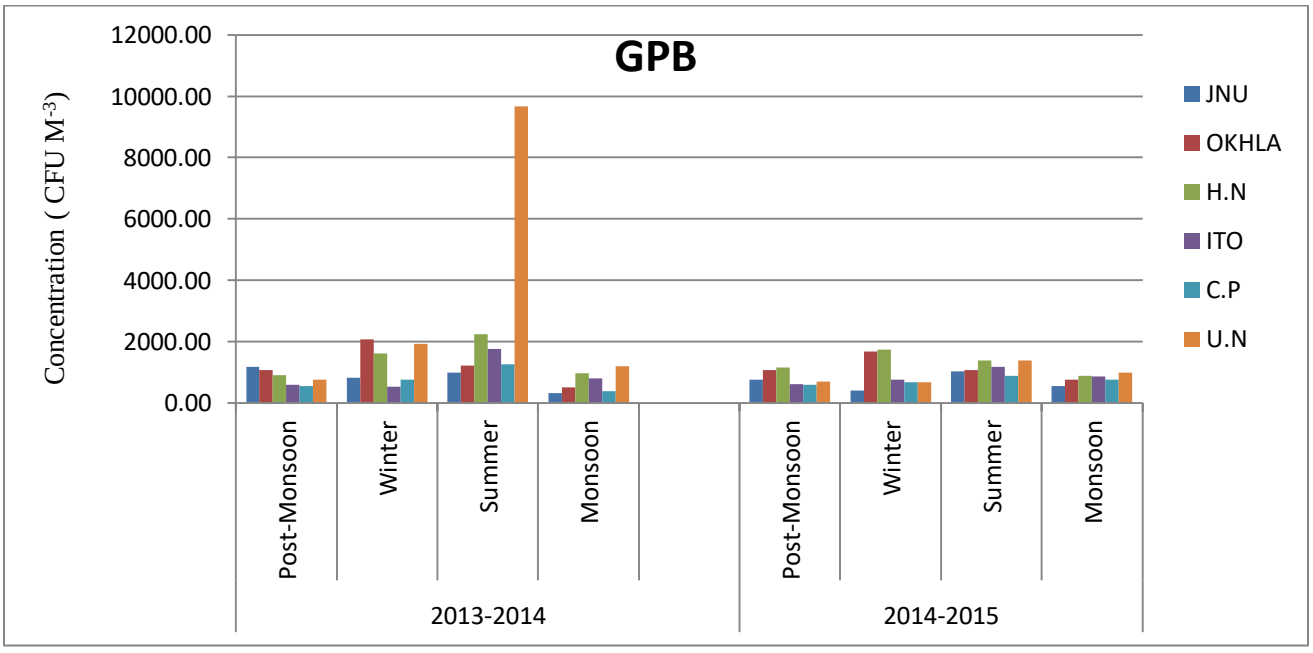

Figure 6: Concentration of GPB bioaerosol at different seasons at different sites

3.2 The concentration of total bioaerosol at different seasons at different sites:

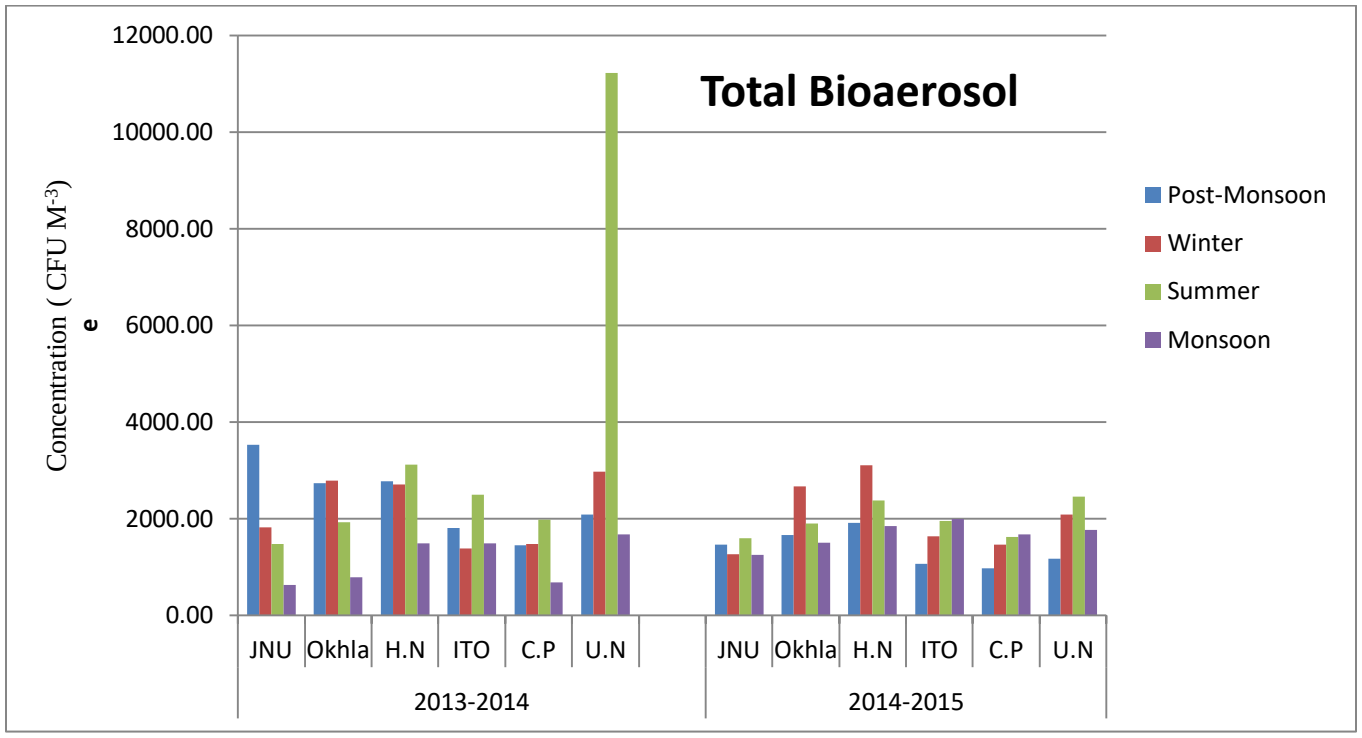

Figure 7: Concentration of total bioaerosol at different seasons at different sites

As seen in figure 7, In 2013-14, the total bioaerosol concentration ranged from (11227.91CFU M^{-3} to 629.42 CFU M^{-3}) with a maximum at Uttam Nagar in summer season and minimum at JNU in monsoon season. At JNU site, the maximum total bioaerosol concentration (3529.15CFU M^{-3}) was found at post monsoon season due to the temperature and relative humidity is more favorable for the growth of bioaerosol in that season and minimum at monsoon season (629.42 CFU M^{-3}) due to rain washout of maximum bioaerosol present in the environment. At Okhla site, the maximum total bioaerosol concentration (2787.10 CFU M^{-3}) in winter season due to the presence of landfill site in nearby sampling site and minimum at monsoon season (786.22CFU M^{-3}) due to washout of bioaerosol particle with the help of rain from the ambient environment. At Hazrat Nizamuddin site, maximum total bioaerosol concentration (3121.32 CFU M^{-3}) in summer season due to aerosolization of bioaerosol in the ambient environment and minimum at monsoon season (1488.52CFU M^{-3}) due to rain washout of maximum bioaerosol present in the environment. At ITO site, maximum total bioaerosol

concentration (2499.99 CFU M^{-3}) in summer season due to aerosolization of bioaerosol in the ambient environment and minimum at winter season (1388.29CFU M^{-3}) due to the presence of heavy traffic at surrounding sampling site. At Connaught Place site, maximum total bioaerosol concentration (1978.80 CFU M^{-3}) in summer season due to aerosolization of bioaerosol in the ambient environment and minimum at monsoon season (689.05CFU M^{-3}) due to rain washout of maximum bioaerosol present in the environment. At Uttam Nagar site, maximum total bioaerosol concentration (11227.91CFU M^{-3}) in summer season due to the presence of nearby market and movement of people in that area as well as aerosolization of bioaerosol in the ambient environment and minimum at monsoon season (1682.85CFU M^{-3}) due to rain washout of maximum bioaerosol present in the environment.

In 2014-15, the total bioaerosol concentration ranged from (3100.71CFU M^{-3} to 971.73CFU M^{-3}) with a maximum at Hazrat Nizamuddin in winter season and minimum at Connaught Place in post monsoon season. At JNU site, the maximum total bioaerosol concentration (1596 CFU M^{-3}) was found at summer season due to aerosolization of bioaerosol particle in the air and minimum at monsoon season (1254.42 CFU M^{-3}) due to rain washout of bioaerosol particle. At Okhla site, the maximum total bioaerosol concentration (2667.84 CFU M^{-3}) at winter season due to the presence of nearby landfill site and a government hospital and minimum at (1506.18 CFU M^{-3}) at monsoon season due to washout of a maximum number of bioaerosol particle in that season. At Hazrat Nizamuddin site, the maximum total bioaerosol concentration (3100.71 CFU M^{-3}) in winter season due to the presence of vegetation as well as sewer flowing in the nearby area and minimum total bioaerosol concentration (1855.12 CFU M^{-3}) in monsoon season due to rain washout is the primary cause of low bioaerosol concentration. At both ITO and Connaught Place site, the maximum total bioaerosol concentration (1996.47 CFU $M^{-3}$and 1678.45 CFU M^{-3}) in monsoon season and minimum total bioaerosol concentration (1065.96 CFU M^{-3} and 971.93 CFU M^{-3}) in post monsoon season. At Uttam Nagar site, the maximum total bioaerosol concentration (2461.72 CFU M^{-3}) in summer season due to the presence of nearby market as well as Uttam Nagar Metro Station and Bus Terminal in the close vicinity of the sampling site and minimum total bioaerosol concentration (1171.97 CFU M^{-3}) in post monsoon season.

3.3 Concentrations of fractions of bioaerosol at different seasons:

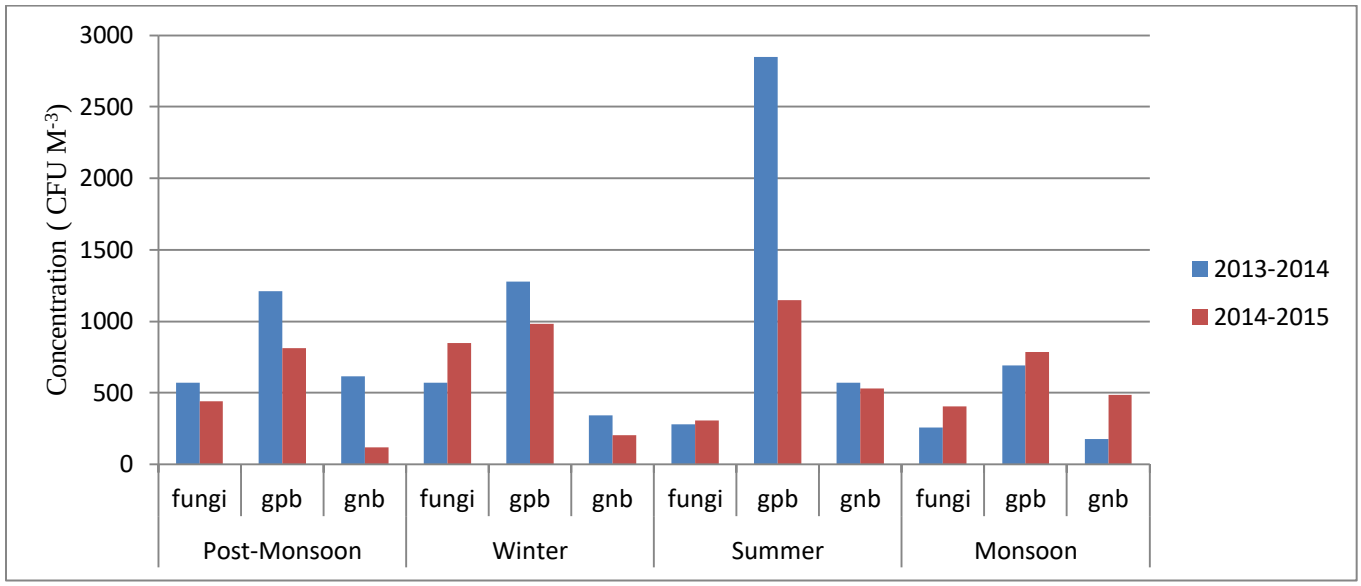

Figure 8: Concentrations of fractions of bioaerosol at different seasons

As seen in figure 8, The fungal bioaerosol concentration was higher in the 1st year (2013-14) than the 2nd year (2014-15) in post monsoon season while at all the remaining season i.e. winter, summer and monsoon season, the fungal bioaerosol concentration was higher in the 2nd year (2014-15) than 1st year (2013-14) due to factors like dwellers, transporters, services, domestic animals, water spray, and putrefactions were found the major contributors in microbial concentration in ambient environment. **(Pathak and Verma, 2009)**. In the case of Gram-Positive Bacteria(GPB), bioaerosol concentration was higher in the 1st year (2013-14) than the 2nd year (2014-15) in post monsoon, winter and summer season respectively. However, there was an exception of high concentration of GPB in the 2nd year (2014-15) than 1st year (2013-14) in the monsoon season. Similarly, the Gram-Negative bacteria(GNB) bioaerosol concentration was higher in the 1st year (2013-14) than the 2nd year (2014-15) in post monsoon, winter and summer season respectively but there was an exception of high GNB bioaerosol concentration in the monsoon season of the 2nd year (2014-15) than 1st year (2013-14).

3.4 Size Segregations of Fungi over two years:

The stage wise size segregation of fungal bioaerosol has been shown in figure 9(a) to figure 9(h) of all the six sites. The fungal bioaerosol follows a typical unimodal distribution of bioaerosol in the atmosphere that means the concentrations of fungal bioaerosol is increasing respectively from stage 1 to stage 4 and then decreasing from stage 4 to stage 6. This shows the fungal bioaerosol concentration has its highest concentration at stage 4. This also shows that in the atmosphere the maximum viable fungal bioaerosol concentration presents in the size ranges of 2.1-3.3µ which resembles the size of stage 4 in the viable cascade impactor, and which is also resembles the secondary bronchi of the human respiratory system. In the 1st year (2013-14), as seen in Figure 9(a) which shows the fungal bioaerosol concentration of the post monsoon season. In post-monsoon, the fungal bioaerosol has a high concentration at stage 4 at Hazrat Nizamuddin site followed by JNU, Okhla, ITO, Uttam Nagar, and Connaught Place sites except for Okhla site which also has a high concentration at stage 2 due to the presence of larger bioaerosol particle in the form of a cluster. Figure 9(b) shows the size segregations of fungal bioaerosol in the winter season, In Winter season, the fungal bioaerosol has a high concentration at stage 4 at JNU site followed by Hazrat Nizamuddin, Connaught Place, Okhla, ITO, and Uttam Nagar site. Figure 9(c) shows the size segregations of fungal bioaerosol in the summer season, In Summer season, Uttam Nagar site has the high fungal bioaerosol concentration followed by the ITO, Connaught Place, Hazrat Nizamuddin, JNU, and Okhla sites. Figure 9(d) shows the size segregations of fungal bioaerosol in the monsoon season, In Monsoon season, the fungal bioaerosol has a high concentration at stage 4 at ITO site followed by the Hazrat Nizamuddin, Uttam Nagar, Connaught Place, JNU, and Okhla sites.

In the 2nd year(2014-15), as seen in Figure 9(e) which shows the fungal bioaerosol concentration of the post monsoon season. In Post monsoon season, stage 4 has the highest concentration of fungal bioaerosol and Hazrat Nizamuddin site has the high concentration followed by Okhla, JNU, Uttam Nagar, ITO, and Connaught Place sites. Figure 9(f) shows the size segregations of fungal bioaerosol in the winter season, In Winter season the fungal bioaerosol concentration has a maximum at stage 4 at Hazrat Nizamuddin site followed by Connaught Place, Uttam Nagar, Okhla, JNU, and ITO sites. Figure 9(g) shows the size segregations of fungal bioaerosol in the summer season, In Summer season, Hazrat Nizamuddin has the highest fungal bioaerosol concentration at stage 4 followed by Okhla, Uttam Nagar, JNU, Connaught Place, and ITO sites.

Figure 9(h) shows the size segregations of fungal bioaerosol in the monsoon season, In Monsoon season, ITO site has the maximum fungal bioaerosol concentration at stage 4 followed by other sites Hazrat Nizamuddin, Okhla, Uttam Nagar, Connaught Place, and JNU sites.

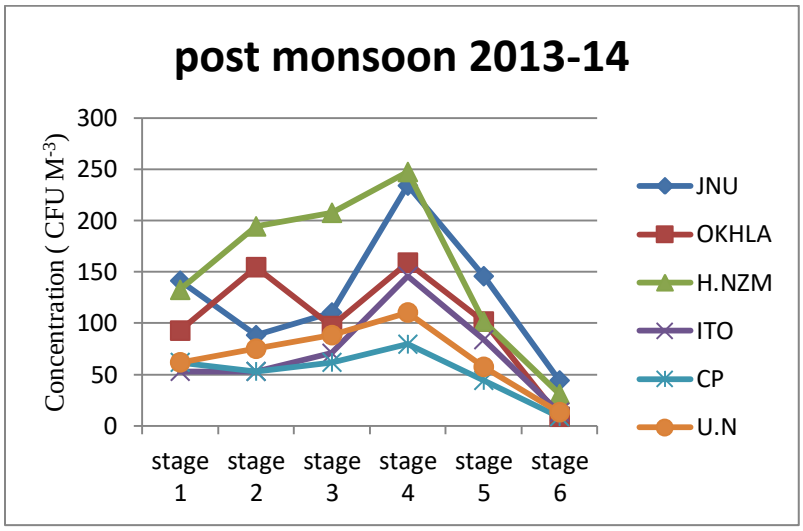

Figure 9(a): Size segregation of fungal bioaerosol in Post Monsoon season of 1st year (2013-14)

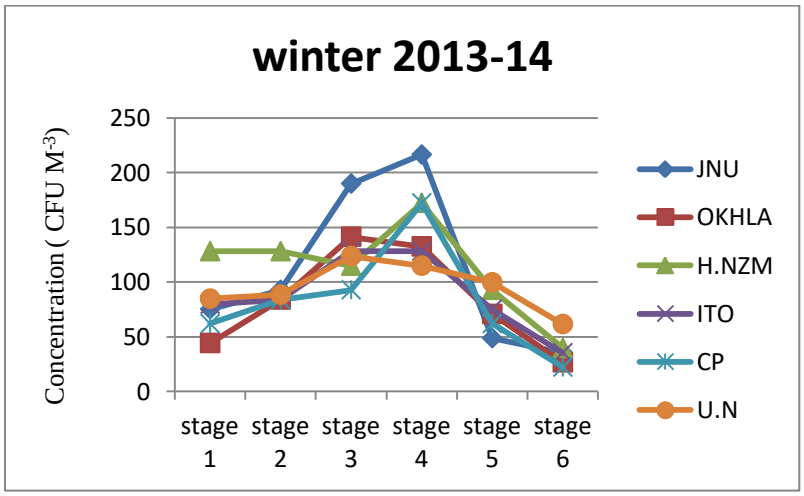

Figure 9(b): Size segregation of fungal bioaerosol in Winter season of 1st year (2013-14)

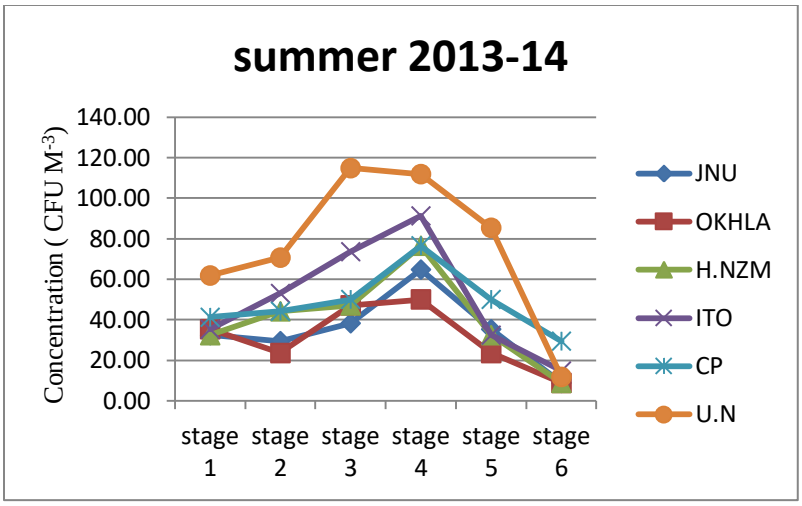

Figure 9(c): Size segregation of fungal bioaerosol in Summer season of 1st year (2013-14)

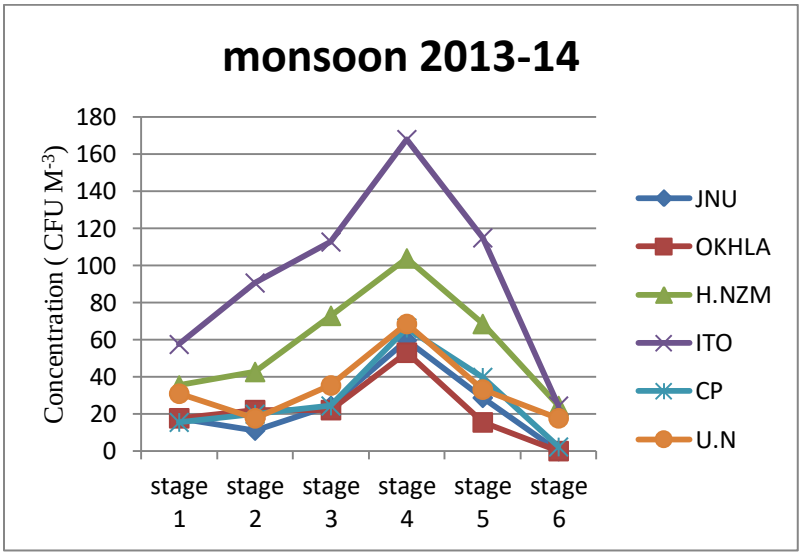

Figure 9(d): Size segregation of fungal bioaerosol in Monsoon season of 1st year (2013-14)

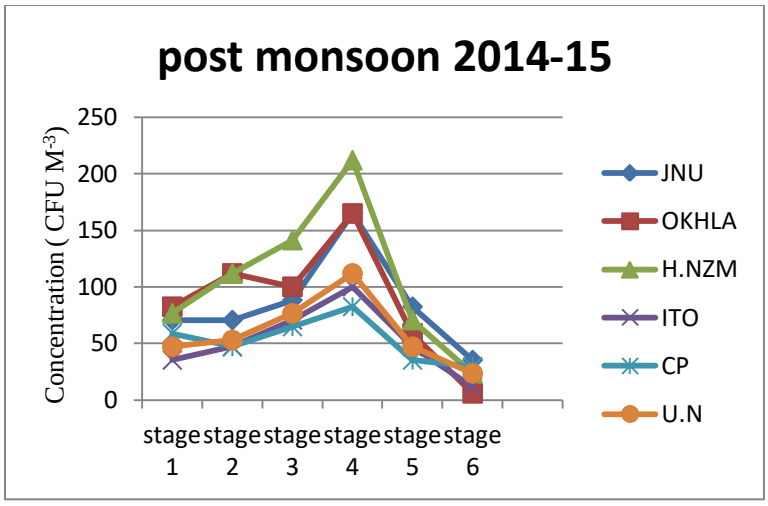

Figure 9(e): Size segregation of fungal bioaerosol in Post Monsoon season of 2nd year (2014-15)

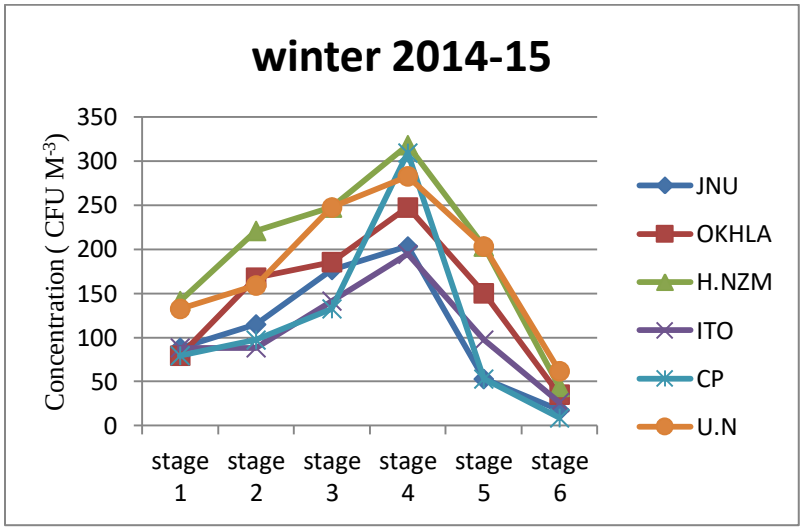

Figure 9(f): Size segregation of fungal bioaerosol in Winter season of 2nd year (2014-15)

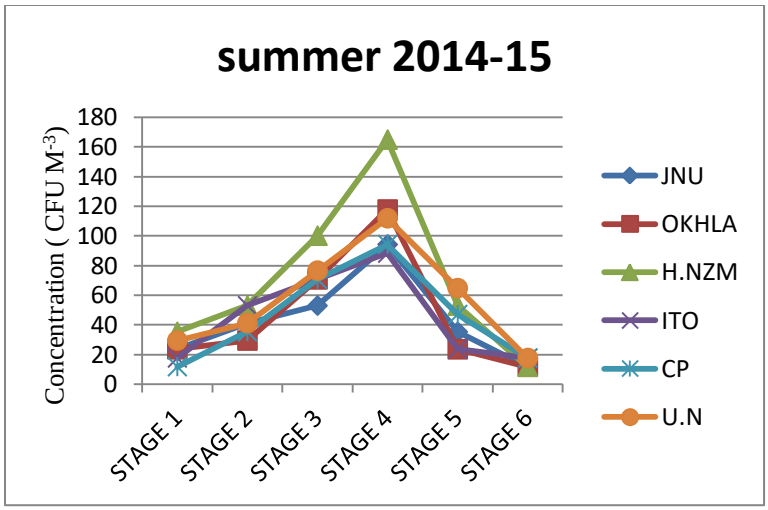

Figure 9(g): Size segregation of fungal bioaerosol in Summer season of 2nd year (2014-15)

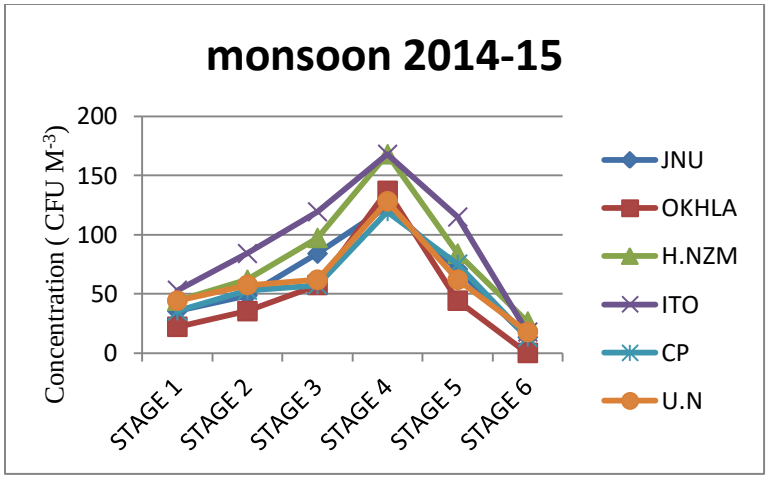

Figure 9(h): Size segregation of fungal bioaerosol in Monsoon season of 2nd year (2014-15)

3.5 Size Segregations of Gram-Positive Bacteria (GPB) over two years:

The size segregations of Gram-Positive Bacteria (GPB) are shown in all the seasons of 1st year (2013-14) and 2nd year (2014-15) from figure 10(a) to figure 10(h). The gram-positive bacteria bioaerosol does not follow a typical pattern of distribution of bioaerosol in the ambient environment. The gram-positive bacteria bioaerosol has shown the irregular pattern of distribution in the atmosphere due to the presence of mainly in the form of the cluster at all sizes. This may be the reason that bacteria bioaerosol found at all sizes. The dominance of Gram-positive bacteria in the air including Staphylococcus and Micrococcus has been reported in classrooms (Liu et al., 2000), residential rooms (Pastuszka et al., 2000; Simard et al., 1983) and health care facilities (Sarica et al., 2002; Shintani et al., 2004). In this study, Gram-positive bacteria were dominant. Since mostly GPB bioaerosol are constituents of the normal human skin flora (CEC, 1993), it was likely that the airborne bacteria in the concourse originated from pedestrians' skin flora. In the 1st year (2013-14), the size segregation of GPB bioaerosol has a high concentration in summer season due to the presence of warm windy weather followed by the winter season, post monsoon season, and then monsoon season. However, in the 2nd year (2014-15) the GPB bioaerosol has a high concentration in winter season due to agglomeration of a larger particle in the atmosphere followed by summer season, post monsoon season, and then monsoon season.

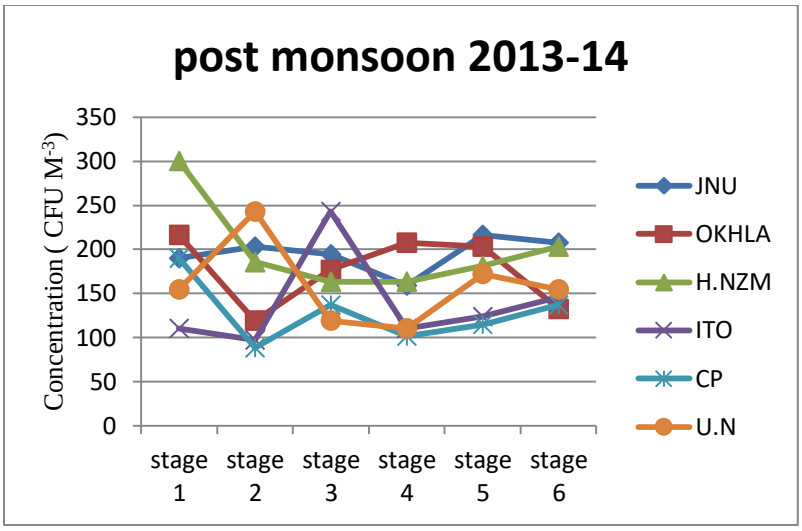

Figure 10(a): Size segregation of GPB bioaerosol in Post Monsoon season of 1st year (2013-14)

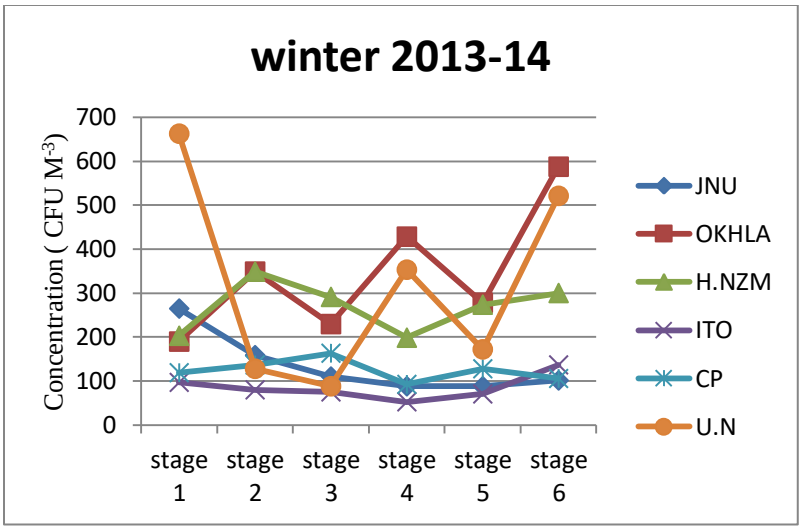

Figure 10(b): Size segregation of GPB bioaerosol in Winter season of 1st year (2013-14)

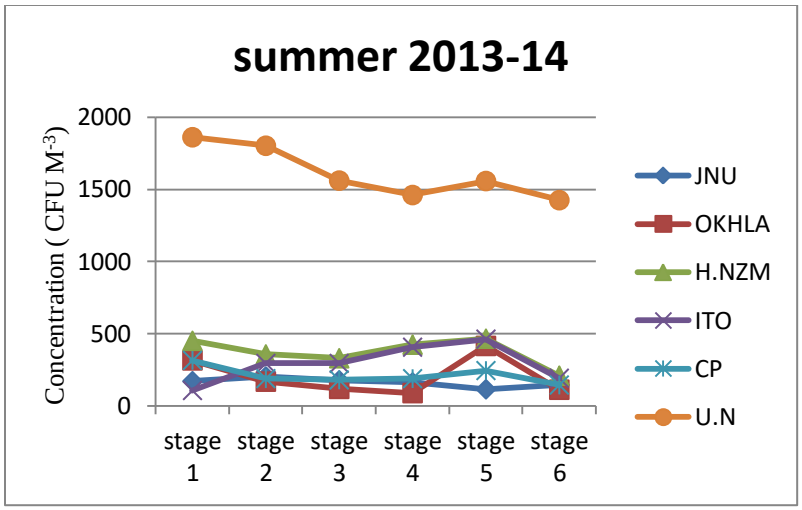

Figure 10(c): Size segregation of GPB bioaerosol in Summer season of 1st year (2013-14)

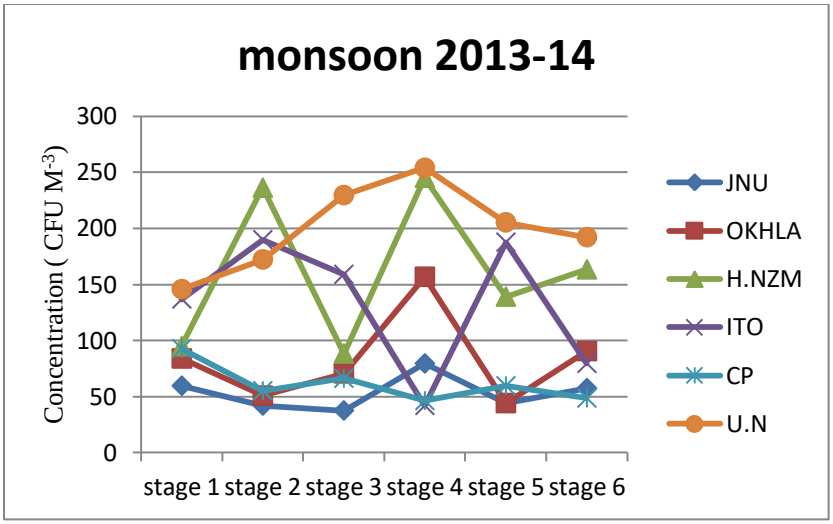

Figure 10(d): Size segregation of GPB bioaerosol in Monsoon season of 1st year (2013-14)

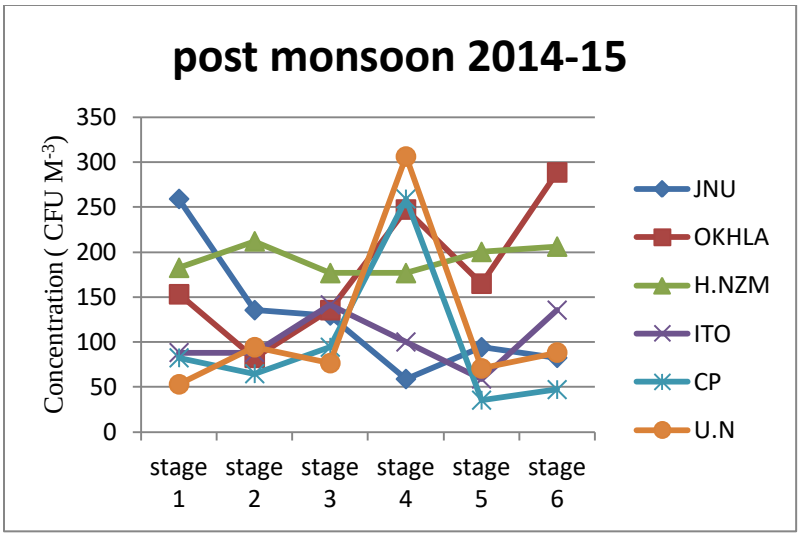

Figure 10(e): Size segregation of GPB bioaerosol in Post Monsoon season of 2nd year (2014-15)

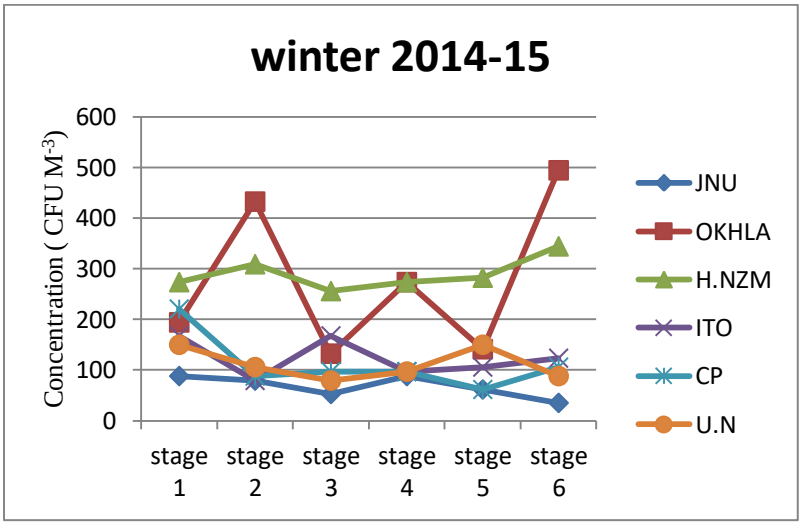

Figure 10(f): Size segregation of GPB bioaerosol in Winter season of 2nd year (2014-15)

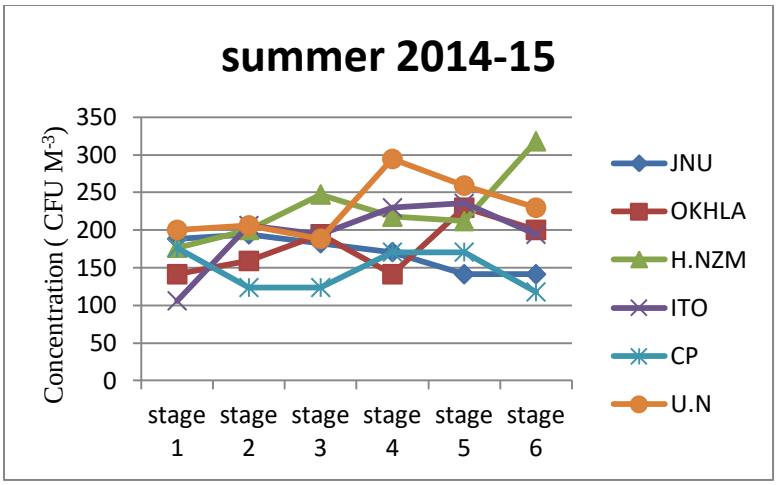

Figure 10(g): Size segregation of GPB bioaerosol in Summer season of 2nd year (2014-15)

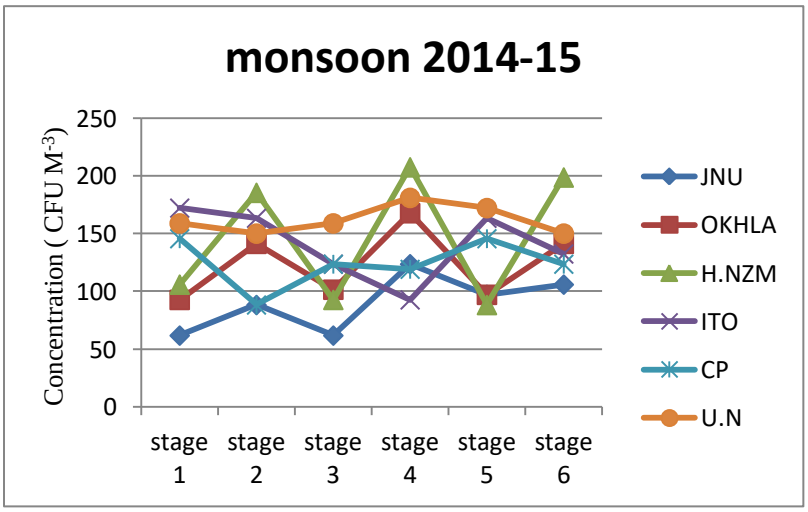

Figure 10(h): Size segregation of GPB bioaerosol in Monsoon season of 2nd year (2014-15)

3.6 Size Segregations of Gram-Negative Bacteria (GNB) over two years:

The size segregations of Gram-Negative Bacteria (GNB) are shown in all the seasons of 1st year (2013-14) and 2nd year (2014-15) from figure 11(a) to figure 11(h). Like GPB, GNB bioaerosol also does not follow a typical pattern of distribution of bioaerosol in the ambient environment. The gram-negative bacteria bioaerosol also showed the irregular pattern of distribution in the atmosphere due to the presence of mainly in the form of the cluster at all sizes. This may be the reason that bacteria bioaerosol found at all sizes. The GNB bioaerosol also reporting in the indoor air of industrial factories (Dutkiewicz et al., 2001), swine house (Bilic et al., 2000; Martin et al., 1996) and animal sheds (Andersson et al., 1999). In the 1st year (2013-14), the GNB bioaerosol has shown high concentration in summer season due to the presence of warm windy weather followed by post-monsoon season, winter season, and monsoon season. However, in the 2nd year (2014-15) the GNB bioaerosol concentration has shown high concentration in monsoon season followed by summer season, winter season, and then post monsoon season.

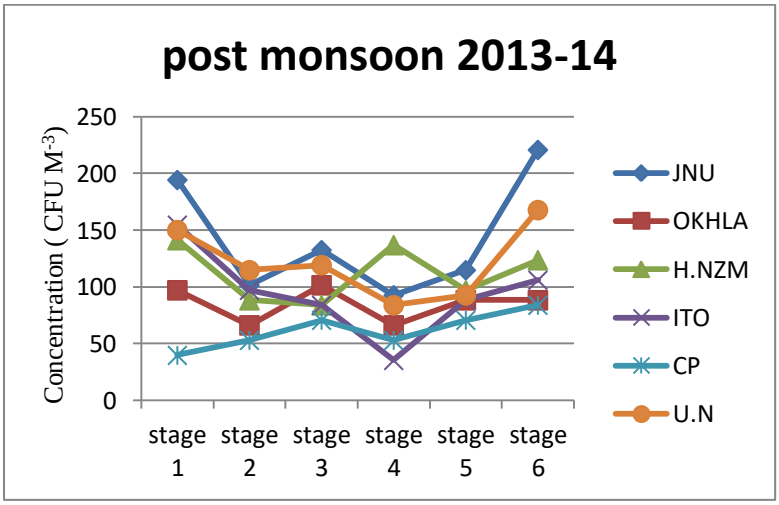

Figure 11(a): Size segregation of GNB bioaerosol in Post Monsoon season of 1st year (2013-14)

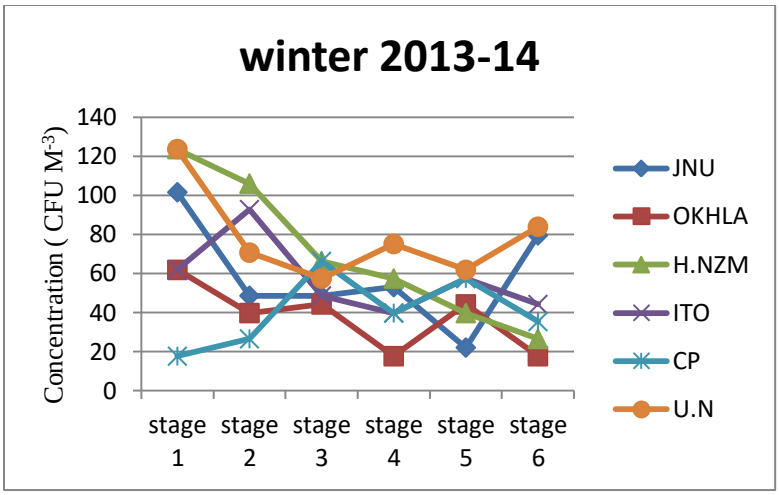

Figure 11(b): Size segregation of GNB bioaerosol in Winter season of 1st year (2013-14)

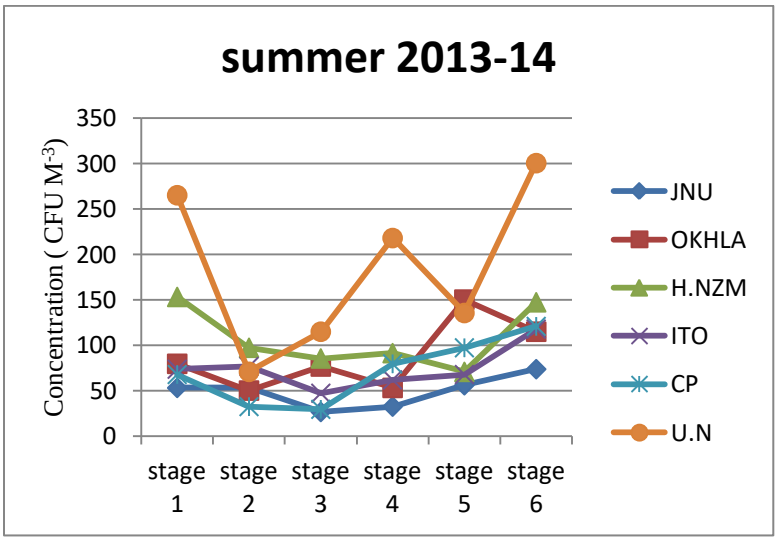

Figure 11(c): Size segregation of GNB bioaerosol in Summer season of 1st year (2013-14)

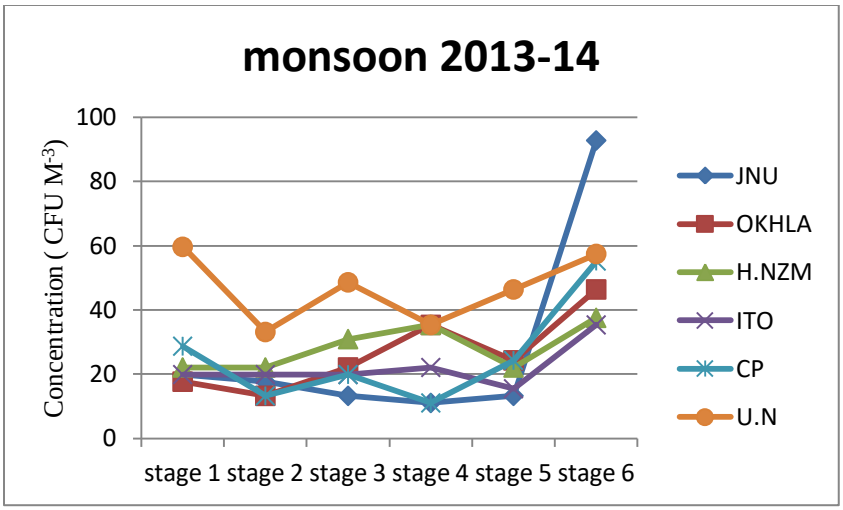

Figure 11(d): Size segregation of GNB bioaerosol in Monsoon season of 1st year (2013-14)

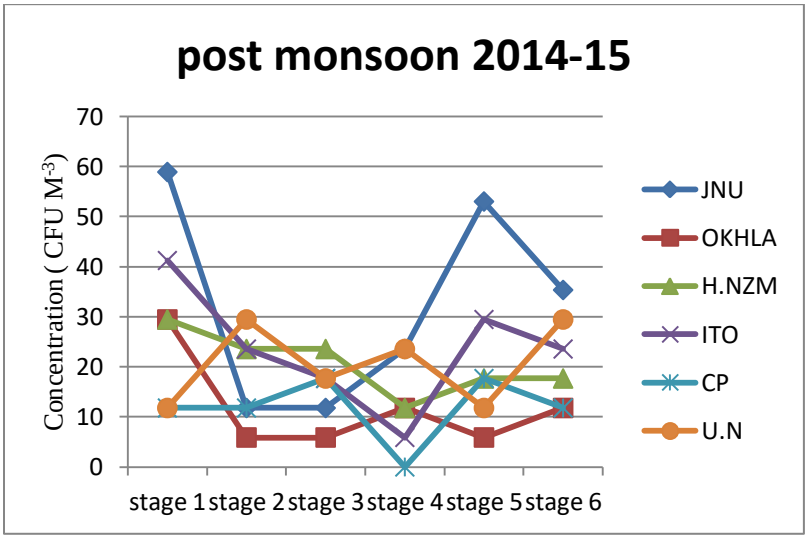

Figure 11(e): Size segregation of GNB bioaerosol in Post Monsoon season of 2nd year (2014-15)

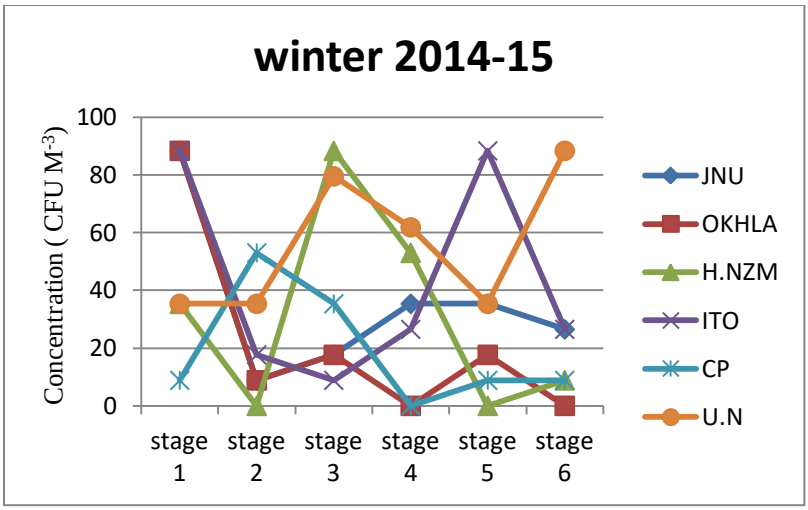

Figure 11(f): Size segregation of GNB bioaerosol in Winter season of 2nd year (2014-15)

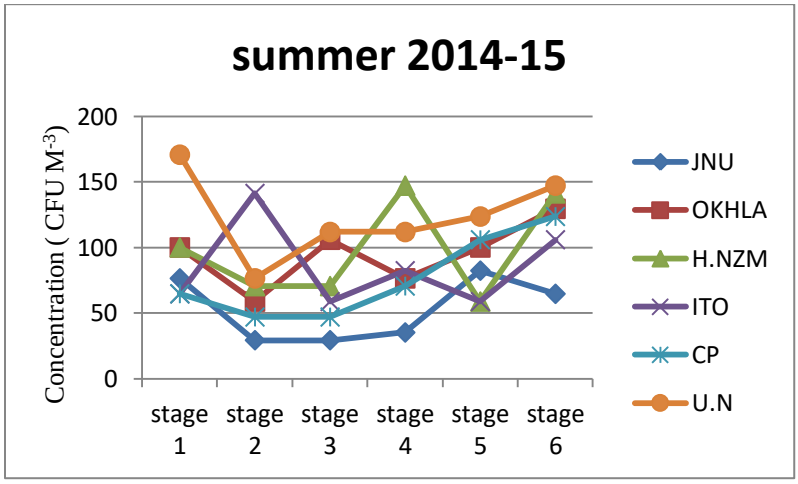

Figure 11(g): Size segregation of GNB bioaerosol in summer season of 2nd year (2014-15)

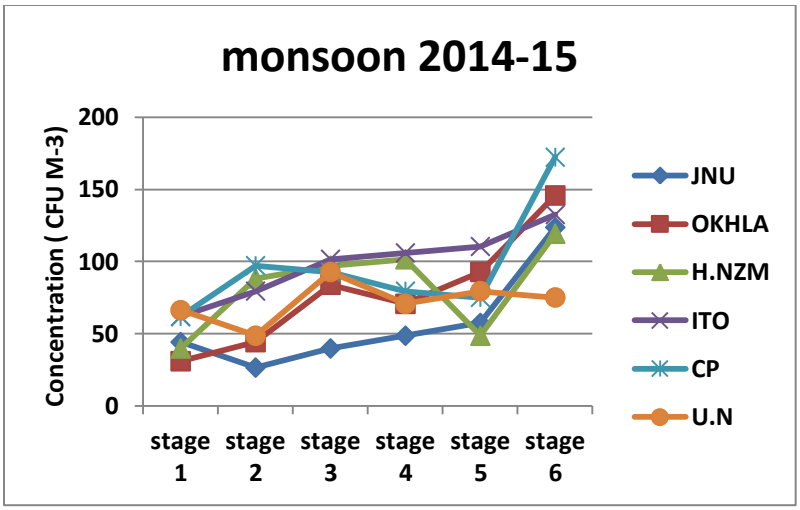

Figure 11(h): Size segregation of GNB bioaerosol in Monsoon season of 2nd year (2014-15)

3.7 Identification of Fungal bioaerosols

In total 9 fungal species were identified and they are tabulated in Table 3 with their species name. It is important to mention that all these species were present at all the sites. Out of them three are immunotoxic, three are allergic while three are harmless.

Table 3: Table of characterized fungal bioaerosol.

Sampling Sites / Types Of Fungi	JNU	Okhla	Hazrat Nizamuddin	Connaught Place	ITO	Uttam Nagar
Aspergillus ≠	+	+	+	+	+	+
Fusarium ×	+	-	+	+	+	+
Dreshelia ≠	+	-	-	+	-	+
Ulocladium ˣ	+	-	+	-	+	-
Curvularia ≠	+	+	+	+	+	+
Penicillium ˣ	+	+	+	+	+	+
Alternaria ×	+	+	+	+	+	+
Mucor ˣ	+	+	+	+	+	+
Rhizopus ×	+	+	+	+	+	+

× IMMUNOTOXIC ˣHARMLESS ≠ ALLERGIC

3.8 Linear and multiple regression for bioaerosol concentration:

Linear regression and multiple regression analyses were carried out between environmental parameters (temperature and RH) and bioaerosol concentration (fungus and bacteria). Here, R represents coefficient of correlation.

In case of fungal concentration, from the close study of the results shown in table 4, it was evident that both RH and temperature had a weak correlation with fungi ($R= 0.352$, $p< 0.05$; $R= 0.444$, $p< 0.05$). Though not much, yet, a substantial improvement could be seen on combination of both the regressors ($R= 0.457$, $p< 0.05$). Although weakly correlated yet, the linear regressions studies were found to be significant ($p< 0.05$) for fungi.

In case of GPB concentration, it was evident that both RH and temperature had a weak correlation alone as well as in combination. The correlation of temperature ($R= 0.192$, $p< 0.05$) was found to be almost double in comparison to that of RH ($R= 0.052$, $p> 0.05$) and also increased when combined ($R= 0.283$, $p < 0.05$). However, apart from RH the remaining two linear regression studies were found to be significant.

Similarly in case of GNB, both RH and temperature had a weak correlation alone as well as in combination. The correlation of RH ($R= 0.270$, $p< 0.05$) was found to be almost double in comparison to that of temperature ($R= 0.102$, $p> 0.05$) and also increased when combined ($R= 0.280$, $p < 0.05$). However, apart from temperature the remaining two linear regression studies were found to be significant.

Table 4: Statistical results of linear regression for fungal and bacterial concentration

	Regression		
Parameters	**Fungi R(p)**	**GPB R(p)**	**GNB R(p)**
Temperature	0.444(0.000)	0.192(0.024)	0.102(0.236)
R.H	0.352(0.000)	0.052(0.544)	0.270(0.001)
Temperature and R.H	0.457(0.000)	0.283(0.004)	0.280(0.004)

Chapter 4

Conclusions

From the present study the following important conclusions can be drawn:

1. In the 1st year (2013-14), the fungal bioaerosol concentration ranged from (914.31-130.30 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at Okhla site. Similarly, in the 2nd year (2014-15), the fungal bioaerosol concentration ranged from (1174.91- 259.13 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at JNU site. This shows that in both the year, Hazrat Nizamuddin site shows maximum fungal bioaerosol due to the presence of more vegetation in that area.

2. In the 1st year (2013-14), the Gram-Negative bacterial bioaerosol concentration ranged from (1104.24-132.49 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at ITO site. In the 2nd year (2014-15), the Gram-Negative bacterial bioaerosol concentration ranged from (1107.18-70.67 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at Okhla and Connaught Place site. This shows that in both the year, Uttam Nagar site has the maximum GNB bioaerosol concentration due to the presence of Uttam Nagar Metro Station, Uttam Nagar Bus Terminal as well as the presence of market in the surroundings of the sampling area.

3. In the 1st year (2013-14), the Gram-Positive bacterial bioaerosol concentration ranged from (9667.26-320.24 CFU M^{-3}) with the maximum at Uttam Nagar site while the minimum at JNU site. In the 2nd year (2014-15), the Gram-Positive bacterial bioaerosol concentration ranged from (1740.28-406.36 CFU M^{-3}) with the maximum at Hazrat Nizamuddin site while the minimum at JNU site. The high GPB concentration at Uttam Nagar in 1st year (2013-14) and Hazrat Nizamuddin site in the 2nd year (2014-15) shows that the both sites are the crowded sites with movements of pedestrians are uncountable at daytime and the human are the most suitable source of bacterial bioaerosol as they move, they transport bacterial bioaerosol in the ambient environment.

4. The concentration of total bioaerosol is high in the summer season of 1st year (2013-14) due to presence of warm windy weather which help in creating the stressed environment for the growth of bioaerosol and it helps in sporulation of bioaerosol and it is easier for bioaerosol to get carried away in that environment while in the 2nd year (2014-15), the concentration of total bioaerosol was high in winter season at two sites namely, Okhla and Hazrat Nizamuddin sites due to presence of favorable temperature for the growth of bioaerosol as well as in winter season the relative humidity is also high than in summer season.

5. As far as the all the three fractions of bioaerosol considered, the GPB bioaerosol concentration remained high throughout the both the sampling year followed by GNB and Fungal bioaerosol concentration.

6. Concentration of fungal bioaerosol found in different stages at each site seems to follow a typical pattern in all three season with highest concentration at stage 4, whose diameter ranges from 2.1 µm to 3.3 µm (synonym to the secondary bronchi of the lungs in human body) and lowest concentration at stage 6 with diameter ranging from 0.6 µm to 1.1 µm. Hence, the majority of the immunotoxic and allergic fungi found at this stage are mostly prone to affect the secondary bronchi in human lungs when inhaled.

7. Unlike fungi, a gram-positive bacteria seems to follow no pattern at all. The gram-positive bacteria bioaerosol has shown the irregular pattern of distribution in the atmosphere due to the presence of mainly in the form of the cluster at all sizes. This may be the reason that bacteria bioaerosol found at all sizes. Maximum concentration found in stage 2, 3, 4 and 6 reveals the fact that pharynx, trachea, primary and secondary bronchi are mostly affected by gram-positive bacteria.
8. Like gram positive bacteria, gram negative bacterial concentration too does not follow any typical pattern. Since all the three stages are in synonym to the tracheal region with primary bronchi and secondary bronchi, thus high concentration at these stages reveals that the above mention parts of lungs are more prone area to be infected by gram-negative bacteria.

9. The combined effect of both Temperature and R.H was found to be significant ($p < 0.05$) with both the factors are responsible to play an important role in the emission of bioaerosol in the atmosphere.
10. Out of nine fungal genera identified *Rhizopus, Aspergillus* and *Penicillium* were found in abundance at all sites. 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 also found in abundance.

References

ACGIH (1999). Particle Size-Selective Sampling for Health-Related Aerosols (Vincent JH, editor). American Conference of Governmental Industrial Hygienists, Air Sampling Procedures Committee, Cincinnati, OH, USA. ISBN 1-1882417-30-5.

Andersson, A. M., Weiss, N., Rainey, F., & Salkinoja-Salonen, M. S. (1999). Dust-borne bacteria in animal sheds, schools and children's day care centres. *Journal of applied microbiology*, *86*(4), 622-634.

Bilić, V., Habrun, B., Barač, I., & Humski, A. (2000). Distribution of airborne bacteria in swine housing facilities and their immediate environment. *Arhiv za higijenu rada i toksikologiju*, *51*(2), 199-205.

Bovallius, A., Bucht, B., Roffey, R., and Anäs, P. (1978). Three-year investigation of the natural airborne bacterial flora at four localities in Sweden. *Applied and Environmental Microbiology*, 35(5), 847-852.

CEN (1993). Workplace Atmospheres - Size Fraction Definitions for Measurement of Airborne Particles. European Standard EN 481, European Standardization Committee (CEN), Brussels.

Chen, H., Goldberg, M. S., Villeneuve, P. J., (2008). "A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases". Reviews on environmental health 23 (4): 243–97.

Commission of the European communities, (CEC), 1993. Indoor air quality and its impact on man. Report No.12 Biological particles in indoor environment. Luxembourg.

D'amato, G., Stanziola, A. A., Cocco, G., & Melillo, G. (1984). Mold allergy: a three year investigation (1980-1982) of the airborne fungal spores in Naples, Italy. *Annals of allergy*, *52*(5), 363-367.

Dutkiewicz, J., Krysińska-Traczyk, E., Prazmo, Z., Skórska, C., & Sitkowska, J. (2000). Exposure to airborne microorganisms in Polish sawmills. *Annals of agricultural and environmental medicine: AAEM*, *8*(1), 71-80.

Fang, Z. G., Ouyang, Z. Y., Hu, L. F., Lin, X. Q., and Wang, X. K. (2004). [Granularity distribution of airborne microbes in summer in Beijing]. *Huan jing ke xue Huanjing kexue/[bian ji, Zhongguo ke xue yuan huan jing ke xue wei yuan hui" Huan jing ke xue" bian ji wei yuan hui.]*, *25*(6), 1-5.

Ghosh, B., Lal, H., and Srivastava, A. (2015). Review of bioaerosols in indoor environment with special reference to sampling, analysis and control mechanisms. *Environment international*, 85, 254-272.

Gregory, P.H. (1973). The Microbiology of the Atmosphere. 2nd ed. Leonard Hall.

Hassan, M. S., and Lau, R. W. M. (2009). Effect of particle shape on dry particle inhalation: study of flowability, aerosolization, and deposition properties. *AAPS PharmSciTech*, 10(4), 1252-1262.

Ho, J., and Duncan, S. (2005). Estimating aerosol hazards from an anthrax letter. *Journal of Aerosol Science*, 36(5), 701-719.

Huffman, J. A., Treutlein, B., and Pöschl, U. (2010). Fluorescent biological aerosol particle concentrations and size distributions measured with an Ultraviolet Aerodynamic Particle Sizer (UV-APS) in Central Europe. *Atmospheric Chemistry and Physics*, 10(7), 3215-3233.

ICRP (1994). Human respiratory tract model for radiological protection. International Commission on Radiological Protection Publication 66. Annals of the ICRP 24(1-3). Elsevier Science Ltd., Oxford, UK.

ISO (1995). Air Quality - Particle Size Fraction Definitions for Health-related Sampling. ISO Standard 7708. International Organization for Standardization (ISO), Geneva.

Jaenicke, R., Matthias-Maser, S., and Gruber, S. (2007). Omnipresence of biological material in the atmosphere. *Environmental Chemistry*, 4(4), 217-220.

LalI, H., PuniaII, T., GhoshI, B., SrivastavaI, A., & JainI, V. K. (2013). Comparative study of bioaerosol during monsoon and post-monsoon seasons at four sensitive sites in Delhi region. *International Journal of Advancement in Earth and Environmental Sciences*, *1*(2), 1-7.

Lave, L., and Seskin, E.(1978). Air Pollution and human health. Baltimore.

Li, M., Qi, J., Zhang, H., Huang, S., Li, L., and Gao, D. (2011). Concentration and size distribution of bioaerosols in an outdoor environment in the Qingdao coastal region. *Science of the Total Environment*, 409(19), 3812-3819.

Lighthart, B., and Shaffer, B. T. (1994). Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmospheric Environment*, 28(7), 1267-1274.

Lin, W. H., & Li, C. S. (2000). Associations of fungal aerosols, air pollutants, and meteorological factors. *Aerosol Science & Technology*, *32*(4), 359-368.

Liu, L. J. S., Krahmer, M., Fox, A., Feigley, C. E., Featherstone, A., Saraf, A., & Larsson, L. (2000). Investigation of the concentration of bacteria and their cell envelope components

in indoor air in two elementary schools. *Journal of the Air & Waste Management Association*, *50*(11), 1957-1967.

Liu, M. M., Qi, J. H., Gao, D. M., Qiao, J. J., Shi, J. H., and Gao, H. W. (2008). Distribution characteristics of bioaerosol in Qingdao coastal region in Fall 2007. *Ecology and Environment*, 7(2), 565-571.

Martin, W. T., Zhang, Y., Willson, P., Archer, T. P., Kinahan, C., & Barber, E. M. (1996). Bacterial and fungal flora of dust deposits in a pig building. *Occupational and Environmental Medicine*, *53*(7), 484-487.

Pastuszka, J. S., Paw, U. K. T., Lis, D. O., Wlazło, A., & Ulfig, K. (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmospheric Environment*, *34*(22), 3833-3842.

Pathak, A. K., & Verma, K. S. (2010). Aero-bacteriological study of vegetables market at Jabalpur. Iran. J. Environ. Health. Sci. Eng., 6: 187-194.

Pöschl, U. (2005). Atmospheric aerosols: composition, transformation, climate and health effects. *Angewandte Chemie International Edition*, 44(46), 7520-7540.

Rao, C. Y., Burge, H. A., & Chang, J. C. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association*, *46*(9), 899-908.

Sarica, S., Asan, A., Otkun, M. T., & Ture, M. (2002). Monitoring indoor airborne fungi and bacteria in the different areas of Trakya University Hospital, Edirne, Turkey. *Indoor and built Environment*, *11*(5), 285-292.

Shintani, H., Taniai, E., Miki, A., Kurosu, S., & Hayashi, F. (2004). Comparison of the collecting efficiency of microbiological air samplers. *Journal of Hospital Infection*, *56*(1), 42-48.

Simard, C., Trudel, M., Paquette, G., & Payment, P. (1983). Microbial investigation of the air in an apartment building. *Journal of hygiene*, *91*(02), 277-286.

Srikanth, P., Sudharsanam, S., and Steinberg, R. (2008). Bio-aerosols in indoor environment: Composition, health effects and analysis. *Indian journal of medical microbiology*, 26(4), 302.

Stanley, R.G. and Linskins, H.F. (1974). Pollen: biology, chemistry and management. Springer Verlag, Berlin.

Stetzenbach, L. D., Buttner, M. P., and Cruz, P. (2004). Detection and enumeration of airborne biocontaminants. *Current opinion in biotechnology*, 15(3), 170-174.

Taylor, E.J. (1988). Dorland's Medical Dictionary. W.B. Saunders Co., Philadelphia.

Thompson, W.A.R. (1981). Black's Medical Dictionary. 33rd ed. Adam and Charles Black.

US Environmental Protection Agency. (2004). Air quality criteria for particulate matter. *National Center for Environmental Assessment-RTP Office*

WHO 2014. http: //www.who.int/mediacentre/news/releases/2014air-pollution/en/

Xu, W. B., Qi, J. H., Jin, C., Gao, D. M., Li, M. F., Li, L., ... and Zhang, H. D. (2011). Concentration distribution of bioaerosol in summer and autumn in the Qingdao coastal region. *Environmental Science*, 32(1), 9-17.