IDENTIFICATION AND CHARACTERIZATION OF SIZE-SEGREGATED BIOAEROSOLS AT DIFFERENT SITES IN DELHI

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CERTIFICATE

This is to certify that the research work embodied in the dissertation entitled **"Identification and characterization of size-segregated bioaerosols at different sites in Delhi"** has been carried out in this school for the partial fulfillment of the award of the degree of Master of Philosophy. This work is Original and has not been submitted in part or in full, for any other degree or diploma of the university.

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CHAPTER:1

INTRODUCTION

lation is responsible for quick urbanization and industrialization. This increasing rialization has been creating many problems, especially different types of ıent. Bioaerosols is one of the pollutants which have received attention in recent :h mould contamination, threat of bioterrorism and associated health effects [1].

as airborne particles consisting of living organisms such as microorganisms or rganisms, such as metabolites, toxins or fragments of microorganisms. Bacterial nents, fungal spores and by-products of microbial metabolism, present as ıtile organic compounds may be components of bioaerosols [2]. Bioaerosols can ɔess that involves biological materials and generates enough energy to separate arger substances, such as wind, water, air, or mechanical movement. Plants, soil, ıding humans) all serve as sources of bioaerosols, that is why bioaerosols are ıces where any of these sources live. Given that these sources are ubiquitous in bioaerosols are just about everywhere!

number of microorganisms, acting as a medium for their transmission or gestion and dermal contact are the routes of human exposure to airborne ɔn being the predominant. The particles in a bioaerosol are generally 0.3 to 100 r, the respirable size fraction of 1 to 10 μm is of primary concern [3]. Bio- ɔetween 1.0 to 5.0 μm generally remain in the air, whereas larger particles are ıces [4].

microorganisms are found to be associated with suitable range of suspended The US Environmental Protection Agency (EPA) has defined four terms for different sizes according their diameter ultrafine <0.1 μm, fine 0.1 -2.5 μm, percoarse > 10 μm (EPA 2002). The shape and density of the particle affect its nd thus the aerodynamic diameter characterizes the filtration, respiratory of the particle [5]. Instead of using the terms "fine" and "coarse" particles, the ıcic" and "respiratory" particles are used in occupational hygiene. Particles of the respiratory system, with the particle size serving as a principal factor in ɔn location. Relative to deposition concerns, ISO [1995] uses the following s:

(1) Inhalable fraction – the mass fraction of total airborne particles that is inhaled through the nose and mouth.

(2) Thoracic fraction – the mass fraction of inhaled particles penetrating beyond the larynx.

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

The 50% cut-off diameter for the thoracic fraction is 10 μm and for the respirable fraction it is 4 μm (CEN 1993). The inhalable fraction of total airborne mass fraction consists of particles that are inhaled through the nose and mouth. Particles greater than approximately 50 μm in diameter can enter the nose and mouth, and particles >10 μm are deposited on the ventilation pathway surfaces above the trachea. Fine particles gain entry to the alveolar region of the lungs [6]. The concentrations and size distribution of trace metals are governed by the nature of emission to the atmospheric, as well as by rates of wet and dry deposition, cloud processing exchange of air between the boundary layer and free troposphere, and chemical transformations [7]. The distribution of trace metals within atmospheric particles over, continental Europe has been studied by many investigators [7].

Exposures to bioaerosols, unlike exposure to chemicals, do not have threshold limits to assess health impact/toxic effects, due to the complexity in their entity. Variations in human response to their exposure and difficulties in recovering microorganisms that can pose hazard during routine sampling is also a major problem. The role in various industrial settings has been well studied [1], but at the same time the role of these airborne microorganisms in healthcare settings is poorly understood. Increasing incidences of nosocomial and occupational diseases due to bioaerosol exposure [2,8,9] indicate the need for a thorough knowledge in this respect.

Research on bioaerosols has mostly focused on their detections and enumerations related to public health hazard. Several methods for identification and characterization have been developed in recent years. Bioaerosol monitoring includes the measurement of viable (culturable and nonculturable) and nonviable microorganisms in both indoor (e.g., industrial, office or residential) and outdoor (e.g., agricultural and general air quality) environments. In general, indoor bioaerosol sampling need not to be performed if visible growth is observed. Monitoring of bioaerosols in the occupational environment is one of the many tools, the industrial hygienists use in the assessment of indoor environmental quality, infectious disease outbreaks, agricultural health, and clean rooms [10].

Several papers on the topic of bioaerosols have been concentrating upon bioaerosols sampling and assay methods. Some relates to laboratory evaluations of such methodologies, whilst other describes application

in the field. One of the major problems associated with sampling and characterizing bioaerosol is in defining what the bioaerosol is and what it looks like in air. The most common methods of sampling are based on the impaction, either in the single or multistage (cascade) form. As for as bioaerosol detection is concerned, five different method are adopted (i) growth techniques (culturable number). (ii) Microscopy (total number and shape), (iii) rapid biochemical techniques, (iv) immunological techniques, and (v) molecular biological techniques [11].

Bioaerosols can be analysed by scanning electron microscopy, but relatively little, mostly occasional, works have been reported on the characterization of bioaerosol by electron microscopy. To our knowledge, a comprehensive overview on the shape and morphology of bioaerosols does not exist till date. Recently, a premier research centre for environment and health, GSF, revealed the merits of the Scanning Electron Microscopy for explaining impaction and regrowth processes which takes place during sampling of aerosol matter in impactors [12].

In the biological defense arena, the standoff detection of biological contamination is desirable because it provides an early warning of possible attack, thereby enabling defensive measure to be taken. The traditional methods included the use of a dichotomous sampler (DS), aerodynamic particle sizer, assay of viable cells, and scanning electron microscopy. The laser cloud mapper (LCM) was tested to determine the lower detectable concentration limit of the system and its mapping capability of the bioaerosol cloud.

Microorganisms and odour emission from composting facilities are regarded as a nuisance, especially for residents living close to such facilities. Composting facilities and waste treatment facilities in general can emit high number of pathogenic and non-pathogenic microorganism of different group and taxa [13]. Studies have been carried out under so called 'normal-case', i.e. typical local climate condition and working activities, and 'real worst-case' condition ('drainage low' condition) being characterized by the translocation of cold air mostly at night, and containing large amount of bioaerosols. Highest concentrations of microorganisms were observed during turning of composting [13].

The presence of undesirable bio-aerosols is often associated with Sick Building Syndrome (SBS) and Building Related Illnesses (BRI). Sources include furnishings and building materials; fungal contamination within wall, ceiling, and door cavities by movement of cells, spores, and cell fragments via wall openings and gaps at structural joints [14]. Lack of fresh air due to increased insulation of buildings, poorly maintained or operated ventilation systems, poorly regulated temperature and relative humidity levels contribute to the presence and multiplication of bioaerosols [15].

Biological hazards to human beings arise from exposure to high concentrations of unfamiliar forms of bio-aerosols and three major groups of diseases associated with bio-aerosol exposure are infectious diseases, respiratory diseases and cancer [1]. Current knowledge is unclear regarding risk to cancer whether these risks occur from exposures to biological agents or are due to various chemicals used in industries [1].

Infectious diseases arise from viruses, bacteria, fungi, protozoa and helminthes, and involve the transmission of an infectious agent from a reservoir to a susceptible host through airborne transmission. Infectious disease may be categorized into bacterial, fungal and viral diseases [16].

Various bacterial diseases such as Legionellosis, Tuberculosis and Anthrax are linked to cause significant public health concern due to their low infectious dose [17]. *Legionella pneumophila* causes human legionellosis and become airborne often as a result of active aerosolizing processes (aeration of contaminated water) and may inhabit various water environments including man-made water systems, often form biofilms in cooling towers and air conditioning systems [18]. The transmission of tubercle bacilli occurs through the inhalation of aerosolized bacilli in droplet nuclei of expectorated sputum-positive tuberculosis patients during coughing, sneezing and talking. Several outbreaks of multidrug resistant tuberculosis in UK have highlighted the potential for transmission within the hospital environment [19]. The transmission of Anthrax occurs due to inhalation of the spores of *Bacillus anthracis* and outbreaks are often linked to bioterrorism that are spread through intentionally contaminated mail, apart from occupational exposures [20].

Airborne fungi causing respiratory infections and allergic reactions include *Penicillium, Aspergillus, Acremonium, Paecilomyces, Mucor* and *Cladosporium* [21]. Most infections, commonest being *Aspergillosis* can occur in immunocompromised hosts or as a secondary infection, following inhalation of fungal spores or the toxins produced by them. Volatile products of fungal metabolism are capable of inducing sensory irritation to eyes and upper respiratory tract [22]. *Aspergillus* species that can grow indoors include *Aspergillus fumigatus* and *Aspergillus flavus* and can cause nosocomial infections [23], allergic broncho-pulmonary Aspergillosis (ABPA) and sinusitis. Chronic asthmatics may progress to have their bronchial passages colonized by Aspergillus *fumigatus*, *Bipolaris hawaiiensis*, or *Wangiella dermatitidis* [24]. Constant allergic response maintains the fungal colonisation, and first-line therapy with steroids, brings down the level of inflammation and may result in elimination of the colonising organism [24].

Viruses readily transmitted by airborne route include, Severe Acute Respiratory Syndrome (SARS) virus [25], enteric viruses of intestinal origin produced at sewage treatment facilities, Respiratory Syncytial Virus (RSV), Hantavirus from rodent faeces [26], varicella - zoster virus, measles, mumps and rubella viruses. SARS, caused by novel corona virus, is a highly contagious and responsible for respiratory illness of significant morbidity and mortality, and may also cause very severe atypical pneumonia [27, 28]. The use of aerosol generating procedures (such as endotracheal intubation, bronchoscopy, and treatment with aerosolized medication) in hospitals may amplify the transmission of SARS [29].

Hypersensitivity pneumonitis or Extrinsic Allergic Alveolitis (EAA) is an inflammatory airway disease caused by an unusual immune response to antigens like fungi (i.e. Farmer's lung), bird excreta (i.e. pigeon breeder's disease), and microbial contaminants in grain dust [30]. Organic Dust Toxic Syndrome (ODTS) occurs within hours of a high dose inhalation of endotoxins, fungal spores and mycotoxins [31], which may lead to Chronic Obstructive Pulmonary disease (COPD) [32]. Bioallergens are potent allergens and include enzymes derived from fungi and bacteria produced by biotechnological companies, and plant pollens [33].

Bioaerosols may also have a significant impact on climate, acting as cloud condensation nuclei and ice nuclei which can initiate cloud formation and precipitation [34].

It is currently thought that the majority of these airborne microorganisms are not in viable state while they stay in atmosphere. However, current researches have shown that certain groups of bacteria are capable of performing basic metabolic activities within cloud water [35]. Group of the small organism clump up and enhance survival while they are airborne. Due to evaporation of water, bacterial cells usually die when they become airborne but under high humidity condition bioaerosol levels are increased. Fungal cells such as spores, mold, yeast can be activated at low humidity level and high or low temperature.

The last 3-4 decades has been characterized by a significant increase of the world wide scientific database on bioaerosols. Inspite of the tremendous scientific progress which has been taking place mainly in developed countries like Europe and USA, the state of knowledge about biologically originated air pollution in many other countries including India seems to be still relatively narrow and insufficient. Air measurement is only carried out by scientific projects and hence their frequency is low. If bioaerosol appear as a center of interest, it is usually related to the occupational matter and to cases of health

complaint. Such a passive attribute towards bioaerosols is increased by lack of internationally recognized criteria for assessing exposure to biological factors [36].

In mid 1990s, first modern complex bioaerosol investigation of dwelling were initiated in Poland. The Bioaerosol Group at the Institute of Occupational Medicine and Environmental Health, Sosnowiec, in co-operation with the Department of Occupational Biohazards at the Institute of Agricultural Medicine, Lubin, as a first research unit initiated comprehensive measurement in dwelling [37]. In many other countries, the presence of elevated level of bioaerosol is still a hidden problem. Limited studies performed on air indicated that microbiological quality of the air could be potential causative agent of health complaint of inhibition.

In India, few works related to bioaerosols have been done. Most of them have concentrated on the effect of pollen triggering allergic reactions. The other is aerobiology, epidemiology and forecasting of fungal diseases found in certain crops in north- eastern India. The current status of airborne pollen grains with special reference to allergic significance has been studied in Kolkata [38]. The pollen and fungal spora of Miranmar beach in Goa has been studied. It showed that the variation in the composition of airspora during post monsoon, pre monsoon, and monsoon was due to variation in meteorological parameter. In Delhi, the national capital, few studies related to aerobiological studies have been done. Some of these include, study of fungal spora in various part of Delhi region [39, 40].

However, any elaborate study on the characterization of various bioaerosols in India, specially Delhi, have not been done so far. This is why the present study has been undertaken with the following objectives;

1. To estimate the concentration of different fraction of bioaerosols in various six size range.
2. To find out the relationship between the different fractions of size segregated bioaerosols with the meteorological parameters.
3. To check the seasonal variation of bioaerosols at Delhi
4. To identify and characterize various toxic fungi collected.

CHAPTER:2

MATERIAL AND METHOD

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2.1 Study Area

Bioaerosol sampling was carried out in Delhi (28°25' latitude and 76° 50' longitude), the capital city of India, situated in Northern India. It lies 160 km south of Himalayas at an altitude of 213.3 to 305.4 m above mean sea level. It is in the close vicinity of Thar Desert (Rajasthan) in the west and hot plain of central India. Delhi is a rapidly growing city, with its area extending over 1,483 km^2. Its population has rapidly increased from 3.5 million in 1970 to over 16 million presently (Census of India 2011). The climate of Delhi is sub-tropical with hot summers and moderately cold winters. The monthly mean temperature varies between 14.3°C (minimum 2°C) in January, which is the coldest month and about 34.5°C (maximum 48°C) in June, the hottest month (Balachandran et al., 2000).

2.2 Sampling Sites

Sampling was carried out at four different sites in Delhi. Sampling sites were selected on the basis of a previous on site survey. Among the four sites, two were inside the Jawaharlal Nehru University (JNU), while the third was in the close vicinity to JNU. The fourth one was faroff ($\simeq$ 24 km) from JNU. The four sites have been illustrated in figure 1 and their detailed descriptions are as follows;

2.2.1 Garbage Site (JNU)

Garbage site of JNU is situated in western part of the campus. The site is surrounded by dense scrubby vegetation on three sides. Hostels and residential areas are in close the vicinity (10-15m) to the garbage site. JNU, in general is situated in the bush forest on the ridge of Aravalli Hills. It is the south-west of Delhi with a sprawling area of over 1000 acres. There are 5500 students, 550 faculties over 1000 supporting staff.

2.2.2 Jhelum Mess (JNU)

Sampling was done in the dining hall of the Jhelum hostel which is interconnected with the kitchen. The dining hall has three large doors and two windows. The windows remain close for most of time. Out of the three, two doors remain open for entire day while the third is blocked by a big air Cooler. Around 390 residents of the hostel visit the dining hall thrice a day for their daily meal. The mess is surrounded by replete green vegetation cover and has 20-25 workers who prepare meals.

2.2.3 Munirka

Munirka, heavily populated residential area in the close vicinity of JNU, is a very congested area and has enormous number of building and houses. Sampling was done at the balcony of a three-story building which is around 200 m away from the main road. The site is characterised by an open area just in front of the building.

2.2.4 Kaushambi (Ghaziabad)

The sampling site was a colony named Siddharth Niketan in Kaushambi Ghaziabad. It is a residential colony near a busy highway (NH-24). A large dumping site, about 400 m away, was also in the vicinity.

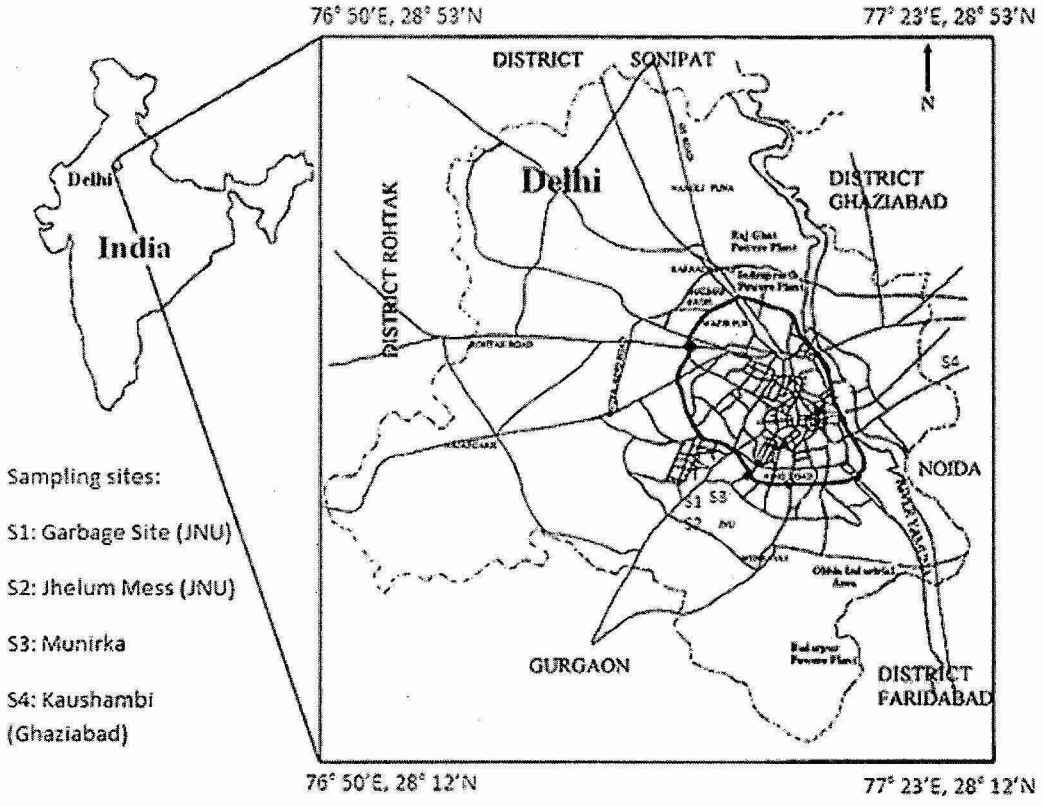

Figure:1 Location of sampling sites (not to scale)

2.3 Bioaerosol Sampling

Sampling was done with the help of a six stage viable cascade impacter, manufactured by Tisch Environmental USA (figure 2). The sampling was carried out for three different fractions of bioaerosols

viz. fungi, gram positive and gram negative bacteria during August 2010 to April 2011, these nine months(September-April) cover more or less all the three different seasons of India i.e. rainy, monsoon and winter.

Sampling was done for 30 minutes during summer and for 1-10 minutes for winter.

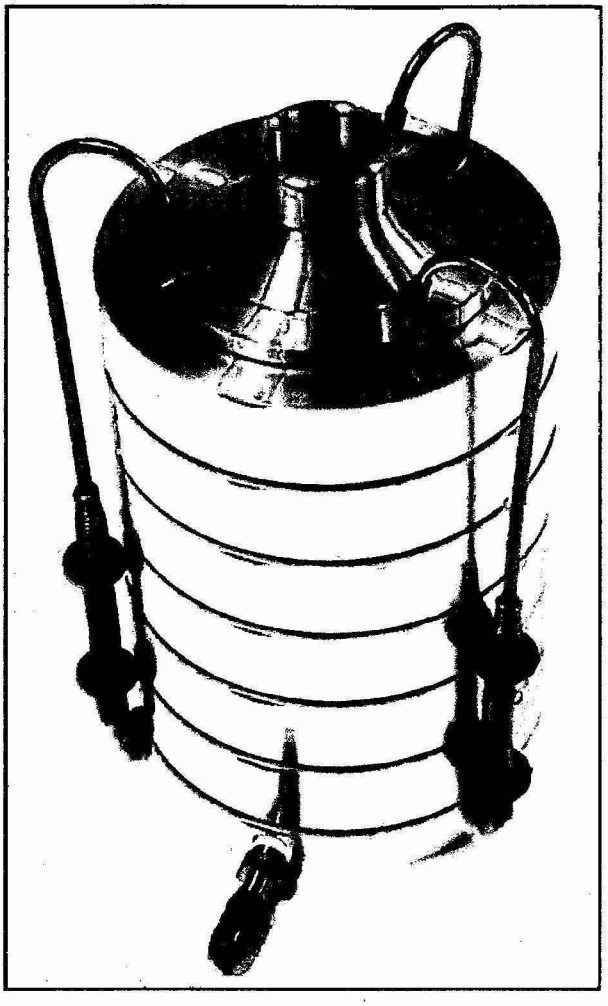

Figure: 2 Tisch six-stage viable sampler

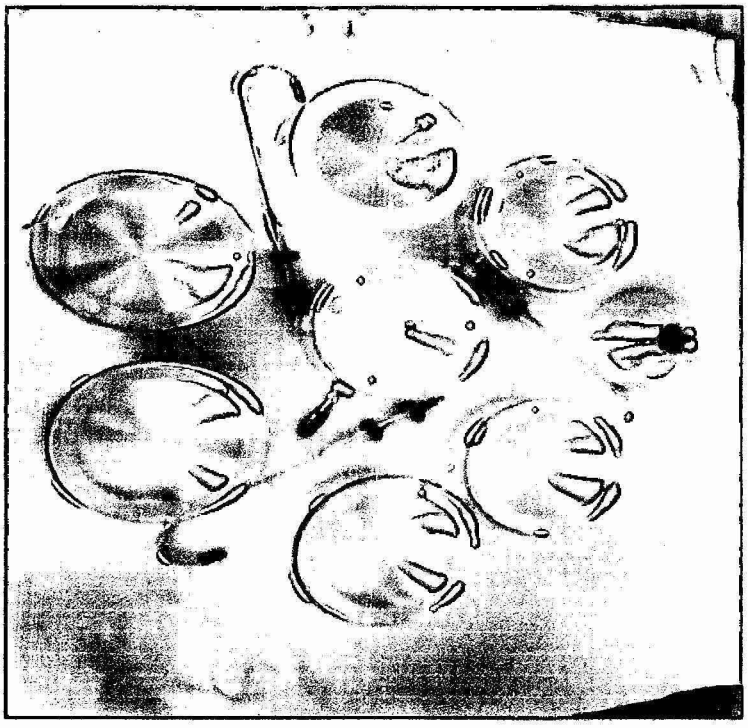

Figure: 3 Six stages of sampler

The sampler, a Cascade impactor consists of a stack of impaction stages (figure 3): each stage consists of one or more nozzles and a target or substrate. The air stream passes through the nozzles and particles larger than a particular aerodynamic size will be impacted onto a collection surface while smaller particle proceed through the stages. The nozzles may take the form of holes. The target may consist of a growth media (agar) contained in petri dishes. Each succeeding stage collects smaller particles (Figure 4). The details of nozzle diameter and particle diameter for six stage impactor are given in Table 1.

Table 1: The jet orifice dimensions and particle size ranges for each stage

Stage	Orifice Diameter (mm)	Range of Particle Sizes (μm)
1	1.18	>7.0
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

US Environmental Protection Agency (EPA) has defined four terms for categorizing particles of different sizes according their diameter ultrafine <0.1 μm, fine 0.1 -2.5 μm, coarse 2.5 -10 μm and supercoarse > 10 μm (EPA 2002).

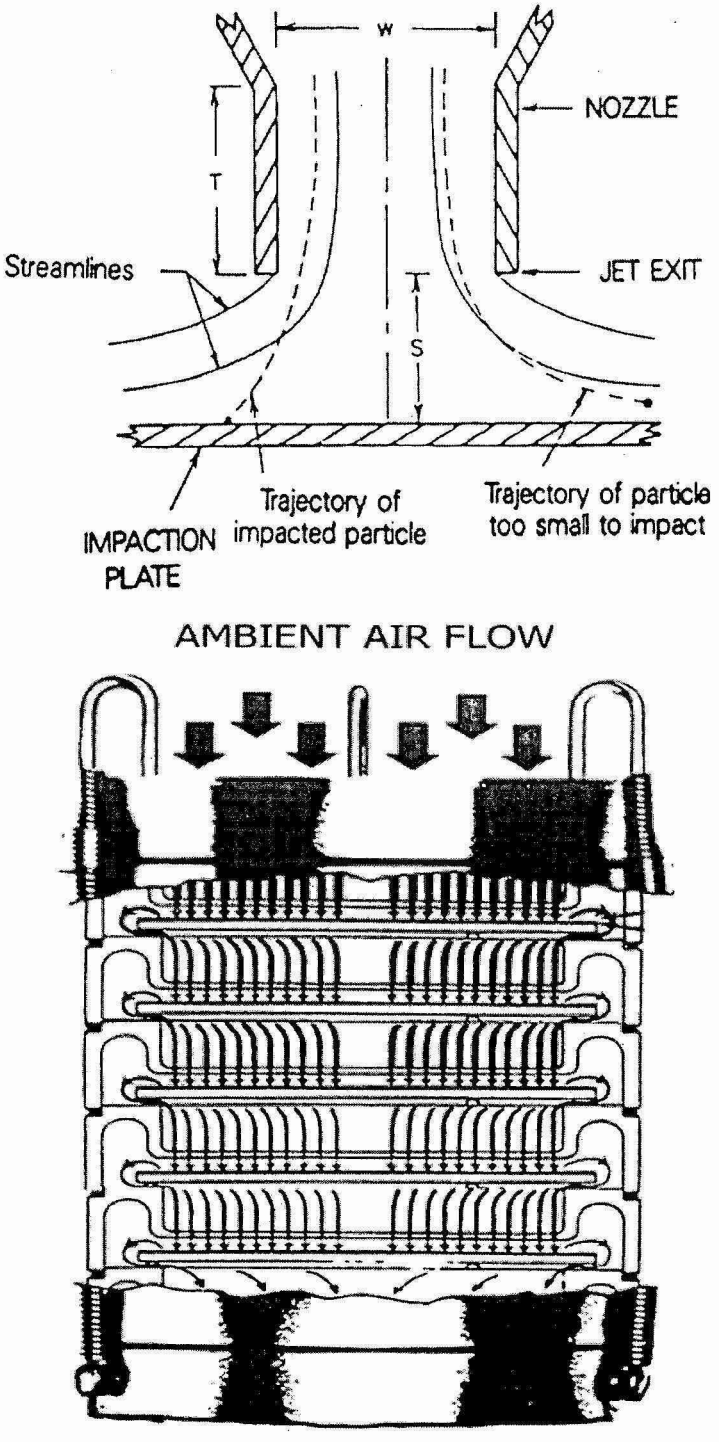

Figure: 4 Schematic of impactor stage

Design of the Tisch Viable sampler was evolved from the human respiratory system tract which 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 the physical properties (size, shape, density) of particles. 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. Large particles deposit primarily in the nasal-pharyngeal area, whereas particles of sub-micrometer size particles deposit mainly in the pulmonary area (Figure 5).

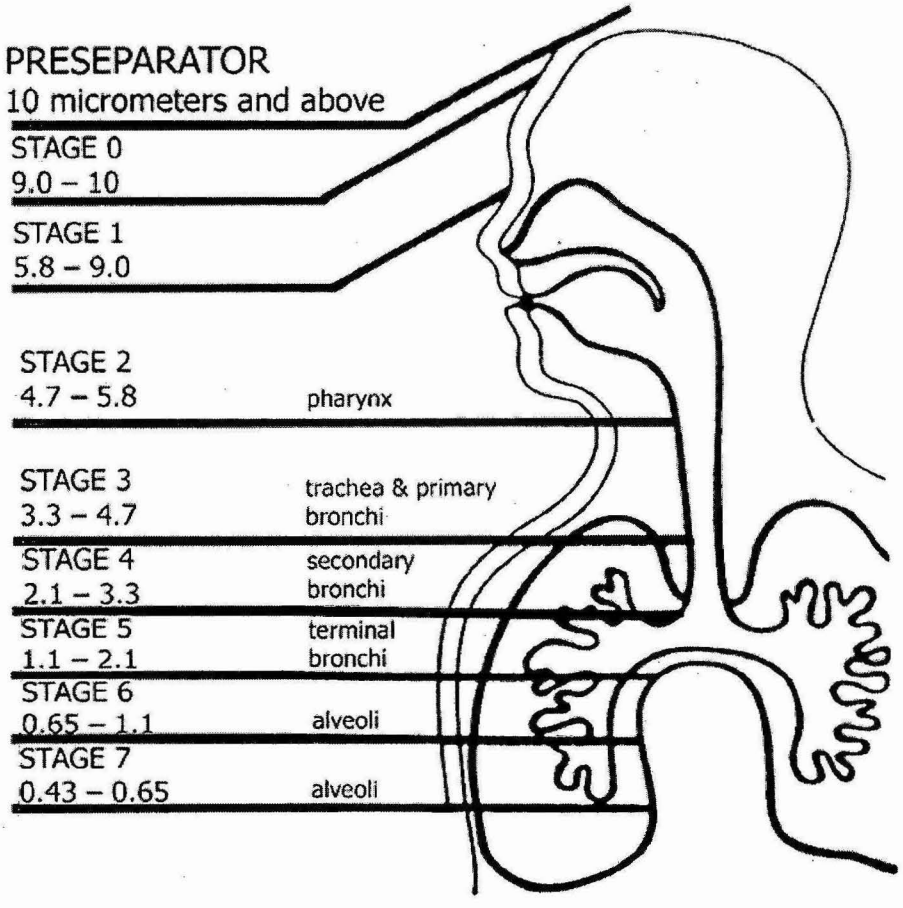

Figure: 5 Tisch sampler simulates the human respiratory system

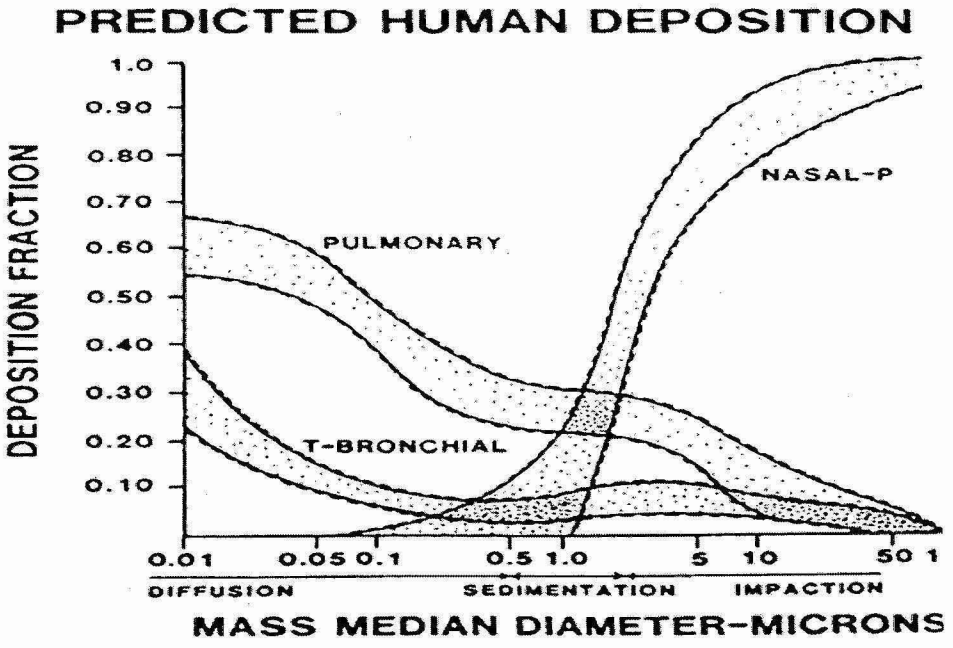

Figure: 6 Graphical representation of predicted human deposition

2.3.1 Sampling consideration

2.3.1.1 Safety

Sampler, culture plates, equipments etc. were handled aseptically to prevent contamination of the samples, and more importantly, to prevent the spread of potential human pathogens to the worker or the work environment. But most of the surfaces, including hands, laminarflow etc. were properly sterilized. However, not all objects could be sterilized, while disinfection with an oxidizing chemical or alcohol destroys most vegetative cells, these agents do not destroy all spores. Sampler was sterilized after each sample collection. Special care was given to sampler with convoluted inlets or air pathways where microorganisms may accumulate. Media prepared and glassware used in experiment was pre-autoclaved.

2.3.1.2 Monitoring of metreological parameters and CO_2

Taking in to account the strong dependence of survival and growth of bioaerosols on the meteorological conditions and CO_2, the monitoring of various meteorological parameter and CO_2 was carried out.

Temperature, relative humidity and wind velocity was recorded using ENVIRO-METER (Fisher Scientific). Whilst CO_2 was monitored with the help of EXTECH INSTRUMENTS CO200, CO_2 monitor.

2.3.2 Culture Media

For detection and enumeration of fungi and bacteria media were used on the collection plates. Fungal fraction of bioaerosol was collected over Potato dextrose agar media (figure 7). 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 inhibits the growth of bacteria [38].

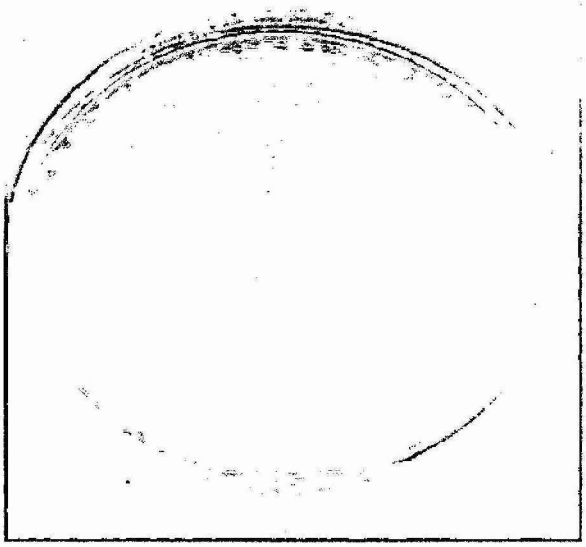

Figure: 7 Potato dextrose agar media.

Bacterial (gram negative) fraction of bioaerosol was collected on Eosin Methylene Blue (ENM) agar media. This is selective-differential plating medium for the detection and isolation of gram-negative bacteria (Figure 8).

Holt, Harris, Teague first developed Eosin Methylene Blue agar. The eosin dye inhibits growth of gram-positive bacteria and combines with the methylene blue indicator to produce a color change whenever lactose or sucrose is fermented.

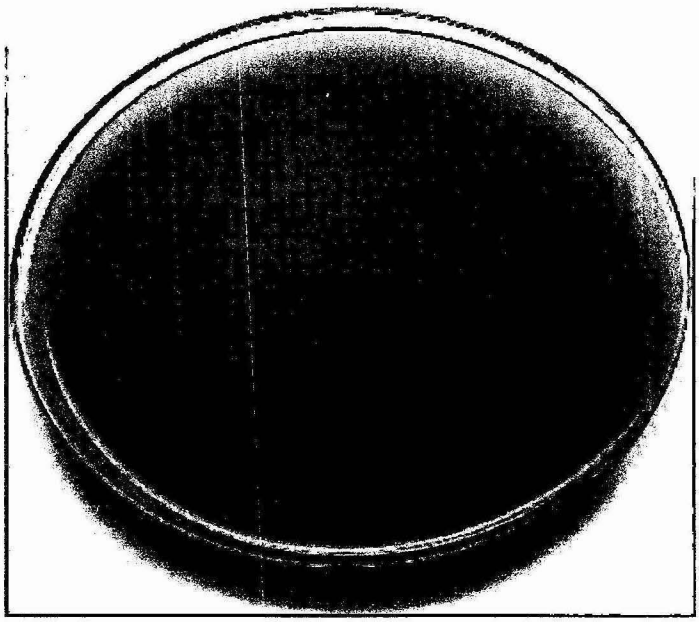

Figure: 8 EMB Agar media (for gram negative bacteria)

Gram positive bacteria were collected on blood agar. Blood Agar Media is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms. Blood agar bases are typically supplemented with 5 - 10% sheep, rabbit, or horse blood for use in isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, blood agar bases can be used as general purpose media (Figure 9).

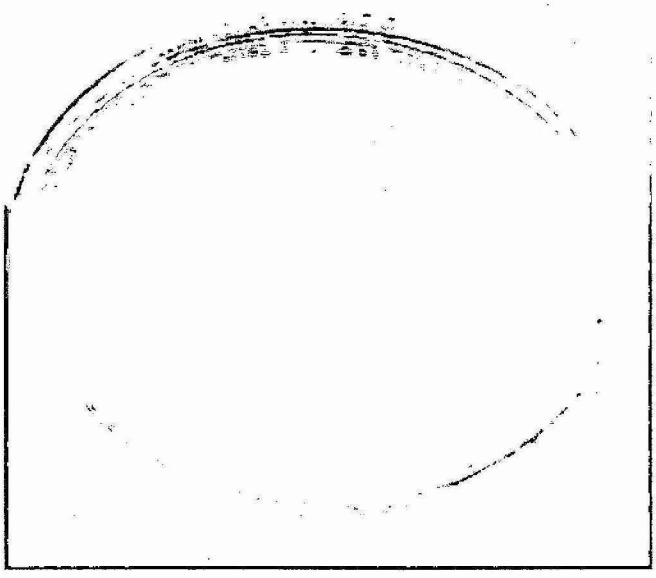

Figure: 9 Blood Agar media (for gram positive bacteria)

To ensure that our work environment and processes completely clean and devoid of any kind of microbial contaminations, laboratory media blanks were prepared. Laboratory media blanks were unexposed, fresh media samples. They were incubated in the same manner as sampling media but they were not taken in to the field.

2.4 Bioaerosol Characterization

2.4.1 Sample Preparation

Inoculated agar plates were incubated at the appropriate temperature for times ranging from hours for a fast-growing bacterium to days for a fungus to develop into a visible colony. As a rule, plates are incubated at the temperatures shown in Table 2

Table 2: Incubation temperatures and conditions for viable (culturable) Microorganism

Fungi	25 ^{0}C or Room temperature
Bacteria, environmental	25 to 30 ^{0}C
Bacteria, human-source	35 to 37 ^{0}C

2.4.2 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.

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

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

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

2.4.3 Identification

A small portion of fungal colony is taken with the help of inoculums loop and placed on to a slide containing 4% of NaCl. A drop of lactophenol cotton blue stain is added over it immediately and left for about 1-2 minutes. The area is then covered by a cover slip and it is ready for microscopic examination

and visual 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.

2.4.4 Statistical analysis

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

CHAPTER:3

RESULT AND DISCUSSION

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3.1 Size segregated distribution of various fractions of Bioaerosols

Concentration of fungal bioserosol found in different stages at each site seems to follow a typical pattern in all three season. As seen in Figure 10, 11 and 12 there is an increasing trend in concentration from stages 1-4 with a further decreasing order from 4-6. In all three figures the highest concentration of fungus is found at stage 4 (diameter ranging from 2.1 μm to 3.3 μm) and lowest concentration at stage 6 (diameter ranging from 0.6 μm to 1.1 μm). The typical pattern of concentration depicts that majority of the fungal species have similar diameter as that of stage 4 which is in synonym to the secondary bronchi of the lungs in 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.

Table 3: Number of Colony Forming Unit (Fungal) on different stages at each site during monsoon period

Stages of the Sampler	No. Of Colonies (CFU)				
	Sites / Months	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	August	36	36	64	9
	September	40	32	62	9
	Average	***38***	***34***	***63***	***9***
II	August	49	63	56	5
	September	41	60	51	4
	Average	***45***	***61.5***	***53.5***	***4.5***
III	August	156	256	100	15
	September	142	257	103	15
	Average	***149***	***256.5***	***101.5***	***15***
IV	August	180	346	199	65
	September	160	340	194	67
	Average	***170***	***343***	***196.5***	***66***
V	August	24	255	10	2
	September	18	270	7	2
	Average	***21***	***262.5***	***8.5***	***2***
VI	August	0	12	2	0
	September	0	10	1	0
	Average	***0***	***11***	***1.5***	***0***

Table 4: Concentration (CFU m^{-3}) of Fungal Bioaerosol on different stages at each site during monsoon period

Concentration in $CFUm^{-3}$				
Sites / Stages Of the Sampler	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	44.76	40.05	74.20	10.60
II	53	72.44	63.02	5.30
III	175	301.53	119.55	17.67
IV	200	404	230.86	77.74
V	24.5	309.19	10.01	2.36
VI	0	12.96	1.77	0
Total	497.51	1140.17	499.41	113.67

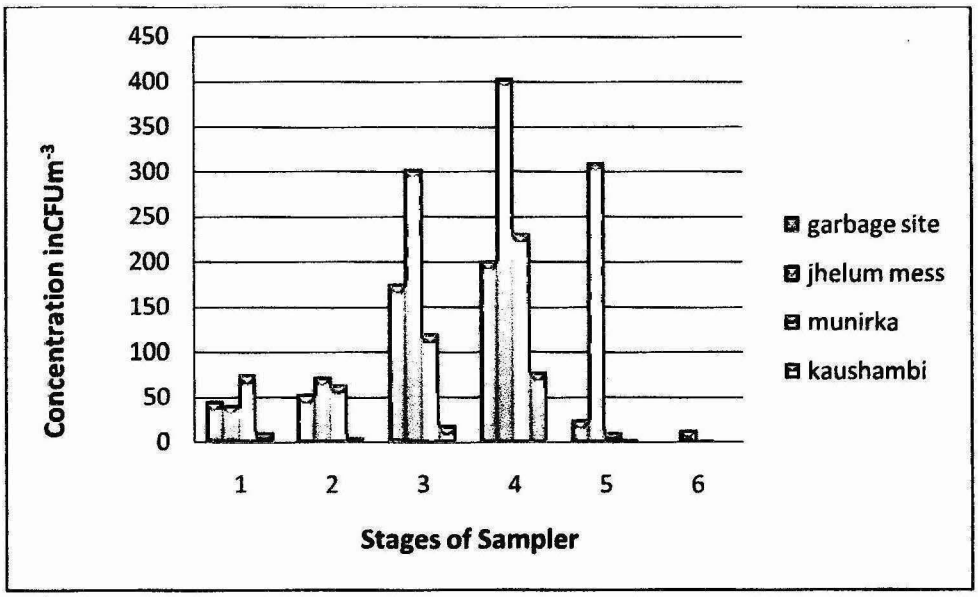

Figure 10: Total Fungal concentration at each stage of the sampler in monsoon period

Table 5: Number of Colony Forming Unit (Fungal) on different stages at each site during post- monsoon period

Stages of the Sampler	No. Of Colonies (CFU)				
	Sites / Months	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	December	11	2	5	3
	January	10	3	15	19
	Average	***10.5***	***2.5***	***10***	***11***
II	December	21	4	9	6
	January	22	2	19	16
	Average	***21.5***	***3***	***14***	***11***
III	December	71	18	32	12
	January	75	15	48	46
	Average	***73***	***16.5***	***40***	***29***
IV	December	96	84	61	26
	January	97	43	112	94
	Average	***96.5***	***63.5***	***86.5***	***60***
V	December	24	42	16	4
	January	26	16	33	46
	Average	***25***	***29***	***24.5***	***25***
VI	December	0	0	0	0
	January	0	0	0	0
	Average	***0***	***0***	***0***	***0***

Table 6: Concentration ($CFUm^{-3}$) of Fungal Bioaerosol on different stages at each site during post-monsoon period

Concentration in $CFUm^{-3}$				
Sites / Stages Of the Sampler	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	175	41.67	166.67	183
II	358	50	233.33	183
III	1216.67	275	666.67	483.33
IV	1608.33	1058.33	1441.67	1000
V	416.67	483.33	425	416.67
VI	0	0	0	0
Total	3774.67	1908.33	2933.34	2266

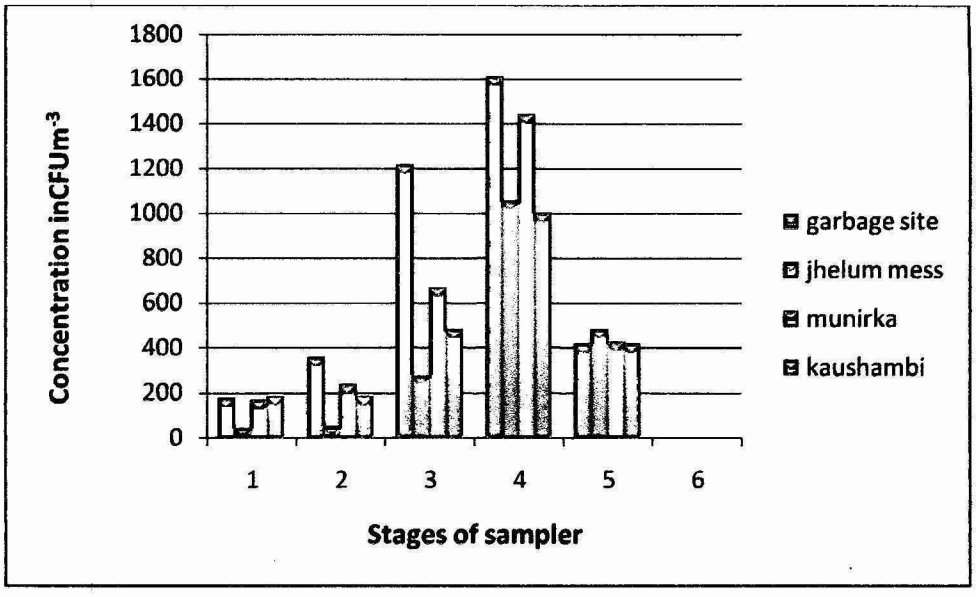

Figure 11: Total Fungal concentration at each stage of the sampler in post- monsoon period

Table 7: Number of Colony Forming Unit (Fungal) on different stages at each site during pre- monsoon period

Stages of the Sampler	No. Of Colonies (CFU)				
	Sites / Months	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	April	73	45	62	74
	May	68	42	60	70
	Average	***70.5***	***43.5***	***61***	***72***
II	April	107	77	78	102
	May	110	69	71	100
	Average	***108.5***	***73***	***74.5***	***101***
III	April	193	152	146	166
	May	184	144	138	178
	Average	***188.5***	***148***	***142***	***172***
IV	April	283	322	182	210
	May	256	298	192	209
	Average	***269.5***	***310***	***187***	***209.5***
V	April	91	141	94	94
	May	88	133	89	87
	Average	***89.5***	***137***	***91.5***	***90.5***
VI	April	8	5	6	9
	May	9	6	7	6
	Average	***8.5***	***5.54***	***6.5***	***7.5***

Table 8: Concentration ($CFUm^{-3}$) of Fungal Bioaerosol on different stages at each site during pre-monsoon Period

Concentration in $CFUm^{-3}$				
Sites / Stages Of the Sampler	Garbage Site	Jhelum Mess	Munirka	Kaushambi
I	83.04	51.24	71.85	84.81
II	127.80	85.98	87.75	118.96
III	222.03	174.32	167.26	202.59
IV	317.43	365.14	220.26	246.76
V	105.42	161.37	107.77	106.66
VI	10.01	6.48	7.66	8.83
Total	865.73	844.53	662.55	768.61

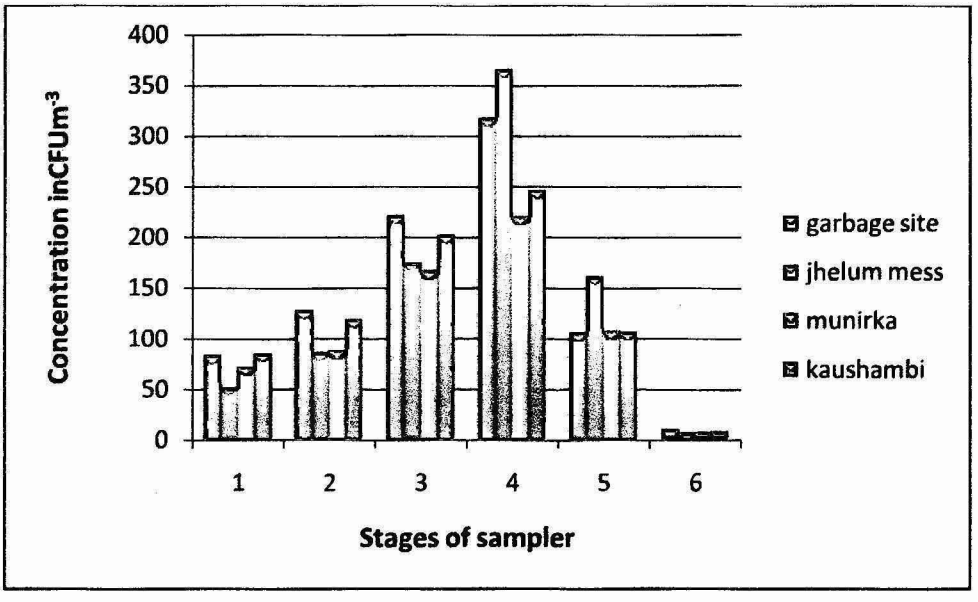

Figure 12: Total Fungus concentration at each stage of the sampler in pre- monsoon period

Unlike fungi, a gram positive bacterium seems to follow no pattern at all. As seen in fig. 13 maximum concentration is found in stage 4 at most of the sites with an exception at Jhelum Mess, where maximum concentration found to be in stage 6 (0.65 μm- 1.1 μm). Minimum concentration is different at different sites. In case of Jhelum mess and Munirka the minimum concentration zone is stage 6 while for Garbage site and Kaushambi it is stage 5 and 6 respectively. High degree of concentration found in stage 4 reveals the fact that secondary bronchi are mostly affected during monsoon season. Fig. 14 reveals different

pattern of distribution at different sites in post monsoon season. Maximum concentration of gram positive bacteria in Jhelum Mess & Munirka is found to be on stage 3 (3.3 μm-4.7 μm), while in case of Garbage site and Kaushambi it is stage 2 and 6 respectively. In most of the sites the minimum concentration is seen to be in stage 6 with an exception at Jhelum Mess in stage 4. Maximum concentration found in stage 2, 3 and 4 reveals the fact that in post- monsoon period pharynx, trachea, primary and secondary bronchi are mostly affected by gram positive bacteria.

Table 9: Number of Colony Forming Unit (gram positive bacteria) on different stages at each site during monsoon period

Stages of the Sampler	**No. Of Colonies (CFU)**				
	Sites / **Months**	**Garbage Site**	**Jhelum Mess**	**Munirka**	**Kaushambi**
I	August	39	47	52	100
	September	37	45	52	103
	Average	***38***	***46***	***52***	***101.5***
II	August	45	30	36	63
	September	43	32	33	61
	Average	***44***	***31***	***34.4***	***62***
III	August	31	111	87	88
	September	37	100	86	90
	Average	***34***	***110.5***	***86.5***	***89***
IV	August	100	154	110	190
	September	120	149	100	185
	Average	***110***	***151.5***	***105***	***187.5***
V	August	25	73	37	113
	September	22	77	34	111
	Average	***23.5***	***75***	***35.5***	***112***
VI	August	52	165	57	17
	September	48	160	50	12
	Average	***50***	***162.5***	***53.5***	***14.5***

As seen in table 28 in case of gram positive bacteria, very good correlation exists between stages 1-5 with the total concentration as well as among different stages such as stage 1 with stages 2, 3 and 5; stage 2 with stage stages 3. 5 and 6; stage 3 with stage 5 and 6 as well as stage 4 with stage 5. Good correlation exists among different stages such as stage 1 with 4 and 6; stage 2 with stage 4; stage 3 with stage 4; stage 5 with stage 6 and stage 6 with the total concentration.

Table 28: Correlation matrix between different size fraction for gram positive bacteria

	Stage 1	*Stage 2*	*Stage 3*	*Stage 4*	*Stage 5*	*Stage 6*	*Total 7*
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

In case of gram negative bacteria correlation among different stages are represented in table 29 unlike fungi and gram positive bacteria very good correlation exists between stage 3-6 with the total. In case of correlation among the stages, very good correlation exists between stage 1 and stage 4; stage 2 and stage 3; stage 4 and stage 6 as well as stage 5 and stage 6. Good correlation is seen between stage 1 and stage 2,3 and total; stage 2 and stage 4 and total; stage 3 and stages 4,5 and 6 as well as between stage 4 and stage 5. Stage 1 with stage 6 and stage 2 with stages 5 and 6 are seen to be averagely correlated.

Table 29: Correlation matrix between different size fraction for gram negative bacteria.

	Stage 1	*Stage 2*	*Stage 3*	*Stage 4*	*Stage 5*	*Stage 6*	*Total 7*
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

3.5 Identification of fungal bioaerosol at different sites

Nine genera of fungal bioaerosol were identified at all site during three different season are given in table 30. Among the nine genera identified, the 3 genera which has been found in maximum number in all the season are *Rhizopus*, *Aspergillus* and *Pencillium*. Remaining genera form very small part of total concentration of fungal bioaerosol. In monsoon period major finding were *Rhizopus*, *Aspergillus* and *Pencillium*. In Post-monsoon period major concentration of *Fusarium, Rhizopus, Aspergillus* and *Pencillium* were found at each site. In Pre- monsoon period *Alterneria* was found in bulk.

Penicillium species are present in the air and dust of indoor environments. The fungus can be readily transported from the outdoors, and grow indoors using building material or accumulated soil to obtain nutrients for growth. *Penicillium* growth can occur indoors even if the relative humidity is low, as long as there is sufficient moisture available on a given surface. Ochratoxin A, a toxin produced by various species of *Aspergillus* is one of the most abundant food-contaminating mycotoxins in the world. Human exposure occurs mainly through consumption of improperly stored food products. Ochratoxin A is potentially carcinogenic to humans and has been shown to be weakly mutagenic, possibly by induction of oxidative DNA damage. Different species of *Aspergillus* genera also produces aflotoxins, which is harmful for human and animal health. Major disease caused by this genera is Aspergillosis. Rhizopus is parasite on animal. Alterneria, *Ulocladium* and *Curvuleria* are plant pathogen and may be a common allergens for animals and human and may cause hay fever or hyper sensitivity reaction that sometimes lead to asthama. Fuserial infection may occur in the nails and in the cornea (keratomycosis). In human whose immune systems are weakend in particular way, aggressive fuserial infections penetrate entire body and blood stream.

Table 30: Fungal Genus characterized at different sites

Sampling Sites	Garbage Site			Jhelum Mess			Munirka			Kaushambi		
Sampling Season / Types Of Fungi	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon	Monsoon	Post-Monsoon	Pre-Monsoon
Asperigillus ≠	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium ×	–	+	–	–	+	–	–	+	–	–	+	–
Dreshelia ≠	+	–	–	+	–	–	–	–	–	+	–	–
Ulocladium ×	–	–	+	–	–	+	–	–	+	–	–	+
Curvularia ≠	+	–	+	+	–	–	–	–	–	+	–	–
Pencillium ˣ	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria ×	–	+	+	–	+	+	–	+	+	–	+	+
Mucor ˣ	+	–	–	+	–	–	+	–	–	+	–	–
Rhizopus ×	+	+	+	+	+	+	+	+	+	+	+	+

× IMMUNOTOXIC ˣHARMLESS ≠ ALLERGIC

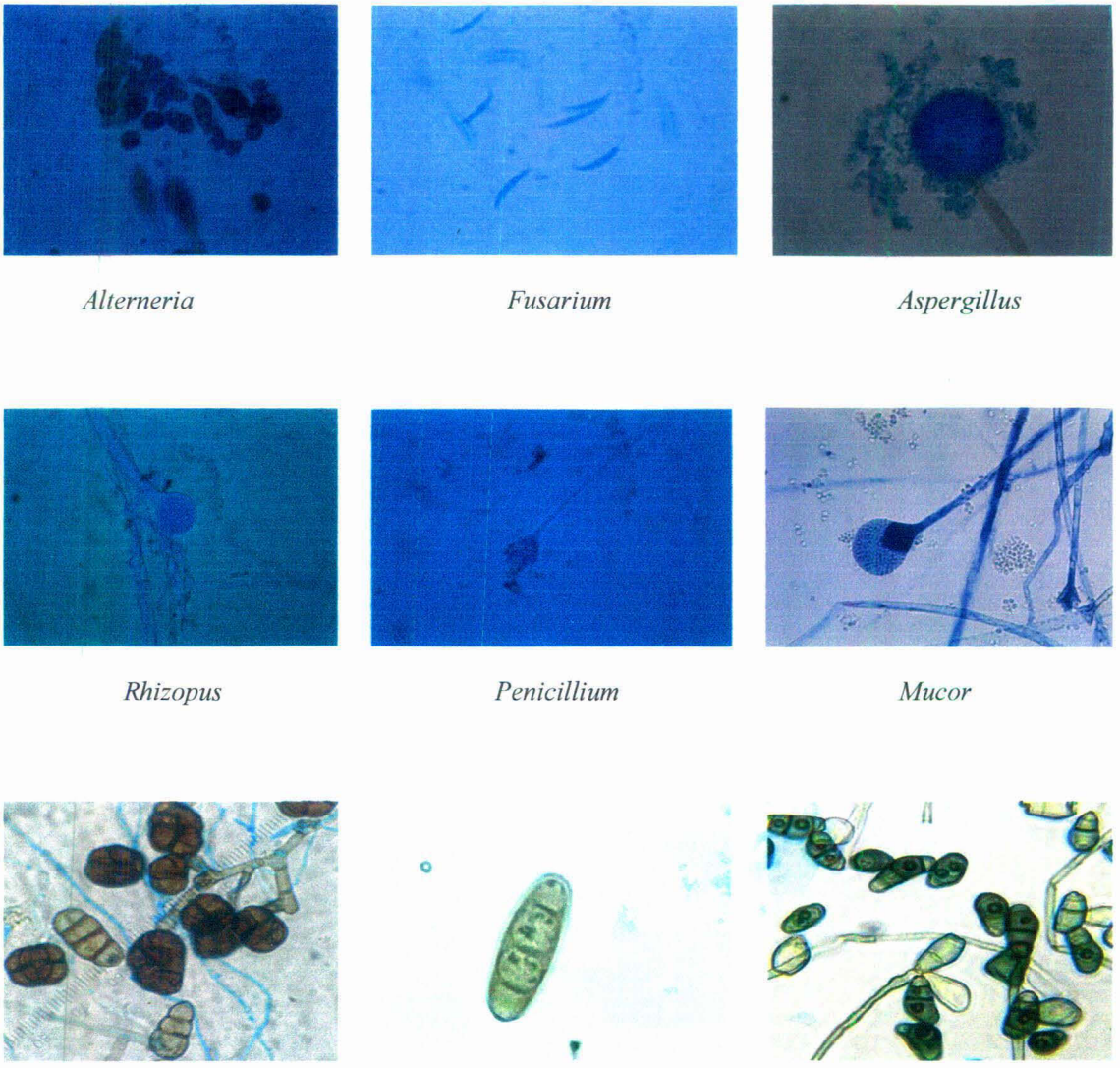

Figure 25: Fungal genus isolated at different sites in Delhi

CHAPTER:4

CONCLUSION

CONCLUSION

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

1. Concentration of fungal bioserosol 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, majority of the immunotoxic and allergic fungi found at this stage are mostly prone to affect the secondary bronchi in human lungs when inhaled.

2. Unlike fungi, a gram positive bacterium seems to follow no pattern at all. During monsoon season maximum concentration is found in stage 4 at most of the sites with an exception at Jhelum mess, where maximum concentration found to be in stage 6. In post- monsoon season maximum concentration in indoor sites such as Jhelum mess & Munirka is found to be in stage 3, while in case of outdoor sites like Garbage site and Kaushambi it is stage 2 and 6 respectively. In case of pre-monsoon sampling a totally different story reveals that maximum concentration is found to be at stage 3 for all sites. 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.

3. Like gram positive bacteria, gram negative bacterial concentration too does not follow any typical pattern. In monsoon and post- monsoon season maximum concentration is found in stage 3 and stage 4. While in pre-monsoon season maximum concentration of gram negative bacteria is found in stage 3 only. Since all the three stages are in synonym to 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.

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

In case of pre-monsoon season high temperature and correspondingly low relative humidity justifies the reason of less bioaerosol concentration.

5. 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 potential to produce carcinogenic Ochratoxin A and Aflatoxins) and *Rhizopus* is immunotoxic in nature. *Fusarium* and *Alternaria* are found in abundance in post- monsoon and pre-monsoon season respectively.

6. Good regression coefficient of regression of fine over coarse in case of all three different type of bioaerosol suggest that variation in the concentration of fine fraction of bioaerosol affects the variation of concentration of coarse fraction of bioaerosol.

7. Good correlation exists between different fractions of fungal bioaerosol with each other and with total. The only exception is stage 6, which is not at all correlated with any of the stages.

8. Similarly, more or less good correlation exists between fraction of gram negative and gram positive bacteria with each other and total.

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