CHARACTERIZATION OF BIOAEROSOLS IN AND AROUND A LANDFILL SITE IN DELHI

Dissertation submitted to Jawaharlal Nehru University

in partial fulfillment of the requirements

for the award of the degree of

MASTER OF PHILOSOPHY

RICHA VERMA

SCHOOL OF ENVIRONMENTAL SCIENCE

JAWAHARLAL NEHRU UNIVERSITY

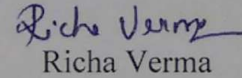
NEW DELHI-110067 INDIA

JULY 2016

जवाहरलाल नेहरू विश्वविद्यालय
Jawaharlal Nehru University
SCHOOL OF ENVIRONMENTAL SCIENCES
New Delhi - 110067, INDIA

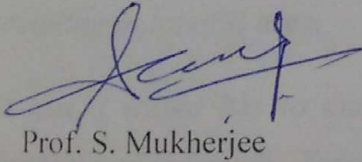
CERTIFICATE

This is to certify that the research work embodied in the dissertation entitled "**Characterization of Bioaerosols in and around a landfill site 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.



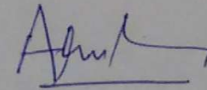
Richa Verma

(Candidate)



Prof. S. Mukherjee

(Dean)



Dr. Arun Srivasatava

(Supervisor)

Prof. Saumitra Mukherjee
Dean
School of Environmental Sciences
Jawaharlal Nehru University
New Delhi-110067

Scanned by CamScanner

ACKNOWLEGEMENTS

I take this opportunity to express my gratitude to the School of Environmental Sciences, Jawaharlal Nehru University for providing me an opportunity to perform this project work. This thesis is not an outcome of my single effort but in fact there has been a significant effort from different people in completion of this thesis work.

Firstly, I would like to express my deep sense of gratitude to my esteemed supervisor ***Dr. Arun Srivastava*** *for their keen interest, valuable guidance, constructive suggestion and providing constant encouragement throughout the course of the present investigation and providing me all the necessary facilities. I am also grateful to entire faculty members of School of Environmnetal Sciences, for imparting me with education, knowledge and wisdom. These ideas and knowledge helped me prepare my thesis work in a relevant way.*

I would like to thank My Lab Senior ***Mr. Himanshu Lal and Ms Bipasha Ghosh*** *for their valuable support, assistance and guidance****, Mr. Naba Hazarika, Ms Sunita Maharia, Mr. Akash Singh, Mr. Anshul Srivastava*** *for their continuous suggestion and cooperation during entire period of my research work.*

I would like to thank ***my parents*** *and all family members who have been a constant source of encouragement and motivation.*

I would also like to convey my gratitude to ***Mr. Nimesh*** *(J.E. Okhla Landfill) for facilitating sampling in landfill area.*

At last, I would like to thank everyone who has been involved in this project work with me, directly and indirectly. You all have made this project work a success and without your help, this project work might not have been completed.

Thank You.

CONTENTS

Certificate

Acknowledgement

List of Tables i

List of Figures ii

Chapters

Chapter 1 Introduction 1

Chapter 2 Materials and Methods 11

- Study Area 11
- Sampling Sites 13
- Bioaerosol Sampling 14
- Bioaerosol Characterization 19

Chapter 3 Results and Discussion 20

- Size segregated distribution of various fractions of fungal bioaerosol 20
- Seasonal variation of fungal bioaerosol concentration at each site 28
- Respirable Fraction 31
- Regression Analysis 33
- Correlation Analysis 38
- Analysis of Variance (ANOVA) 44
- Identification 49

Chapter 4 Conclusion 52

LIST OF TABLES

Table 2.1: The jet orifice dimension 15

Table 3.1: Fungal Bioaerosol Distribution in relation to meteorological parameters in Site 1, Site 2 and Site 3 30

Table 3.2: Fungal Bioaerosol Distribution in relation to meteorological parameters in Site 5, Site 6 and Site 7 30

Table 3.3: Fungal Bioaerosol Distribution in relation to meteorological parameters in Office Indoor Site (Site 4) 30

Table 3.4: Output of multiple regression analyses between fungal concentration and meteorological parameters 33

Table 3.5: Significance test of multiple regression analyses 34

Table 3.6: Results of regression analysis for four models 36

Table 3.7: Correlation matrix between different size fractions for fungal bioaerosols over the complete year 39

Table 3.8: Correlation matrix between different size fractions for fungal bioaerosols in the monsoon season 40

Table 3.9: Correlation matrix between different size fractions for fungal bioaerosols in the autumn season 41

Table 3.10: Correlation matrix between different size fractions for fungal bioaerosols in the winter season 41

Table 3.11: Correlation matrix between different size fractions for fungal bioaerosols in the spring season 41

Table 3.12: Correlation matrix between different size fractions for fungal bioaerosols in the summer season 42

Table 3.13: Correlation among meteorological conditions and fungal bioaerosol concentration 43

Table 3.14: Mean fungal concentration difference among various stages of Sampler 44

Table 3.15: Mean fungal concentration difference among various sampling sites 45

Table 3.16: Results of analysis of variance (ANOVA) showing concentration difference of fungal bioaerosols within the six stages of Anderson Sampler 46

Table 3.17: Results of analysis of variance (ANOVA) showing concentration difference of fungal bioaerosols at all the sites among various seasons 48

Table 3.18: Fungal genus characterized at different sites 51

LIST OF FIGURES

Figure 2.1 (a): Location of sampling sites 12

Figure 2.1 (b): Diagrammatic representation of distance between each sampling site 12

Figure 2.2: Anderson Six stage Cascade impactor 14

Figure 2.3: Six stages of Anderson Sampler 15

Figure 2.4: Schematic of impactor stage 16

Figure 2.5: Bioaerosol sampler stimulating the human respiratory System 17

Figure 2.6: Potato dextrose agar media 18

Figure 3.1: Total fungal concentration in each stage at all the sites in monsoon season 21

Figure 3.2: Particle size distributions of airborne fungi at each site in monsoon season 21

Figure 3.3: Total fungal concentration in each stage at all the sites in autumn season 22

Figure 3.4: Particle size distributions of airborne fungi at each site in autumn season 23

Figure 3.5: Total fungal concentration in each stage at all the sites in winter season 24

Figure 3.6: Particle size distributions of airborne fungi at each site in autumn season 24

Figure 3.7: Total fungal concentration in each stage at all the sites in spring season 25

Figure 3.8: Particle size distributions of airborne fungi at each site in spring season 26

Figure 3.9: Total fungal concentration in each stage at all the sites in summer season 27

Figure 3.10: Particle size distributions of airborne fungi at each site in summer season 27

Figure 3.11: Comparison of total fungal concentration at different sites in different seasons 29

Figure 3.12: Respirable Fraction of fungal bioaerosols at all the sites during all the seasons 32

Figure 3.13: Relationship plot between fungal concentration and temperature 35

Figure 3.14: Relationship plot between fungal concentration and Relative Humidity (%) 35

Figure 3.15: Relationship plot between fungal concentration and wind speed (m/sec) 36

Figure 3.16: Fungal genus characterized at different sites 50

INTRODUCTION

Air quality is one among many factors that have an impact on our environment (Burkowska et al. 2011). Rapid urbanization and industrialization have deteriorated the air qualities in cities. Air pollution is a continuing threat to our health and welfare.

Air contamination is not a new problem. Many natural sources of air contaminants have always existed. Examples of natural air pollution include spores and pollen released by plants, ash from volcanoes explosion, smoke from forest fire and windblown dust. Human activities added to the frequency and intensity of some of these natural air pollutants (Lutgens et al. 2004).

Air quality is determined by the presence of air pollutants. Air pollutants are airborne particles and gases that occur in concentration that have adverse effect on health of organisms or disrupt the orderly functioning of ecosystem. Pollutants are of two types: primary and secondary. Primary pollutants are those pollutants which emit directly from identifiable sources. It includes particulate matter, sulfur dioxide, nitrogen oxides, volatile organic compounds, carbon monoxide and lead. Whereas secondary pollutants are those which are released by the chemical reactions among primary pollutants in the atmosphere, for example smog (product of coal burning coupled with high humidity) (Lutgens et al. 2004).

Air pollutants can be of both chemical as well as biological origin. Chemical natured air pollutants mainly are of three types: Gaseous pollutants (ozone, carbon monoxide, nitrogen dioxide, dioxins etc.), heavy metals (mercury, lead etc.) and particulate matter with different sizes (Kampa et al. 2008). Biologically originated contaminants of air include Bioaerosols. Bioaerosols are aggregation of naturally or artificially produced biological particles, dispersed in air. Bioaerosols may consist of living as well as non-living cells of microorganisms (Fraczek et al. 2014).

Bioaerosols can occur as single cells, aggregation of single cells as well as conglomerates with minute dust particles or with water droplets called “nuclei droplets”. Dust acts as carriers of both naturally as well as artificially generated biological particles (Gorny et al. 1999).

Bioaerosols are formed by minute liquid droplets or solid matter particles that include bacteria, fungus, virus, independently floating microorganisms and pollen in gaseous medium.

Technically, bioaerosols are colloids which are systems of 2 or 3 components that are dispersed in dispersion medium i.e., air. Particles size in bioaerosols, ranges from 0.3 μm to 100 μm. Bioaerosol is a changeable system, its impermanent nature is due to its capability of coagulation. Individual components tend to combine with each other due to difference in electric charges. Longevity of bioaerosols depends on the chemical composition of bacterial capsules. Bacteria with protein capsules are most durable bioaerosols (Burkowska et al. 2011). Bioaerosols are released by microbial decomposition of organic materials in naturally occurring processes and also by atmospheric dispersion of bioaerosols (Kummer and Thiel, 2008).

5% to 34% of air pollution in indoor environment is due to bioaerosols. The respirable fraction of bioaerosols ($PM_{2.5}$) is of primary health concern as this size of bioaerosols can easily reach to deeper parts of respiratory system (Mandal et al. 2011). In non-industrial indoor environment, human presence is one of the most important sources of bioaerosols. Sensitive environment of bioaerosols includes animal housing (Millner, 2009), dumping and composting sites (Shantha et al. 2009), ceiling and walls of indoor surfaces (Horner et al. 2004) and food processing and manufacturing plants (Fischer et al. 2003).

Airborne bacteria are generated by various human activities for example, walking, talking, sneezing, coughing, toilet flushing and washing. House dusts, carpets, pets, textiles, wood materials, flower pots and house plants occasionally release various types of fungal spores into the air (Cox and Wathes, 1995). Indoor bioaerosol level depends on meteorological parameters like temperature, relative humidity, wind speed, moisture content of building material along with other factors like outdoor concentration of bioaerosols, number of people and presence of pets. Indoor environment are usually considered to be protective as compared to outdoor environment but they can also become more contaminated and can cause more serious health risks than outdoor sometimes, when their concentration exceed recommended maximum limit. These are 1000 CFU/m^3 for total number of airborne microbes set by National Institute Occupational Safety and Health (NIOSH), 1000 CFU/m^3 set by the American Conference of Government Industrial Hygienist (ACGIH) with the culturable count for total bacteria not to exceed 500 CFU/m^3 (Kalogerakis et al. 2005).

Generally, bioaerosol concentration is higher in warmer climate compared to colder climate (Mandal et al. 2011). Temperature range of 18 ºC to 32 ºC is most favourable for fungus growth. Fungi can however survive in a wide range of temperatures from -5 ºC to 60 ºC. They flourish when relative humidity is more than 65% and pH ranges from 1 to 9 with calm wind conditions (Codina et al. 2008). Composting facilities and waste treatment plants in general emit higher number of pathogenic and non-pathogenic microorganisms of different species (Hryhorczuk et al. 2001).

Fungal bioaerosols are very common and there is essentially no fungus free environment surrounding us. Fungal spores are the dominant component of the air and contribute about 4% to 10% of organic carbon and 2% to 5% of PM_{10} (Bauer et al. 2008).

United States Environmental Protection Agency (USEPA) has defined four terms for categorizing particles of different sizes according to their diameter: Ultrafine <0.1 μm, Fine 0.1-2.5 μm, Coarse 2.5-10 μm and Super Coarse >10 μm (USEPA, 2002). As we inhale, depending upon the size of airborne particles, they deposit in different parts of our respiratory system. International standardization organization (ISO 7708, 1995) uses the following particles size classifications:

Inhalable Fraction- It is the mass fraction of bioaerosols that is inhaled through the nose and mouth.

Thoracic Fraction- It is the mass fraction of inhaled particles penetrating beyond larynx.

Respiratory Fraction- It is the mass fraction of inhaled particles that reach alveoli.

The 50% cut off diameter for respirable fraction is 4 μm and for the thoracic fraction it is 10 μm (CEN, 1993). Airborne particles of size greater than 50 μm diameter can enter nose and mouth, and particles less than 10 μm are deposited on the ventilation pathway surface above the trachea. Finer particles can easily enter the alveolar region of lungs (Rodes and Wiener, 2001).

Persistence and disease causing ability of microbes depends on type of species, meteorological and microclimatic conditions such as temperature, solar radiation, wind speed and direction, relative humidity, rainfall, atmospheric pressure. Health is affected by the inhalation of various biological particles. Health effects of bioaerosols depend on their chemical composition, size,

microbiological properties and also the place on which these biological particles get deposited in the respiratory system. Particles of diameter less than 5 µm can cause allergic inflammation and other diseases as they can reach to pulmonary alveolus (Fraczek et al. 2014).

Infectious, saprophytic or mixed bioaerosols have adverse effect on air quality and can cause infections not only in humans, animals and plants but also medicinal products, food products and may even cause bio-corrosion of building materials (Burkowska et al. 2011). Respirable fractions of bioaerosols are easy to disperse and can easily enter into the respiratory system even up to pulmonary alveoli and hence they are very important concerning the study (epidemiology) of infectious diseases (Kazmierczuk et al. 2014).

Elevated level of air pollutants can lead to decreased functioning of lungs and can cause respiratory symptoms like cough, shortness of breath, wheezing and asthma attacks, chronic obstructive pulmonary disease, cardiovascular diseases and lung cancer (WHO, 2002). Almost 80 genera of fungi are associated with symptoms of respiratory tract allergies (Horner et al. 1995). *Cladosporium, Alternaria, Aspergillus, Fusarium* are the most common allergy causing genera of fungi. Volatile products secreted by them can cause sensory irritation in eyes and upper respiratory tract (Allard et al.1994). *Aspergillus* species that can grow indoors, including *Aspergillus fumigates* and *Aspergillus flavus* which in turn can cause nosocomial infections (Robert and Sherertz, 1987), Allergic Broncho-Pulmonary Aspergillosis (ABPA) and Sinusitis. 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) and microbial contaminants in grain dust (Edward et al. 1970). High dose of inhalation of endotoxins lead to Organic Dust Toxic Syndrome; fungal spores and mycotoxins causes Chronic Obstructive Pulmonary Disease (COPD) (Aven, 1989).

Fungal exposures may also be responsible for non-specific building related symptoms (BRS) (Harrison et al. 1992). BRS refers to symptoms that cannot be associated with an identifiable cause but they appear to be building related, including headache, irritation of the eyes, nose and throat irritation, lethargy, nausea, dizziness, and chest tightness (WHO, 1983; ACGIH, 1989).

Bacterial bioaerosols are also responsible for many diseases. Examples include, *Bordetella pertussis* causes whooping cough, which is also recognized as prolonged cough illness (Senzilet

et al. 2001). Meningitis is caused by gram negative bacteria *Neisseria meningitides* (Stephens et al. 2007). Diphtheria is caused by *Cornibacteriumulcerans* (Wagner et al. 2010). Pneumonia is a condition of inflammation of lungs caused by gram positive bacteria *Streptococcus pneumonia* (Tettelin et al. 2001). Tuberculosis is a lung disease caused by *Mycobacterium tuberculosis* (Cole et al. 1998). It is more common when there is a contact with the source, in crowded places and in urban residence (Lienhardt, 2001). All these bacteria are airborne in nature and spread the respective diseases through air.

Microbiological air quality is highly affected by the presence of objects like municipal landfill sites, sewage treatment plants, animal farms and composting plants. Their impact on surrounding environment and the degree of atmospheric contamination may differ depending upon how they are being utilized site (Burkowska et al. 2011). Even if the landfill sites are well protected, besides their positive role on our environment, they have negative impact on public health. They are the source of a variety of air contaminants like chemicals, bioaerosols and odours. Odour arises due to the decomposition process and secretion of biogas that occurs there. Physical and chemical contamination in air for example NO, CO, heavy metals, hydrocarbons, takes place due to site functioning and the contamination is further enhanced by the lorries which carry waste to the site (Burkowska et al. 2011).

Most important source of bioaerosols near the landfill sites is municipal waste that includes, remains of food materials, personal hygiene materials, cleaning agents, faeces of domestic animals. Another source of microorganisms includes dehydrated sewage sediments (Burkowska et al. 2011).

Bioaerosols may be emitted during different steps of waste management like transportation, unloading of waste, sweeping, leveling, compressing, storage, sorting and dumping (Burkowska et al. 2011). The concentration of microorganisms in air is also affected by dust pollution. Factors that increase dust pollution in landfill sites such as waste delivery, compacting and covering of waste, can release bioaerosols in air with high microbial concentration (Burkowska et al. 2011). Concentration of microorganisms depends on the amount of organic waste present in landfill. Distribution of bioaerosols depends on the weather and the microclimatic conditions. Most common fungi present in landfill site are: *Aspergillus, Alternaria, Penicillium,*

Cladosporium, Fusarium, Rhizopus, Mucorand Trichoderma. Some opportunistic pathogen bioaerosols are also present in landfill sites that can cause diseases when the immunity system of host fails (Fraczek et al. 2014).

Municipal landfill site workers are more susceptible to respiratory diseases as compared to other people (Burkowska et al. 2011). Bioaerosols may cause various respiratory diseases in workers and also may have other health issues in neighbouring residents. A large fraction of bioaerosols isolated from landfill site is within a respirable size range that includes 80% of the fungi and the 40% of the bacteria (Rahkonen et al. 1990).

Wind is the most important factor for transporting microorganisms from landfill to the atmosphere. Survival of bioaerosols depends on many factors like their resistance, weather conditions, duration they will be residing in atmosphere and air pollution (Kazmierczuk et al. 2014).

Other than air pollution, landfills also cause a major pollution problem that is landfill leachate. Leachate is dark colored liquid residue resulting from various biological, physical and chemical processes taking place within the landfill. It is generated as a result of precipitation; ground water intrusion and surface run off percolating through a landfill and inherent water content of waste themselves along with biochemical processes. It may contain a large amount of ammonia-nitrogen, organic matter, heavy metals and organic and inorganic salts. Leachate is a great threat to surrounding soil and water resources (Bhalla et al. 2012).

Bioaerosol sampling device is based on three different principles namely impaction, impingement and filteration. Each of these principles is described below (Mandal et al. 2011):

a) Impactors: Impaction samplers are based on collecting bioaerosols on solid media such as agar. Its major advantage is that it is cheap and easy to handle (Zollinger et al. 2006). For example, Anderson sampler and Rotorod sampler.
b) Impingers: Impinger samplers are based on drawing air by suction through a narrow tube into flask containing collection liquid media. Generally used impinger sampler is "Bio-Sampler" liquid impinger. The AGI-30 sampler is also popular. It is cheaper but less efficient in sampling bioaerosols (Lin and Li, 1999).

c) Suction sampler: Particle collection by suction sampler is based on the suction of certain volume of air with a known velocity and for an opted duration. Burkard personal slide sampler, Burkard seven day volumetric sampler, Hirst automatic volumetric sampler, Burkard Petriplate sampler are few examples of suction sampler based on suction principle (Hirst, 1952).

d) Filtration samplers: This method is suitable for smaller aerosol particles and also where ambient velocities are very low. In this method, particles are trapped by suction filters of definite pore mesh size. Filtration samplers are less convenient than impactors and also they may cause dehydration stress in trapped bioaerosols and dehydration prevents from determining colony forming units (CFU), but molecular analysis techniques can be used to overcome this problem (Yao and Mainelis, 2007).

Both the number and the size of the particles can be determined by Laser particle counters. Determination of particle size is based on optical particle counting by light scattering, reflection, refraction and diffraction from single particles flowing out of a nozzle (Mandal et al. 2011).

To determine the presence of bioaerosols and perform their characterization many analytical techniques have been developed. Following two approaches can be used for enumeration of bioaerosols:

1. Cultivation approach: In this method aerobic microbes are collected on agar plate and then cultured under particular conditions. Cultivation approach includes several identification techniques (Mandal et al. 2011):
    - Microscopy: In this method, the spores from the cultured fungus are transferred on glass slides and then examined under microscope after staining with suitable dye. Fungus is identified by the special morphological features of the spore of a particular fungus.
    - Plate counts: This method involves collection of micro-organisms from the environment on an agar plate and then cultured under particular conditions. After incubation period, distinct colonies of microorganisms present on nutrient media are counted and are expressed as colony forming units (CFU). This method is simple, easy to use and relatively cheap. However, this method can only be

applied for those microorganisms that are viable and culturable. Only 10% microbes of all microorganisms are culturable (Torsvik et al., 1994). Further, this method is not representative of all the microorganisms contributing to bioaerosols. For example, thermophilic microorganisms such as Thermoactinomyces sp. and Saccharopolyspora sp. prefer culture temperature over 50 °C.

2. Non cultivation approach: This includes the following identification techniques (Mandal et al. 2011).
    - Raman Spectroscopy: This technique is based on inelastic scattering of monochromatic light. A typical vibrational "fingerprint" is observed depending on chemical composition of cell (Cabredo et al. 2009).
    - Polymerase chain reaction (PCR): By this method, amplification of specific regions of genome is done. This method is used for detection and identification of non-culturable microbes (Dungan and Leytem, 2009).
    - Fluorescence in situ hybridization: In this method, airborne microbes are detected by specific molecular probes that are bound to ribosomal RNA of the intact cell (Mandal et al. 2011).

The last 3-4 decades have been characterized by significant increase of the world wide scientific database on bioaerosols. Inspite of tremendous scientific progress, which has been taking place mainly in the 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 awaits a serious look due to the relatively narrow and insufficient experiments.

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 cooperation with the Department of Occupational Biohazards at the Institute of Agricultural Medicine, Lubin, as a first research unit initiated comprehensive measurements in dwelling (Gorny et al. 1998). Effect of landfill sites on the aerosol concentration in atmosphere and its impact on the surrounding environment has also been carried out in developed countries. Rahkonen et al. (1987) analyzed airborne dust at five sanitary landfills to determine bioaerosol, total dust and heavy metal concentration. Landfill workers,

contacted to bioaerosols and dust exposure were also studied. It was found that bioaerosols concentration was maximum in summer in warm windy weather. It was also suggested that that landfill workers should try to work upwind (Rahkonen et al. 1987).

A wide 3-yr study in southern Taiwan in closed municipal landfill site was done to analyze seasonal distribution of bioaerosols by Huang et al. (2001). In that study, it was found that levels of bioaersols (bacteria and fungi) were all above 1000 CFU/m^3. The bioaerosol concentration was higher in winter than in other seasons. According to the study, this might be because of geographic characteristics of that area and also the absolute water content in the air was low resulting in higher concentration of fungi: *Cladosporium* and *Alternaria.*

Danuta et al (2004) evaluated the influence of municipal landfills on the microbiological air quality in offices on landfill sites. It was found throughout the study that both indoor and outdoor air was highly contaminated with bioaerosols (bacteria and fungi). The microbial concentration was higher in summer than in cold autumn. The respirable fraction was found to be lower in summer season.

Kalwasinska et al. (2013), analyzed the air quality in work place of municipal landfill site in Torun. Sampels were collected in both the indoor and outdoor spaces using impaction method. Highest fungal concentration was found outdoor sites. It was found that microbial concentration in the outdoor air depended on the season ($p < 0.05$) but did not depend on site. For indoor air, concentration was depended on the sampling site ($p < 0.05$) but did not depended on season.

Statistical analysis of bioaerosol concentration in Tarnow landfill site was done by Fraczek et al. (2014). In this study, it was found that there is a significant difference in the bioaerosol concentration determined at different sampling sites located at different distances from the landfill. It was because; bioaerosols rose up with air to upper layers of atmosphere and start sedimenting after travelling some distance.

Significantly less work related to bioaerosols has been done in India. The impact of bioaerosols on the organic materials stored and present in the food grain godowns, library buildings and bakeries has earlier been done in Gwalior (Jain, 2000). In Delhi, viable bioaerosol assessment was carried out within the campus of Jawaharlal Nehru University. This study identified that

fungal bioaerosols are associated with immunotoxin diseases such as sick building syndrome in respirable fraction (Srivastava et al. 2011). Influence of seasonal variation on fungal bioaerosol concentration was analyzed at sewage treatment plant in Delhi by Maharia et al. (2014). In that study, maximum fungal concentration was found in pre monsoon season while minimum in summer season.

Even though landfill sites are a major source of bioaerosols, but no detailed work have been done so far in India to understand the bioaerosol level at these sites. In this work, we carry out a detailed characterization of bioaerosols in and around landfill sites of Delhi. To the best of our knowledge, this is the first scientific study to characterize bioaerosols in landfill sites of Delhi. The present study has been carried out with the following objectives:

1. To estimate the concentration of fungal bioaerosols in different six size ranges.
2. To find out the relationship between the different sized fungal bioaerosols and the meteorological parameters.
3. To check the seasonal variation of fungal bioaerosols in landfill site.
4. To identify and characterize the different types of collected toxic fungi.

MATERIAL AND METHODS

2.1 Study Area

Fungal bioaerosol sampling was done in New Delhi, the capital city of India. This city lies 160 km south of Himalayas at an altitude of 216 m above mean sea level and at latitude of 28º25΄ and longitude of 76º50΄. It is in the close vicinity of Thar Desert (Rajasthan) in the west and hot plains of central India. Delhi is a speedily growing city, with its area extending over 1,483 km^2. Its population has readly increased from 3.5 million in 1970 to over 16 million presently (Census of India 2011). The climate of Delhi is subtropical with moderately cold winters and hot summers. The monthly mean temperature varies between 14.3ºC (minimum 2ºC) in January, which is the coldest month and about 34.5ºC (maximum 48ºC) in June, the hottest month (Balachandran et al, 2000).

2.2 Sampling Sites

Sampling for the presented research was done at the Okhla Landfill site in Delhi. This site is a controlled open dump. It is operated by the Municipal Corporation of Delhi. Okhla sanitary landfill site was commissioned in the year1996. It has a capacity of processing 2000 metric ton of waste per day. 700 metric ton of this garbage is from Najafgarh area and rest of the waste is daily building rubbish and ash from Waste-Energy plants. The waste is delivered by approximately 300 trucks per day and is handled by roughly 100 workers employed at the site. Landfill area is about 32 Acre. The landfill site is surrounded by urban area. There is a printing factory and residential area to the north, an Inland Container Depot (ICD) is present in the east. Green belt is present in west and south of Okhla landfill.

Seven sites were identified for sampling of fungal component of bioaerosols. Four sampling sites lie within the landfill premises while three other lie in close vicinity of the landfill. Sampling sites selection was based on a previous on-site survey. All the seven sites have been illustrated in figure 2.1.

Figure 2.1 (a): Satellite image of sampling sites in landfill

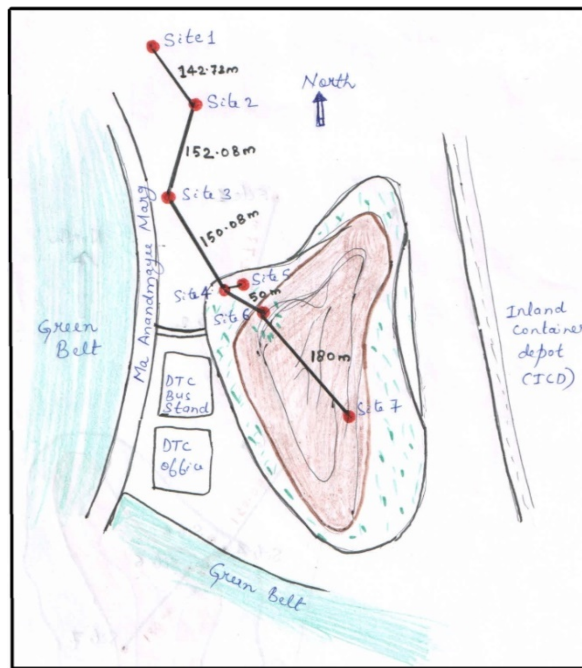


Figure 2.1 (b): Diagrammatic representation of distance between each sampling site

The detailed description of the sites is as follows:

2.2.1 SITE 1

Site 1 is residential as well as factory area. It is farthest from landfill and is situated in the northern side of landfill.

2.2.2 SITE 2

Site 2 is also a residential and factory area and is at about 142 m in distance from Site 1 towards landfill. It is also situated in northern side of landfill.

2.2.3 SITE 3

Site 3 is situated near an Employee State Insurance Hospital (ESI). It is at about 152 m in distance form Site 2 towards landfill.

2.2.4 SITE 4

Site 4 is at about 150 m in distance from site 3. It is inside the landfill office room. It is closed room with no windows, one door and attached bathroom. Its environment is affected by both outdoor as well indoor (due to the presence of bathroom and number of people working inside) environments.

2.2.5 SITE 5

Site 5 is situated outside the landfill office.

2.2.6 SITE 6

Site 6 is at about 50 m in distance from site 4. It is situated near leachate drainage system. It is an open area with sporadic plants.

2.2.7 SITE 7

Site 7 is at a distance of about 180 m from site 4. It is present near the top of landfill. More than 300 trucks per day come to this site for dumping garbage. Because of these trucks, there is a very high level of dust in the surrounding air. There are no trees near this sampling site.

2.3 Bioaerosol Sampling

Fungal bioaerosol sampling was done with the help of Anderson six stage viable cascade impacter, manufactured by Tisch Environmental USA (figure 2.2). Bioaerosol (Fungi) sampling was done from July 2015 to May 2016. This sampling duration covers almost all five seasons of Delhi i.e. Monsoon (July-September), Autumn (October- November), Winter (December-January), Spring (February- March) and Summer (April-June). Timings of sampling were between 9 to 11 am. Sampling was done for 2 minutes in each site. For measurement, samplers were kept, 1.2 -1.5 m above the ground to parallel the human breathing zone (Huang et al. 2002).

Figure 2.2: Six stage Cascade impactor

The sampler, a Cascade impactor consists of a stack of impaction stages. Each stage involves one or more nozzles and substrate. The air stream passes through the nozzles and particles bigger than a particular aerodynamic size would be impacted onto a collection agar media while smaller particles get through the stages. The nozzles may take the form of holes. The substrate consists

of a growth media contained in petri dishes. Each following stage collects smaller particles. The details of nozzle diameter and particle diameter for six stage impactor are given below in table 2.1 (Thermo Scientific, Instruction Manual, 2009):

Table 2.1: The jet orifice dimension

Stage	Orifice diameter	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

Figure 2.3: Six stages of Anderson Sampler

The six stage viable cascade impactor consists of six aluminium stages that are supported by three spring clamps and sealed with O-ring gaskets. Each impactor stage involves multiple drilled orifices. As air is drawn through the sampler, multiple jets of air in every stage direct air borne microbes toward the agar containing petridish in the stage. The jet orifices size is uniform

within each stage, but is smaller in following stage. The range of airborne particle sizes collected on its stage depends on jet velocity of the stage and cut off of the antecedent 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 involves 400 orifices with diameter ranging from 1.81 mm on the first stage to 0.25 mm on the sixth stage (Thermo Scientific, Instruction Manual, 2009).

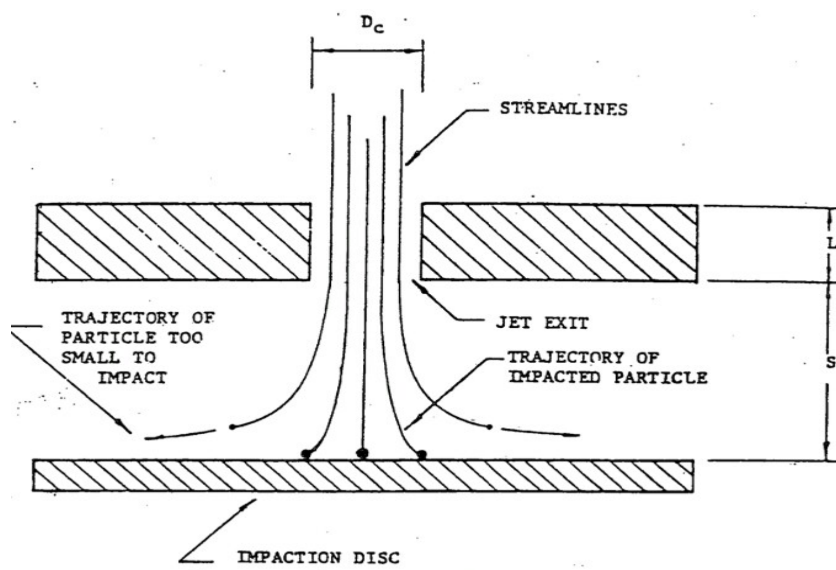


Figure 2.4: Schematic of impactor stage (Thermo Scientific, Instruction Manual, 2009)

Design of the Tisch Viable sampler is based on the human respiratory system tract which is an aerodynamic system of classification of airborne particles. This sampling device can reproduce the lung penetration by airborne particles and can be used as a substitute for the respiratory tract as a collector of bioaerosols. The physical properties (size, shape, density) of inhaled particles determine their fraction retained in the respiratory system and their site of deposition. Using a standard model of sampler, the stage distribution of the collected material will indicate the extent to which the airborne particle would have penetrated the respiratory system. Particles of sub-micrometer size deposit primarily in the pulmonary area whereas larger particles deposit mainly in the nasal area (Thermo Scientific, Instruction Manual, 2009).

Many small round jets provide a sharper cutoff of particle size in each stage of inertial impactor, improving impaction efficiency. The Anderson six stage viable sampler has 400 small round jets per stage and therefore can enable efficient collection of bioaerosols (Thermo Scientific, Instruction Manual, 2009).

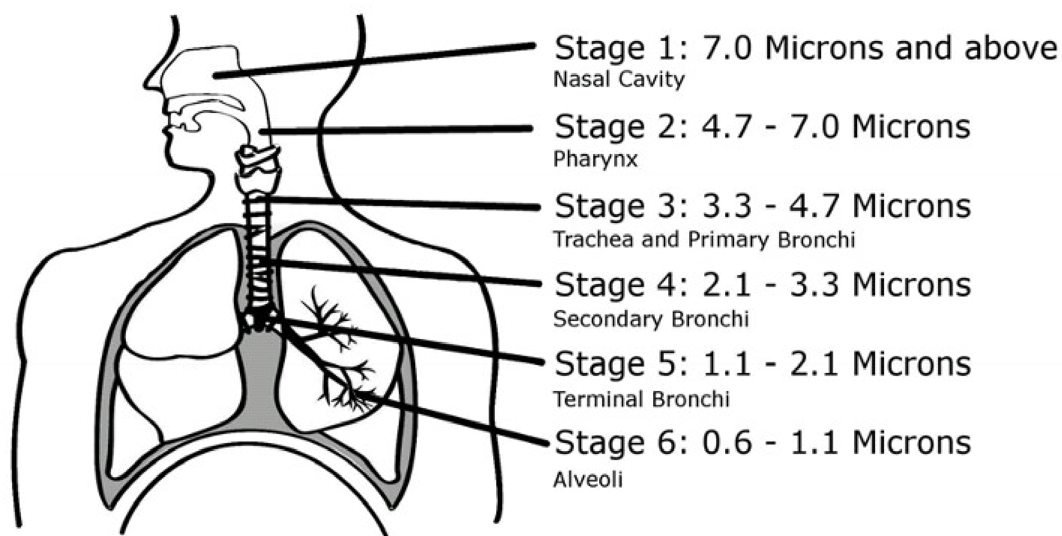


Figure 2.5: Bioaerosol sampler stimulating the human respiratory system (Thermo Scientific, Instruction Manual, 2009)

2.3.1 Sample Consideration

2.3.1.1 SAFETY

To prevent the spread of potential human pathogens to the workers or to the work environment, sampler, culture plates, equipment etc. were handled aseptically and most surfaces including hands, laminar flow etc. were properly sterilized. However, not all objects could be sterilized. Disinfection with an oxidizing chemical or alcohol destroys most vegetative cells, these sterilizing agents do not destroy all spores. The sampler was sterilized after each sample collection. Special care was given to sampler with convoluted inlets or air pathways where microorganisms may accumulate. Pre-autoclaved media and glassware were used in the experiments.

2.3.1.2 MONITORING OF METEREOLOGICAL PARAMETERS

Survival and growth of bioaerosols strongly depends on the meteorological conditions, therefore various meteorological parameters (such as temperature, relative humidity and wind velocity) were monitored during the sampling using ENVIRO-METER (Fisher Scientific).

2.3.2 CULTURE MEDIA

For detection and enumeration, fungal fraction of bioaerosol was collected over Potato dextrose agar media. Potato dextrose agar is common microbiological media made from potato infusion, and dextrose (corn sugar). Potato infusion and carbohydrate promote the growth of fungi while low pH and antibiotic present inhibit the growth of bacteria.

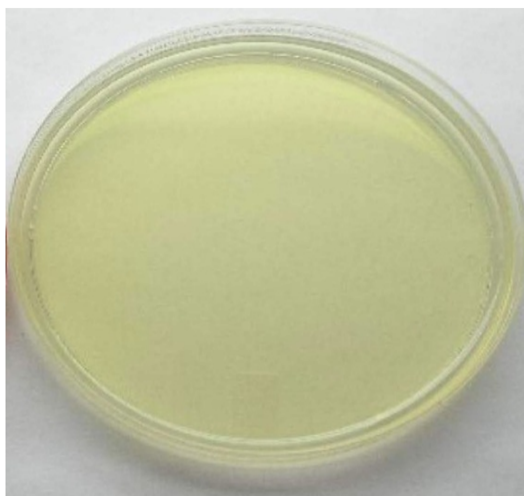


Figure 2.6: Potato dextrose agar media

To ensure that our work environment and processes completely clean and devoid of any kind of microbial contaminants, 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 into the field.

2.4 Bioaerosol Characterization

2.4.1 SAMPLE PREPARATION

Inoculated agar plates were incubated at 25^{0}C for three to five days.

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.

$$Bioaerosol\ Conc. \left(\frac{cfu}{m^3}\right) = \frac{No.of\ colonies}{Flow\ rate\ \times Sampling\ duration\ (min)}$$

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

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 is ready for microscopic examination and visual identification. Identification was done comparing the fungal spores of the samples with the existing results viz. published papers, available literature and images available on the internet.

2.4.4 STATISTICAL ANALYSIS

Statistical analysis was carried out with the help of Microsoft Office Excel 2007 and SPSS 16.

RESULT AND DISCUSSION

Results obtained from bioaerosol samples collected at various sites in different seasons at Okhla landfill sites were analyzed for size distribution, seasonal variability, and correlation between different sizes and with the meteorological parameters. Apart from that, Analysis of Variances (ANOVA) was also applied to understand the variability in the mean fungal concentration based on sites and sizes as well as the microscopic identification of fungus bioaerosols was done. The findings are reported and discussed as follows:

3.1 Size segregated distribution of fungal Bioaerosols in different seasons

3.1.1 Monsoon Season

In monsoon season, airborne fungal concentration at each site in different stages had been found to follow a typical pattern with few exceptions. As seen in figure 3.1, there is a decrease in concentration from stage 1 to stage 3; while a significant increase is observed from stage 4 to stage 6 as seen in sites 1, 4, 5 and 7. Highest concentration of fungal bioaerosols was found at Site 1 and Site 7 in stage1 (>7.0 μm) while at site 2, 3, 4, 5 and 6 the highest concentration was found in stage 6 (0.65 μm -1.1 μm).

Bioaerosols in the size range of 0.65 μm -1.1 μm can reach up to the alveoli of lungs and therefore such a high concentration of fungal bioaerosols of this size range possess serious threat to the health of people working in landfill as well as those inhabiting in nearby residential areas.

Highest concentration of total fungal bioaerosol was found in Site 2 (1101 cfu/m^3), followed by site 1 (986 cfu/m^3) and site 7 (875 cfu/m^3) and lowest bioaerosol concentration was found at Site 6 (533 cfu/m^3). Site 1 and 2 are surrounded by residential area, so there might be probability that these sites have their own source of bioaerosol in addition to those coming from landfill.

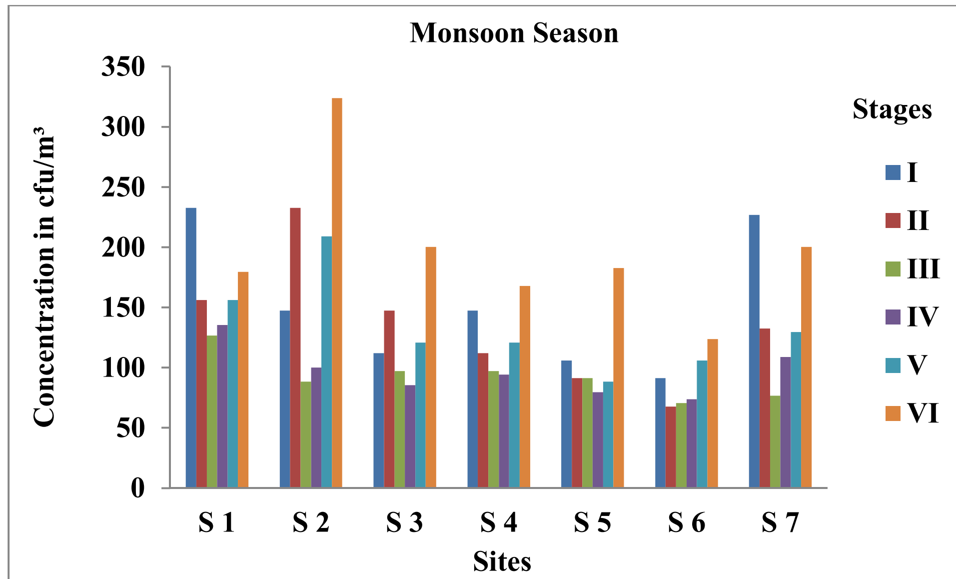


Figure 3.1: Total fungal concentration in each stage at all the sites in monsoon season

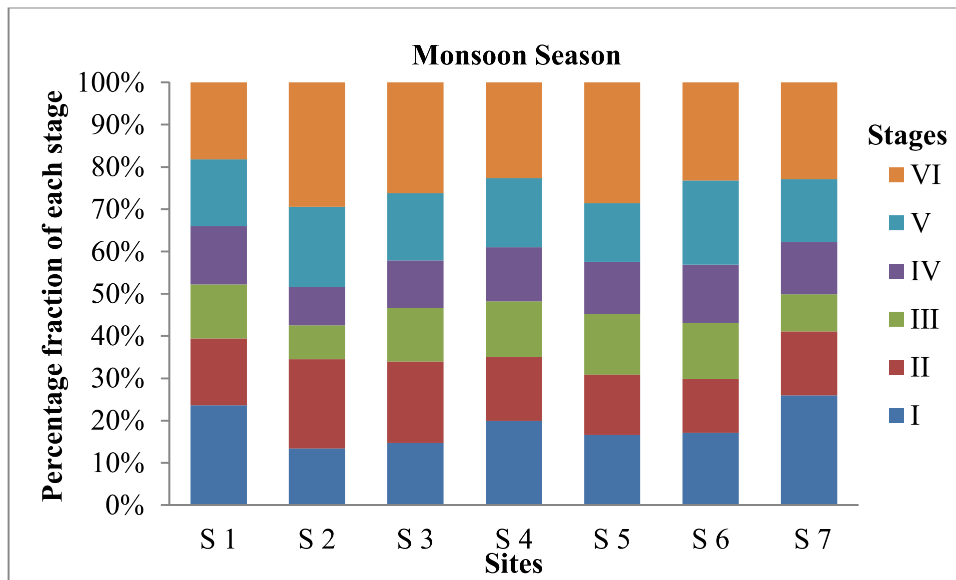


Figure 3.2: Particle size distributions of airborne fungi at each site in monsoon season

3.1.2 Autumn Season

In autumn season, no typical pattern is observed in the concentration of fungal bioaerosols at all the sites in different stages. As observed in figure 3.3, highest fungal concentration was found in stage 4 at Site 1; in stage 5 at Site2; in stage 6 at Sites 3, 4 and 5; in stage 1 at Site 7; in stages 2 and 6 at Site 6. Lowest fungal concentration was found in stage 5 at Site 1; in stage 3 at Sites 2 and 3; in stage 2 at Sites 4 and 7; in stage 5 at Site 5; in stage 4 at Site 6.

Total airborne fungal concentration was highest at Site 2 (577 cfu/m^3), followed by Site 7 and 1 (483 cfu/m^3) and lowest at Site 6 (312 cfu/m^3).

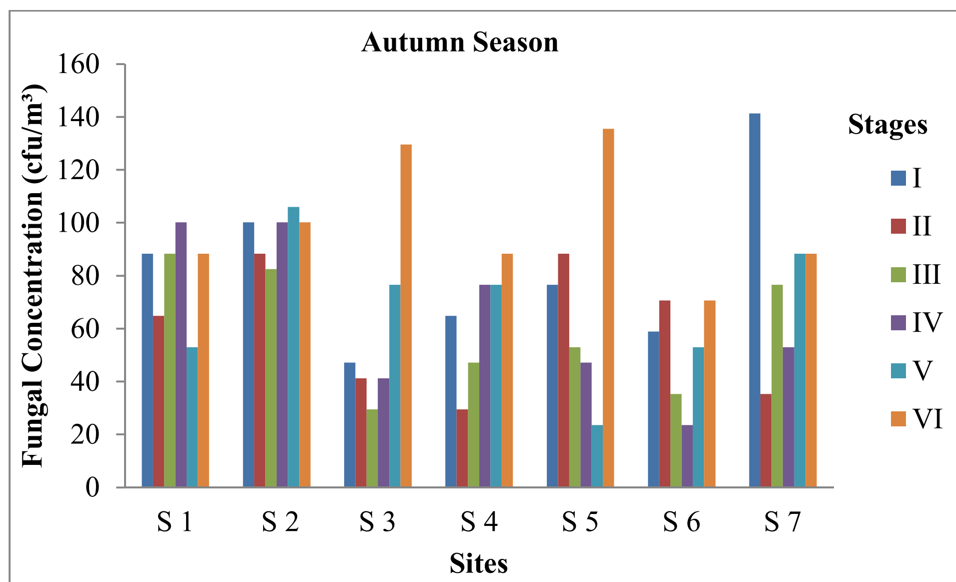


Figure 3.3: Total fungal concentration in each stage at all the sites in autumn season

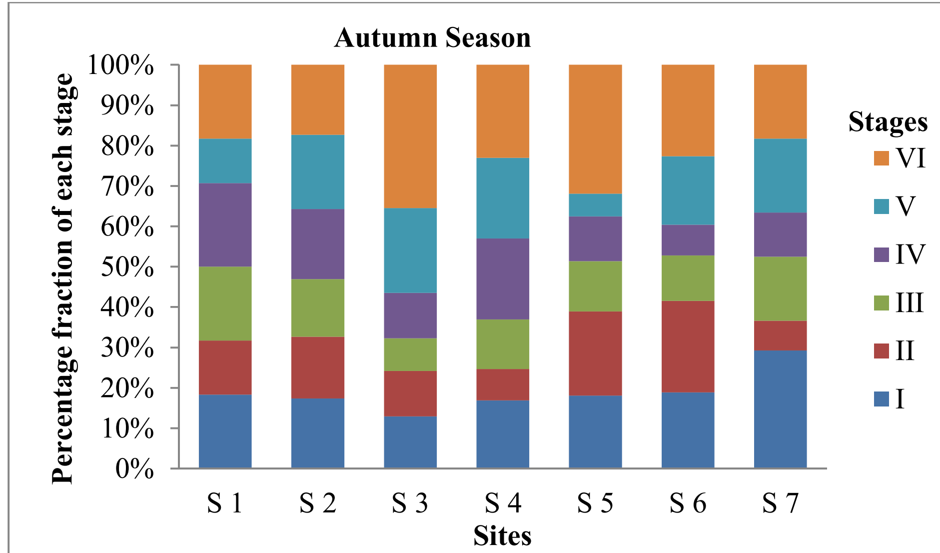


Figure 3.4: Particle size distributions of airborne fungi at each site in autumn season

3.1.3 Winter Season

Fungal concentration at each site in all the different stages did not follow a typical pattern in winter season. As seen in figure 3.5, maximum concentration was found in stage 5 at Site 1, in stage 6 at Sites 2, 3, 6 and7 while, at Site 4, it was found to be in stage 3 and at Site 5 in stage 1. Lowest fungal concentration was found in stage 3 at Sites 1 and 2; in stage 4 at Sites 3, 4 and 7; in stage 5 at Site 5.

Highest total fungal concentration was found at Site 1 (768 cfu/m^3), followed by Site 2 and 7 (521 cfu/m^3); while lowest concentration was found at Site 5 (221 cfu/m^3). Site 1 is surrounded by residential area, thus, this site has its own source of bioaerosols in addition to those coming from the landfill.

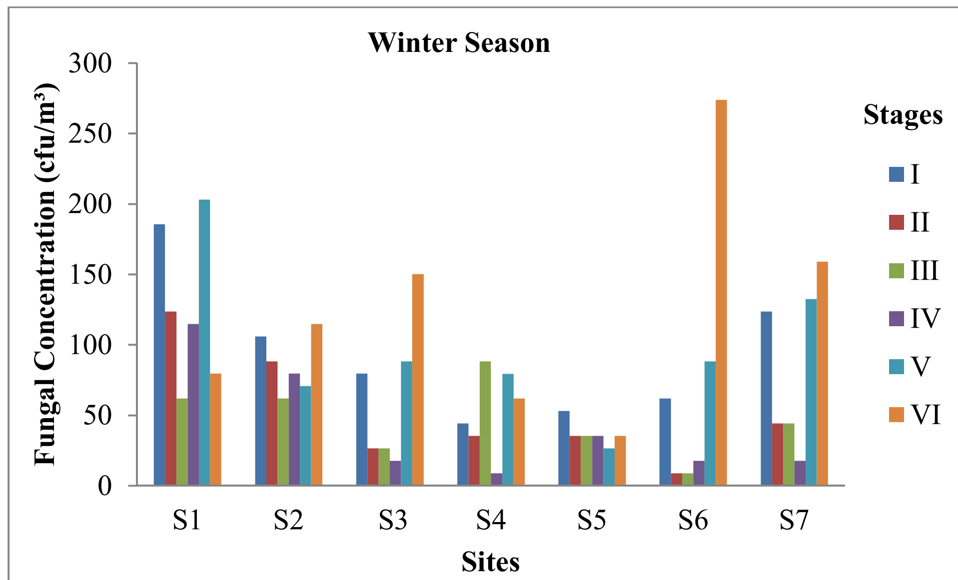


Figure 3.5: Total fungal concentration in each stage at all the sites in winter season

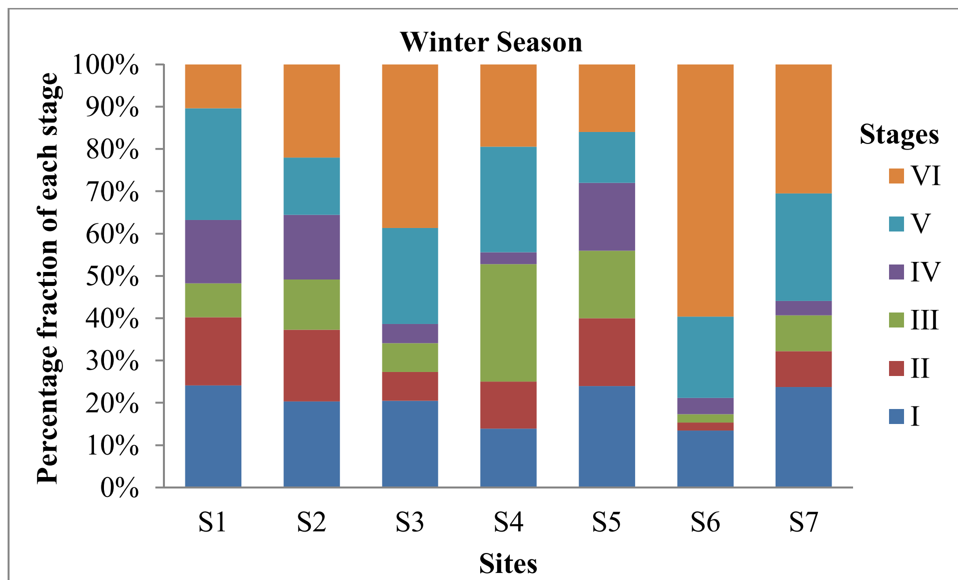


Figure 3.6: Particle size distributions of airborne fungi at each site in autumn season

3.1.4 Spring Season

In spring season, concentration of fungal bioaerosols at each site in different stages had not been found to follow any typical pattern. As seen in figure 3.7, at most of the sites highest fungal bioaerosol concentration was found in stage 6 (0.65 µm -1.1 µm) except at sites 2 and 3, where maximum concentration was found in stage 5 (1.1 µm-2.1 µm). Minimum fungal bioaerosol concentration was found in stage 6 at Site 2; in stage 4 at site 5; in stage 3 at Site 7 while at Sites 1, 2 and 3 lowest airborne fungal concentration was found in stage 2.

Highest total fungal concentration was found at site 3 (836 cfu/m^3), followed by Site 4 (636 cfu/m^3) and Site 7 (548 cfu/m^3). Site 3 is near a hospital, Site 4 is inside the landfill office and site 7 is on the top of landfill. Lowest total fungal concentration was found at Site 5 (294 cfu/m^3), which is outside the office.

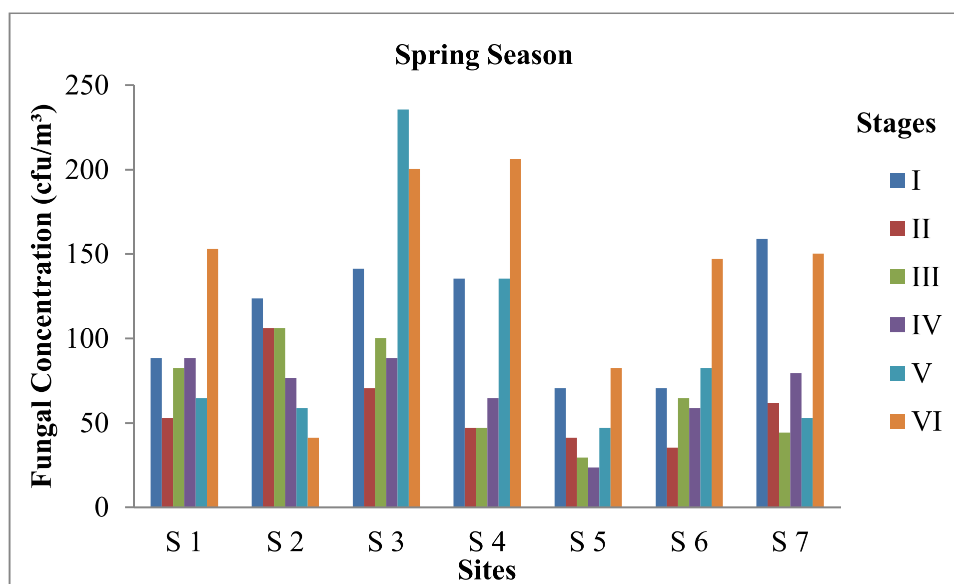


Figure 3.7: Total fungal concentration in each stage at all the sites in spring season

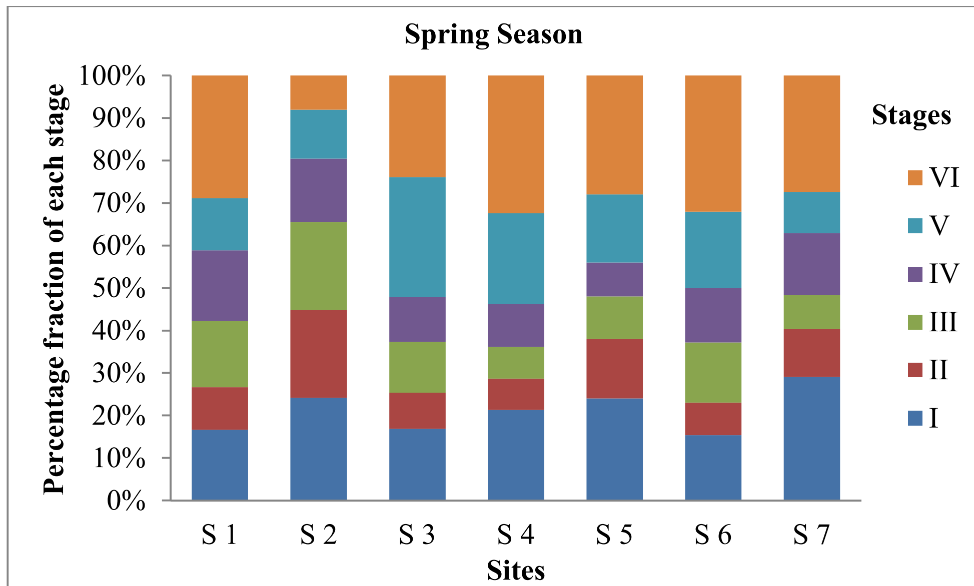


Figure 3.8: Particle size distributions of airborne fungi at each site in spring season

3.1.5 Summer Season

In summer, fungal bioaerosols did not follow any pattern at all. Maximum concentration was found in stage 1 at Sites 3, 4, 5 and 7; in stage 6 at Sites 1 and 2; in stage 5 at Site 6. Minimum concentration was found in stage 4 at Sites 1, 2, 6 and 7; in stage 5 at Sites 3, 4 and 5 (Figure 3.9 and 3.10).

As seen in figure 3.9, highest bioaerosol concentration was found in site 4 (1237 cfu/m^3) followed by site 7 (1201 cfu/m^3). Site 4 is indoor site (landfill office). The probable reason of highest concentration in landfill office site was presence of both indoor and outdoor fungal bioaerosols. Important sources of indoor fungal bioaerosols were the attached bathroom and people working inside. Outdoor bioaerosol concentration from landfill also added to this value.

Minimum fungal concentration was found at Site 3 (536 cfu/m^3), which is near bus stop. Various chemical pollutants released by vehicles and these chemical pollutants might be the reason behind the low fungal concentration at Site 3. This might be because air pollutants such as ozone,

CO, SO_2, NO_X and PM_{10} are negatively correlated with fungal counts as reported by Glikson et al., (1995).

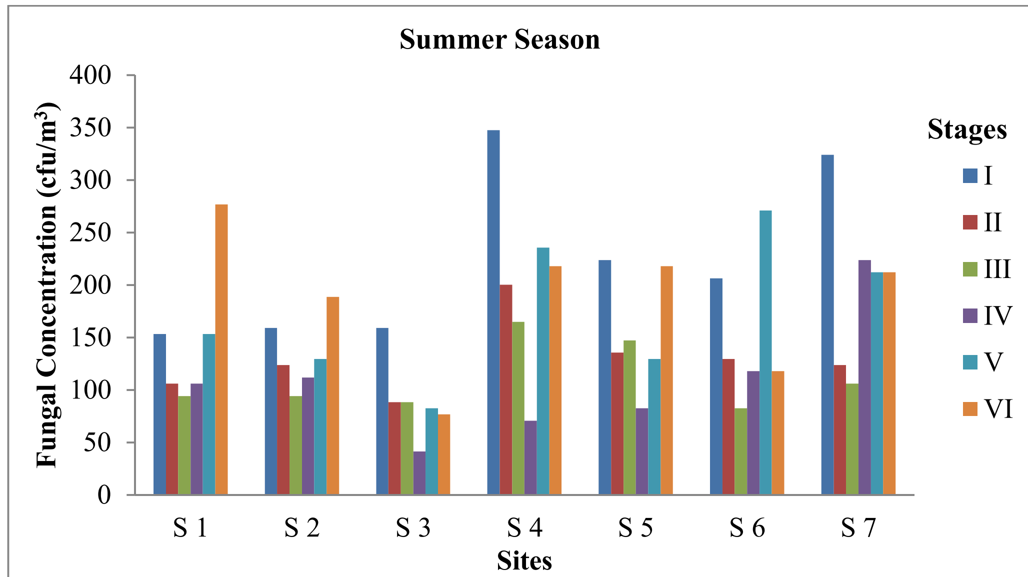


Figure3.9: Total fungal concentration in each stage at all the sites in summer season

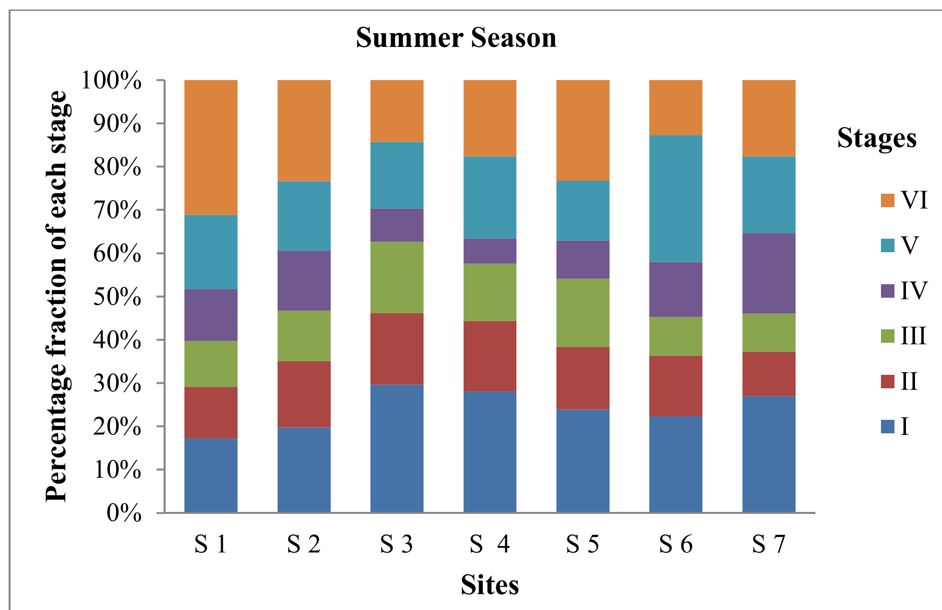


Figure 3.10: Particle size distributions of airborne fungi at each site in summer season

3.2 Seasonal variation of fungal Bioaerosol concentration at each site

Figure 3.11 and tables 3.1, 3.2 and 3.3, show the seasonal variation of fungal concentration at each site with respect to meteorological parameters. Highest fungal concentration (986 cfu/m^3 to 1101 cfu/m^3) was found at Site 1 and Site 2 in monsoon season at a temperature range of 35.5 to 36.6 ºC and wind speed of 1.4 to 1.9 m/sec. At Site 3, maximum fungal concentration (836 cfu/m^3) was at temperature 25.9 ºC and wind speed 0.6 m/sec. At Site 4, Site 5, Site 6 and Site 7 the highest airborne fungal concentration (925 cfu/m^3 to 1237 cfu/m^3) was found in summer at the temperature range of 33.7 ºC to 36.1ºC and wind speed range of 0 m/sec to 1.8 m/sec.

As seen in figure 3.11, high temperatures could cause increased dryness of substratum (in this case the heap of dump), thereby accelerating the probability of higher release of fungal spores and fragments in air. Additionally, in this study, the wind speed was also quite high enough to disperse off the microbial propagules, thereby leading to higher concentration in summer. In two sites, bioaerosol concentration was higher in monsoon season; this might be because of poor monsoon in the year 2015, though the numerical monsoon data is not officially available yet.

In Site 1, Site 3, Site 6 and Site 7, minimum fungal concentration (312 cfu/m^3 to 483 cfu/m^3) was found in autumn season at temperature range of 27.4 to 31.7 ºC and wind speed range of 0.2 to 0.8 m/sec. In Site 4 and Site 5, lowest fungal concentration was found in winter season at temperature range of 21.9 to 23 ºC and wind speed range of 0 to 0.2 m/sec. However, in Site 2, lowest airborne fungal concentration was found in spring season at temperature 24.3 ºC wind speed 0.3 m/sec. This might be due to the fact that lower temperature and lower wind speed does not favor microbal growth in winter season.

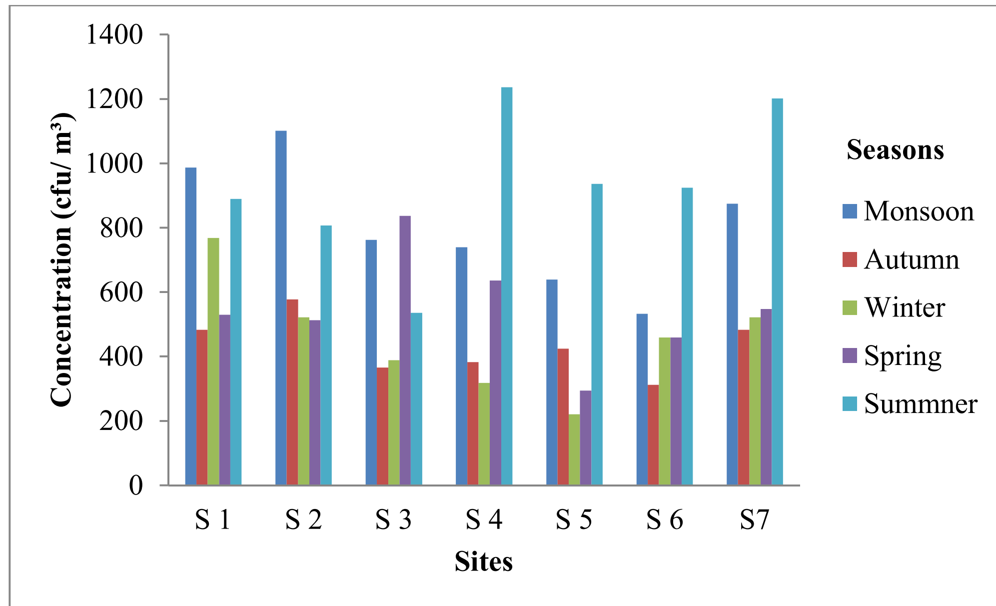


Figure 3.11: Comparison of total fungal concentration at different sites in different seasons

Table 3.1: Fungal Bioaerosol Distribution in relation to meteorological parameters in Site 1, Site 2 and Site 3

Sites / Seasons	Site 1				Site 2				Site 3			
	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)
Monsoon	36.5	49.9	1.4	986.46	35.5	50.5	1.9	1101.29	38.7	46.7	1.7	762.66
Autumn	27.36	48.83	0.2	482.92	26.77	50.83	0.5	577.15	29.2	44.37	0.3	365.14
Winter	17	60.2	0.1	768.55	19.1	53	0.4	521.2	21.2	46.6	0.1	388.69
Spring	23.4	48.8	0.3	530.04	24.3	48.7	0.3	512.37	25.9	44.9	0.6	836.28
Summer	31.9	37.2	1.5	889.28	32.8	35.9	1.1	806.83	33.6	33.6	0.7	535.92

Table 3.2: Fungal Bioaerosol Distribution in relation to meteorological parameters in Site 5, Site 6 and Site 7

Sites / Seasons	Site 5				Site 6				Site 7			
	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (Cfu/m^3)
Monsoon	38.4	46.6	1	638.98	37.6	44.6	1.3	532.97	38.2	44.3	1.5	874.56
Autumn	30.67	48.53	0.2	424.03	31.27	41.47	0.2	312.13	31.7	38.8	0.8	482.92
Winter	23	41.2	0.2	220.85	22.7	46.5	0.9	459.36	22	44.6	2.5	521.2
Spring	26.4	45.4	0.7	294.46	26.8	44	0.9	459.36	26.4	43.5	1.7	547.7
Summer	34.7	32.3	0.9	936.39	34.5	31.4	1.6	924.62	36.1	29.7	1.8	1201.41

Table 3.3: Fungal Bioaerosol Distribution in relation to meteorological parameters in Office Indoor Site (Site 4)

Site 4 / Seasons	Temp (°C)	R.H. (%)	W.S. (m/sec)	Conc. (cfu/m^3)
Monsoon	37.4	49.7	0	739.1
Autumn	29.5	47.53	0	382.8
Winter	21.9	54.5	0	318.02
Spring	27.1	46.8	0	636.04
Summer	33.7	34.1	0	1236.75

3.3 Respirable Fraction

Respirable fraction is defined as the fraction of bioaerosols less than 4.7 μm in aerodynamic diameter (Wang, 2011). Respirable fraction of bioaerosol is important to study as these sized particles can easily penetrate into the human trachea and bronchioles (Kim and Kim, 2007). By adding stages 3, 4, 5 and 6 of Anderson Sampler, we get concentration of fungal bioaerosols that comes under 4.7 μm in diameter.

$$R_f = \frac{C_3 + C_4 + C_5 + C_6}{C_f} \times 100\%$$

,where R_f denotes the respirable fraction; C_3, C_4, C_5 and C_6 are concentration of fungal bioaerosols in 3rd, 4th, 5th, and 6th stage of Anderson Sampler respectively;C_f is total concentration of fungal bioaerosols in all the stages of Anderson Sampler.

As seen in figure 3.12, at S 1 site respirable fraction was highest in spring season (73.3%) and lowest in winter season (59.77%) whereas at S 2 site it was highest in autumn season (67.36%) and lowest in spring season (55.17%). At S 3 and S 4 site R.F. was maximum in autumn season (75.83% and 75.32% respectively) and minimum in summer season (53.85% and 55.71% respectively). At S 5 site it was highest in monsoon season (69.13%) and lowest in winter season (60%). At S 6 site, respiratory fraction was maximum in winter season (84.62%) whereas minimum in autumn season (58.49%) while at S 7 site, it was highest in winter season (67.79%) and lowest in monsoon season (58.92%).

At three sites 2, 3 and 4, maximum R.F. was found in autumn season, at two sites 6 and 7, maximum R.F. was found in winter season. In monsoon and spring season R.F. was found to be maximum at site 5 and site 1 respectively. Therefore it can be concluded that in autumn and winter seasons, there are maximum probability of getting respiratory diseases in people working in landfill and living in nearby residential areas. Lower level of respirable fraction in summer is may be due to higher dust emission during the summer as the soil is dry and temperature is high with no rainfall (Danuta et al. 2004).

It can be seen that while considering all the sites and seasons, R.F. varies from 53.85% to 84.62%. It is very high value and thus can cause severe respiratory problems.

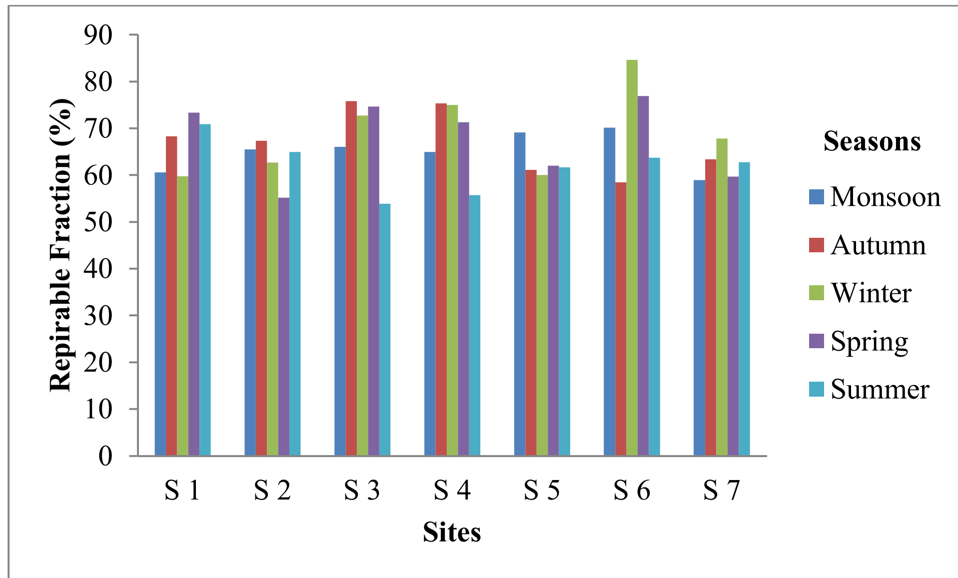


Figure 3.12: Respirable Fraction of fungal bioaerosols in all the sites during all the seasons

3.4 Regression Analysis

Regression is a statistical measure to determine the strength of relationship between one dependent variable and a series of changing independent variables. There are two basic types of regressions, linear regression and multiple regression. Linear regression uses one independent variable to predict the outcome of dependent variable whereas multiple regression analysis uses several independent variables predicting the dependent variable. In this study we have performed both linear regression as well as multiple regression.

Regression analyses were carried out between fungal concentration and meteorological parameters i.e. Temperature, Relative Humidity and Wind Speed. In this case dependent variable is fungal concentration and independent variables are temperature, relative humidity and wind speed.

Multiple regression was performed between fungal concentration and all the three meteorological parameters (temperature, relative humidity and wind speed). Table 3.4 shows the output of multiple regression analysis. R square is coefficient of determination. It is an indicator of how well the model fits the data. According to table 3.4, in our case R-square is 35%. This means that close to 35% of the variation in the concentration of fungal bioaerosols (dependent variable) is explained by meteorological parameters i.e. Temperature, Relative Humidity and Wind Speed (independent variables).

Table 3.4: Output of multiple regression analyses between fungal concentration and meteorological parameters

Regression Statistics	
Multiple R	0.59
R Square	0.35
Adjusted R Square	0.29
Standard Error	222.49
Observations	35.00

According to table 3.5, the significant value (p) = 0.003 < 0.05 (α), we conclude that the regression model is a significantly good fit; i.e. there is only a 0.36% possibility of getting a correlation this high (Multiple R = 0.59) assuming that the null hypothesis is true. Therefore we conclude that the effect of weather conditions (Temperature, Relative Humidity and Wind Speed) on the concentration of fungal bioaerosols is significant and cannot be neglected.

Table 3.5: Significance of multiple regression analyses

ANOVA

	Df	SS	MS	F	Significance F
Regression	3	826082.8	275360.9	5.56	0.003
Residual	31	1534493	49499.77		
Total	34	2360576			

Linear regression was carried out between bioaerosol concentration and meteorological parameter (temperature and relative humidity and wind speed) individually. As seen in fig. 3.13, 3.14 and 3.15, no good regression is found between fungal concentration and temperature (R^2 = 0.28), fungal concentration and relative humidity (R^2 = 0.13) and fungal concentration and wind speed (R^2 = 0.19) respectively, yet the effect of temperature, relative humidity and wind speed on the fungal bioaerosol concentration is significant as the significat values (p value) is less than 0.05 i.e. (0.001, 0.03 and 0.008 respectively).

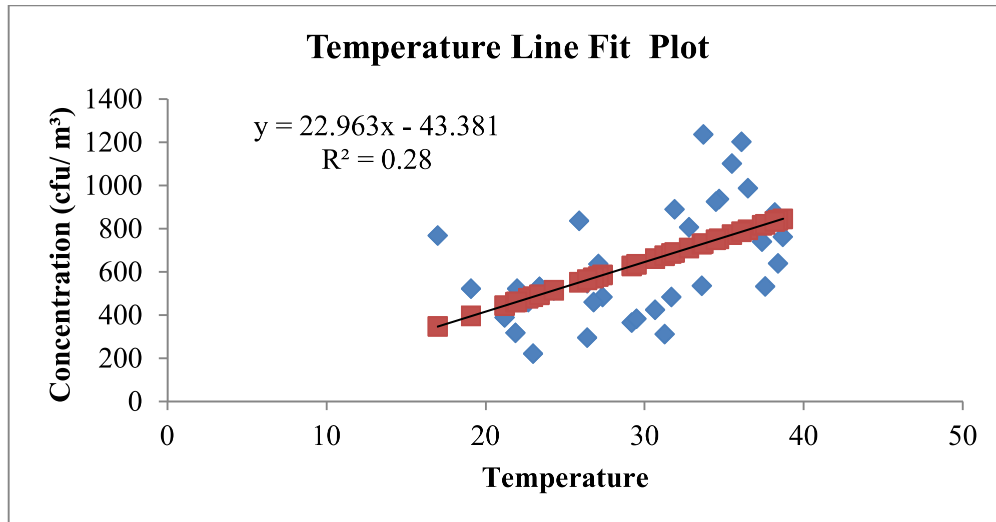


Figure 3.13: Relationship plot between fungal concentration and temperature

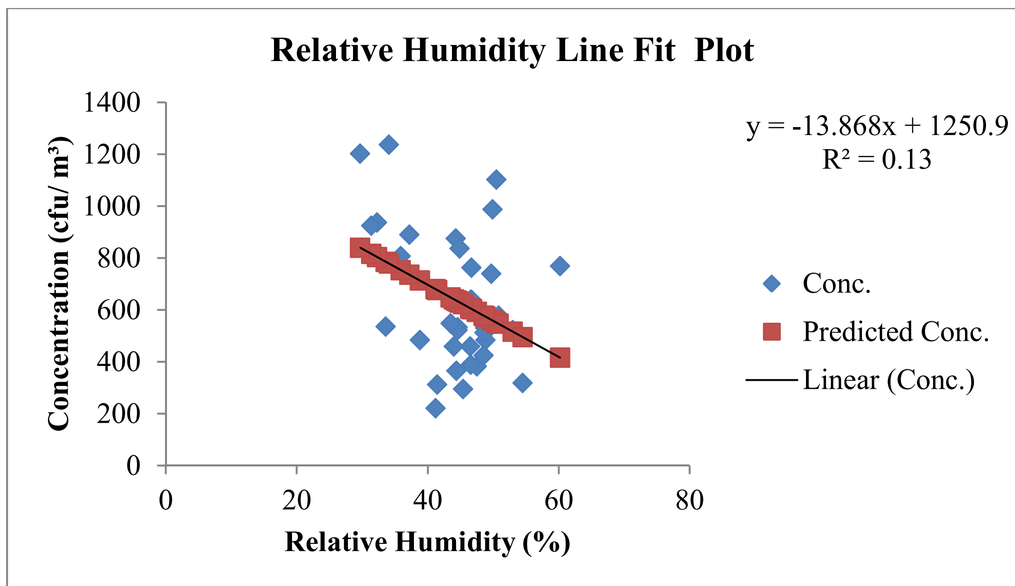


Figure 3.14: Relationship plot between fungal concentration and Relative Humidity (%)

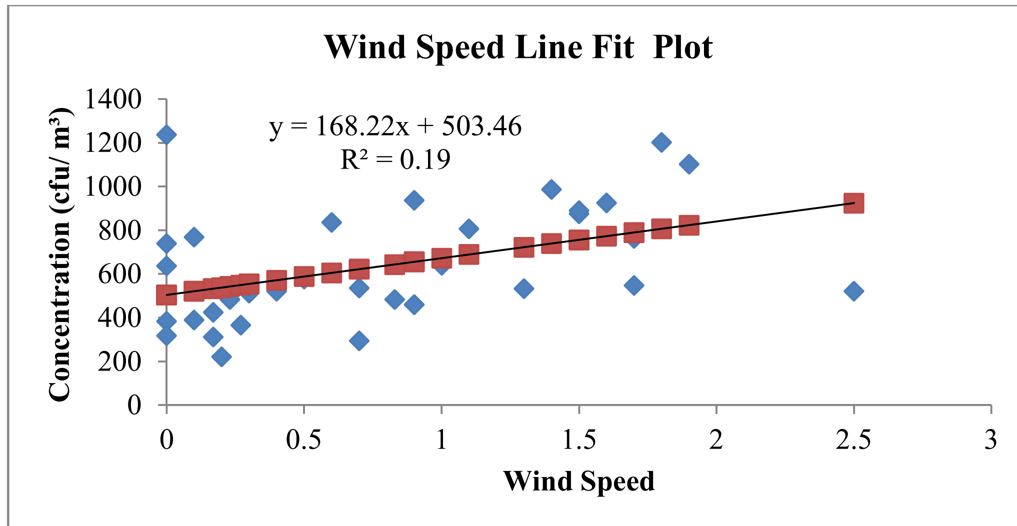


Figure 3.15: Relationship plot between fungal concentration and wind speed

When regression analyses were carried out, these four models were found to fit (Table 3.6). The best one was that which considered all the three meteorological parameters with a determination coefficient (R square) of 0.35.

Table 3.6: Results of regression analysis for four models, where T is temperature, R is relative humidity and W is wind speed

Models	R-square (p value)	Equations
T+R+W	0.35 (0.003)	226.04+16.75T-3.63R+96.91W
T	0.28 (0.001)	22.963T-43.381
W	0.19 (0.008)	168.22W+503.46
R	0.13 (0.03)	-13.868R+1250.9

Thus we can conclude that although temperature, relative humidity and wind speed in a combined way do not show good regression (R square=0.35) yet its effect is significant (p = 0.003) on the concentration of fungal bioaerosol. This poor regression is may be due to the presence of many other factors that affect bioaerosol concentration for example presence of human beings, stray animals in the sites, bioaerosols emitted from the factories surrounding our

sites and atmospheric conditions like radiations, chemical composition of air (chemical pollutants).

3.5 Correlation Analysis

Correlation is used to test the degree of association between variables. Pearson correlation coefficient measures the strength of the linear association between two variables.

3.5.1 Correlation between different size fractions of fungal bioaerosols over the complete year

Correlation was carried out between fungal concentration of every stage and between each stage over the complete year. Table 3.7 shows the correlation matrix between different size fractions for fungal bioaerosol. According to table 4.1, there is a strong relationship between all the different stages, except stage 6 which is only correlated with stage 5. Strong relationship means that changes in one variable are strongly correlated with changes in the second variable.

All Pearson's r values are positive. It means there is positive correlation among different stages. As one variable (bioaerosol concentration in one stage) increases in value, the second variable (bioaerosol concentration in the second stage) also increases in value. Similarly, as one variable decreases in value, the second variable also decreases in value.

For statistically significant correlation, significant (2-tailed) value i.e. p should be less than 0.05 ($p < 0.05$). Therefore all stages are statistically significant correlated with each other except stage 6 with stage 4. That means increases or decreases in one variable do significantly relate to increases or decreases in second variable except in correlation in stage 6 with stage 4 where increases or decreases in one variable do not significantly relate to increases or decreases in second variable.

Table 3.7: Correlation matrix between different size fractions for fungal bioaerosols over the complete year

		Stage1	Stage2	Stage3	Stage4	Stage5	stage6
Stage1	Pearson Correlation	1					
	Sig. (2-tailed)						
Stage2	Pearson Correlation	**0.705****	1				
	Sig. (2-tailed)	0					
Stage3	Pearson Correlation	**0.699****	**0.715****	1			
	Sig. (2-tailed)	0	0				
Stage4	Pearson Correlation	**0.671****	**0.582****	**0.538****	1		
	Sig. (2-tailed)	0	0	0.001			
Stage5	Pearson Correlation	**0.720****	**0.624****	**0.507****	**0.560****	1	
	Sig. (2-tailed)	0	0	0.002	0		
Stage6	Pearson Correlation	0.406*	0.464**	0.269	0.321	**0.510****	1
	Sig. (2-tailed)	0.015	0.005	0.118	**0.06**	0.002	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

3.5.2 Correlation between stages of the sampler in different seasons

Correlation analysis was carried out among fungal concentrations in different stages in different seasons. In this study, more or less a poor or no correlation exist among all the stages in all the seasons except in few cases such as, in monsoon season a very good linear relationship was found between stage 4 and stage 1 (r = 0.93); stage 5 and stage 2 (r = 0.93); stage 6 and stage 2 (r = 0.93) and stage 6 and stage 5 (r = 0.83) (Table 3.8). Existence of good correlation was also found between stage 3 and stage 1 (r = 0.78); stage 4 and stage 3 (r = 0.78) in autumn season (Table 3.9). Similarly in winter season, a good correlation was observed between stage 2 and 1 (r = 0.84); stage 5 and stage 1 (r = 0.88) and stage 4 and 2 (r = 0.95) (Table 3.10). Table 3.11, shows the correlation matrix between different size fractions for fungal bioaerosol in the spring season. There was good correlation between stage 3 and stage 2 (r = 0.72); stage 4 and stage 3 (r = 0.70). Table 3.12, shows the correlation matrix between different size fractions for fungal bioaerosols in the summer season. In summer season there was good linear relationship between stage 2 and stage 1 (r = 0.76); stage 3 and stage 2 (r = 0.82).

Table 3.8: Correlation matrix between different size fraction for fungi in Monsoon season

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Stage 1	1.00					
Stage 2	0.37	1.00				
Stage 3	0.44	0.32	1.00			
Stage 4	**0.93**	0.50	**0.69**	1.00		
Stage 5	0.41	**0.93**	0.25	**0.54**	1.00	
Stage 6	0.16	**0.93**	0.05	0.23	**0.83**	1.00

Table 3.9: Correlation matrix between different size fraction for fungi in Autumn season

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Stage 1	1.00					
Stage 2	-0.03	1.00				
Stage 3	**0.78**	0.25	1.00			
Stage 4	0.33	0.14	**0.78**	1.00		
Stage 5	0.38	-0.33	0.28	0.39	1.00	
Stage 6	-0.25	0.21	-0.24	-0.12	-0.27	1.00

Table 3.10: Correlation matrix between different size fraction for fungi in winter season

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Stage 1	1.00					
Stage 2	**0.84**	1.00				
Stage 3	0.18	0.52	1.00			
Stage 4	**0.79**	**0.95**	0.29	1.00		
Stage 5	**0.88**	**0.63**	0.19	**0.55**	1.00	
Stage 6	-0.07	-0.44	-0.69	-0.32	0.10	1.00

Table 3.11: Correlation matrix between different size fraction for fungi in Spring season

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Stage 1	1.00					
Stage 2	**0.51**	1.00				
Stage 3	0.18	**0.72**	1.00			
Stage 4	**0.59**	0.49	**0.70**	1.00		
Stage 5	0.40	0.07	0.40	0.38	1.00	
Stage 6	0.34	-0.46	-0.10	0.37	**0.68**	1.00

Table 3.12: Correlation matrix between different size fraction for fungi in Summer season

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Stage 1	1.00					
Stage 2	**0.76**	1.00				
Stage 3	**0.67**	**0.82**	1.00			
Stage 4	0.39	-0.06	-0.21	1.00		
Stage 5	**0.61**	**0.59**	0.15	0.43	1.00	
Stage 6	0.25	0.33	0.43	0.32	0.11	1.00

3.5.3 Correlation among meteorological conditions and fungal bioaerosol concentration

According to table 3.13, there is more or less poor correlation between fungal concentration and meteorological parameters. Average positive correlation exists between temperature and fungal concentration (Pearson coefficient is 0.53). Negative poor correlation is present between relative humidity and fungal concentration (Pearson coefficient is -0.36) whereas poor positive correlation exists between wind speed and fungal concentration (Pearson coefficient is .44).

All the meteorological parameters are significantly related to airborne fungal concentration as all the p values are less than 0.05.

Table 3.13: Correlation matrix among the fungal concentration and various meteorological parameters

		Fungal Concentration	Temperature	Relative Humidity	Wind Speed
Fungal Concentration	Pearson Correlation	1			
	Sig. (1-tailed)				
Temperature	Pearson Correlation	.53**	1		
	Sig. (1-tailed)	0.001			
Relative Humidity	Pearson Correlation	-.36*	-.47**	1	
	Sig. (1-tailed)	0.016	0.002		
Wind Speed	Pearson Correlation	.44**	.39**	-.33*	1
	Sig. (1-tailed)	0.004	0.009	0.025	

**. Correlation is significant at the 0.01 level (1-tailed).

*. Correlation is significant at the 0.05 level (1-tailed).

3.5 Analysis of Variance (ANOVA)

Statistical analysis of variance of the results was performed using SPSS 16 software. The one-way analysis of variance (ANOVA) is used to determine whether there are any significant differences between the means of three or more independent (unrelated) groups. The difference was considered statistically significant when significant value $p < 0.05$ (chosen alpha is 0.05), and a confidence level of 95%.

3.5.1 ANOVA within stage by concentration

The fungal bioaerosol concentration difference among all the stages of the Anderson Sampler was calculated using one-way ANOVA test. According to table 3.14, the result is found to be statistically significant as the significant value (p) is 0.00 which is less than 0.05 ($p<0.05$). Therefore the null hypothesis is rejected in support of the conclusion that the mean concentration of airborne fungi vary significantly across the different stages of Anderson Sampler. The mean of the fungal concentration in all the stages is significantly different.

Table 3.14: Fungal concentration difference among various stages of sampler

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	172744.2	5	34548.8	10.6	0.00
Within Groups	663066.0	204	3250.3		
Total	835810.2	209			

3.5.2 ANOVA within site by concentration

The concentration difference of airborne fungi among all the seven sites of sampling was calculated with one-way ANOVA test. In table 3.15, Statistical analysis showed that the observed p-value in the sites is higher (0.786) than chosen alpha of 0.05. So the null hypothesis is accepted, in support of the conclusion that the mean concentration of fungal bioaerosols does not vary significantly across the sites. The means of concentration of fungi of all the sites are

similar. No significant difference is observed. This may be due to the fact that all sites have also their own source of bioaerosols in addition to landfill source.

Table 3.15: Fungal concentration difference among various sampling sites

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	715016.0	6	119169.3	0.5	0.78
Within Groups	2.056E7	91	225922.0		
Total	2.127E7	97			

3.5.3 ANOVA within stage by season

Table 3.16 shows the output of the ANOVA analysis to determine the mean fungal concentration difference in each stage of Anderson Sampler with respect to season. The Significant value (p) is less than 0.05 in all the stages. So, we can conclude that there is statistically significant difference in the fungal concentration in each stage with respect to season i.e., in all the seasons, airborne fungi concentration was different in a particular stage of Anderson Sampler.

Table 3.16: Results of analysis of variance (ANOVA) showing concentration difference of fungal bioaerosols within the six stages of Anderson Sampler.

		Sum of Squares	Df	Mean Square	F	Sig.
Stage 1	Between Groups	93012.0	4	23253.0	8.0	0.00
	Within Groups	87057.7	30	2901.9		
	Total	180069.7	34			
Stage 2	Between Groups	47541.5	4	11885.3	8.6	0.00
	Within Groups	41083.7	30	1369.4		
	Total	88625.2	34			
Stage 3	Between Groups	19044.4	4	4761.1	6.8	0.00
	Within Groups	20775.7	30	692.5		
	Total	39820.1	34			
Stage 4	Between Groups	19849.3	4	4962.3	3.6	0.01
	Within Groups	40706.5	30	1356.8		
	Total	60555.8	34			
Stage 5	Between Groups	45629.4	4	11407.3	3.9	0.01
	Within Groups	87622.9	30	2920.7		
	Total	133252.3	34			
Stage 6	Between Groups	47344.1	4	11836.0	3.1	0.03
	Within Groups	113398.4	30	3779.9		
	Total	160742.5	34			

3.5.4 ANOVA within sites by season

As seen in table 3.17, mean fungal concentration difference of all the sites among different seasons was calculated using ANOVA. It is observed that at all the sites, significant value (p) is more than 0.05 (our chosen alpha) thus the null hypothesis is accepted. There is no significant difference in the mean fungal concentration at all the sites among various seasons.

Table 3.17: Results of analysis of variance (ANOVA) showing concentration difference of fungal bioaerosols at all the sites among various seasons

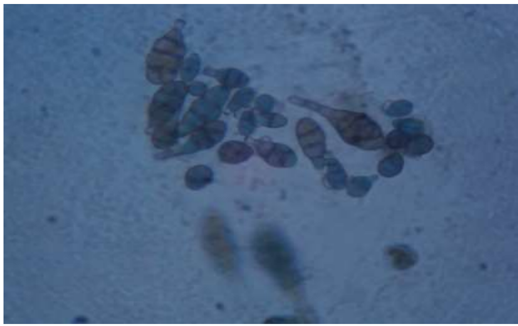
		Sum of Squares	Df	Mean Square	F	Sig.
Site 1	Between Groups	579472.5	4	144868.1	0.8	0.55
	Within Groups	1625379.2	9	180597.6		
	Total	2204851.7	13			
Site 2	Between Groups	728230.0	4	182057.5	0.8	0.53
	Within Groups	1952983.5	9	216998.1		
	Total	2681213.5	13			
Site 3	Between Groups	512887.3	4	128221.8	1.1	0.40
	Within Groups	1019230.6	9	113247.8		
	Total	1532118.0	13			
Site 4	Between Groups	1472504.3	4	368126.0	.5	0.74
	Within Groups	6756181.6	9	750686.8		
	Total	8228685.9	13			
Site 5	Between Groups	921710.8	4	230427.7	1.8	0.20
	Within Groups	1114177.1	9	123797.4		
	Total	2035887.9	13			
Site 6	Between Groups	632070.2	4	158017.5	1.5	0.25
	Within Groups	896658.7	9	99628.7		
	Total	1528728.9	13			
Site 7	Between Groups	1097973.0	4	274493.2	1.9	0.18
	Within Groups	1249443.3	9	138827.0		
	Total	2347416.4	13			

3.6 Identification

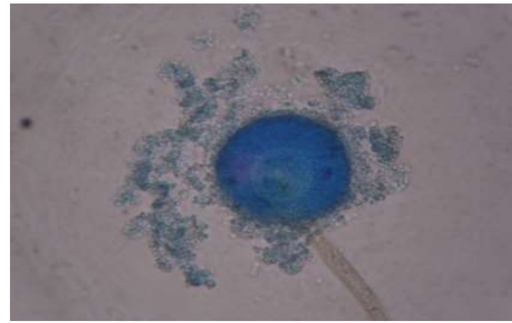
Eight genera of fungal bioaerosol were identified, at all the seven sites of Okhla Landfill during five different seasons given in table 3.18. Among the eight genera identified, the three genera which had been found in maximum number in all the seasons are *Aspergillus, Rhizopus* and *Pencillium.* In monsoon period major fungal bioaerosol concentration consists of *Aspergillus, Penicillium* and *Rhizopus* in all the sampling sites. In autumn and winter season, major findings were *Aspergillus, Fusarium, Penicillium* and *Rhizopus.* In spring and summer season *Alternaria* was found in bulk. *Alternaria* was not found in winter season. Among the eight genera identified, 3 were immunotoxic, 3 were allergic and 2 were harmless.

Various species of *Aspergillus* produce a mycotoxin called Ochratoxin A. It causes contamination in food and makes it toxic. Human exposure occurs through consumption of improperly stored food products. Ochratoxin A is potentially carcinogenic to humans. Aflatoxins are also produced by some species of *Aspergillus*. It is harmful for human and animal health.

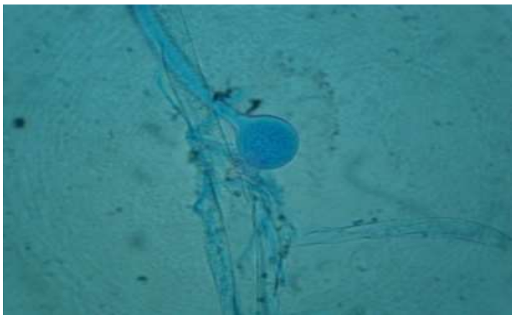
Aspergillus flavus causes Aspergillosis. It invades the arteries of lungs and brain and causes infarction (Kumar et al. 2005). *Alternaria* is plant pathogen and is common allergen for animals and human. It may cause fever or hypersensitive reaction that may even lead to asthma. *Fusarium* causes fusarial infection which may occur in the nail and in the cornea (Keratomycosis). *Cladosporium* can cause pulmonary infections and sinusitis in human. It is found on both live and dead plant material.



Alternaria sp



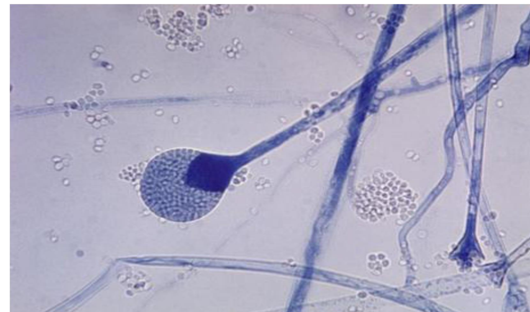
Aspergillus sp.



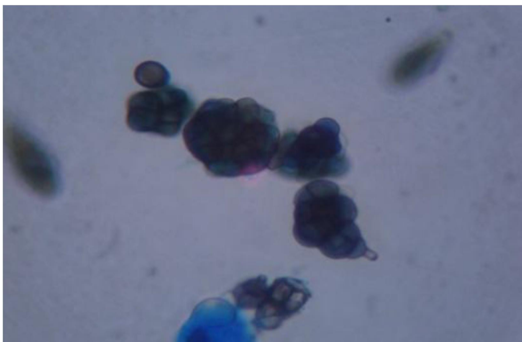
Rhizopus sp.

Fusarium sp.

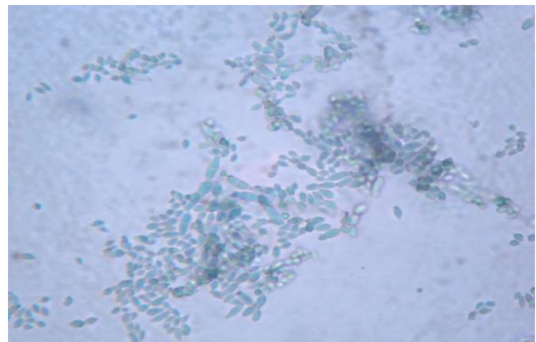
Penicillium sp.



Mucor sp



Ulocladium sp.



Cladosporium sp.

Figure 3.16: Fungal genus characterized at different sites

Table 3.18: Fungal genus characterized at different sites

Sampling sites	1					2					3					4					5					6					7				
Sampling season / Fungi	M	A	W	Sp	Su	M	A	W	Sp	Su	M	A	W	Sp	Su	M	A	W	Sp	Su	M	A	W	Sp	Su	M	A	W	Sp	Su	M	A	W	Sp	Su
Asper-gillus #	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhizop-us X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
Penicil-lium *	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Uloclad-ium *	-	-	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-
Alterna-ria X	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	-	-	+
Mucor *	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusari-um X	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-
Cladospor ium #	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-

(X) - Immunotoxic **(*) - Harmless** **(#) - Allergic**

M – Monsoon Season **(+) - Present** **(-) - Absent**

A – Autumn Season

W – Winter Season

Sp – Spring Season

Su – Summer Season

CONCLUSION

From the present study the following conclusion can be drawn:

- It was found that in monsoon season fungal bioaerosols concentration seems to follow typical pattern at most of the sites. Concentration decreases from stage 1 to stage 3 and then increases from stage 3 to stage 6. At maximum sites, fungal concentration was highest in stage 6. In stage 6 smallest size of bioaerosols particles (0.65µm- 1.1µm) are present thus these particles can pose serious threat to respiratory organs.
- In autumn, winter, spring and summer season, it was found that fungal bioaerosol concentration does not follow any pattern. At most of the sites fungal bioaerosol concentration was highest in stage 6 in autumn, winter and spring season whereas in summer season highest concentration was found in stage 1 at most of the sites.
- Seasonal variation of fungal bioaerosol analysis was also done in this study. It was found that at most of the sites fungal bioaerosol concentration was maximum in summer season and minimum in autumn season. Fungal growth is highly dependent on the surrounding temperature. Temperature in summer is favourable for fungal growth.
- It was found that at most of the sites respirable faction was highest in autumn and lowest in summer season. The more the respiratory fractions, more are the chances of getting respiratory diseases.
- In this study both multiple as well as linear regression analysis was performed. It was observed that combined effect of all meteorological parameters (temperature, relative humidity and wind speed) had more effect on fungal bioaerosol concentration than that of individual parameters. Although the R-square value in multiple regression analysis was moderate yet its impact was significant. Similarly when linear regression was applied between fungal concentration and temperature, fungal concentration and relative humidity and fungal concentration and wind speed, R-square value was poor but its effect was significant and therefore the impact of meteorological parameters cannot be neglected.
- Correlation analysis was carried out between fungal concentration of every stage and between each stage over the complete year. There was a strong relation between stage 2

and stage 1; stage 3 and stage 2; stage 5 and stage 1. It was found that all stages are statistically significant correlated with each other except stage 6 with stage 4. When correlation between stages of the sampler was performed, it was observed that there exists a more or less poor correlation between different stages in all the seasons except with few exceptions.

- Correlation analysis was also performed between fungal concentration and meteorological parameters. It was found that there is an average positive correlation between concentration and temperature, average negative correlation between concentration relative humidity and positive poor correlation between concentration and wind speed.
- One way ANOVA was performed to determine the significant differences between the means of fungal concentration. It was observed that mean concentration of airborne fungi vary significantly across the different stages of Anderson Sampler and does not vary significantly across the site. When ANOVA was performed by the season, it was found that there is statistical difference in the fungal concentration in each stage with respect to season and no difference in mean fungal concentration at each site with respect to season.
- Among the eight fungal genera identified in Okhla landfill site *Penicillium, Aspergillus and Rhizopus* were found in abundance at all the seven sites in all the five seasons. Out of these three, *Aspergillus* is allergic, *Penicillium* is harmless and *Rhizopus* is immunotoxic in nature. *Alternaria* and *Fusarium* were found in abundance in spring, summer and winter seasons whereas *Cladosporium* in the season of autumn and winter. *Aspergillus* was present in all the seasons.

REFERENCES

ACGIH, 1989. Committee on Bioaerosols, eds. In: Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, OH: American Conference of Governmental Industrial Hygienists. Step two: On-site investigation, pp. 1-8; Fungi, pp. 1-10; Bacteria, pp. 1-7.

Allard A.C., Endris, J.J., Miller, J.G., Baker, T.C., 1994. Identification of volatile compounds from fungus-infected date fruit that stimulate upwind flight in female Ectomyeloisceratoniae. Entomologia Experimentaliset Applicata; 72: 233-238.

Bauer H., Schueller E., Weinke G., Berger A., Hitzenberger R., Marr L.L., Puxbaum H., 2008. Significant contributions of fungal spores to the organic carbon and to the aerosol mas balance of the urban atmospheric aerosol. Atmospheric Environment; Vol. 42, Issue 22, Pages 5542-5549.

Bhalla B., Saini M.S., Jha M.K., 2012. Characterization of leachate from municipal solid waste (MSW) landfilling sites of Ludhiana, India: A comparative study. International journal of engineering research and application ISSN: 2248-9622 Vol. 2, Issue 6, pp.732-745.

Burkowska A., Brzezinska M.S., Kalwasinska A., 2011. Impact of the municipal landfill site on microbiological contamination of air. Chapter 5, Contemporary Problems of Management and Environmental Protection, No.9.

Cabredo S., Parra A., Saenz C., Anzano J., 2009. Bioaerosols chemometric characterization by laser-induced fluorescence: Air sample analysis. Talanta; 77:1837-42.

Codina, R., Fox, R,w., Lockey, R.F., DeMarco, P., Bagg, A., 2008. Typical Levels of Airborne Fungal Spores in Houses without obvious Moisture Problems during a Rainy Season in Florida, USA. Journal of Investigational Allergology and Clinical Immunology. Vol. 18 (3): 156-162

Cole, R., Brosch, J., Parkhill, T., Garnier, C., Churcher, D., Harris, S. V., Gordon, K., Eiglmeier, S., Gas, C. E. Barry., 1998. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence, Nature 393, 537-544.

Cox C.S., Wathes, C.M., 1995. Bioaerosols handbook. New York: Lewis Publishers.

Danuta O., Krzysztof U., Agnieszka W., Jozef P., 2004. Microbial air quality in offices at municipal landfills. Journal of occupational and environmental hygiene; Vol. 1, Issue 10.

Dungan R.S., Leytem A.B., 2009. Qualitative and quantitative methodologies for determination of airborne microorganisms at concentrated animal-feeding operations. World J Microbiol Biotech; 25:1505-18.

Edward, F.B., Walter H.T., Jordan N.F., 1970. Hypersensitivity Pneumonitis Due to Contamination of an Air Conditioner, N Engl J Med; 283,271-276.

Fischer, G., Dott, W., 2003. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Arch. Microbial; 179(2): 75-82

Fraczek K., Rozycki H., Ropek D., 2014. Statistical analyses of bioaerosol concentration at municipal landfill site. Ecol Chem Eng S; 21(2):229-243.

Glikson M., Rutherford S., Simpson R. W., Mitchell C.A., Yago A., 1995. Microscopic and submicron components of atmospheric particulate matter during high asthma periods in Brisbane, Queensland, Australia. Atmospheric Environment 29:549-562.

Gorny R.L., Dutkiewicz J., Krysinska-Traczyk E., 1999. Size distribution of bacterial and fungal bioaerosols in indoor air. Ann Agric Environ Med; 6: 105-113.

Gorny R.L., 1998. Characterization of particulate aerosols and bioaerosols in the dwellings located on the territory of the upper Selisia Conurbation. Ph.D. thesis Selisian Medical Academy, Katowice (in Polish)

Hirst J.M., 1952. An automatic volumetric trap. Ann Appl Biol; 39:73-8.

Horner A., Helbling J.E., Salvaggio and Lehrer S.B., 1995. Fungal allergens.Clin.Microbiol. Vol. 8 No. 2 161-179.

Hryhorczuk D., Curtis L., Scheff P., Chung J., Rizzo M., Lewis C., 2001. Bioaerosol emission from a suburban yard composting facility. Ann Agric Environ Med; 8: 177-185.

Huang C.Y., Lee C.C., Li F.C., Ma Y.P., Jenny Su H.J., 2002. The seasonal distribution of bioaerosols in municipal landfill sites: a 3-yr study. Atmospheric Environment; 36: 4385-4395.

Jain., 2000. Survey of bioaerosol in different indoor working environments in central India.Aerobiologia; 16: 221–225.

Kalogerakis N., Paschali D., Lekaditis V., Pantidou A., Eleftheriadis K., Lazaridis M., 2005. Indoor air quality- bioaerosol measurements in domestic and office premises. Aerosol Science; 36: 751-761.

Kalwasinska A., Burkowska A., Brzezinska M.S., 2014. Exposure of workers of municipal landfill site to bacterial and fungal aerosol. Clean- soil, air, water; Vol. 42, Issue 10, Pages 1337-1343.

Kampa M., Castanas E., 2008. Human health effects of air pollution. Environmental Pollution; 151: 362-367.

Kazmierczuk M., Bojanowicz-Bablok A., 2014. Bioaerosol concentration in the air surrounding municipal solid waste landfill. Environmental protection and natural resources; Vol.25, No. 2(60): 17-25.

Kim K.Y., Kim C.N., 2007. Airborne microbiological characteristics in public buildings of Korea. Building and environment; Volume 42, Issue 5, Pages 2188-2196.

Kummer V., Thiel W.R., 2008. Bioaerosols- Sources and Control measures. International Journal of Hygiene and Environmental Health; Volume 211, Issues 3–4, 15 July 2008, Pages 299–307.

Lienhardt, C., 2001. From Exposure to Disease: The Role of Environmental Factors in Susceptibility to and Development of Tuberculosis. Epidemiologic Reviews; Vol. 23, No. 2.

Lin W.H., Li C.S., 1999. Evaluation of impingement and filtration methods for yeast bioaerosol sampling. Aerosol Sci Technol; 30:119-126.

Lutgens F.K., Tarbuck E.J., Tasa D., 2004. The atmosphere: An introduction to meteorology. Ninth edition. Prentice Hall, Upper Saddle River, New Jersey 07458.

Maharia S., Srivastava A., 2014. Influence of seasonal variation on concentration of fungal bioaerosol at a sewage treatment plant (STP) in Delhi. Aerobiologia; Vol. 31, Issue 2, pp 249-260.

Mandal J., Brandl H., 2011. Bioaerosols in indoor environment- A review with special reference to residential and occupational locations. The open environmental & biological monitoring journal; 4: 83-96.

Millner P.D., 2009. Bioaerosols associated with animal production operations. Bioresource Technol; 100(22): 5379-5385.

Rahkonen P., Ettala M., Laukkanen M., Salkinoja-Salonen M., 1990. Airborne microbes and endotoxins in the work environment of two sanitary landfills in Finland. Aerosol Science and Technology; 13: 505-513.

Robert J. and Sherertz M.D., 1987. Impact of air filtration on nosocomial *Aspergillus* infections: Unique risk of bone marrow transplant recipients. The American Journal of Medicine; 83: 703-718.

Rodes C.E. and Wiener R.W., 2001. Indoor Aerosols and Exposure Assessment. In P.A. Baron and K.Willeke (Eds.), Aerosol Measurement: Principles, Techniques and Applications John Willey & Sons, Inc. New York, NY, USA. 859-885.

Senzilet L., Halperin S., Spika J., Alagaratnam M., Morris A., Smith B., 2001. Pertusis is a frequent cause of prolonged cough illness in adults and adolescents. Oxford Journals; Vol. 32, Issue 12, Pp 1691-1697.

Shantha R., Sarayu K. and Sandhya S., 2009. Molecular identification of air microorganisms from municipal dumping ground. World Appl. Sci. J; 7(5): 689-692.

Srivastava A., Singh M., Jain V.K., 2012. Identification and characterization of size-segregated bioaerosols at Jawaharlal Nehru University, New Delhi. Natural Hazards; Vol. 60:485-499.

Stephens D., Greenwood B., Brandtzaeg P., 2007. Epidemic meningitis, meningococcaemia, and *Niesseria meningitides*. Lancet; 369: 2196-210.

Tettelin., Nelson K.E., Paulsen I.T., Eisen J.A., Read T.D., Heidelberg S.P.J., DeBoy R.T., Haft D.H., Dodson R.J., 2001. Complete Genome Sequence of a Virulent Isolate of Streptococcus pneumonia. Science; 293, 498.

Thermo-scientific Instruction Manual. Six and two stage viable samplers, 2009. Part No. 100072-00, Series 10-800.

Torsvik V., Goksoyr J., Daae F.L., Sorheim R., Michalsen J. and Salte K., 1994. Use of DNA analysis to determine the diversity of microbial communities. In: Beyond the biomass: compositional and functional analysis of soil microbial communities, Ritz, K., Dighton, J. and Giller, K.E., Editors. John Wiley & Sons Ltd: Chichester, UK; 39-48.

Wagner J. M., White N. S., Crowcroft S., Martin D.E., Mann G. and Efstratiou A., 2010. Diphtheria in the United Kingdom, 1986–2008: the increasing role of Corynebacteriumulcerans. Epidemiology and Infection: Vol. 138, Issue 11; 1519-1530.

Wang Y.F., 2011. Sesonal difference of airborne bacteria and fungi in commuter buses. Environmental engineering science; Vol. 28, No. 6.

Yao M.S., Mainelis G., 2007. Use of portable microbial samplers for estimating inhalaton exposure to viable biological agents. J Exposure Sci Environ Epidemiol; 17:31-8.

Zollinger M., Krebs W., Brandl H., 2006. Bioaerosols formation during grape stemming and crushing. Sci Total Environ; 363:253-9.