Spatial Distribution of Bio-Aerosols and Determinant factors in a built Environment

By

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
DC	Direct Current
GIS	Geographic Information System
PMD	Pakistan Meteorological Department
rpm	Revolution per minute
V	Voltage
RH	Relative Humidity
GHI	Global Horizontal Irradiance

ABSTRACT

Atmospheric Bio-Particulates, mostly pollen and fungus concentrations, are altered by a number of environmental factors and spatial variables. This study aimed to analyze the spatial distribution of pollen and fungus at a local scale. Therefore, NUST H-12 sector with an area 3.28 sq.km was selected as a study area. For the aero-biological survey, low cost air samplers were developed and placed at six sites to obtain the primary data. Pollen and fungus count were obtained through microscopic examination. Thirty-three different kinds of pollen and fourteen fungus species were identified on basis of morphological characterization of these biological particulates. The concentration values at six sites showed that significant spatial heterogeneity exist at a regional scale. The maximum concentration of pollen was found at an open space with value 1985 per meter cube while for fungus, it was high with concentration value of 3234 per meter cube at University entrance gate. The difference in trap height greatly influenced the pollen concentration as concentration decreases with height .The results of individual fungus species showed that *Alternaria* and *Aspergillus* were the dominant allergens in this region making up to 70% of the data in total fungus concentration at all sites. Land use land cover maps of radius 2 km surrounding the pollen traps were generated. The results showed that shrubs and grasses contributed significantly as covering up to approximately 60% of the Total Land Cover area. Thus, by combining aerobiological data with the proximity analysis, we have interpreted the immediate surrounding vegetation cover responsible for pollen load at a regional scale. In the light of our study, it is recommended that for both fungus and pollen monitoring, multi-site aerobiological monitoring should be preferred at a height close to ground within a city. Aerobiological data, integrated with GIS technique, could be used for determining the local vegetation sources responsible for pollen load in the specific region.

Chapter1

INTRODUCTION

In the recent decades attention has been diverted to investigate the spatial heterogeneity of biological particulates in the atmosphere because of changes in climate, increased urbanization, excessive tree planting and increased number of allergic cases across the globe. The content of non-biological aerosols is higher as compared to biological aerosols therefore the behavior of biological aerosols have not been observed well for air quality models .However, It has been found that atmospheric pollution holds about 22% of microbial content in a populated urban environment (Jones et al., 2004).Some of these bio-particulates present in the air are involved in causing allergic symptoms. Among these, pollen and fungal spores are predominant allergens. Though much research has been conducted on air pollutants and factors effecting its dispersal, however, the interrelationship of air borne biological particles with meteorological variable and land use and land lover is not yet well established.

The present research focused on analyzing the geographical distribution of fungus and pollen levels at six different sites within H-12 Sector of Islamabad. For aerobiological sampling, low cost portable volumetric sampler was designed. Primary data was gathered using these five devices at six sites. Total Pollen and fungus count was derived from microscopic examination. Further, all the pollen and molds types that were found in the study area, were identified.

Moreover, the association of meteorological variables with the identified pollen and fungus species were also studied. Pearson test was performed to find correlation of biological particulates with global horizontal irradiance, wind speed and temperature.

Maps of land use land cover within radius of 200 meter surrounding the volumetric trap were created in order to find the major source for pollen within the region.

1.1 RATIONALE

Numerous pollen and fungus particulates have been reported as allergenic components causing various respiratory diseases. The severity of an allergic reaction largely depends on the amount of biological spores inhaled by the human system. Therefore, spatial distribution and continuous monitoring of these biological pollutants have become essential for understanding the interaction of these biotic pollutants with the surrounding environment. The importance of aerobiological survey has grown with increased tree planting in the urban cities as well. Further, due to changing climate, many spatial and meteorological factors are affecting pollen and fungus concentration levels (Jones & Harrison, 2004).This study would add knowledge about the prevalence of bio-particulates concentration in relation to local weather patterns. The rapid advance in urbanization has largely affected the atmospheric air quality. This study helped in investigating the dominant spatial variables that have impact on atmospheric bio allergens concentration.

1.2 RELEVANCE TO NATIONAL NEEDS

Islamabad is among the cities with highest pollen counts in the world (Abbas et al., 2012) .Many types of grasses, weeds and trees found in Islamabad produce allergic pollens. Every year the pollen and mold concentration levels reaches peak values in different months, eventually increasing the number of allergic patients.

1.3 OBJECTIVES

The main objective of the study was to investigate the spatial distribution of bio-aerosols in a built environment.

This objective was achieved through the following sub-goals:

- Designing a low cost volumetric trap;
- Identification of pollen grains at aperture level and fungus species at genus level;
- Analyzing the impact of meteorological factors and built-up area on the concentration of Bio-aerosols.

The study attempted to address the following research question:

How sampling location and meteorological factors contribute in altering the concentration levels of biological aerosols in a built environment?

Chapter 2

LITERATURE REVIEW

The chapter deals with the review of previous studies that has been done on this topic. Pollen and fungus acting as air pathogens are now considered as a part of air pollution. Two types of aerobiological traps are generally used for fungus and pollen air monitoring. The distribution pattern of pollen and fungus in air is altered by local wind speed and wind direction. Recent studies find an association of local vegetation type with pollen flux. The other factors that could influence the pollen and fungus concentration at a specific location is the sampling height.

2.1 BACKGROUND

The air we breathe often carries pathogenic materials causing infections and allergens. The bio particulates involved in causing allergy symptoms are insect debris, animal dander, pollen grains, house dust mites, fungal spores, chemicals, food etc. (Valsan et al., 2015).

Among all these agents, pollen grains and mold spores are the most predominant allergens in the air. Therefore continuous exposures to ambient bio aerosols have substantial effect on the human respiratory system (Singh et al, 2012; Kallawicha et al., 2015). Inhalation of these pollen allergens directly affects the respiratory system (Kallawicha et al., 2015) . In the recent decades, due to the growing increase in cases of allergy, hay fever and pollen-related respiratory diseases, aerobiological research in arboreal pollen and mold diversity and seasonal alterations in their concentration have become increasingly important (Rojo et al., 2016).

Aerobiological studies have become important in urban areas due to change in climate, degradation of land cover data and hazardous effects of air pollution. The society in urbanized territories is more sensitive to pollen impact due to pollution particles stick to pollen. Thus, chemical and biological air pollution acting together poses new challenges for environment quality strategy shaping and control (Ribeiro et al., 2015; Sauliene et al., 2012). Knowledge of types of pollen circulating in air and interpretation of airborne pollen is of great help in the environmental and public health sectors (Rojo et al., 2015).

Continuous monitoring of microscopic aerosols is conducted within the city using various sampling devices. Fixed samplers that are located in outskirts of city are mostly used for aerobiological monitoring (Gonzalo-Garijo et al., 2006). Pollen dispersed through wind in air is an important class of allergens with adverse effect on human health .There are three main sources of airborne pollen: trees, grasses and weeds with each category having a distinct temporal distribution. Tree pollen is a large contributor of airborne pollen spectrum, although not all tree pollens are allergenic.

Pollen has been well discussed from clinical view point, however, there has been little knowledge on the spatial and temporal spread of pollen and its determinant factors within cities. Many environmental risks are effecting the urban population. To reduce the air pollution, massive urban tree planting program has resulted in increased pollen influx. Weinberger (2015) States that understanding the spatial variation of pollen concentration is important in the light of two factors that have potential to increase the pollen exposure in the coming decades. Massive Urban tree planting program and Climate change.

On the other hand spores presence in air is an indicator of level of biological pollution in air. Spores are a dominant microorganism of indoor environment but recent researches have revealed that molds and spores are an important outdoor air borne aerosol and is considered as an important indicator of level of air borne biological pollution (Grinn-Gofroń et al., 2011).

Status in Pakistan

Allergy through air borne pollens and fungus is an emerging problem in Islamabad, Pakistan. Abbas et al. (2012), in collaboration with world Allergy organization, conducted a study in which first pollen calendar for pollen taxa and molds species was formed with clinical significance. The aerobiological study was conducted for three years from January 2005 till December 2007. The statistics showed that *Broussonetia, papyrifera* and *Cannabis sativa* are the seasonal allergens from tree pollen as shown in Figure 2.3 and 2.4.

Of the fungal family, Spores and molds showed continuous presence throughout the year with *Stachybotry, Cladosporium* and *Pithomyces* species as prominent allergens.

2.2 SAMPLING DEVICES

Standard methods for aerobiological monitoring (Hasnain et al., 2007) is necessary for quantifying the concentration of viable and non-viable biological aerosols, airborne particles and aeroallergens (Kimber et al., 2015). The sampling is carried out using active and passive air samplers (Levetin, 2004). The devices differ in design and collect samples by following one of these basic regimes (Juozaitis et al., 1994).

Figure 2. 1. *Broussonetia Papyrifera* along roadside of Islamabad (Source: CABI image by Harry C. Evans)

Figure 2. 2. *Cannabis sativa* (UNODC, 2009)

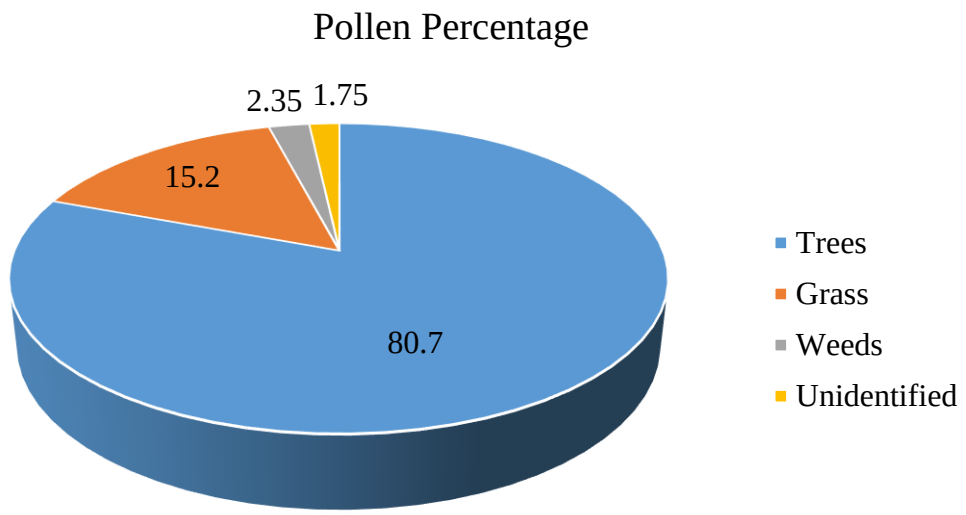


Figure 2. 3. Pollen Percentage in Islamabad For year 2007 (WAO, 2012).

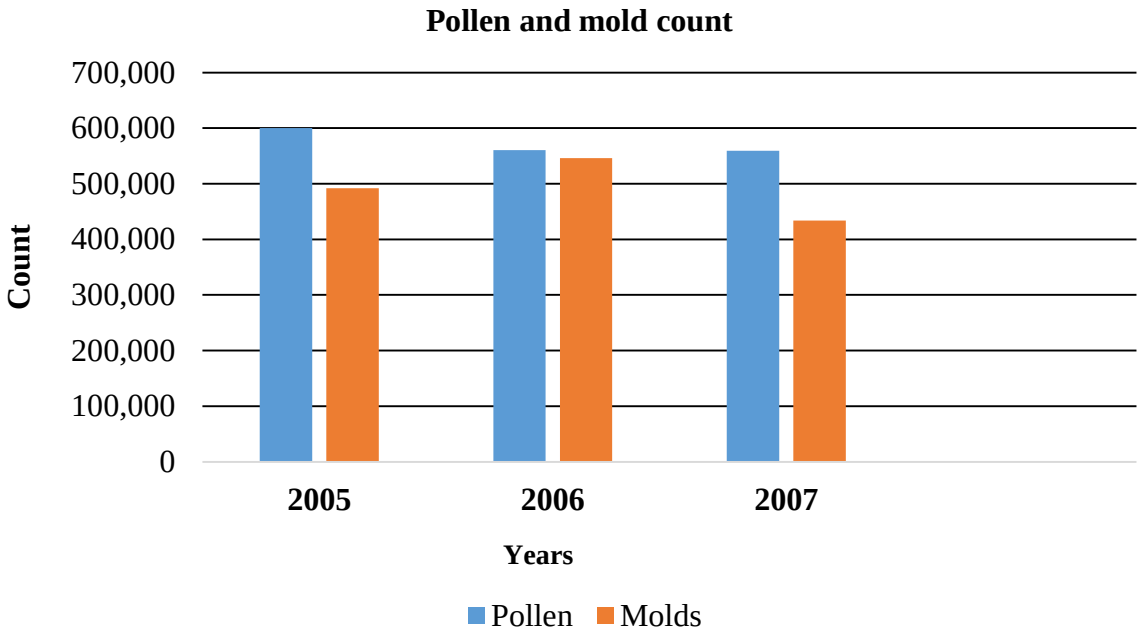


Figure 2. 4. Annual pollen count for years 2005, 2006, and 2007 (WAO, 2012).

- Bio particulates settle down on a surface through gravitational force
- Impaction on a rapidly moving motor
- Suction through certain volume of air
- Filtration
- Immunochemical assays

Conventional stationary aerobiological sampler are designed on the basis of factory or user applied collection medium at deigned air flow rates (Godish et al., 2007). Air samplers have advantages and disadvantages on the basis of power consumption, length of sampling period, collection efficiency (dependent on particle size) and air sample flow rate (Kimber & RBE, 2015). Below is the description of some important aerobiological samplers that are in use across the globe.

2.2.1 GRAVIMETRIC SAMPLER

Gravimetric sampler, as shown in Figure 2.5, is the simplest trap as micro particulates are collected on the settling plate due to gravitational force (Juozaitis et al., 1994).The Durham gravimetric sampler consists of two horizontal discs. The upper disc shields the lower dish from rain and sun while the particles are trapped in silicone gel applied to the lower disc. The collection efficiency of trap depends on particle size and wind speed (Singh et al., 2012). Data obtained though this trap cannot be used for quantification of species or analytical purpose, however, it can be used for identification of species in indoor environment. These devices are no more in use in developed countries while they are still used in places where cost is an issue (Bricchi et al., 2000).

Figure 2. 5. Durham gravimetric sampler.

2.2.2 ROTOROD SAMPLER

Rotating arm Impactor, commonly known as Rotorod, is an aerobiological sampler that has been used extensively in outdoor sampling (Di-Giovanni, 1998). Rotorod collects particles that are larger than 15 µm (Juozaitis et al., 1994). The Rotorod is an impaction trap that operates by DC motor. The motor spins the retracting head, on which two Lucite rods are mounted as shown in Figure 2.6. The rods are coated with an adhesive like silicon grease in order to capture the particles from the air. The rotating head works intermittently, however the spinning duration of the retracting head can be adjusted according to the requirement. This sampler collects pollen at regular intervals. The rate of deposition on slide depends on rotation speed, concentration of particles and surface exposed to pollen (DellaValle et al., 2012; Ghufran et al., 2013; Weinberger, 2015). Numerous scientific studies have been carried out where RotoRod sampler was used for collecting outdoor aeroallergens (Adhikari et al., 2003; Ho et al., 1995; Oh et al., 1998).

Figure 2. 6. RotoRod sampler Model 40.

2.2.3 HIRST TRAP

Hirst Trap (1952) has been in use for aero allergic monitoring for over 60 years. The modified form of Hirst Trap, which is in use these days is named as Burkard volumetric trap shown in Figure 2.7. It can collect data continuously for seven days without any disturbance (Kimber & RBE, 2015; Singh & Mathur, 2012).

In Hirst trap, pollen are collected through suction of certain volume of air. The velocity and duration of rotating sampler should be known. It records the atmospheric concentration of pollen gains, molds, fungal spores and other aerobiological particulates as a function of time. The Hirst trap shows better results for small pollen size that are less than 10 µm.(Adhikari et al., 2004; Galán et al., 2016; Irdi et al., 2002; Rojo et al., 2015).

Figure 2. 7. Burkard volumetric trap.

The advantages of both these active samplers (Hirst and RotoRod) on gravimetric is that it quantifies the concentration of bio particulates in cubic per meter (Stevenson et al., 2015). However, when both these instruments were compared, results showed that Rotorod has a low collecting efficiency than Hirst trap for particles that are less than 10 μm. Another advantage of Rotorod instrument over Burkard is that, it is insensitive to wind speed. Both these volumetric sampler requires an external power source therefore aerobiological sampling at remote areas cannot be done, and they are much expensive as well (Frenz, 1999).

2.3 FACTORS AFFECTING DISTRIBUTION OF BIOLOGICAL AEROSOLS

Airborne aerosol concentration vary from place to place and several factors contribute in altering their concentration level. These factors include geographical location, time of the year, environmental variables, and local vegetation type (Adhikari et al., 2004; Rojo et al., 2015).

2.3.1 Meteorological Variables

In biogeographic studies, environmental factors have greatly shaped the distribution, occurrence and patterns of biological particulates (Vicente et al., 2014). Several attempts have been made in Aerobiological research to numerically analyze the collinearity between pollen spectra and climatic parameters at different regional scale (Minckley et al., 2000). Daily and annual pollen count levels have shown a significant correlation with many meteorological variables. Of the meteorological variables influencing the bio aerosols, the most prominent ones appear to be rainfall, wind speed, temperature and relative humidity. Once pollen is released in air, wind direction, wind speed and rainfall largely alters the composition and count of bio-aerosols (Rojo et al., 2015).

Meteorological condition varies with the geographical location. Therefore, the results of various studies have shown that climatic variables have a different response towards individual specie (Li et al., 2015). Prevalent weather conditions largely influence the composition and spectrum of pollen and fungus load. Therefore, detailed knowledge of local weather conditions is required (Li et al., 2015; Maya-Manzano et al., 2017). The results of Rojo et al. (2015) showed that hourly count data of different pollen taxa are largely influenced by wind direction. Annual pollen count showed a positive association with mean temperature and solar radiations while humidity and rain fall had inverse relation with most of the pollen taxa.

The local weather has a nonlinear relationship with the aeromycoflora as well (Corden et al., 2001). Many studies have been conducted to find the influence of meteorological variables with the fungus species (Hameed et al., 2009; Wu et al., 2007). Of all the variables, dew point, air temperature, relative humidity and mean wind speed

have been found significant in fluctuating the composition and count of airborne fungus (Grinn-Gofroń et al., 2015) but as mentioned earlier ,every fungus species have an inconsistent response towards each meteorological variable. Mitosporic fungi needs strong wind speed for their dispersal. On the other hand, the content of water in atmosphere (dew point and relative humidity) influences concentration of fungus species of basidiomycetes and ascomycetes family. Under favorable humidity levels, the spores are dispersed in the atmosphere. The air temperature plays an effective role in dispersal of spores but contrary to the wind circulation, its role is significant only at the ground level (Rivera-Mariani et al., 2012).

2.3.2 LAND USE LAND COVER

In addition to the regional climatic characteristics, the surrounding vegetation in the proximity of pollen traps greatly influences the total pollen count. The information about air borne pollen spectrum is a key indicator of surrounding vegetation, species type and plant phenology (Maya-Manzano et al., 2017).

The local vegetation type can be determined by identifying the pollen presence within that region. Given the proximity of urban pollen traps to parks and gardens, vegetation cover has a large impact on pollen count. Sampling stations that are at a near distance to vegetative cover gave more pollen count compared to devices where urban development is more (Weinberger, 2015). Widely planted ornamental trees surrounding the aerobiological station have a decisive effect on pollen count. Urban green spaces and broad leaves forest though covering 0.1% of total land Cover have contributed 70% of total pollen of the region (Rojo et al., 2015) .

Contrary to this ,Shendell et al. (2012) in his study investigated the influence of buildup area on the pollen count. Results of pollen concentration at nine different

locations showed that locations surrounded by parks have lower pollen influx compared to the central site of a class building.

Topography of a region could influence the pollen spectrum. It has been found that people living at higher altitudes are more prone to pollen allergy as pollen load is found to be high at high altitudes due to high wind speed resulting in long distance pollen transport (Jochner et al., 2015).

2.3.3 SPATIAL SCALE

Most of the pollen and mold alert values are measured at a single station using a stationary sample. There arises a doubt that "are these levels represents the correct measurements of the whole region"?

There is no adequate information on the number of sampling sites that are needed for the monitoring of airborne aerosols. Sampling at multiple sites have not been well documented. Few researches have been conducted so far.

Adhikari et al. (2004), conducted a study to find whether multiple- sampling sites are preferable over single station for aeroallergen reporting within a city. The results showed a statistically significant variations of total spore concentration ($P< 0.0001$) among five stations.

When sampling of individual taxa is performed, the height of Aerobiological sampler is also a considerable factor. In a study conducted by Chakraborty et al. (2001) , six samplers were installed at different height intervals. The results showed that for most of the pollen taxa count, there is positive correlation with increasing height and for smaller particles like fungus and molds species, higher count was found corresponding to lower heights.

2.3.4 AIR POLLUTANTS

The influence of air pollution and meteorological factors have been well documented. However, few researches have been devoted in recent years, to find the direct influence of air contaminants on biological aerosols and it has been found that there exists a significant correlation between air pollutant and air borne biological aerosols (Grinn-Gofroń et al., 2011; Konishi et al., 2014; Sousa et al., 2008). Ørby et al. (2015), in his study, found that ozone concentration levels coincides both seasonally and diurnally with pollen concentrations. Similarly, some studies have shown that particulate matter could stick to the air borne pollen grain (Duque et al., 2013), altering the morphological characteristics of pollen, which leads to increase in allergic sensitization (Ribeiro et al., 2015).

It has been clear that interactions of biological pollutants with its surrounding environment is complex and needs further investigations. In an urban environment, the spread of pollen in the lower atmosphere could be altered by the degree of obstruction of the buildings, local meteorological conditions, and height of the sampling station.

Chapter3

MATERIALS AND METHODS

This chapter explains the detailed methodology adopted for the study. Chapter has been divided into different sections. For collection of aerobiological data, five portable volumetric sampler were developed and placed at six sites with different land cover characteristics. The samples collected were then examined under light microscope for pollen and fungus identification and counting.

3.1 STUDY AREA

The study was undertaken in one of the sectors of Islamabad, Pakistan. The city of Islamabad lies in the Potohar plateau, northern eastern part of Punjab province, at northern latitudes 33º 49' and longitudes 72º 24' east of Greenwich. The capital is roughly 14 km away from Rawalpindi. To its north, lies the Margalla Hills. The topography of Islamabad contains hilly and plain lands with altitude ranges from 457 to more than 900 meters at the Margalla Hills above the sea level. The city extends to an area of about 906.50 square kilometers. According to the Pakistan Meteorological Department, the mean temperature value range between 3.9°C in the coldest month (January) to 46.1°C in the warmest month (June) with an annual rainfall of about 1141 mm. There are many plant species producing pollen in Islamabad and responsible for causing pollens allergy by. As described earlier, trees, weeds, grasses and molds spores are the main sources of allergic pollens in Islamabad (Abbas et al, 2012).

To investigate the spatial heterogeneity of biological aerosols at a local scale, H-12 sector of NUST campus was chosen as the study area. The campus is located at sector H-12 along Kashmir Highway. It covers a total area of 3.28 square kilometer. The campus has unique landform characteristics. The terrain is uneven with moderately

sloping and rolling hills covered by sparse vegetation and soil while the buildup area of the campus is mostly plain. Besides natural vegetation cover, the campus has many organized and well maintained grassy fields. Thousands of ornamental trees are planted inside the campus at various locations. According to the campus' horticulture department there are over 3000 tress that are spatially distributed within the campus area. They are found along road sides, around parking lots and nearby all the buildings present.

As shown in Figure 3.1 the campus has largest natural vegetative cover of 2.7 square kilometer, while organized grassy field covers an area of 0.5 square kilometer. The building density that contains most of built-up features covers an area of approximately 0.1 square kilometer. Buildings are sparsely distributed with tallest building has the height of 19 feet approximately. The university also has some water bodies in the form of lakes.

The study area was selected as it represents a small built-up urban design with some natural land cover features. Second consideration was the closer vicinity of features that can help to investigate the spatial heterogeneity of airborne bio-aerosols at various sites.

3.2 METHODOLOGY

The methodological approach taken in this study is briefly described in flow diagram (figure 3.2) .For biological aerosol field survey, five portable devices that were similar in mechanical design to Rotorod Sample Model 40 were made. Pollen and fungus data was collected as a primary data that were to be used for further analysis.

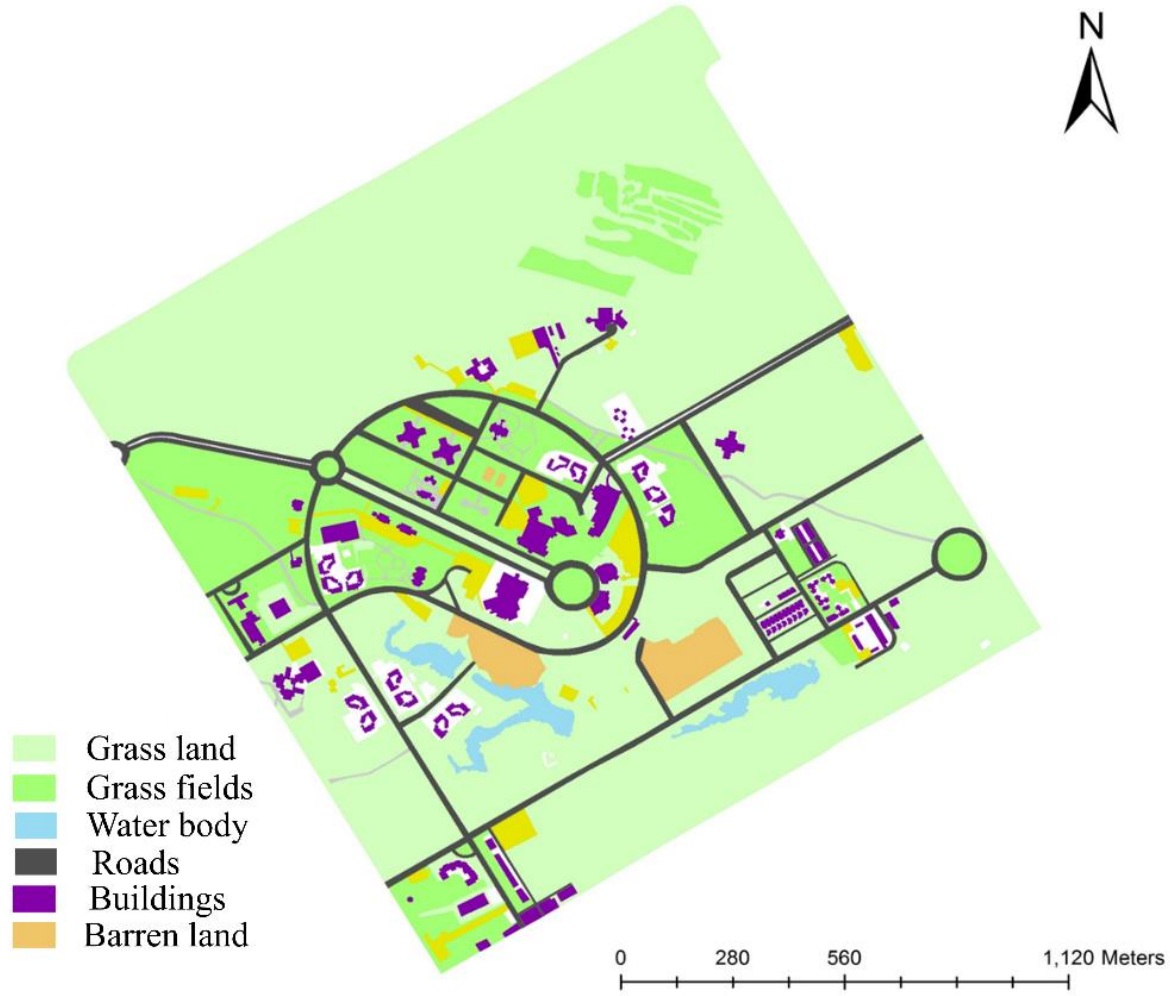


Figure 3. 1. The study area showing H-12 Sector, Islamabad.

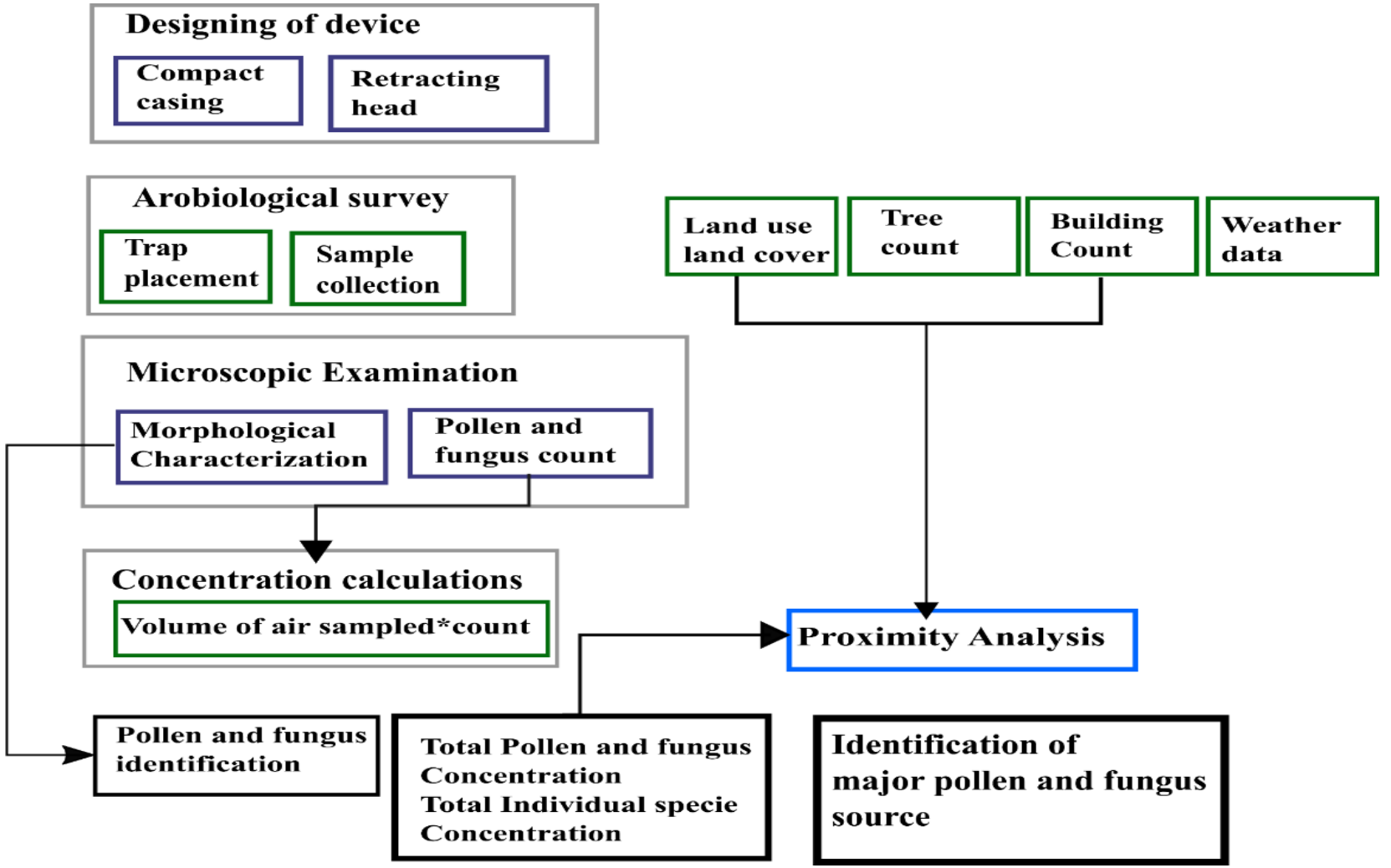


Figure 3. 2. Methodological flow diagram.

3.2.1 DESIGNING AND WORKING OF SAMPLING DEVICE

For capturing the airborne pollen and fungus, the primary data of this research, a sampling device was required. As discussed in literature, Rotorod and Hirst trap are the frequently used mechanical devices for capturing the bio aerosols but the reason that any one of these devices could not be used for research was:

Both the devices are:

- Expensive, therefore multi- site sampling at the same time cannot be conducted;
- Requires an external power supply, thus their use at remote locations not possible;
- Large in size and weight;
- Not locally available.

Keeping in view these reasons and limitations, a portable, low cost device was designed for data sampling at various locations on a local scale.

3.2.1.1 PORTABLE VOLUMETRIC SAMPLER

A low cost portable active impaction sampler has been designed for quantitative sampling of pollen concentration. The basic principle of the device resembles that of a rotating impaction sampler commonly known as Rotorod (Raynor et al., 1961) with few changes in dimensions and material used for its making. The general specification of the device are given in the Table 3.1.

The device incorporates a 12V battery (DC power source), that spins the motor at a nominal rate of 2700 rpm (Cox et al., 1995). As the sampling was to be done at regular intervals, therefore, digital programmed timer was installed in the device that

controlled the spinning of the retracting head by time mechanism as shown in Figure 3.3. The retracting head consists of a fixed rotating metallic head mounted with a glass rods for pollen collection. The compact casing also includes relay, transformer and automatic charging circuit for regulating the voltage and charging of the battery.

3.2.2 COLLECTING SURFACE AND ADHESIVE

Rectangular rods, bars, slides or tapes have been used as a collecting surface for impaction samplers. The materials commonly used for making the rods are either plastic or glass (Juozaitis et al., 1994; Levetin, 2004; Stevenson et al., 2015).Therefore, glass rods were used as a collecting surfaces for bio aerosol capturing as shown in Figure 3.4. Glass rods were mounted vertically on the retracting head. The dimensions of the rods are: length=0.15cm, length=0.15cm and height=3.5 cm (Brown, 1993; Adhikari et al 2003). Glass rods are coated with an adhesive (silicone gel) so that particles in air could stick to the surface (Raynor et al., 1961). The silicone gel is tacky as well as transparent thus making the rods visible for microscopic examination.

3.2.3 FUNCTIONING AND PERFORMANCE OF PORTABLE SAMPLER

For the aerobiological sampling, five such portable devices were made. The rpm of the device was tested using tachometer. The device could operate continuously for two hours without external power supply. For a 10 percent duty cycle i.e. spinning the motor for every ten minutes interval, it can operate continuously for 7 to 8 hours thus making the device capable of collecting biological aerosols at remote locations. The device needs to be charged, depending upon the time for which it has been in operation. It requires maximum half an hour time for recharging.

3.2.4 SAMPLER PLACEMENT

The bio aerosol sampling was conducted in National University of Science and Technology, Islamabad campus. Six sampling sites were selected on the basis of feasibility, availability of conveyance and several other factors like proximity to water bodies, proximity to road, elevation and land cover characteristics. These sampling sites are shown in Figure 3.5.

Samples were collected from 4th April 2016 till 20th April 2016. At stations 1,2,3,4 and 6 sampling was conducted during morning hours between 10:00 -13:00, while at station 5 the data was collected from 15:00 P.M - 18:00 in the afternoon hours. Among the survey sites, five were outdoor Locations while one site which is site 6 was an indoor place in order to compare the results between outdoor and indoor locations. The location of each site was recorded using GPS. . Daily samples were collected from the six stations during working days of week while on some days samples were not collected limited charging of portable sampler. Landform characteristics with their geographical location of each site are summarized in Table 3.2. Placement height of volumetric trap is same for sites one to four but different for site 5 and 6. The two sites of different heights were selected in order to find the influence of height on the pollen concentration.

Figure 3. 3. Portable volumetric sampler.

Figure 3. 4. Pollen collecting surface

Table 3. 1. General specification of Portable Volumetric sampler.

Specifications	
Height Width Length	12 cm 6 cm 17 cm
Weight	500 gm
Sampling duration	10% duty cycle
Motor	DC
Battery	12 V

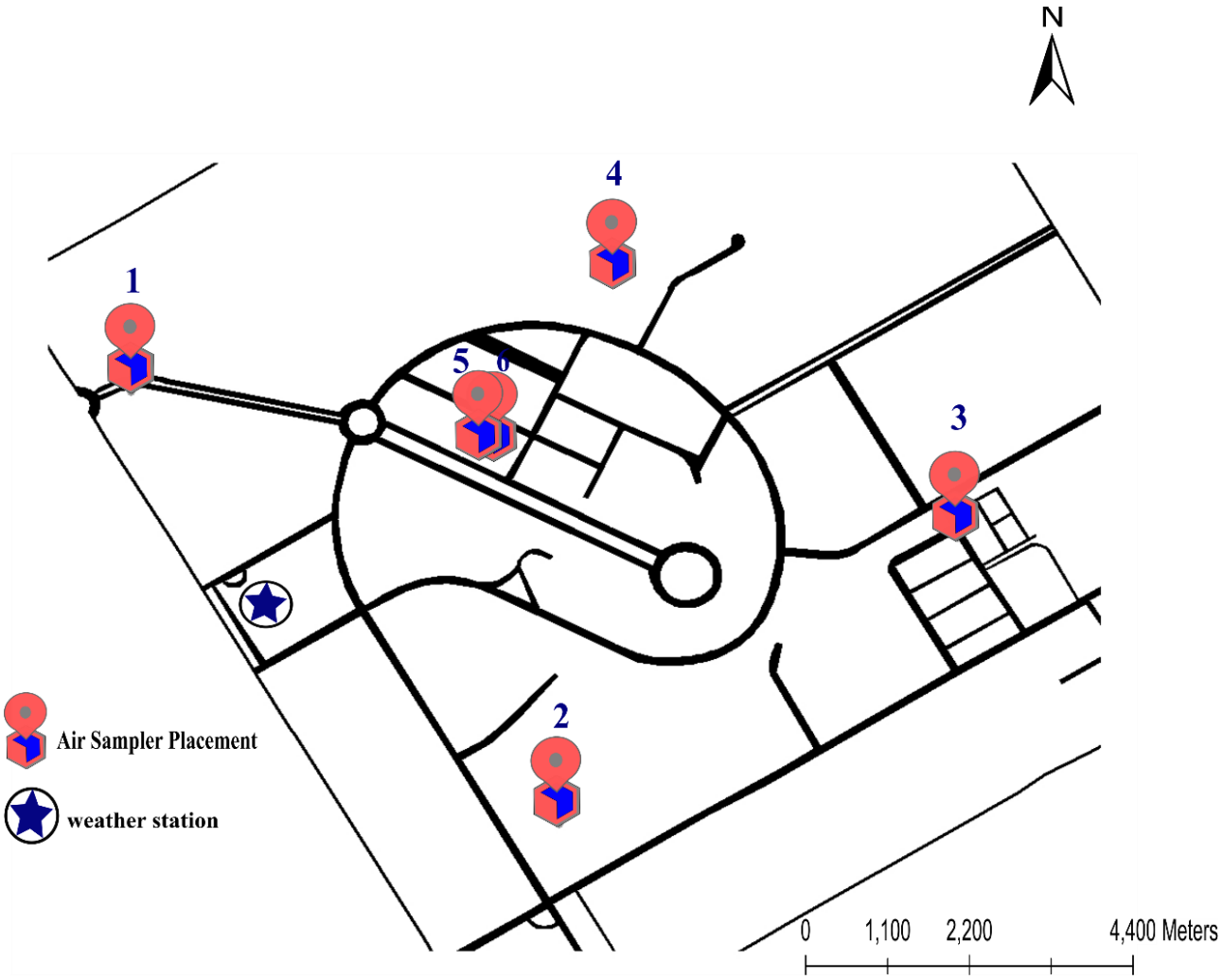


Figure 3. 5. Volumetric sampler placement.

Table 3. 2. Characteristics of sampling sites.

Sampling Site	Location		Site Characteristics	Height of Sampler
	Latitude	Longitude		
1	312751.1	3724730.74	More trees, University main entrance gate, grassy lands, near road with continuous anthropogenic activities.	3 ft. above ground
2	313562.3	3723863.38	Lake, shrubs and sparse vegetation and road at a near distance	3 ft. above ground
3	314315	3724436.4	Apartments, building, grassy lands, sparse vegetation	3 ft. above ground
4	313662.4	3724946.29	Ongoing construction work, dumping site, shrubs and grassy land, polluted water stream.	3 ft. above ground
5	313427.1	3724611.55	Rooftop of a building	50.25 ft. above ground
6	313416.5	3724603.88	Placed at window of a room which has cabinets, papers and electronic gadgets.	25.125 ft. above ground

Site 1

Site 2

Site 3

Site 4

Site 5

Site 6

Figure 3. 6. Volumetric Trap placement at six sites.

3.2.5 MICROSCOPIC EXAMINATION

Storing of samples was a cruicial step as these samples were to be examined after the two weeks of survey, therefore, it was necessary to prevent the coated rods from being drying out. Within limited resources, an air tight plastic box containig a molding material (dough) was placed at the bottom and coated gels were employed vertically on dough as shown in Figure 3.7. The samples were stored at room temperature.

Total of 144 samples were collected which were then taken to the Environmental Microbiology lab. The samples were examined using direct microscopy technique. This technique is a non-viable approach that has been widely used for outdoor samples (Burge et al., 1987; Levetin, 2004; Singh & Mathur, 2012).The rods were exposed directly under compound microscope. No stain was applied to rods as the stain couldn't retain on rods due to in availability of rods holder (see Figure 3.8). After careful observation of magnifications available, the pollens and fungus were both identified at 40X. Entire surface of the rods were examined in order to attain maximum accuracy.

Coated rods contained dust particles, molds, spores and pollen, therefore, each sampling rod took approximately one hour of examination. Images of different species of these biological aerosols were captured directly from the Microscope.

3.3 METEOROLOGICAL DATA

The meteorological data were collected from ESMAP Tier1 Meteorological Station, Islamabad. The weather station was installed by U.S.- Pakistan center for

Figure 3. 7. Sampler storage box.

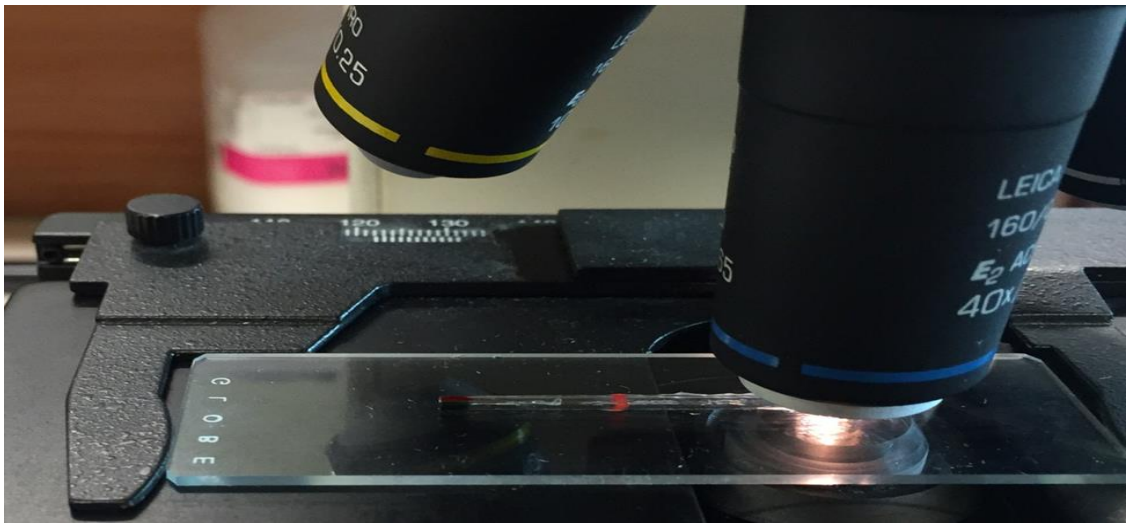


Figure 3. 8. Exposed rods examined at 40X.

advanced studies in energy (USPCAS-E), NUST. The station is installed at latitude 33.64191 °N and longitude 72.9838 °E with an altitude of 500 m. The weather station is capable of monitoring ten minute interval, daily average and hourly data of meteorological variables. Temperature (maximum, mean, minimum), Wind speed, relative humidity, global horizontal irradiance, wind gust and wind direction are measured by the station. Accuracy of anemometer is greater than 0.1 meter per second over 5 to 25 meter per and that of wind vane is < 1%. The details are shown in Table 3.3.

As biological sampling duration was from 10 A.M. – 6 P.M, therefore, daily averages of climatic attributes were not taken. 10 minute data for each meteorological parameter was averaged out to find more precise result. Data were extracted for the complete sampling period. The meteorological data was further used for finding the correlation with pollen and fungus concentration .the wind direction values were also incorporated to generate the wind rose plot in order to understand the direction of local winds in the region during the specified time.

Table 3.3. ESMAP Tier1 specifications.

Measured values	
GHI	Global Horizontal Irradiance in W/m
DHI	Diffuse Horizontal Irradiance in W/m², both measured with K&Z CMP21 pyranometer
T_{amb}	Ambient air Temperature in °C
RH	relative humidity in %, measured with Campbell CS215
WS	Wind speed in meter per second m/s
WS_{gust}	maximal measured wind speed within the time interval
WD	Wind direction in °N (to East), measured with NRG 200 Wind Direction Sensor.
WD_{StDev}	Standard deviation of wind direction within measurement interval.
BP	Air pressure from Campbell CS100 barometric pressure sensor
Cleaning	Cleaning of sensors: A cleaning event is marked with a "1".
Error	Error message recorded by the data logger. Full operation of the equipment is marked with a "0".

Chapter 4

RESULTS AND DISCUSSION

4.1 RESULTS

The aerobiological sampling performed by portable sampler captured the desired bio aerosols. Thirty three different kinds of pollen grains and fourteen species for fungus were identified with *Aspergillus* and *Alternaria*, as the dominant fungus specie in the region. The Proximity analysis of LULC with pollen and fungus concentration showed that nearby grasses and shrubs are the contributing factor in pollen and fungus load. The results of the study showed that spatial scale, height, local vegetation, wind direction influence the distribution of pollen and fungus.

4.1.1 VOLUMETRIC AEROBIOLOGICAL SURVEY

The aerobiological survey using portable sampler provided us with primary data of our research i.e. pollen and fungus count. Through the microscopic pictures of various kinds of pollen and fungus, it was possible to identify the biological particulates that were dominant in the sector H-12.

4.1.1.1 SAMPLER DESIGN AND PERFORMANCE IN THE FIELD

The volumetric sampler was capable of capturing the air borne pollen and mold. The spores with size less than 4 µm are captured by this device. In comparison to the costs of commercially available devices where Hirst trap is $5,500 and Rotorod Model 20 is $822 approximately (Stevenson et al., 2015), this device was made in 6000 pkr ($56) which proves to be a cost effective approach. This portable device with low weight and size was easily taken in to the fields and can be replicated in comparison

with the inertial devices therefore it was possible to take measurements at remote locations.

However, there are some limitations of this device that leads to inefficiency of the portable sampler. The device was not water proof so the main risk involved was the electrical hazard that could damage the device. Daily sampling became a little hectic as after using it in the field, the device needs to be charged as well. With limited human resources and equipment, daily charging of five devices became difficult at times and sampling could not be performed the very next day at few sites.

4.1.2 IDENTIFICATION OF BIO PARTICULATES

Biological aerosols, which are present in sufficient amount in the ambient air are characterized on the basis of their unique physical surface features. Microscopic observation of our samples revealed that portable sampler, being a non-selective, captured dust particles, insect fragments, pollens and fungus species. Among these only pollen and molds were considered for identification and absolute counting.

The bio aerosols may be present either in groups like clusters or as a single particle. Pollen and fungus are identified on the basis of their various physical and morphological characteristic (Galán et al., 2016).

4.1.2.1 MORPHOLOGICAL CHARACTERIZATION OF POLLEN

Pollen form different taxa share similar features when observed under light microscope. Since this was a generalized attempt, therefore for pollen identification, few morphological characteristics, which were observable at 20X were considered. The identification has been done on the basis of, (Kapp et al., 2000) classification. Twenty four different kinds of pollen were identified in our study as shown in palette below.

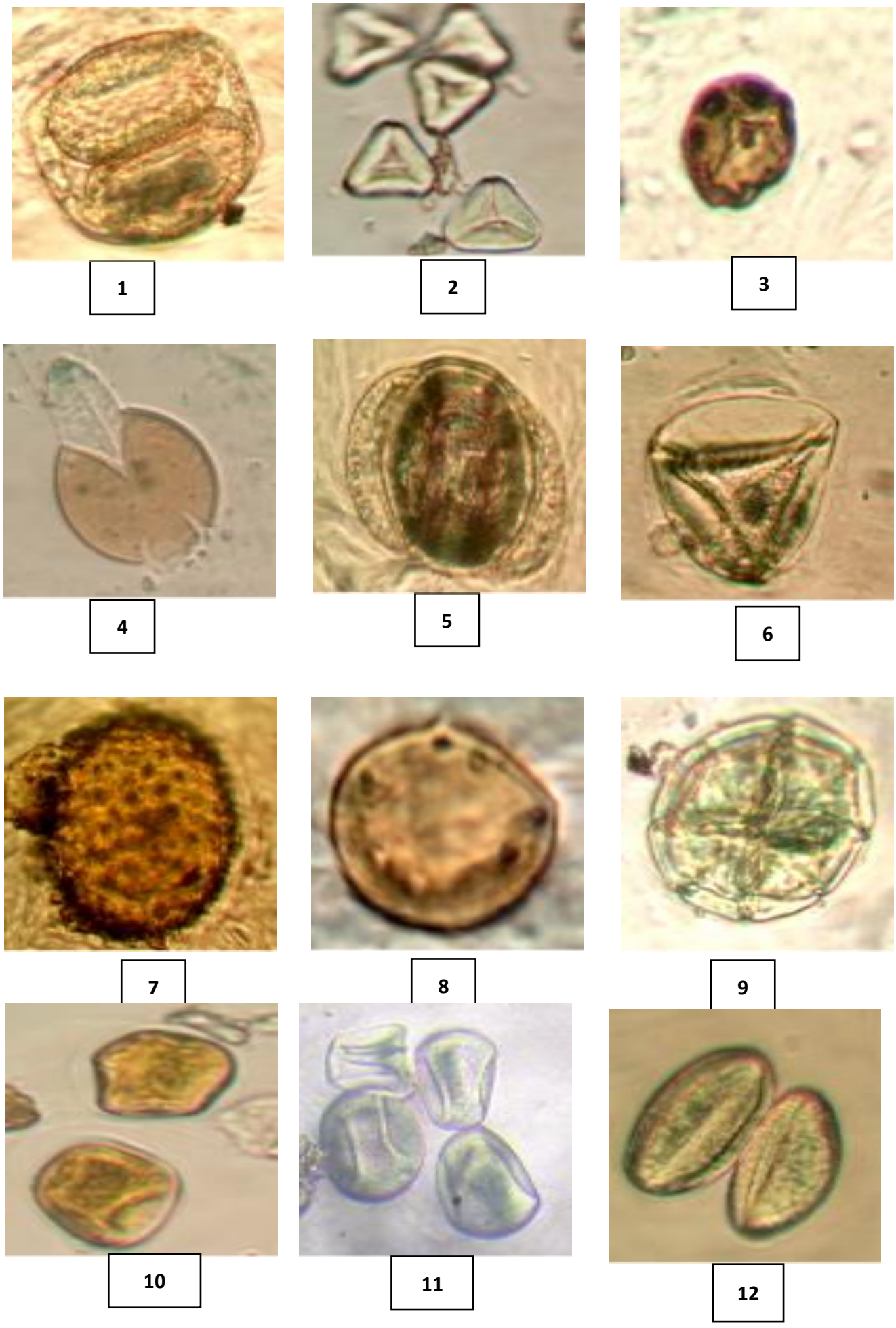
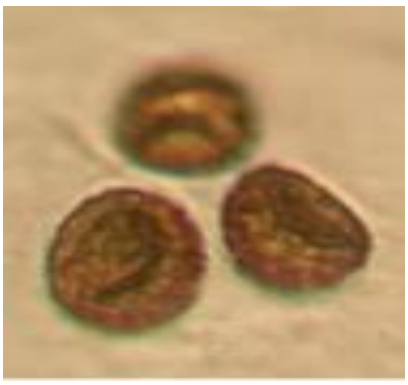


Figure 4. 1. Types of pollen photographed at 40X



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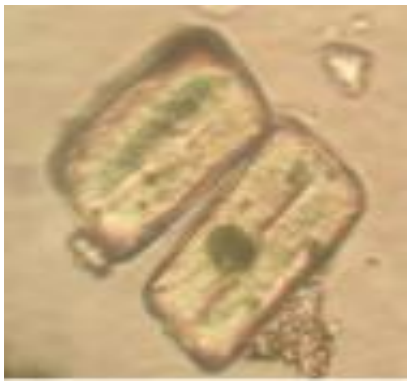

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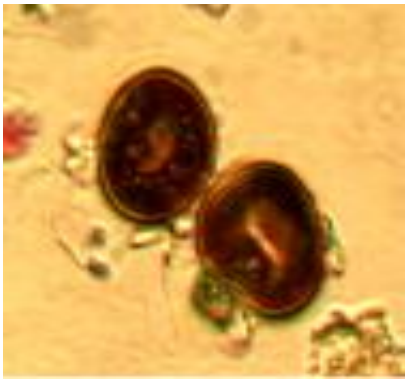

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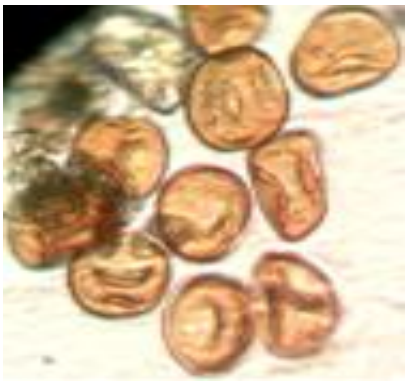

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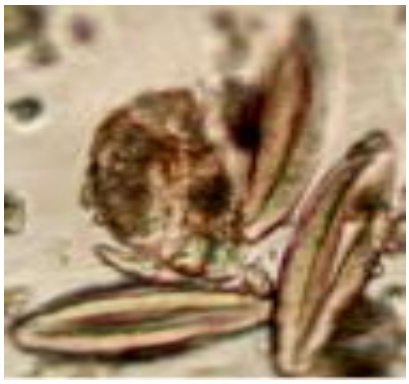

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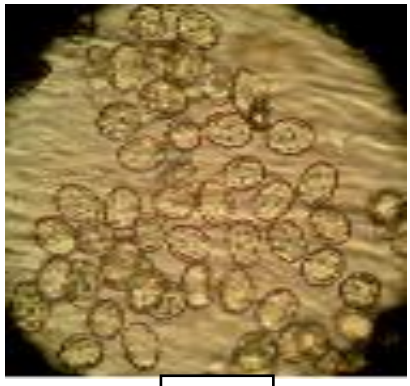

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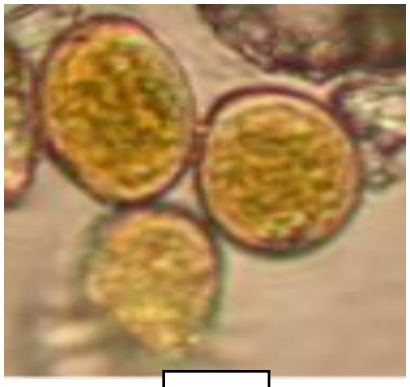

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Table 4. 1. Classification of pollen grains on basis of morphological characteristics.

No	View	Sculpture	Surface	Exine	Pollen class	Cell type	Shape
1	Equatorial	Granulate	Aporate	thick	Dicolpate	Diyad	Circular
2	Polar	Psilate	Aporate	thick	Tricolporate	Monad	Sub angular
3	Polar	Echinate	Periporate	Thin	Periporate	Monad	suboblate
4	Polar	Psilate	Aporate	thick	tricolpate	Monad	spheroidal
5	Polar	Psilate	Finely regulated	thick	Saccate	Monad	saccate
6	Polar	Psilate	Porate	thick	Tricolporate	Monad	sub angular
7	Equatorial	Reticulate	Periporate	thick	Inaperturate	Monad	spheroidal
8	Polar	Psilate	Periporate	thick	Inaperturate	Monad	spheroidal
9	Polar	Granulate	Periporate	thick	Octacolporat	Monad	Circular
10	Polar	Psilate	Aporate	thin	Tetracolpate	Monad	oblate spheroidal
11	Equatorial	Psilate	Aporate	thin	Dipcolporate	Monad	Suboblate
12	Equatorial	Psilate	Aporate	thick	Tricoplorate	Monad	prolate
13	Polar	Granulate	Aporate	thick	tricolpate	Monad	Peroblate
14	Polar	Psilate	Aporate	thin	Tetracolpate	Monad	oblate spheroidal
15	Equatorial	Psilate	Aporate	thick	Monocolpate	Monad	rectangular
16	Polar	Psilate	Aporate	thick	Inapperturate	Monad	subprolate
17	Polar	Psilate	Porate	thick	Dicolporate	monad	apple
18	Polar	Psilate	Aporate	thick	Dicolporate	monad	spheroidal
19	Equatorial	Psilate	Aporate	thick	Monocolpate	Monad	perprolate
20	Polar	Psilate	Aporate	thick	Dicolporate	monad	apple
21	Polar	Echinate	Porate	thick	tetracolporate	Monad	oblate spheroidal
22	Polar	Granulate	Aporate	thick	tricolpate	Monad	Peroblate
23	Equatorial	Psilate	Aporate	thick	Dicolpate	Monad	tubular
24	Equatorial	Psilate	Aporate	thin	Inaperturate	Monad	Spheroidal

The twenty four different kinds of pollen found in the region showed that the local vegetation is contributing in pollen flux as the same pollen grains were found at all the six sites with few exceptions as we identified the pine pollen at roof top. This indicate that pine pollen could transport to a long distance, though there is no presence of pine trees in H-12 sector .The morphological characteristics showed that most of the pollen found were monad with varying shapes like circular, triangular, spheroidal etc. Ten pollen classes were identified on the basis of their aperture structures. The results of physical characterization showed that region consists of various types of pollen taxa that could be further identified at the family level.

4.1.2.2 MORPHOLOGICAL CHARACTERIZATION OF FUNGUS SPECIES

Fungus were identified on the basis of these two following characteristics

- Hyphae (septate/non-septate)
- Characteristics spores

Based on these characteristics, fungus were identified at genus level as shown in Figure 4.2. Fourteen different kinds of fungus were observed in the region of which few were found in abundance. Four different kinds of *Alternaria* was found along with *Aspergillus* spores that were in abundance. Among these *Alternaria*, *Cladosporium, Aspergillus* and *Penicillium* are prominent indoor and outdoor allergens (Heseltine et al., 2009).

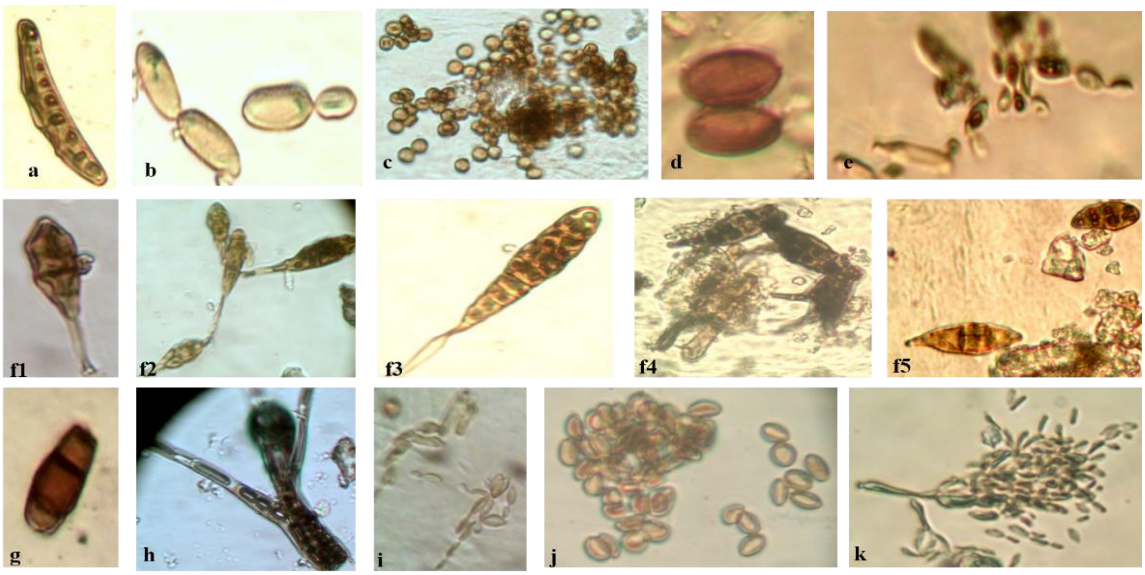

Figure 4. 2. Genera identified from non-viable mold sampling counts. a. *Drechslera sp.* b. *yeast cell* c. *Aspergillus spores* d. *Emericella* sp. e. *Penicillium sp.* f (1-5). *Alternaria sp.* g. *Curvularia* sp. h. *Tetraploa sp*. I. *Cladosporium* sp. j. *Emericella* k. *Fusarium.*

4.1.3 POLLEN AND SPORE CALCULATIONS

Average concentration of pollen and spores in atmosphere should be calculated in particles per cubic meter. Concentration of particles is reported in this unit in order to standardize the atmospheric count and to compare samples of different areas or equipment.

In order to determine the pollen and spore calculations, Ted Brown, the originator of Impaction Sampler technology, developed the conversion factors and calculations. (Levetin, 2004). Using these calculations, pollen and spores concentrations in air were calculated. It involves three basic steps

- Calculate number of particles in the sample
- Calculate total volume of air encountered by rods
- Calculate the number of particles per cubic meter.

4.1.3.1 TOTAL VOLUME OF AIR SAMPLED

Number of particles were counted using direct microscopy method. The volume of air encountered by rods in particle per meter cube is calculated using the following formula

$V\ (m^3) = \text{Rod area}\ (m^2) \times D \times p \times RPM \times t$ ……. (1)

Where,

V is volume of air, Rod area = width of rod x length of the rod x 2 (both rods), D is the diameter of the Rotorod head, RPM is rotation per minute, t is minutes sampled per day (Adhikari et al., 2003; Oh et al., 1998).

To calculate the total pollen and spore concentration in air, divide the total number with volume of air sampled. Hence pollen and spore count was obtained in particle per meter cube

Concentration of particle (**C**) = $\frac{\mathbf{N}}{\mathbf{V}}$ …… (2)

Where,

N= Number of particles counted

V= volume of air sampled

Hence concentration levels are then represented as particle per cubic meter in table 4.2 and 4.3.

4.1.4 ABSOLUTE POLLEN COUNT

From the concentration data of pollen, total pollen count for 12 days for each site was calculated as shown in Figure 4.3. Data values showed that spatial heterogeneity exists at different sites. The results of daily concentration and total concentrations of pollen showed that maximum high values for pollen were found at site 2 which was an open space. Daily data values showed that during 12 days of sampling period, open space sampling site has highest pollen count for four days while for other eight days site 1 and site 3 showed the highest concentration value respectively.

The lowest values were observed for site 6, an indoor location, throughout the sampling period with an exception on date 12, 13 and 18 where site 5 showed lowest values.

4.1.5 ABSOLUTE FUNGUS COUNT

Contrary to pollen concentration results, highest concentration count for fungus was observed at site 1 which is the University Entrance as shown in Figure 4.3. For five days site 1 showed daily peak spore concentration values. Critical evaluation of fungus data showed that site 6 though showed lowest count in most of the sampling days but the concentration data was significant.

Table 4. 2. Spore Concentration in meter per cube.

Spore Concentration data						
Date	**1**	**2**	**3**	**4**	**5**	**6**
4/4/2016	360	320	86	0	0	202
4/5/2016	41	123	51	468	266	20
4/6/2016	78	26	165	71	332	0
4/7/2016	825	272	321	362	317	44
4/8/2016	259	47	43	173	0	11
4/12/2016	323	176	263	252	110	151
4/13/2016	354	338	929	392	136	293
4/14/2016	291	148	392	248	211	118
4/15/2016	170	254	85	195	121	80
4/18/2016	247	89	78	9	104	74
4/19/2016	71	98	304	65	67	134
4/20/2016	215	314	147	120	138	39

Table 4. 3. Pollen concentration per meter cube.

Pollen Concentration data						
Date	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
4/4/2016	32	489	13	0	0	9
4/5/2016	154	76	13	46	21	4
4/6/2016	80	123	148	46	78	0
4/7/2016	229	163	163	342	246	21
4/8/2016	340	84	123	199	0	11
4/12/2016	110	201	83	281	21	177
4/13/2016	128	296	95	285	26	52
4/14/2016	58	61	95	105	72	23
4/15/2016	54	224	51	103	26	7
4/18/2016	135	47	45	3	16	49
4/19/2016	8	45	209	10	23	19
4/20/2016	88	176	103	11	15	5

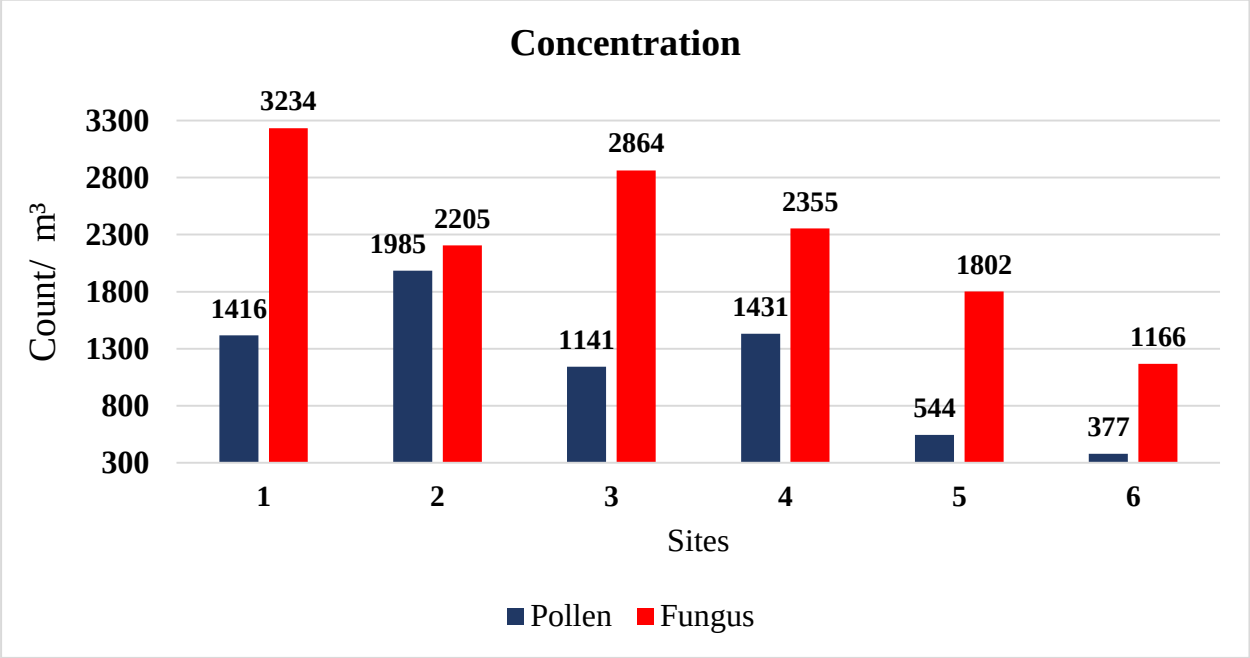

Figure 4. 3. Total pollen and fungus count at six sites.

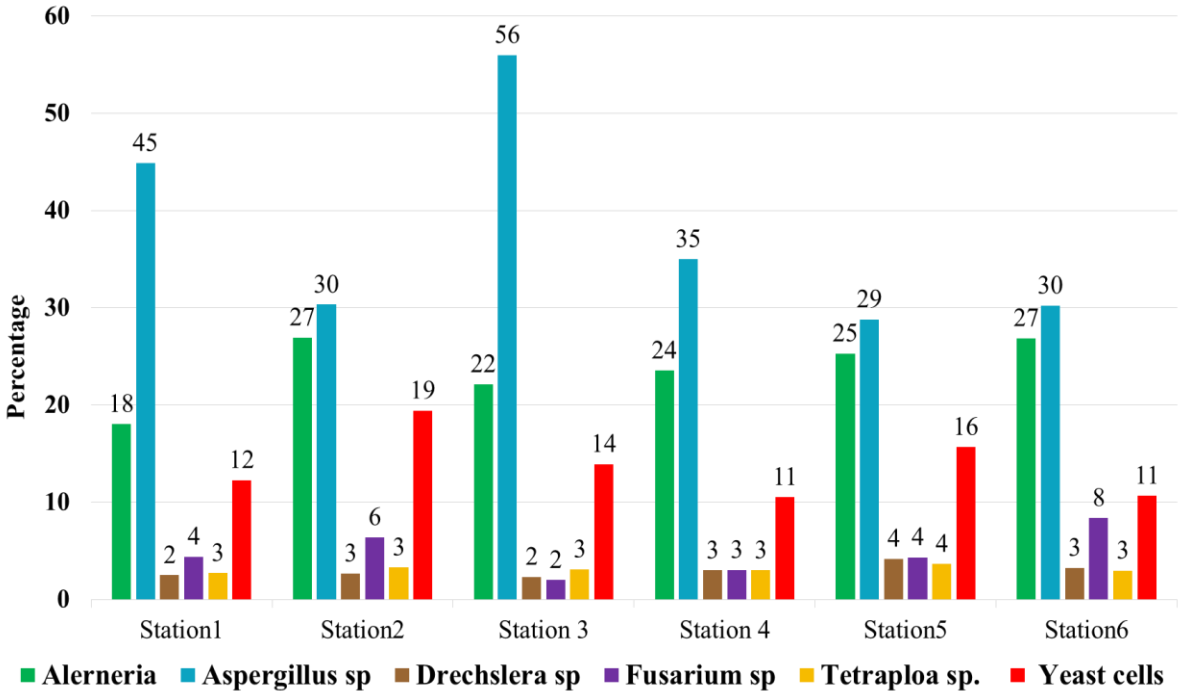

Figure 4. 4. Percentage contribution of abundantly occurring fungus species.

4.1.6 PERCENTAGE CONTRIBUTION OF INDIVIDUAL FUNGUS SPECIE

Percentage values of individual taxa have shown results that differ with respect to total fungus count. The percentage contribution results have showed that almost 70-80 % of data comprises of *Alternaria* sp. and *Aspergillus* as shown in figure 4.3. As station 1 has the highest count for total fungus however the individual taxa percentage contribution has revealed that at site 4 *Alternaria* has the highest contribution in the total fungus count and percentage contribution of *Aspergillus* is high at site 3.

From individual specie percentage results it is found that *Aspergillus* and *Alternaria* are making the maximum fungus concentration as shown in Figures 4.5 and 4.6. The results of *Alternari*a at different sites showed that it has highest values at station 1 and station 2. However in case of *Aspergillu*s, maximum amount is fount at site 1 and site 3. Another observation about these two spores is that the count decreases with the height.

4.1.7 CORRELATION OF METEOROLOGICAL VARIABLES WITH POLLEN AND FUNGUS COUNT

Among environmental parameters, meteorological variables were correlated with pollen and fungus count at six sampling sites. The variables considered for finding correlation with pollen and fungus count were temperature, relative humidity, and wind speed, wind gust and global horizontal Irradiance. Ten minute data was available for these meteorological variables. The results showed different relation with pollen and fungus count. Pearson correlation results as described in table six showed that site 2 has a significant positive correlation of 0.6 with wind speed. The results (Table 4.4 and 4.5) have shown that overall no significant correlation was found at all sites except for site 2.

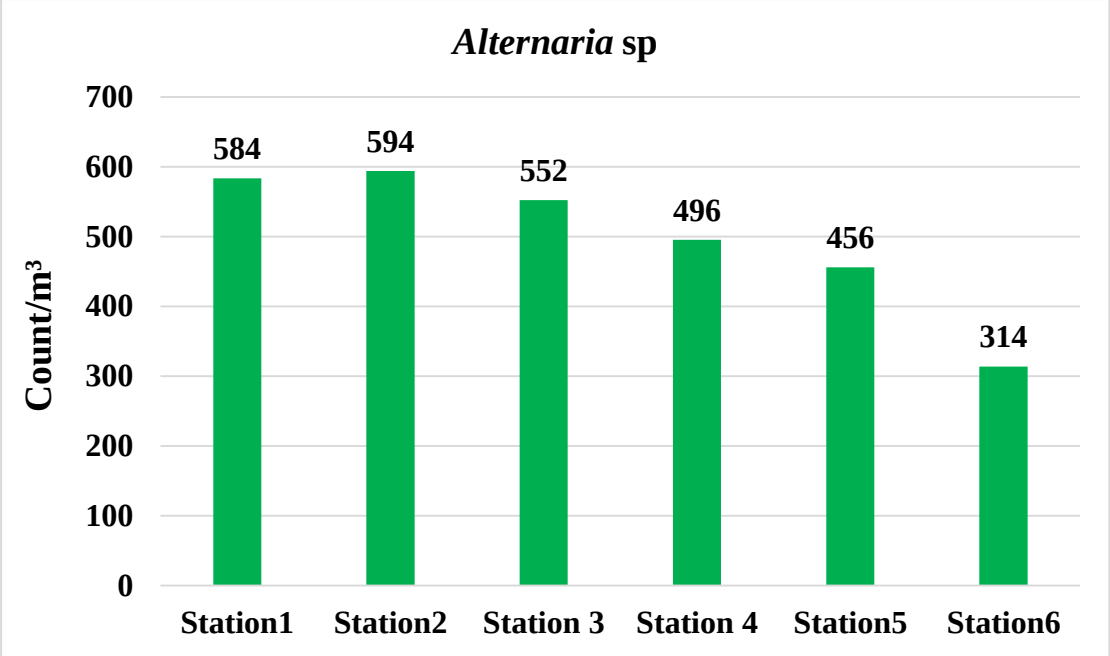

Figure 4. 5. Concentration variation of Alternaria.

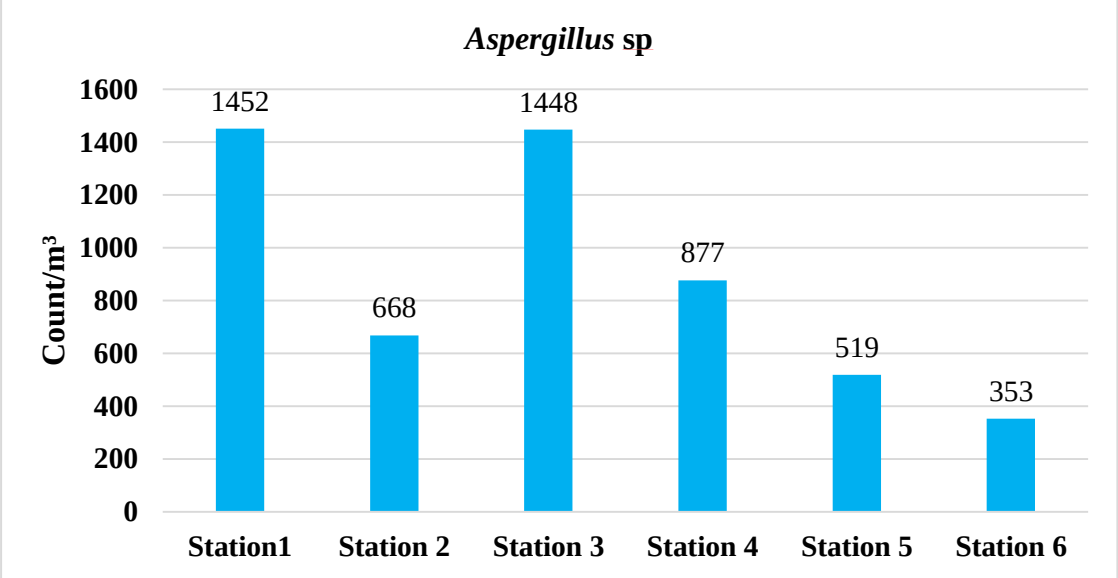

Figure 4. 6. Concentration variation of Aspergillus Spores.

Table 4. 4. Correlation between meteorological variables and pollen count.

Sampling Sites	Wind Speed	Wind Gust	RH	Temperature	GHI
1	-0.52	-0.47	-0.12	-0.061	0.19
2	0.60	0.56	0.46	-.0143	0.54
3	0.22	0.35	0.09	-0.385	-0.63
4	-0.04	0.04	-0.12	-0.020	0.26
5	-0.15	-0.04	0.04	-0.263	-0.20
6	0.32	0.32	0.09	-0.018	0.16

Table 4. 5. Correlation between meteorological variables and fungus count.

Sampling Sites	Wind Speed	Wind Gust	Temperature	RH	GHI
1	0.2	0.2	-0.1	0.1	0.3
2	0.6	0.7	0.2	0.0	0.7
3	0.4	0.5	0.0	-0.1	0.1
4	-0.5	-0.5	0.2	-0.3	0.3
5	-0.2	-0.2	-0.4	0.0	-0.3
6	0.6	0.6	0.1	0.1	0.5

4.1.8 WIND DIRECTION AND POLAR PLOT

The wind rose plot was drawn in order to find the maximum direction from which the wind was coming during our sampling period (Figure 4.7). The results showed that during 1st April till 20th April of sampling duration, most of the winds came from the west and northwest direction. The wind direction explains well that what other environmental variables could be attributed to the non-significant relation of wind speed at five of the sampling sites.

4.1.9 PROXIMITY ANALYSIS

In the recent years, the software of Geographic Information System has been integrated alongside aerobiological data for analytical analysis (Maya-Manzano et al., 2017). The proximity analysis is a basic technique of GIS that was used to find the relationship of pollen flux with the surrounding vegetation. Land use land cover maps of area 200 meter in radius were created surrounding the volumetric traps. Tree count, building density was also calculated within the proximity region. The vegetation of the region was divided into two main classes i.e. grass lands and grass fields. The percentage area for each land cover class was calculated as shown in Figure 4.9 and the area coverage was then analyzed with pollen and fungus concentration in order to find the potential sources for these two biological aerosols (Table 4.6 and 4.7).

4.1.10 INDOOR AND OUTDOOR VARIATION OF A SINGLE BUILDING (I/O)

In order to find how much of the pollen grain and fungus spore could penetrate inside the building the ration for Indoor to Outdoor variation was found (Figure 4.8). The values for total concentration for rooftop and office window was used to find the I/O ratio. The results showed values of 0.69 and 0.64 for pollen and fungus respectively.

The fraction value shows that almost 60 – 70 % of outdoor pollen and fungus spores could reach near the room window and penetrate into indoor environment.

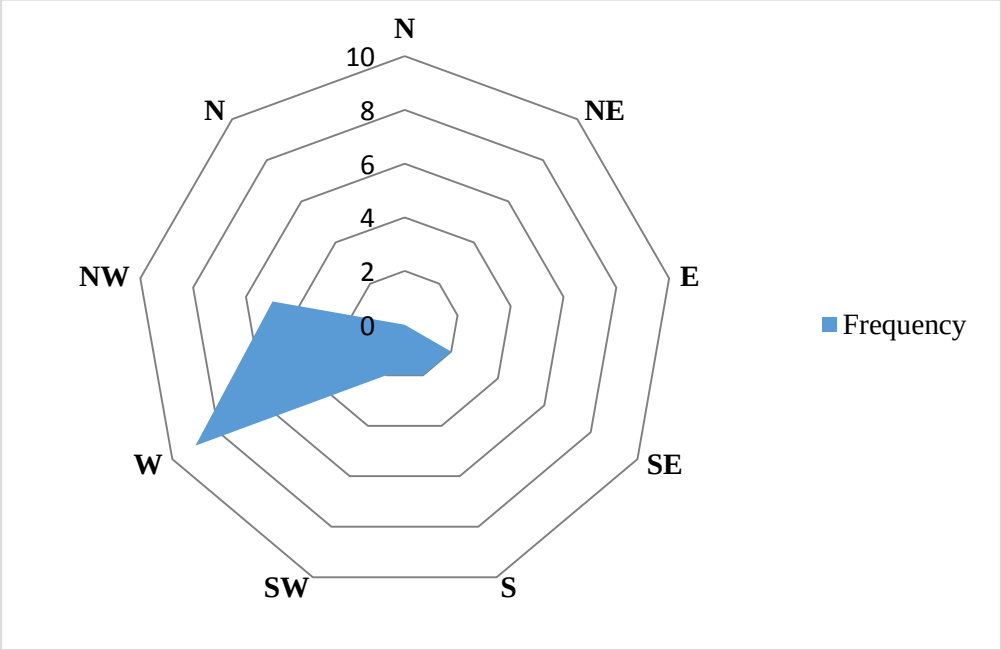

Figure 4. 7. Wind direction plot.

Figure 4. 8. Site of a single building.

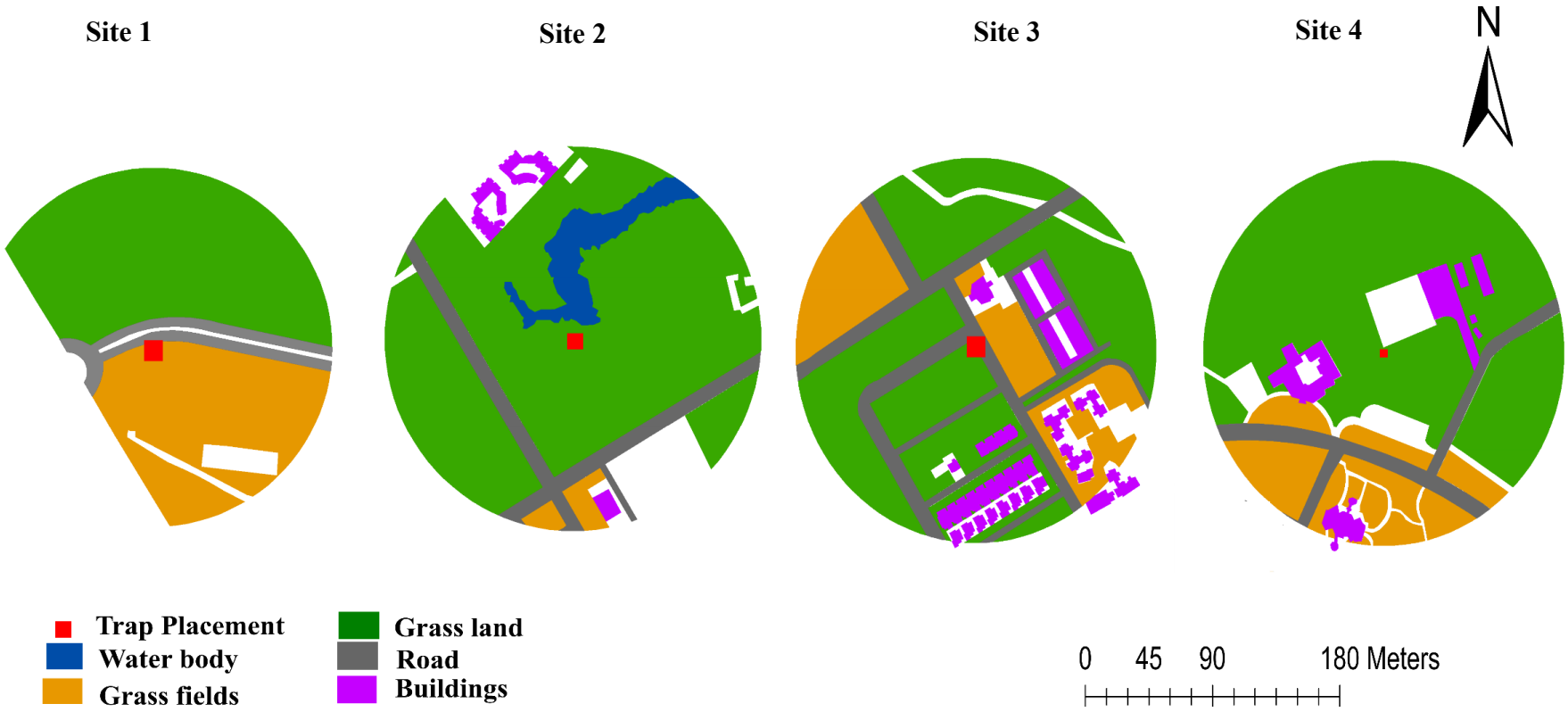

Figure 4. 9. Land use land cover map of radius 200 m.

Table 4. 6.Proximity analysis interpretation for pollen data.

Sampler Placement	Station Number	Pollen Concentration	Grass Lands	Grass Fields	Building density	Tree count (within 100 m)
Open Space	2	1985	72%	2%	3	18
By Grid station	4	1431	63%	18%	3	36
University Entrance	1	1416	41%	27%	1	78
Near Residential Area	3	1141	49%	18%	11	59

Table 4. 7. Proximity analysis interpretation for fungus concentration.

Sampler Placement	Station Number	Fungus Concentration	Grass Lands	Grass Fields	Building density
University Entrance	1	1985	72%	2%	3
Near Residential Area	3	1431	63%	18%	3
By Grid Station	4	1416	41%	27%	1
Open Space area	2	1141	49%	18%	11

4.2 DISCUSSION

Presence of biological particulates in the air and its interaction with the atmosphere is a complex phenomenon. Air quality can be determined by the presence of ambient biological particulates in a region. Many factors are responsible in altering the concentration of bio aerosols as described in the literature review section. The impact of a single variable affecting the concentration is difficult to determine, therefore, multiple factors were considered for a comprehensive analysis. Considering this approach, an exploratory study was conducted to examine the distribution of pollen and fungus spores at a regional scale. The study included the construction of low cost portable volumetric sample for pollen and fungus collection, an aerobiological field survey at different sites to find the distribution of bio aerosols, statistical analysis with environmental variables and concentration data to find the influence of these variables on pollen and fungus count and proximity analysis in order to determine the land use and land cover features that could alter the concentration of pollen and fungus count

4.2.1 PORTABLE VOLUMETRIC INSTRUMENT

The low cost portable volumetric device that was constructed for capturing the biological aerosols proved to be effective in field survey. In comparison to the commercially available aerobiological sampler, this device was built in 6000 (pkr) thus making it economical. The results of microscopic imagery have showed that the device was capable of capturing the pollen and fungus spores with size less than 4 µm. With three hours of monitoring period, the highest count observed was 929/ m^3 showing that device could capture significant amount of microorganisms. As the device has advantages, it also has some limitations.

One of the main issue with the portable sampler was voltage level of battery. As the device operates at 12V of battery therefore voltage level was checked before going into the field. The battery of three devices were replaced as the voltage level of the battery decreased thus degrading the rotational speed of glass rods. The sampling duration during high wind speed should be kept small in order to avoid overloading of glass rods.

The length and width of the glass rods were same as used in the Rotorod device. However the rpm of our instrument was 2700 while most of the Rotorod devices uses motor of 2400 rpm. There are no standard calibrations for this type of sampler except that rotation rate of the motors were checked with a tachometer and voltage level of battery were observed before going into the fields. The device proved to be a useful instrument for air quality monitoring in a small region.

4.2.2 AEROBIOLOGICAL SURVEY

The aerobiological field survey revealed that significant portion of biological aerosols are present in the study area during the spring season. *Aspergillus, Penicillium, Cladosporium* and *Alternaria are* the dominant air pathogens that are involved in causing hay fever and several other respiratory and allergic diseases. Identification of fungus species have shown that H-12 sector contains some ambient spore types. It was found in the results that *Alterneria* and *Aspergillus* are predominant allergens at all the six locations with high concentration levels. The presence of these spores is an indicator of poor air quality in the region. The main sources for airborne allergens dispersal in the sector could be the dampness of building material, poor sewage system, plant diseases, and excessive cutting of grasses in order to maintain the fields and human activity. The high concentration at university Entrance and near residential areas indicate that dispersal could be due to human movements as well.

As for pollen analysis, twenty four different kinds of pollen grains have been identified. Classification on the aperture level has indicated that the region possesses different species with few pollen grains belonging to the same family. Though all pollen grains cannot be allergenic however, high pollen concentration values poses threat for the allergic and asthmatic patients in spring season. One of the interesting result was the identification of pine tree pollen grain. As there are no pine trees within the University campus, therefore it showed that distant vegetation could also contribute along with the local vegetation of a specific region. The high pollen flux values have also indicated that the area comprises of massive tree planting and occupies large vegetation cover.

4.2.3 TOTAL POLLEN AND FUNGUS COUNT

The multi-site sampling performed, indicated the pattern of spatial distribution within the study area. Sites were approximately 1 km apart from each other. Total pollen count values showed that spatial scale is of significant importance when aerobiological monitoring is to be conducted at a regional scale. Total count values showed that Site 1 has highest fungus count of 3234 and site 2 has highest total count of 1985 for pollen during the 12 days of sampling period. Total pollen count was different for each site indicating that spatial variations are significant at regional scale (Adhikari et al., 2003; Wu et al., 2007).

4.2.4 EFFECT OF METEOROLOGICAL VARIABLES ON TOTAL POLLEN AND FUNGUS COUNT

The correlation of meteorological data with total pollen and fungus count was significant for only one site. The different response towards meteorological variables of each site could be attributed to small sample size. For any statistical technique to be used it has been found that large sample size gives more accurate results. The second

reason could be that meteorological relationship between fungus and pollen count has been well documented but still it has been observed that interaction of biological aerosols have a complex relationship with climatic variables.

4.2.5 PROXIMITY ANALYSIS

The results of the proximity analysis have shown that the major contributor for pollen flux is grass. The pollen concentration is higher for areas with large grass lands. The results for fungus data have shown that for areas with more grass fields have shown higher concentration of fungus. Secondly, the presence of fungus is high in areas with higher anthropogenic activities as compared to other sampling sites.

In case of individual pollen grain type, most of the higher concentration for specific pollen grain was found at open space with type 11, 14, 21 and 24 being prominent. Type 17 was found in highest concentration by grid station. For Individual fungus, *Aspergillus* spores shows highest concentration both at University Entrance site and near residential area, as for *Alternaria*, highest count was seen only at University entrance site.

We infer from proximity analysis that the pollen and fungus content is same at all sites with difference in concentration values, thus indicating that it represent the local vegetation with short range transport. Throughout the sampling period the average wind speed was almost 3 m/s thus giving stable conditions for pollen and spore dispersal. With more grass land at site 4 but less pollen concentration in comparison to site 1 could be due to the barrier of buildings and uneven topography at site 4.

Chapter 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This research aimed to study the daily variations of the pollen and fungus count in an urban environment and factors that contribute in altering the concentration levels of these Bio aerosols. The aerobiological survey highlights that single station sampling is not an ideal approach for quantifying pollen and fungus levels as significant difference in concentration level exists at small regional scale. The identification of pollen grain class and fungus species provided us with the information of dominant taxa and fungus spores presence in the region. Moreover, the prevalence of *Alternaria* and *Aspergillus* molds within the region is an indirect indicator of poor air quality of the region. Though small sampling time, however correlation with the meteorological variables at six sites indicate that biological aerosols have a complex interaction with the local meteorological conditions. Land use Land cover maps within the radius of 200 meter along with wind direction plot indicated that open land with no barriers in the immediate surroundings have shown high concentration of pollens . The proximity analysis indicated that grasses are the major contributor in local pollen flux.

5.2 LIMITATIONS

Aerobiological survey was conducted at six sampling sites for the collection of pollen and spore data for 12 days. One of the limitations of our research is small sampling period that had eventually effected our results where statistical analysis was to be performed. The reason for a small sampling period was the limited human resources and few problems that were encountered with the device in the field.

5.3 RECOMMENDATIONS

The findings of the research suggests the following recommendations.

- As the results showed that concentration levels are higher close to the ground as compared to roof tops of the buildings, therefore aerobiological stations should be installed at a lower height.
- Multi-site sampling should be preferred for aerobiological monitoring with in the city.
- High levels of ambient fungus spores in outdoor environment indicate that all human induced factors that contribute in adding to the concentration of these pathogenic spores should be monitored.
- The portable device with few more changes in material and glass rods design could be used for monitoring the biological air pollution in indoor environment like schools, hospitals during the spring season.

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