"Relationship of Temperature with Pollens and Effects of Paper Mulberry Pollen Grains on Environment and Human Health"

Researcher:

Akhter Ali

Registration No: 75-FBAS/MSES/F09

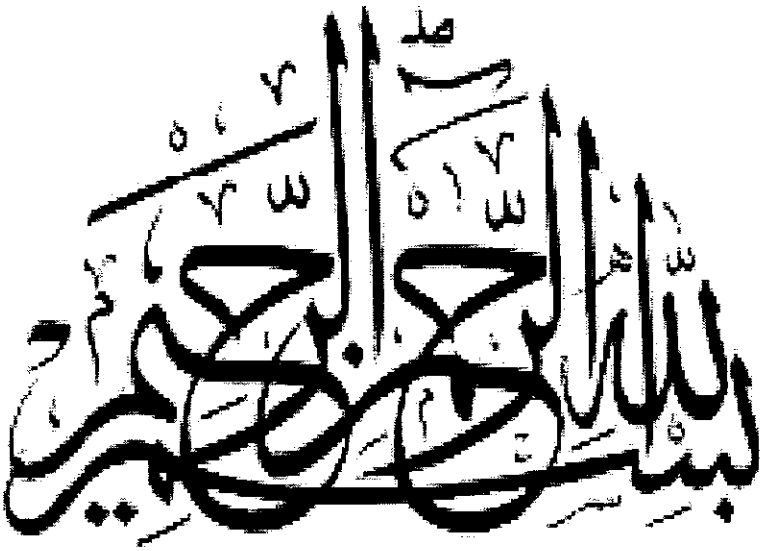
Submitted in partial fulfillment of the requirements for the Master of Philosophy in Environmental Sciences at the faculty of Basic and Applied Sciences

International Islamic University, Islamabad.

Dr. Muhammad Asad Ghufran **2009-2011**

1 - Pollination

2. Pollination by insects



In the name of Allah the Most Merciful, the Most Beneficent

DEDICATION

I dedicate this humble effort to my most loving Mother & Father who always wished to see me glittering high on the skies of success like Galaxies. My loving sisters and brothers whose love and mellifluous affection arten me to achieve success in every share of life My affectionate teacher who always helped and guided me to fulfill my research work.

DECLARATION

The work reported in this thesis was carried by me under the supervision of Dr. Muhammad Asad Ghufran (International Islamic University, Islamabad), for the completion of MS Degree 2009-2011.

I also hereby declare that the substance of this thesis has neither been submitted elsewhere nor is being concurrently submitted for any another degree.

I further declare that the work embodied in this thesis is the result of my own investigation and where work of other investigation has been used, it has been fully acknowledged.

Akhter Ali

75-FBAS-MSES-F-09

Department of Environmental Sciences

International Islamic University, Islamabad

Acceptance by the Viva Voce Committee

Thesis Title: Relationship of Temperature with Pollens & Effects of Paper Mulberry Pollen Grains on Environment & Human Health
Name of Student: Mr Akhtar Ali
Registration No: 75-FBAS/MSES-F09
Accepted by the Faculty of Basic and Applied Sciences, Department of Environmental Sciences, INTERNATIONAL ISLAMIC UNIVERSITY, ISLAMABAD in partial fulfillment of the requirements for the Master of Studies in Environmental Science

Viva Voce Committee

Dean (FBAS)

Dr. Muhammad Irfan Khan
Faculty of Basic & Appl. Sciences

Chairperson (DES)

Dr. Muhammad Irfan Khan
Faculty of Basic & Appl. Sciences

Supervisor

Dr. Muhammad Asad Ghufran
(Assistant Professor, IIU)

External Examiner

23/2/12

Dr. Asma Jabeen
Fatima Jinnah Women University

Internal Examiner

Dr. Ibrar Shinwari
(Assistant Professor, IIU)

Dated: February 23, 2012

Abstract

The current study aims to find out the influence of particular meteorological parameters (Temperature, Relative Humidity and Rainfall) on atmospheric pollen concentration. Along with the subsequent effects of the increased pollen numbers on the allergy patients in Islamabad. The study will help to appraise the seriousness of allergy problems along with other respiratory diseases among the residents of Islamabad, which is enhanced by the excessive Paper Mulberry pollen grains concentration in the atmosphere specifically during spring months. The study is intended to find a relationship between various meteorological parameters and Total Pollen Count (TPC) into the atmosphere during 2009 and 2010. Pollen numbers in the atmosphere is measured by using RotoRod sampler. Extremely high pollen concentrations in the atmosphere were recorded in the month of the march while the lowest concentrations were recorded in the month of December in both years (2009 and 2010). The high and low pollen concentrations into the atmosphere are mainly based on the pollen production from the Paper Mulberry tree. Different metrological parameters like average temperature, relative humidity and rainfall are correlated with total pollen count to draw a relationship which provides useful information for the allergy patients and policy makers. The TPC is also correlated with allergy patients visiting some hospitals throughout the study years (2009-2010). Maximum total pollen number (43,780 grains/m^3) was observed at 17^{th} march, 2009 with average temperature (19.45°C) and Relative Humidity (59%) with zero rainfall. Similarly in case of 2010, Maximum total pollen number (41,800 grains/m^3) was observed at 17^{th} march, 2010 with average temperature (22.55°C) and relative humidity (60%) with no rainfall. The trends from month wise data and by regression analysis showed that the average temperature has a positive correlation with TPC while relative humidity and rainfall generally have negative relationship with it. The change in TPC along with different environmental variables is also supported by linear regression. It was established by ANOVA that the TPC (for years 2009-2010) in relation with different environmental variables was insignificant ($P \leq 0.93$), while in case of paper mulberry pollen the regression values for the month of March, 2009 and March, 2010 were significant with ($P \leq 0.004$) and ($P \leq 0.044$) respectively. Two years data (2009-2010) of pollen patients from two hospital records (PIMS and NIH) and meteorological parameters also studied with TPC. A total of 9952 pollen patients were observed in 2009 while 11607 pollen patients were observed in 2010 which showed that the subsequent increase in TPC also increased pollen allergy patients.

ACKNOWLEDGEMENT

All praises be to ALMIGHTY ALLAH (SWT) who guides us in darkness and helps us in difficulties He bestowed me with the ability to do this research. All respects to our Holy Prophet Hazrat Muhammad (peace be upon him) who is forever a torchbearer of guidance and heart , flourish my thoughts towards achieving high ideas of life.

I am obliged to my worthy supervisor, Dr. Muhammad Asad Ghufran (Assistant Prof. of Environmental Biology), Department of Environmental Sciences, International Islamic University Islamabad, Pakistan whose aluminating suggestions , inspiring guidance and consistent encouragement enabled me to produce this piece of work.

I also express my heartfelt thanks to my dear friends Muhammad Yahya, Sahib Raheem, Mubashar Gardezi, Misbah ud-din, Ali Rehman, Muhammad Waqas and Adnan Saleen for their co-operation, good wishes and moral support.

Last but not the least, words are lacking to express my humble obligation to my loving and affectionate parents, whose hands always raised for my success and escalation, their prayers always helped us in coming out for the complex situation, and my brothers and my sister for their special help and encouragement throughout my studies.

May Allah Almighty bless all of you with His Honorable blessing! (Aameen)

Akhter Ali

TABLE OF CONTENTS

Chapter 1: INTRODUCTION

LIST OF TABLES

LIST OF FIGURES

List of Abbreviation

PM: Paper Mulberry

TPC: Total Pollen Count

CDA: Capital Development Authority

NIH: National Institute of Health

PIMS: Pakistan Institute of Health

PMD: Pakistan Meteorological Department

ANOVA: Analysis of Variance

MR: Multiple Regressions

Forwarding Sheet

The thesis entitled "Relationship of temperature with pollens and Effects of Paper Mulberry Pollen Grains on Environment and Human Health" submitted by Akhtar Ali in partial fulfillment of MS degree in Environmental Sciences has been completed under my guidance and supervision. I am satisfied with the quality of student's research work and allow him to submit this thesis for further process of as per IIU rules and regulations.

Date.....

Signature.....

Name.....

CHAPTER 1

INTRODUCTION

1.1. Background:

Pollen allergy and associated respiratory problems are common all over the world; likewise in Pakistan this is causing various health problems e.g. asthma, skin allergy, hay fever and allergic rhinitis etc. According to Pakistan Meteorological Department (PMD) reports the airborne pollen concentration of pine, cannabis, some introduced ornamental plant species and different grasses shows a very high value in the atmosphere of Islamabad, specially in spring. There is an increase in pollen concentration of paper mulberry and it dominates the other allergic pollen after mid of March. Islamabad is situated at 33°26′N 73°02′E / 33.43°N 73.04°E / 33.43; 73.04 at the Northern edge of the Potohar Plateau, near Himalayan foot-hills called Marghlla Hills. It is located at an altitude of 507 meters i.e. 1663 feet above sea level (asl.). The total area of the capital city is 906 Km^2 (approximately 350 sq. miles). Another 2,717 Km^2 (1,049 sq. miles) area is covered by the Marghlla Hills. The weather ranges between -1°C (30.2°F) minimum in January to 46°C (114.8°F) maximum in June. Islamabad receives average annual rainfall of about 1141 mm. Total population of Islamabad is about 9,50,000 of which 6,30,000 is Urban and 3,20,000 is Rural (GoP, 1998).

The predominant flora on the top mountains and slopes of Marghlla Hills includes *Pinus roxburghii* (chir), *Acacia modesta* (phulai), *A. arabica* (kikar), *Olea ferrugenia* (kohau), *Dodonaea viscosa* (sanatha), *Justicia adhatoda* (baker), *Carisa opaca* (garanda), *Woodfordia fruticosa* (dhavi), *Morus alba* (tout), *Ficus carica* (fig, wild fig), *F. religiosa* (pepal), *F. Bengalensis* (bar), while *Broussonetia papyrifera* (paper

mulberry) trees are found in the foothills or plain areas of Islamabad. Since *Broussonetia papyrifera* (paper mulberry), *Cannabis sativa* (bhang) and *Pinus roxburghii* (chir) along with others grass species are abundantly present in the residential areas of Islamabad. Therefore, they are known to produce serious allergy problems in the residents of the region (Abbas, et al., 2009).

1.2 Problem Statement:

The pollen allergy is among the emerging health problems of Islamabad, Pakistan. There are many plant species which are producing pollens but Paper Mulberry is the most dominating among them. The pollen of this plant produces many problems to pollen sensitive people of Islamabad. Capital Development Authority (CDA) is putting a number of measures to eradicate the plant, specially the cutting of male *Broussonetia* trees in greenbelts and wastelands, but due to its fast growth it is not only widely spreading throughout the Urban area of the Islamabad but still invading the new areas. Keeping in view the plant nature and its extant of occurrence the complete eradication is almost impossible. In the past no more research attention was given to solve this problem. Advance Research is very necessary to solve the problem of this pollen allergy plant, while in current research, an effort was made to study the pollens abundance, its pattern in various seasons in Islamabad as well as pollen victims numbers during these months.

1.3 Global Health Problems of Pollen Grains:

According to WHO, grasses and trees pollens are the most effective inhalant. Airborne pollen allergens in China are major inhalant allergens and cause type-I hypersensitivity such as Asthma, Rhinitis and Hay fever in atopic individuals and

there are about 10,000,000 patients with pollinosis (Liu, *et al.*, 2010). According to current survey, pollinosis affected people in USA are about 2%-10% and currently in Europe 20% of the population are affected. According to a report in India, there was an increase from 10%-30% of allergic problems during last 40 years. In Spain, there were about 24.6% school children victims of Allergic Rhinitis (AR) and around 20%adults (Liang, *et al.*, 2010).

According to the report of International Study of Asthma and Allergies in Childhood (ISAAC), Allergic Rhinitis range between 1.4%-39.7% of the total country population (Breton, *et al.*, 2006).

1.4 Current status of pollen allergy problem in Islamabad:

According to an estimation of Ministry of Health, 120,000 residents are victims of pollen allergy in federal territory of Pakistan. According to a survey held by National Institute of Health (NIH), about 15 % of the population in the industrialized countries suffered from allergies during 1983-1993. According to Pakistan Institute of Medical Sciences (PIMS) sources, 80-110 pollen allergy patients are visiting the hospital for nebulization and oxygen on daily basis during the season. Health physicians believed the first and the most affected sectors of the city is G-6, followed by F-6, F-7 and G-7 and then the remaining sectors of the capital. According to the report of Pakistan Meteorological Department (PMD), the most common tree in Islamabad responsible for pollen allergy is Paper Mulberry, mostly because of its massive pollen production in spring season.

1.5 Brief Introduction of Palynology:

Palynology is an interdisciplinary knowledge and is a branch of life science particularly plant science. Palynology is the science that studies contemporary as well as fossil palynomorph found in various types of deposition and sediments.

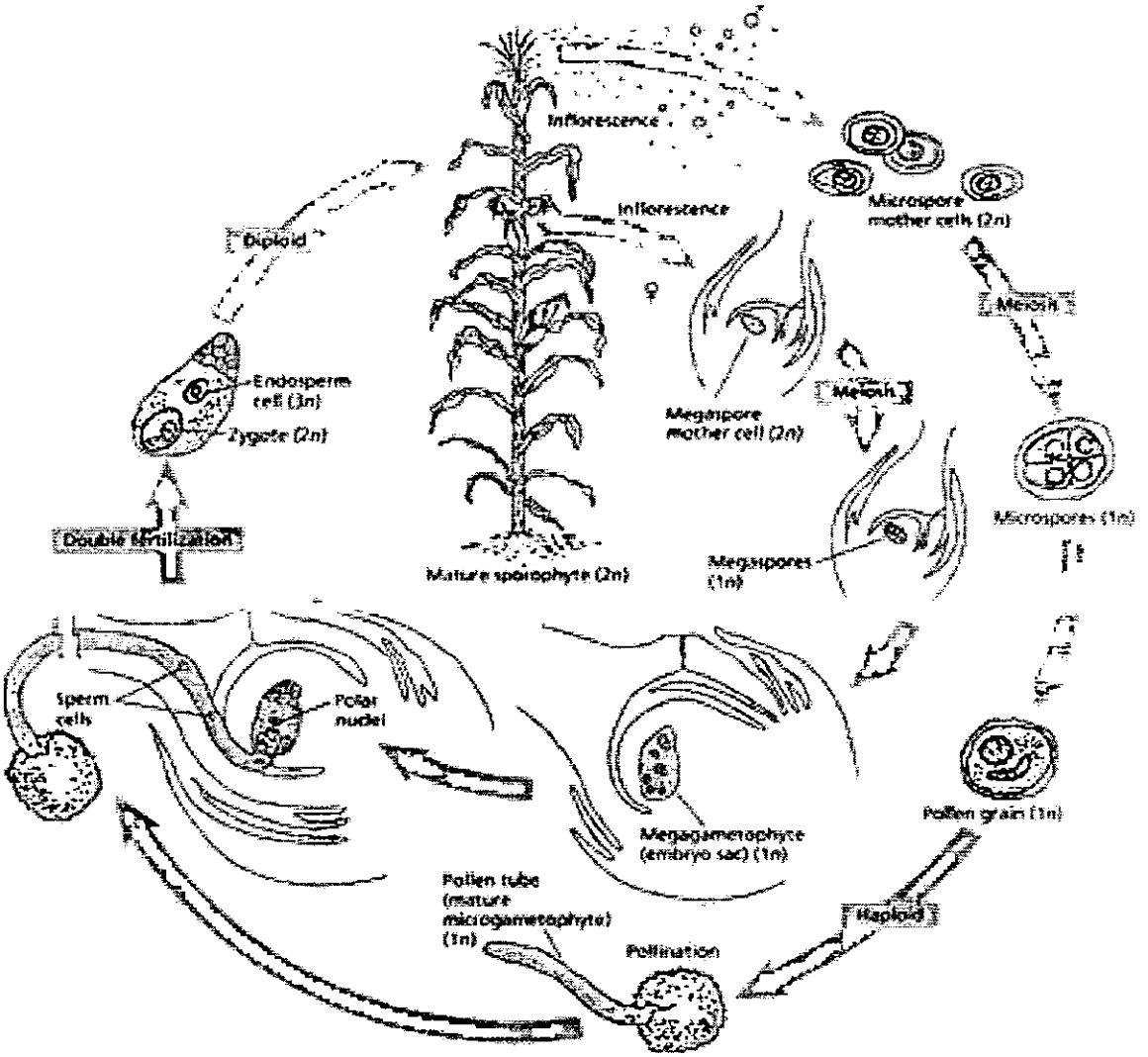
1.6 Pollen:

Pollen is a fine powder produced by certain plants when they reproduce. It contains haploid number of chromosomes to make embryos, seed and finally full plant after fertilization with the haploid ovule.

1.7 Structure of a Typical Pollen Grain:

The microscopic appearance of pollen is determined primarily by its three concentric domains: the (central) protoplast, the intine, and the (outermost) exine. The protoplast contains genetic material and various organelles. Starch granules, especially, can convey a distinctive appearance when they are numerous, as in certain herbs pollens (William, *et al.*, 1984).

Figure 1: Formation and Structure of a typical pollen grain



1.8 Dispersal of Pollens:

Airborne pollens released from flower clusters are carried by air currents. Pollens attached to dry vegetative surfaces are "catapulted" into air turbulent currents. In addition, pollen frequency at any site reflects the strength of upwind air sources both horizontal and vertical (William, *et al.*, 1984).

1.9 Types of Allergenic Pollens:

1.9.1 Grass pollens:

They are typically small sized pollen grains belong to the family Poaceae and their size ranges from 10-35 micrometer in diameter.

1.9.2 Weed Pollens:

They mostly belong to Asteraceae family and include ragweed pollens and their pollen size is about 18 micrometer in diameter.

1.9.3 Tree Pollens:

They belong to many different trees species especially in the higher altitude areas the *Betulaceae* family including Birch pollen, plays an important role toward the pollen allergy. Usually their size is about 30 micrometer in diameter.

1.10 Allergic Diseases:

The Allergic Diseases also known as Atopic Diseases include hay fever, rhino-conjunctivitis, allergic chronic obstructive pulmonary disease, allergic dermatitis, eczema, scabies, angioedema and anaphylactic shock (Bhalla and Singh, 2008).

1.11 Pollen Allergy:

It is mostly defined as bad immune-mediated over reaction to innocuous environmental substances such as allergens (Bhalla and Singh., 2008). Allergy can appear in any form of body response against an allergen like irritation of skin membranes, swellings of skin, lymph nodes and sub-cutaneous layers, or rashes and pain of various types.

1.12 General Allergy Diagnosis:

First of all, sensitization of allergy victims after the exposure to specific allergen. Exposed allergen can start allergic reaction. Then, there is inflammation because of increased IgE levels and de-granulation of mast cells (Bhalla and Singh, 2008).

1.13 Minor Plants causing allergy problems in Islamabad:

The plants which are releasing pollen grains in the atmosphere other than Paper Mulberry include *Acacia, Eucalyptus,* Pines, Grasses, *Cannabis,* Dandelion (*Taraxicum*) and *Alternaria.*

1.13.1 Grass Pollen:

Grass pollen is easily identifiable, characterized by a single pore. Typically, grasses begin pollinating in May, where some attractive and lawn grasses may produce pollen throughout the summer and into the fall, while for many local grasses this is the only pollination period. Like in Islamabad the pollen appearing in Monsoon season (August) is largely based on grass pollens.

1.13.2 Pine Pollen:

Pine pollen is found in forests, a yellow, flourlike material produced in the millions of tons each year all around the world. Divergent to the majority of flowering plants, pine trees are wind-pollinated. That means they rely on the wind carrying the pollens to the pine cone, as opposed to a pollinator to help them to reproduce. Every spring the trees release the pollens from their male catkins.

1.14 Paper Mulberry: *(Broussonetia papyrifera)*

The Paper Mulberry is ancestor of family Moraceae, local to eastern Asia. It is a deciduous tree mounting to 15 meters (49 ft.) tall and is most significant cause of allergy troubles in Islamabad.

Figure 2: A mature *Broussonetia Papyrifera* tree in Sector G-6, Islamabad

1.15 Eucalyptus pollen:

Eucalyptus pollen is an allergen that creates respiratory, skin and eye problems.

Basically two kinds of factors are involved in the making and discharge of pollens in the environment.

Various Biological & Climatic Factors affect the pollen production for different plant species. As concerning with climatic conditions, maturing of pollens inside anthers and their release from anthers directly depends on climatic conditions. There is a positive co-relation of this pollen concentration with increasing temperature and negative co-relation with increasing precipitation (Jato, *et al.*, 2002).

1.16 Pollen as a Bio-indicator of pollution:

Degradation of pollen structure and sub-structure of the external wall of sporoderm or the exine indicate about soil erosion. Modification of the ionic composition of pollen exine indicates about the increase in sulphur and chlorine concentration in the environment (Cerceau, *et al.*, 1996).

1.17 Objectives of the study:

1. To co-relate different meteorological parameters with total pollen grain production and distribution in the atmosphere during different months of the year (2009-2010).
2. To estimate the extent of paper mulberry pollen allergy based on the patients (numbers), which appeared in various hospitals of Islamabad.
3. To appraise the significance of pollen allergy and related health/respiratory problems among the residents of Islamabad.
4. Estimating the effect of various climatic variables (Temperature, Rainfall and Relative Humidity) on paper mulberry pollens during Spring season.

CHAPTER 2

REVIEW OF LITERATURE

During a detailed investigation to evaluate the relationship of pollen numbers and meteorological factors in Islamabad, Haroon and Rasul (2008) proposed a prediction model for total pollen count (TPC) for the month of March in 2007. Rotorod sampler was used for pollen count and data of five years (2003-2007) related to environmental factors and pollen numbers was taken. Maximum pollen numbers were observed in March and minimum in the month of July. Paper mulberry was mainly responsible for high pollen concentration. Pollen concentration varies in atmosphere due to different environmental factors which affects greatly pollen production and dispersion. This relationship was very useful to diagnose pollen patient's numbers and draw pollen forecasting model.

Van Hout, *et al.* (2008) carried out a study to check the relationship of the local (meteorological) environmental parameters including temperature, relative humidity, solar radiation, mean wind speed and turbulence quantities with the diurnal cycle of corn pollen emission. Four RotoRod samplers were used on a pole in the middle of corn field to measure the pollen concentration at the Canopy height. The pollen concentration at Canopy height showed the pollen source length while the pollen concentration above Canopy showed the pollen transportation from Canopy height. Pollen concentration was maximum during morning while it gradually decreased during afternoon and no significant pollen concentration was measured 24h prior to Sunset. Routine monitoring of airborne pollen had marked difference and it generally depends on the time required for the anthers to dry and open, and the strong winds scattered the pollen away from the plants. Atmospheric pollen concentration division

was bi-modal on four consecutive mornings. Pollen concentration at its peak before the direct irradiance peak showed that direct solar irradiation speedily dried the anthers. The subsequent dip in pollen concentration showed a link with mean and turbulent wind conditions. Quadrant-Hole analysis was applied which showed that low values ejection duration fractions were associated with low concentration values, while high values were associated with high concentration values. Pattern of the fraction of pollen that reached twice the canopy height was similar to diurnal pattern of ejection duration fractions, indicating the important effect of large scale co-herent ejections on pollen transport.

Nava, *et al.* (2009) under took an investigation aiming to estimate the effect of high temperature in blooming and pre-blooming periods on the production of Granada Peach and their sexual gametes. Charqueadas Orchard was selected to perform the experiments under measured conditions of Central Depression at Rio Grande do sul state, Brazil. Two experiments were tested: Trees inside the greenhouse under limited ventilation and trees in the Orchard. Pollen grains were evaluated on the basis of their phenology, morphologic constitution, ovule growth, yield, germination and fruit set. Pollen grains dormancy and blooming was projected in high temperature under blooming and pre-blooming periods. Female gametophytes delayed growth under these conditions while male gametophytes formation was enhanced. These factors increased low pollen viability synchrony lack in fertilization therefore caused low percentage of fruit production.

Kawashima, *et al.* (2007) stated that the airborne pollen number has much significance for the social issues like pollinosis and ecological effects of genetically modified plants. Earlier techniques for pollen monitoring were carried out by counting and

categorization of pollen grains that preset to a sampling substance, which require a lot of time and expert labor. Now-a-days, pollen grains are monitored by laser-optics device. This device uses sideways and forward spreading of laser beam in which each pollen grain is recorded for computer processing. In 2005, this method was compared with older Hirst Method.

Mandal, *et al.* (2008) stated that the pulmonary allergic problems such as asthma and allergic rhinitis are mainly caused by pollen grains intake. Clinical features of pollinosis were performed with most common pollen types on local patients to carry out skin tests and occurrence of pollen types were also analyzed produced by meteorological parameters. The main pollen types were Trema (19%), Poacea (13%), Casuarina (6%), Cocos(6%), Azadirachta (4.6%),Peltophorum (4%), Cyperacea (3.6%), Delorix (3%) and Areca(2.6%). There was a positive co-relation of temperature with pollen concentration while there was a negative co-relation with moisture.

Graneau, *et al.* (2006) carried out a detailed study from 1994-2002 to evaluate the meteorological factors influence on Ambrosia pollen numbers and its effects on Montreal residents by causing Allergic Rhinitis. Aerobiology Research Laboratories (Nepean, Ontario) measured the concentration of Ambrosia pollen number. Meteorological service Centre of Canada shared daily data including high, average and low temperature and rainfall. During Ambrosia pollen season, from 1994-2002 about 7667 consultations for Allergic Rhinitis were observed. There was found a strong relationship between pollen levels and medical patients 'numbers. It was observed that consultation rate was higher in lower income residents.

Ribeiro, *et al.* (2009) stated that the allergenic pollens are also produced by trees. Main aim and objective of this study was to recognize the aerobiology of the

Platanus, Acer, Salix, Quercus, Betula and *Populas* pollen and its relationship with monthly urgent condition of hospital patients and to assess the different reactivity levels in sensitized patients. The study was conducted in Porto, Portugal from 2005-2009. Hirst-type volumetric trap was used for sampling of airborne pollen. Monthly data of patients 'suffering from respiratory problems was obtained from the Emergency Room database of Hospital Geral de Santo Antonio. There was found a positive co-relation between Hospital patients numbers and tree pollens production. Hospital admissions were maximum during pollen season in March. The highest allergic reactions were observed with A.negundo, S.babylonica and P. Occidentalis pollen extracts and the lowest reactions with P. Hybrid. Higher risk to allergenic reactions was due to *Acer* and *Platanus* airborne pollen count. Higher pollen allergen reactivity of particular IgE to pollen extracts of most abundant tree pollen present in the environment was observed. Patient sera showed multiple similar allergenic bands shared by the different extract. This multidisciplinary advancement is useful in day-to-day medical practice to help in diagnostic, therapeutic and allergy alerting system adjusting.

Barnes and Baltimore (2006) established through a detailed survey that in different races and ethnic disparities most of the diseases such as allergic rhinitis of asthma cannot be explained only by environmental, economical, social and cultural factor. The most important thing in such disease which entirely depends upon its diagnosis is genetic factor. Current Research Studies proved that frequency of disease occurrence in African Americans, Duerto Ricans and Mexicans Americans is different because of the genetic variations in these races. In these races dissimilar markers are present in different candidate gene (e.g. STAT6, ADRB2, and IFNGR1) for trials such as high

total IgE level observed in resistance to extracellular parasitic disease and asthma supports the common variant model for disease.

In an experiment by Mitsumoto, *et al.* (2009) it was studied that the pollen grains classification can be done by auto-fluorescence image monitoring of different pollen species. Auto-fluorescence images were captured of nine different pollen species with a microscope having digital camera. Ratio of Blue to Red pollen auto-fluorescence spectra (B/R ratio) and pollen size were measured by processing of their images. Different pollen species showed different pollen size and B/R ratio. B/R ratio versus pollen size confirmed the taxonomy of pollen by both parameters. Particle flow image examination and fluorescence spectra established pollen size and B/R ratio. A flow system deliberated scattered light and auto-fluorescence of particles can classify and count pollen grain in real time.

A study of olive pollen germination by Koubouris, *et al.* (2009) showed that the temperature was based to check out olive pollen germination and tube length growth in relation to humidity and genotype for olive varieties of Koroneiki, Kalamata, Mastoidis and Amigdalolia. Pollen samples were subjected to pre-incubation at 10, 20, 30 or 40°C in combination with decreased air relative humidity 80, 40, 30 or 20% respectively for 24 hour stressed temperature that is carried out in vitro culture during pollen dispersal. During second experiment, pollen was exposed at 15, 20, 25 and 30°C for 24 hour in vitro to estimate the optimum pollen germination and tube growth temperatures for each cultivar under the environment of water and nutrition availability. Koroneiki and Mastoids pollen germination was forbidden at pre-incubation temperature (40 °C) while Amigdalolia and Kalamata germination was about 7.6 and 2 % respectively. At temperature 30°C, Koroneiki pollen germination had negative impact (-65%), Kalamata (-20%) and Amigdalolia (-72%) when

compared to (20°C). Pollen tube length was reduced at 40°C, Kalamata (-50%) and Amigdalolia (-52%) respectively. During second experiment, pollen germination was improved after incubation at 25°C, Koroneiki (+6%), Mastoidis (+52%), Kalamata (+10%) and Amigdalolia (+10%) compared to control (20°C). While at 30°C, Mastoids, Kalamata and Amigdalolia germination percentages were 8, 6, and 14% respectively, compared to control (20°C). All of studied cultivars showed increased pollen tube length with incubation temperature. Varieties were classified for high temperature stress based on cumulative stress response index (CSRI). Mastoids and Kalamata were tolerant at 30°C while Koroneiki and Amigdalolia showed intermediate behavior at same temperature, while all cultivars were sensitive at 40. New tolerant olive varieties could be produced by observed strong genotype differentiation in low and high temperature stress.

Jose, *et al.* (2007) established that the aerobiology primarily depends on forecasting airborne pollen concentration due to its application to allergology. Forecasting is carried out by single linear regression and auto-regressive models (ARIMA). Neuro-Fuzzy models were used for pollen forecasting based on Artificial Intelligence. Selected models were applied firstly to check their overall performance then data was segmented into intervals at various concentrations to check their behavior on each interval. Although Neuro-Fuzzy models showed better performance than classical statistical models but till now there is need of more development.

A study was carried out by Wolukau, *et al.* (2004) to find the effect of temperature (10, 25, 35°C) polyamines (Put, Spd, Spm) and Polyamine (PA) synthesis inhibitor (MGBG) on in vitro pollen germination and pollen tube length growth in Prunusmume CV. Zaohua. Among different temperatures pollen germination and pollen tube length

growth showed marked differences in a liquid medium for 24 h in darkness. There were also distinct differences among PAs and PA concentration and PAs, PA synthesis MGBG. At 10°C, Spd stimulated pollen germination was over control except for Spd 0.5-2.5mmol/l. At 25°C, all pollens were germinated but the PAs were stimulated on Spd 0.01-0.05 mmol/l and Spm 0.025mmol/l. While at 35°C, inhibition of PAs on pollen germination was becoming stronger with increasing concentration except for Put 0.25mmol/l, Spd 0.025-0.25mmol/l and Spm 0.025mmol/l. At different PAs concentration there were diverse differences between the percentage of pollen germination and pollen tube growth at different temperatures. Pollen tube was larger than that of control at 10°C with the concentration of Put and Spd at 0.05mmol/l while when the concentrations became higher they would inhibit pollen tube growth. At 25°C, Spm 0.005mmol/l slightly stimulated pollen tube growth except for Put 0.25mmol/l. At higher temperature (35 °C) except for Put at 0.25mmol/l all the PAs tested have inhibitory effect on pollen tube.

In another study Pasken, *et al.* (2005) found out the method for the source-oriented pollen concentration forecasting. Various tests were performed at National Centre for atmospheric Research/Penn state fifth Generation Mesoscale Model (MMS), the National Oceanographic and Atmospheric Administration (NOAA) Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT_4) Model combined with the locations of oak trees and their aerial coverage from biogenic emissions land cover database version 3.1(BELD3). MM5 and HYSPLIT_4 were used for pollen concentration forecasting with 30-min. increments and were tested against 30-min. oak pollen data collected by the St. Louis County Department of Health in Clayton, Missouri, for the month of April 2000. It was confirmed that the combination of MM5 and HYSPLIT_4 with accurate source locations can provide short term forecasts as

indicated by the levels of forecast pollen and actual oak pollen levels, which follow similar profiles for the day. Two example forecasts presented out of 30 individuals pollen concentration forecasts. Pollen collectors were used for the further validation of these results. Pollen concentration forecasts can be further improved by better understanding of biology of pollen release.

Pacini and Hesse (2004) conducted a study aiming to show all the steps involved in anther opening, pollen presentation and its dispersal. The main step involved the mechanisms of anther drying out, pollen dehydration and release of pollen from the anther. Pollens are released with the opening of anthers and the mechanism of anther opening varies in different plants. Pollens release from anthers and its dispersal totally depends on pollen type, water content available, type of carbohydrate, conversion of carbohydrate from insoluble to soluble and vice versa to maintain turgor for environmental condition and other mechanisms to avoid desiccation during pollen release.

In a study Chehregani, *et al.* (2004) said that the polluted cities signify major health problems by allergic symptoms. This study was carried out to check the impacts of air pollutants on pollen structure, proteins and allergenicity. Pollen grains study under scan electron microscope showed that in polluted areas airborne particles stuck on surface of pollen grains changing the shape and tectum of pollen grains. Polluted pollen grains released many vesicles while pollen grain surface was agglomerated by pollen substance. Several protein materials were discovered in mature and immature pollen grains by SDS-PAGE. The bands of polluted and non-polluted pollen grains were nearly same but polluted pollen showed the decline in protein quantity due to air pollutants which caused the release of protein material from pollen grains. This showed that ripened pollen grain is more allergic than un-ripened pollen grain.

Allergic symptoms of polluted pollen grains were observed more severe than non-polluted pollen grains. Allergic symptoms can be induced by air pollutants but when united with allergen pollen grains their allergy causing power is elevated.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials:

In order to determine day to day fluctuations in pollen number, the data was gathered for total pollen count (TPC) on daily basis using a RotoRod sampler technique (Haroon and Rasul, 2008).

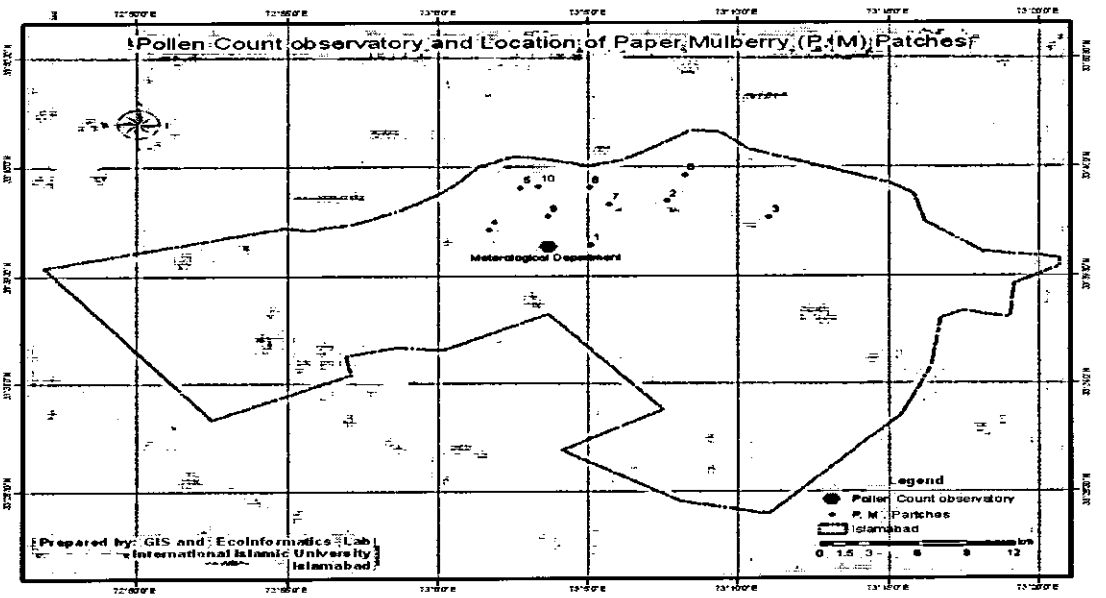
- Rotorod Sampler (Model 40)
- Polystyrene rods
- Silicone Grease
- Staining Solution (Calberla's stain)
- Cover Slip and Glass Slides
- Light Microscope
- Hygrometer
- Rainguage
- Thermograph

3.2 Study Area:

Islamabad is the capital city of Pakistan. It is partially surrounded by small to medium height mountains. There are a number of green belts, vegetation zones and man-made grasses lands all over the area. Most part of the current study was carried out in H-8/2 Sector of Islamabad. It is a very dense vegetation area surrounded by thick vegetation in which different species of plants and grasses produces various types of pollen species. Pollens producing plants are mainly present in G-6, G-7, G-8, H-8, I-8, F-6 and F-7 sectors of Islamabad and at park road area as the major paper mulberry tree location can be seen from Fig. 3 . The pollen grains sampling site at the Pakistan Meteorology Department is shown in (Fig. 3) along with Laboratory. The points represented on map with the numbers show the detail of paper mulberry distribution and abundance zones. Point # 1 shows the paper mulberry presence at shakarparian.

Point # 2 shows the green belt and open areas with paper mulberry located near Rawal Lake. Point # 3 shows the paper mulberry vegetation patches around Rawal Lake and near the stream present at Banni-Gala. Point # 4 shows large number of paper

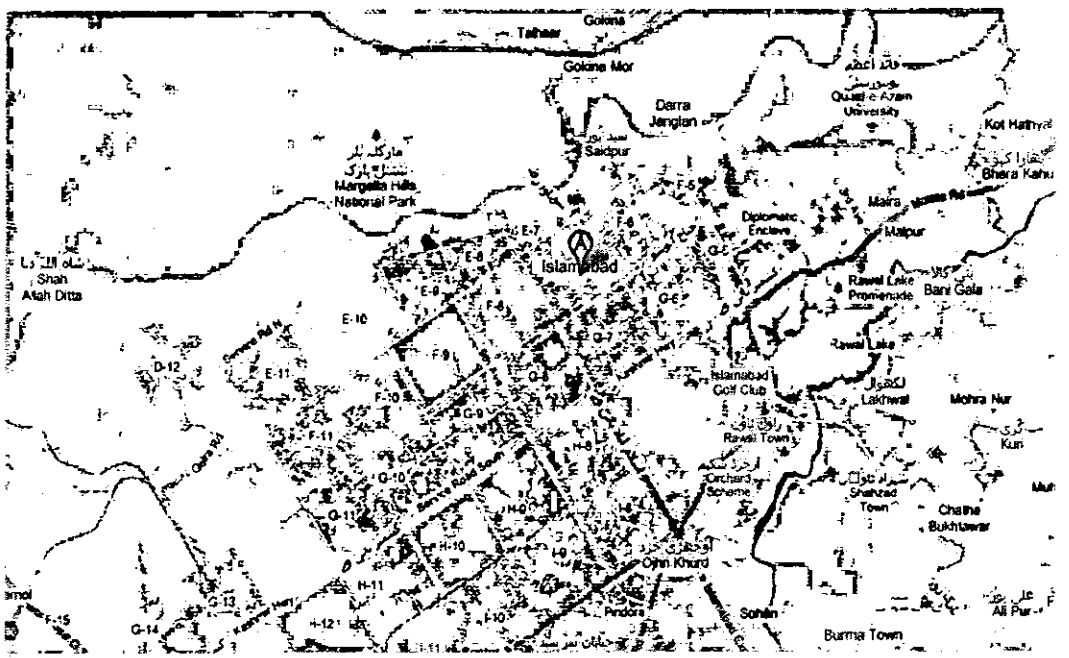
Figure 3: Pollen count Observatory and Location of Paper Mulberry Patches



mulberry patches inside the Fatima-Jinnah Park which include most of the F-9 sector. Point # 5 is the paper mulberry trees near and across the Faisal Mosque. Point # 6 shows the paper mulberry trees presence near Quaid-i-Azam University and Diplomatic Enclave stream. Point # 7 shows the Paper Mulberry vegetation presence in G-6 sector which is among the highly populated sectors in Islamabad. Point # 8 shows the paper mulberry presence into G-8 sector which is also populated area. Point # 9 shows the paper mulberry presence in G-7 sector which is considered among badly pollen effected sectors of Islamabad and is densely populated area. Point # 10 shows the paper mulberry patches present in Marghlla hills at higher altitude which has low human population as compared to other sites. The reason to mark these locations of paper mulberry population in various patches near the sampling site was that the pollen grains of all these paper

mulberry patches were transferred to the sampling site by dispersal to make the TPC at the sampling site.

Figure 4: Map of Islamabad /showing different residential Sectors & Greenbelts



City Coordinates: 33°43'N 73°04'E Latitude °N, Longitude °E (of Map center): (33.666, 73.077)

3.3 Data Collection:

Two years data for various Environmental Parameters (2009-2010) was obtained from Pakistan Meteorological Department on daily basis, during an internship programme at the department. Meanwhile to estimate the pollen concentration into the atmosphere, the pollen data was also gathered on daily basis. Simultaneously, the data concerning to the pollen allergy patients of two years (2009-2010) was taken from two major allergy treatment centers of the city namely, Pakistan Institute of Medical Sciences (PIMS, commonly known as Hospital Complex) and National Institute of Health (NIH), Park Road Islamabad. These pollen data and the allergy patient's records were also gathered in these hospitals on daily basis. In spite of some other pollen allergy clinics in the city like one in G-8 and other in E-7 with limited numbers

of the patients and the record for the patient number visiting on daily or weekly basis was missing, but the patients visiting these clinics were negligible. While the number of the allergy patients was huge in PIMS and NIH, while the pollen patients visiting were observed higher at PIMS as compared to NIH. The data collected from all these sources was then analyzed and managed into Microsoft Word and MS-Excel in tabular form or some data is arranged in lists in order to evaluate statistically by Linear Regression and ANOVA. While line graphs showing relation between pollen numbers and Environmental Parameters (Rainfall, Temperature and Relative Humidity). Table form was made to develop a picture about pollen increase or decrease trends with changing Meteorological Parameters.

3.4 Pollen Sampling:

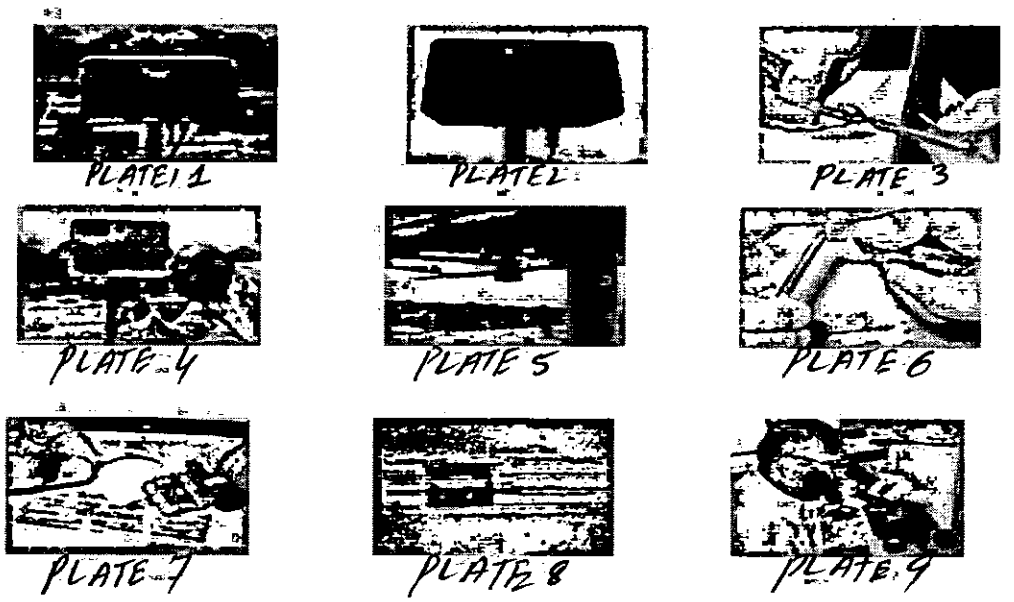
Many types of samplers, including deposition surfaces, stationary and rotating impactors, suction, and filter devices, were used in a wind tunnel and in the field under various meteorological conditions. But for the current study analysis RotoRod sampler technique was used in order to take the sample of pollen grains in the atmosphere. The pollen grains of Paper Mulberry, Acacia, Eucalyptus, Pines, Grasses, Cannabis, Dandelion and Alternaria were present in atmosphere (Eugene, *et al.*, 2004).

The Paper Mulberry pollens were observed in higher concentration than other plant species. The RotoRod sampler is essentially independent of wind speed and direction and has a collection efficiency of approximately 64 percent for the particles in the size range of pollen. It samples approximately 60 L. per minute, but for long-period samples it may be operated intermittently to prevent overloading (Gilbert, *et al.*, 2004).

3.5 Pollen count:

The pollen count is a measure of the number of pollen grains of a certain type per cubic meter of air sampled, averaged over 24 hours. The pollen is collected with a **Rotorod Sampler** (Model 40) operating on a 10% duty cycle. The sampler is located on the roof of **Pakistan Meteorological Department** Building. (Plate 1 & 2)

The sampler collects the pollen on two 1.52 x 1.52 x 32 mm Pre -greased polystyrene rods. A thin coat of Silicone Grease captures the pollen and mold spores on the leading edge of the rod when the spindle is moving and rods are pulled downward due to centrifugal force (Plate 3, 4 & 5). The rods are removed from Rotorod Sampler after 24 hours and are placed in one of the deep grooves of a stage adapter (Plate 6). A few drops of Staining Solution (**Calberla's stain**) are applied and then the rod is covered With a standard 22 x 22 mm coverslip, with the edge of the coverslip about one mm from the distal end of the rod (Plate 7 & 8). All pollen counting is done with a light microscope under 400x Magnification. Only area of the rod under the coverslip is counted (Plate 9).



CHAPTER 4

RESULTS

The current study has been carried out in order to estimate the trends of distribution, abundance and spread of pollen in Islamabad along with some of the localized effects of the increased pollen concentration in Islamabad. The study in part addresses the pollen associated health issues, especially pollen allergy as a component of this survey. The data was also collected regarding number of patients visited various Hospitals and Laboratories of Islamabad for allergy test, checkup and treatment etc.

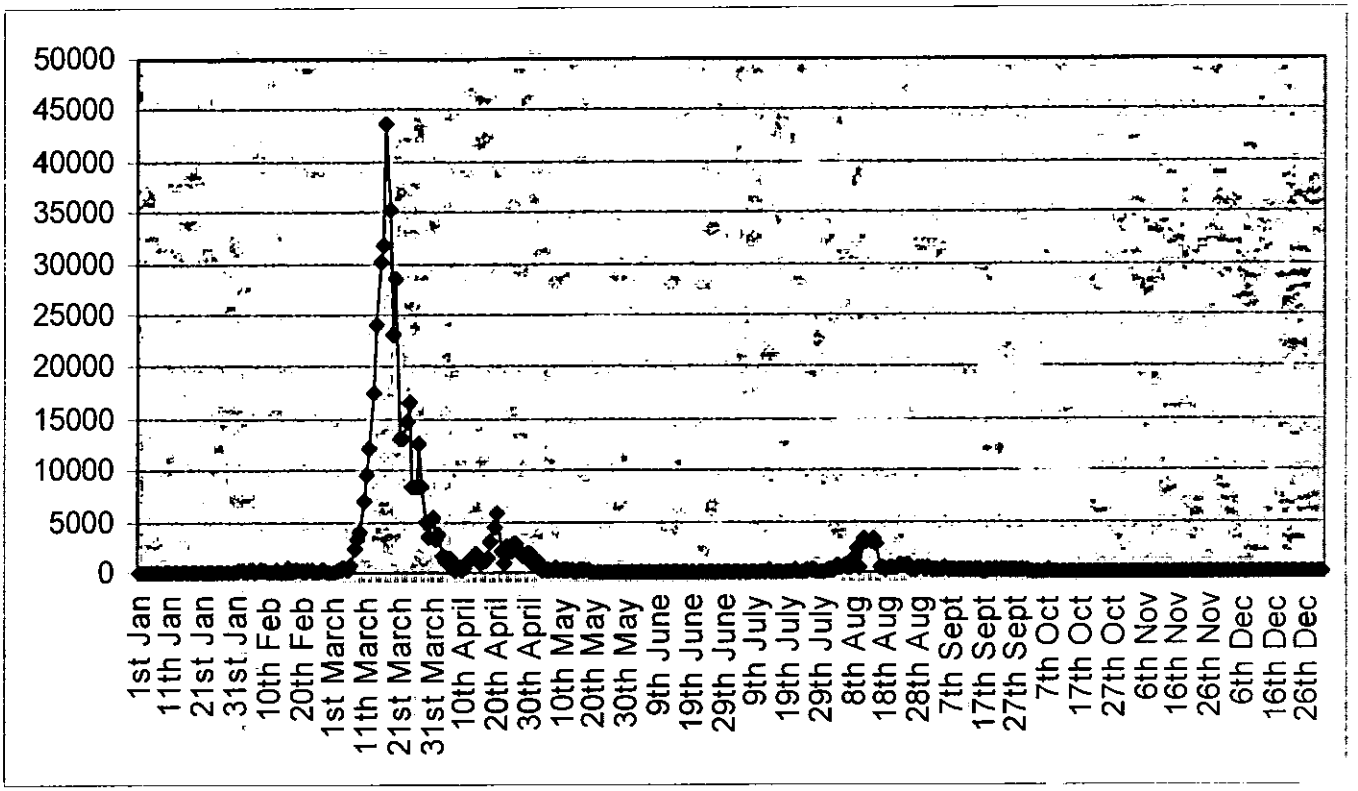
The data regarding patient's number was mostly gathered on daily basis (average was calculated on monthly basis for convenience) and the pollen allergy patient's data was also gathered on the similar lines. In general, month wise data is presented in graphs taking in account the daily temperature, rain pattern, relative humidity and the daily pollen counts. The trends regarding increase or decrease of pollen count in association of these environmental parameters were observed to reach a conclusion regarding pollen increase or decrease phenomenon involved. Patient's numbers compared with the pollen numbers was also considered and is shown in the following tables (1&2).

The major pollen type find out of the various pollen samples was belonging to the Paper Mulberry tree followed by the pollen from cannabis, different exotic ornamental trees and various local grass species.

The total pollen count (TPC) for the year, 2009 is shown in (Fig. 5). Pollen number was observed maximum during spring season, while TPC reached at its peak value (i.e.45,000/ m^3) in the month of March, 2009. The high concentration of pollen grains in atmosphere was observed surely causing allergy problems for the residents of that area. The most prominent diseases caused by high pollen numbers in atmosphere were pollen allergy, eczema and hay fever. The second next annual pollen season was

observed in the month of August and September. As an example of TPC variations the graphical representation of total pollen count during the year, 2009 is shown in Fig. 5.

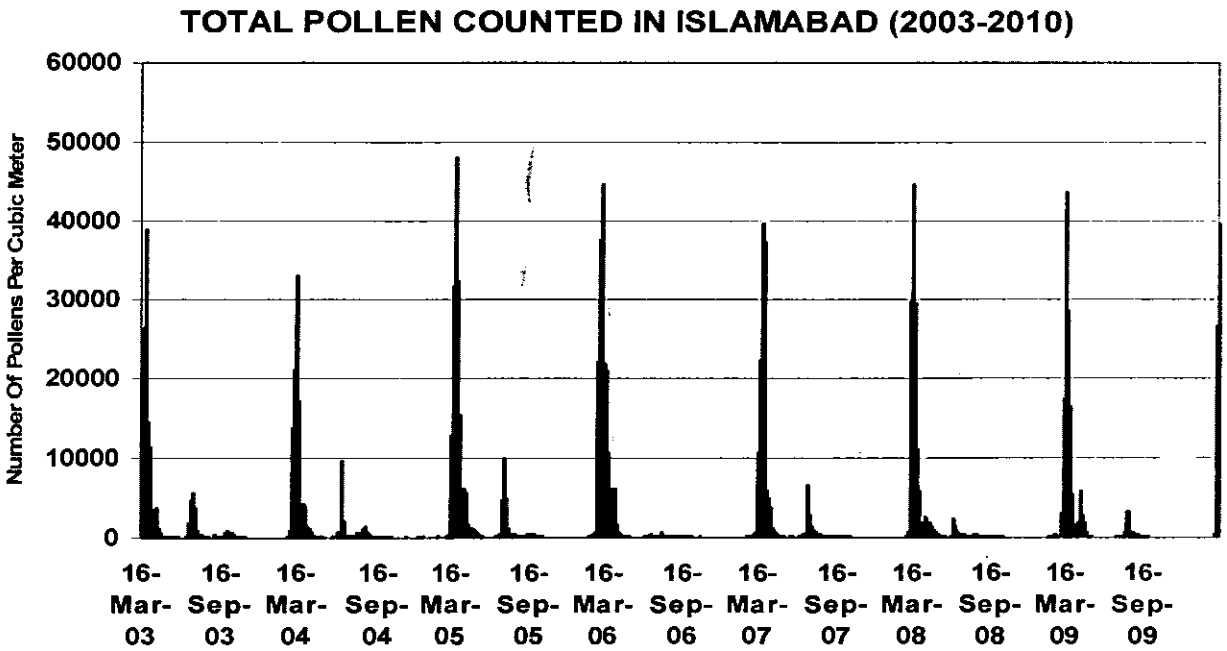
Figure 5: Total pollen count for the year 2009 in Islamabad.



4.1 Total Pollen Distribution Trend in Islamabad during 2003-2010

According to the TPC data acquired from Pakistan Meteorological Department for the years 2003-2009, it has been observed that the peak pollen season in Islamabad starts from the first week of March and ends in the mid April. Extremely high pollen concentrations (above 30,000/m^3) in the month of March usually in 3rd week of March and relatively less high concentration (about 4000 to 8000 pollen per cubic meter) in August were recorded. The highest concentrations of pollens for the month of March were observed in 2005 (48,000/m^3) while the lowest in March, 2004 (32,000/m^3) respectively till 2009 as shown in Fig. 6.

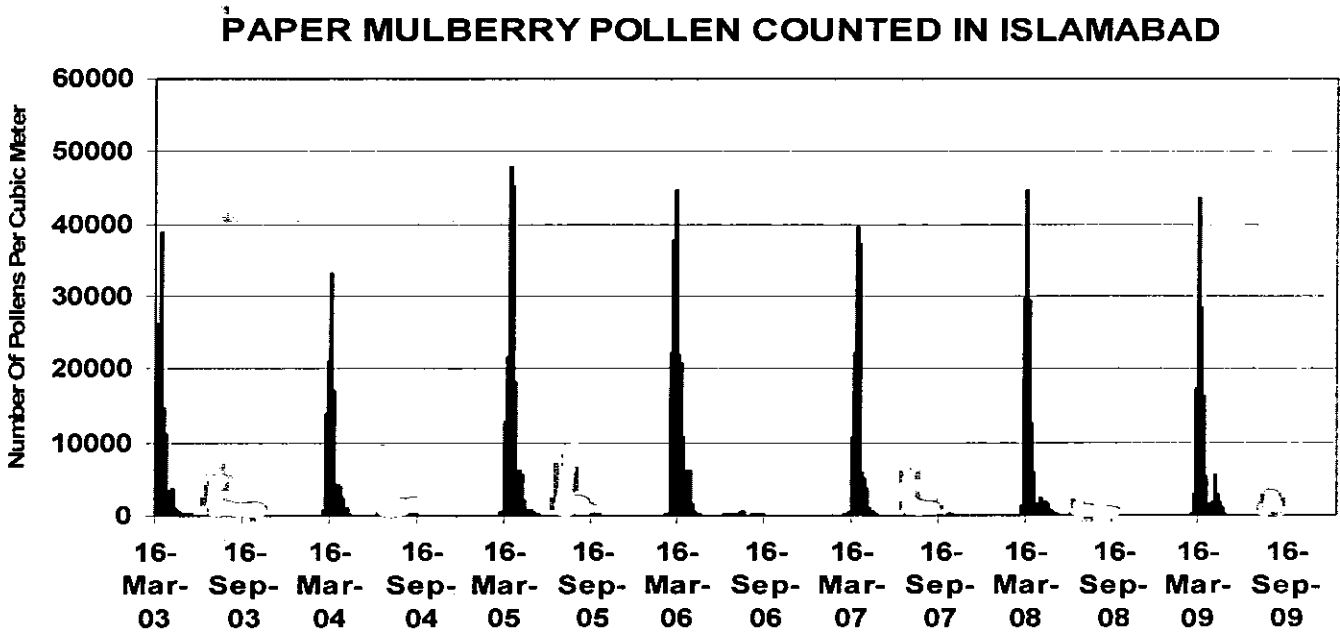
Figure 6: Total pollen distribution in Islamabad 2003 to March, 2010



4.2 Paper Mulberry Pollen Distribution Pattern in Islamabad (2003-2009)

According to Fig. 7 it is interesting to note that the peak paper mulberry pollen season starts in first week of March and usually ends in Mid April. Highest pollen count was in March, 2005 (above 48,000/m^3) which is noticeably higher than all pollen counts of the seven years (2003-2009) while lowest pollen count was observed during March, 2004 (slightly above 30,000/m^3) which is notably lower compared to the other years from 2003-2009 as shown in the Fig. 7.

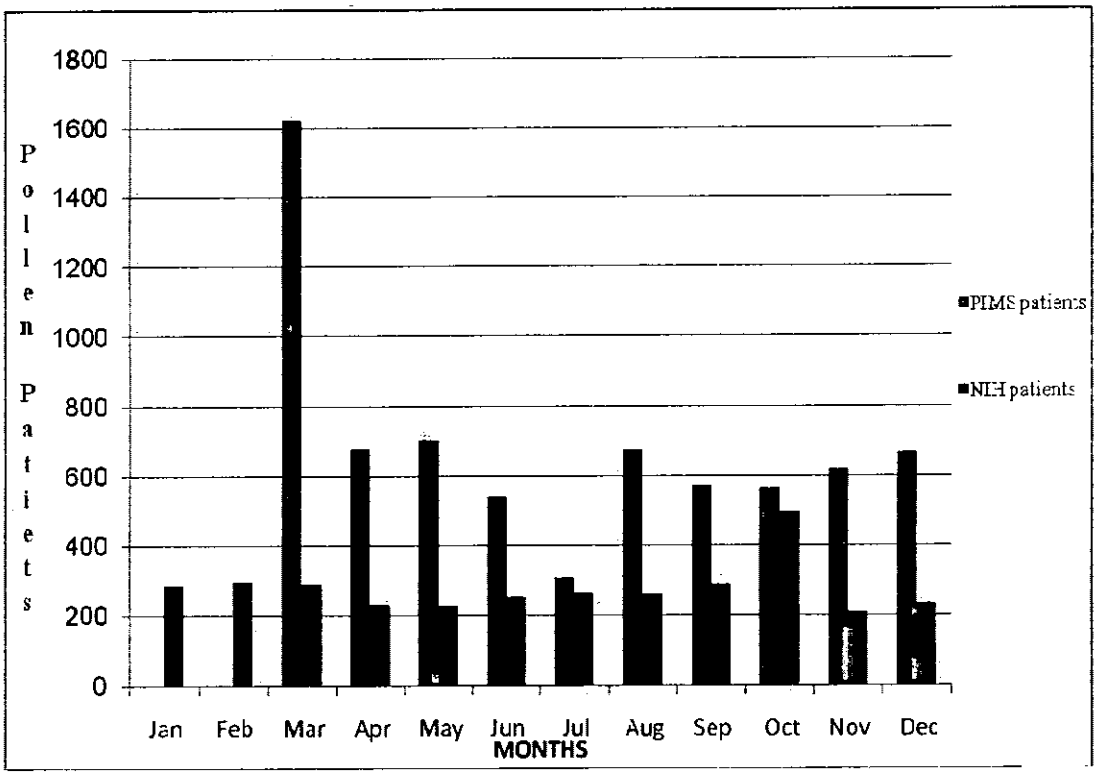
Figure 7: Paper Mulberry Distributions in Islamabad (2003-2009)



4.3 Pollen Patients data from local Hospitals, 2009-2010

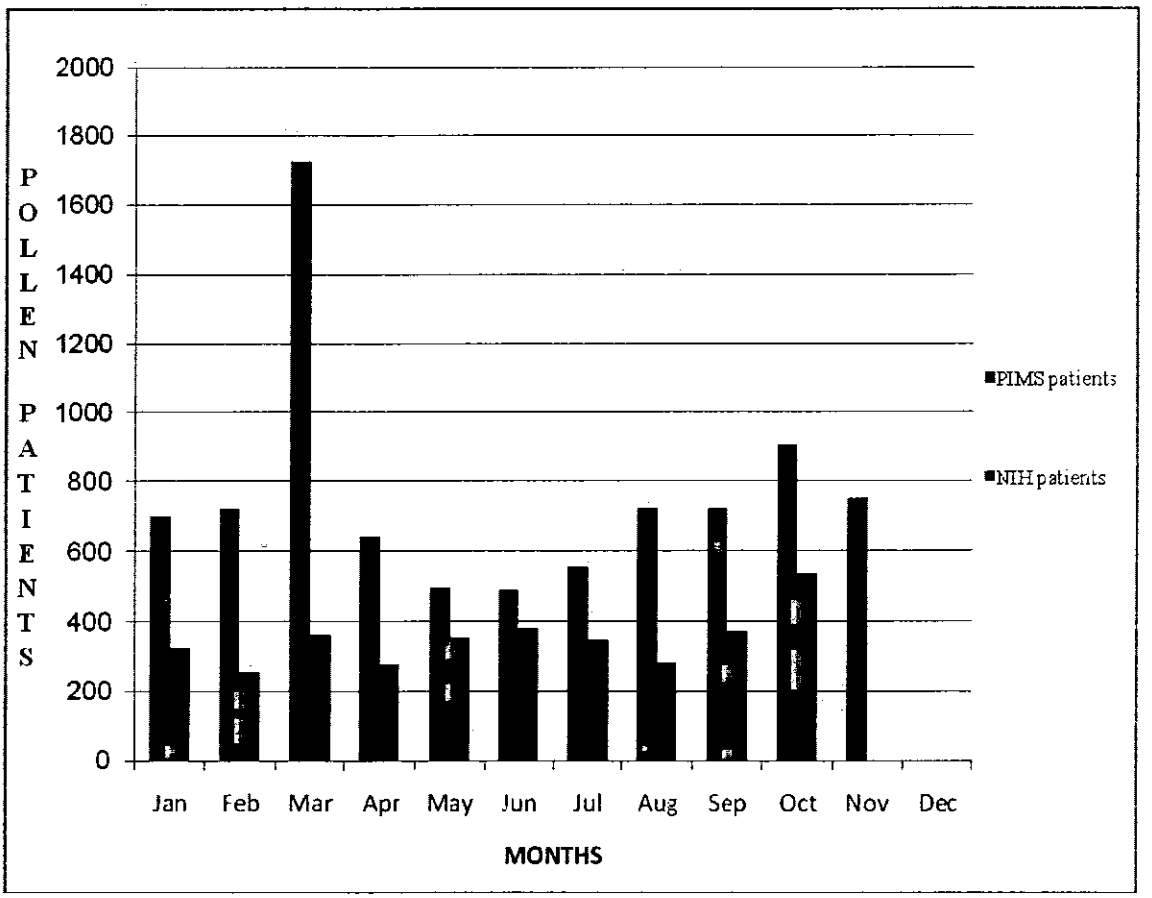
The following chart shows that the maximum number of pollen patients was observed in the month of March, 2009, whereas it was found to be low during the months of June, July, September and October in both hospitals PIMS and NIH. It is also observed that almost equal numbers of pollen patients appearing in the months of April, May, August, November and December in both hospitals. The highest pollen patients were observed in the PIMS hospital i.e.1624 in the month of March, 2009 while minimum pollen patients were about 309 in the month of October, 2009, Similarly the maximum pollen patients numbers observed in NIH centre were about 270 in the month of October, 2009, while minimum pollen patients were observed about 164 in the month of December, 2009.

Figure 8: Pollen Patients comparison between NIH and PIMS during 2009



A similar trend was observed in 2010. Maximum numbers of pollen patients appeared during March followed by October, whereas minimum pollen patients appeared in May, June and July meanwhile almost equal number of pollen patients were appeared in Jan, February, August, September and November which was found nearly comparable with previous year (2009) pollen patients recorded. The highest pollen patients in PIMS hospital were found to be 1724 in the month of March while minimum pollen patients were found equal to 492 during June, 2010. In case of NIH centre the maximum numbers of pollen patients were found to be 486 in the month of October, 2010, while minimum pollen patients were equal to 229 in February, 2010.

Figure 9: Pollen Patients comparison between NIH and PIMS during 2010

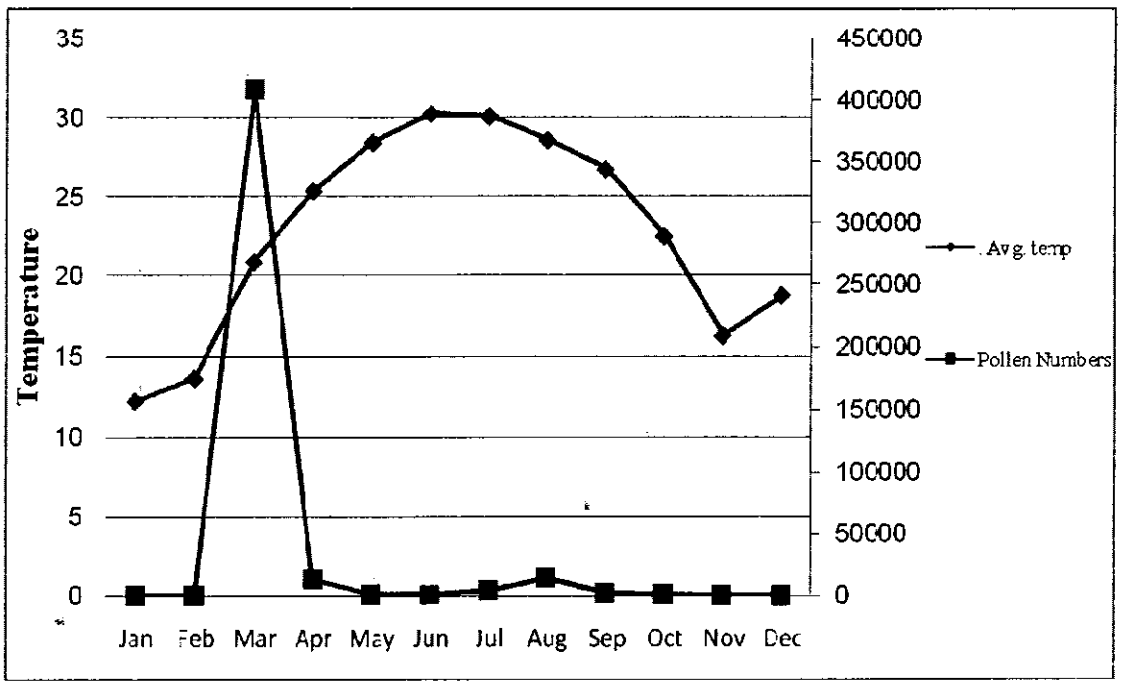


4.4 Effect of temperature on pollen concentration in the atmosphere

In Fig. 10, the month wise pattern of pollen production (by adding daily TPCs for full month) and its relationship with the average temperature for the full month is shown during 2009. During the first month of the year i.e. January, 2009 the pollen numbers were low, which started to increase in the month of February, apparently linked to the gradual rise in the average daily temperature. Total pollen numbers reached to its climax in the middle of March apparently due to optimum temperature i.e. 17.5 ^{0}C. Pollen numbers again fall to minimum from middle of May to mid of July based on very high temperatures for these warm summer months. In the middle of July the total pollen concentration in the atmosphere gradually increased and reached about 30045/m^3 pollen numbers in the month of August. Rest of the months i.e. October,

November and December showed constantly low pollen numbers with gradual rise and fall in average temperature.

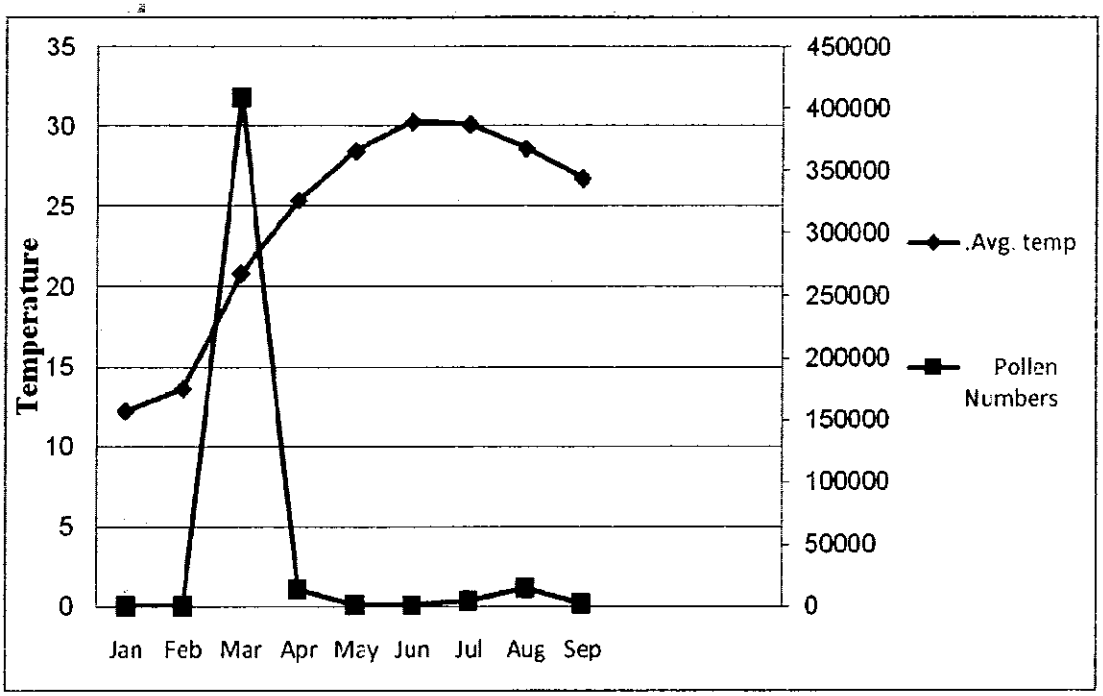
Figure 10: Relationship between full-month TPC and Temperature during 2009



In Fig. 11, the month wise pattern of pollen production (by adding daily TPCs for full month) and its relationship with the average temperature for the full moth shown the similar trends as observed in 2009. The TPC remained low till the mid of February and gradually increased and reached to its peak value (i.e. 4, 09,162/m^3) in the middle of March at average temperature of 20.5^{0}C. TPC then gradually decreased and reached to its lowest value till the middle of May and remained low from mid of May to middle of July. Pollen numbers again gradually increased from middle of July and their number again became low in the mid of September. The Figure for 2010 shows that 20.5^{0}C is the optimum temperature for maximum pollen production and growth in contrast with the 2009 (Fig. 10) where the average optimum temperature for maximum TPC was 17.5 ^{0}C. It is important to note that for 2009 the maximum number of TPC was observed at average temperature of 17.5 ^{0}C while in 2010 TPC

was maximum at average temperature 20.5^{0}C therefore this difference of 3^{0}C indicates that there must be other climatic factors/variables affecting the pollen production like rainfall and relative humidity etc.

Figure 10: Relationship between full-month TPC and Temperature during 2010



4.5 Monthly Pollen Variation (TPC) with Meteorological Parameters

Keeping in view the additional effects of relative humidity and rainfall in addition to the daily temperature, the pattern of pollen production was studied month wise. The month wise data help to understand the effect of temperature on pollen production in all seasons along with some additional information regarding effect of other climatic variables (humidity and rainfall) in order to obtain a better picture regarding change in TPC.

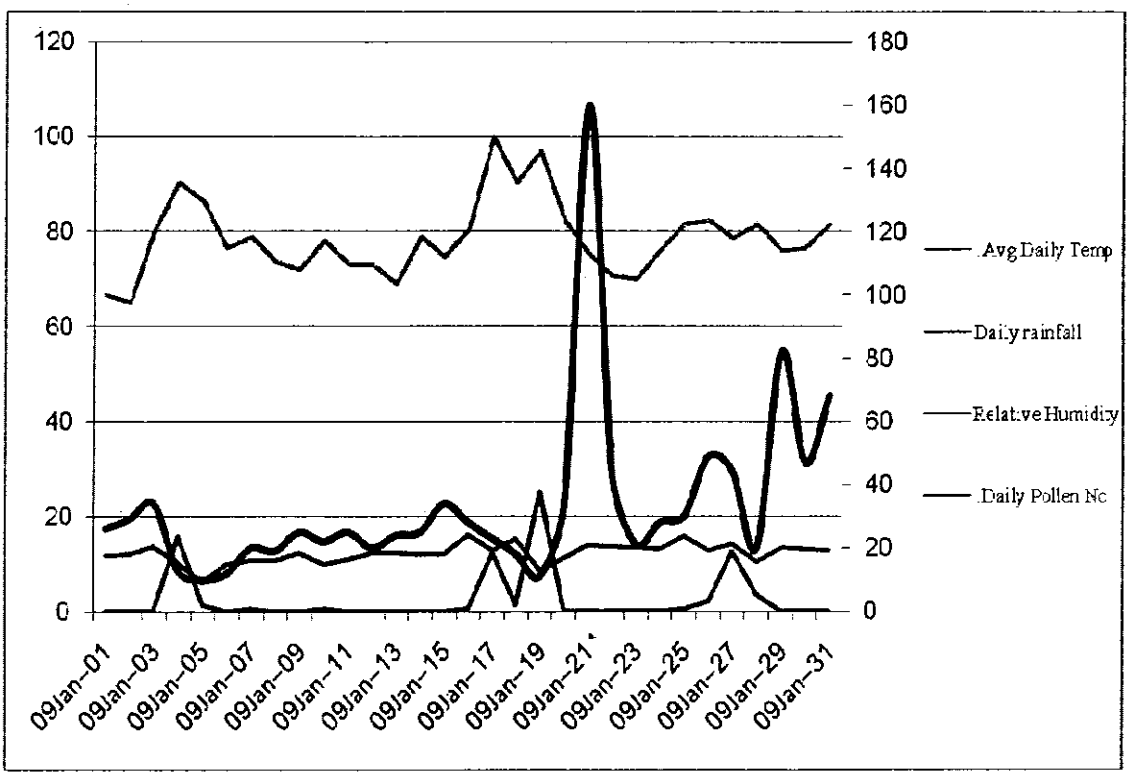
January 2009:

The Fig. 12 shows the co-relation of pollen numbers with Environmental Parameters like daily average temperature, daily rainfall and atmospheric relative humidity. The

X-axis in the following figure shows the days of a particular month at which the readings for average daily temperature, rain fall and relative humidity are plotted on Y-axis (against primary axis i.e. left side of figure). The secondary axis i.e. right side of the Y-axis shows the readings taken for total pollen count.

In the Fig. 12, It is observed that initially when the temperature increased, pollen quantity in atmosphere also increased but at certain temperature (i.e.13.75^{0}C) pollen numbers reached to climax of this month i.e. only 160 grains/m^3 and at the last part of the January pollen numbers showed variations at constant temperature. Primarily, with the increase of rainfall pollen numbers decreased and at no rainfall pollen numbers increased meanwhile at the end of the month rainfall had no significant impact on pollen counts. With the increase of relative humidity pollen numbers decreased but at the middle of the month with low relative humidity a pattern of increases in pollen number was observed.

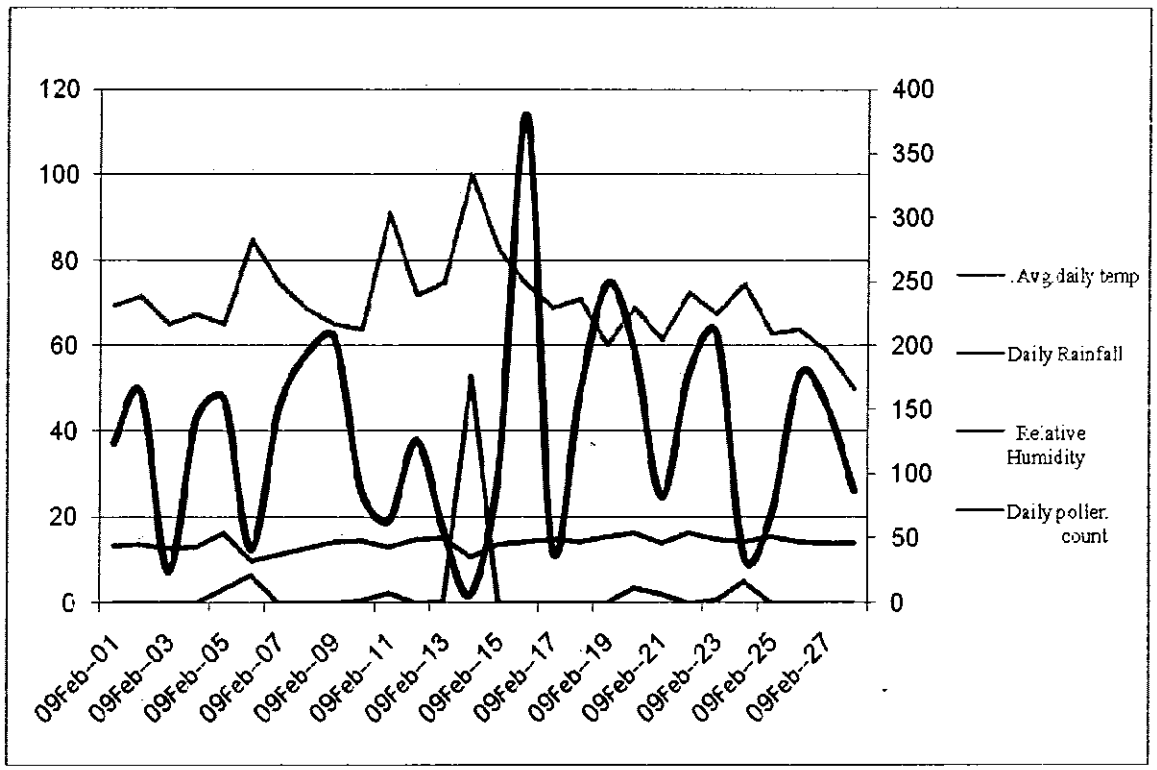
Figure 11: Relationship of TPC with average temperature, relative humidity and rainfall during January 2009.



February 2009:

During the first week of February almost at the constant temperature, pollen numbers shows variations but with the increase in temperature pollen numbers also increased and at the last week of the month pollen numbers repeated the same pattern with increasing and decreasing temperature. With the escalating Rainfall pollen numbers decrease and at the mid of the month when there was a high rainfall the pollen numbers were at its low value. Decreased relative humidity increase pollen numbers in first ten days but at the mid of the month pollen numbers were at high values and at the end of the month pollen numbers show variations with declining relative humidity.

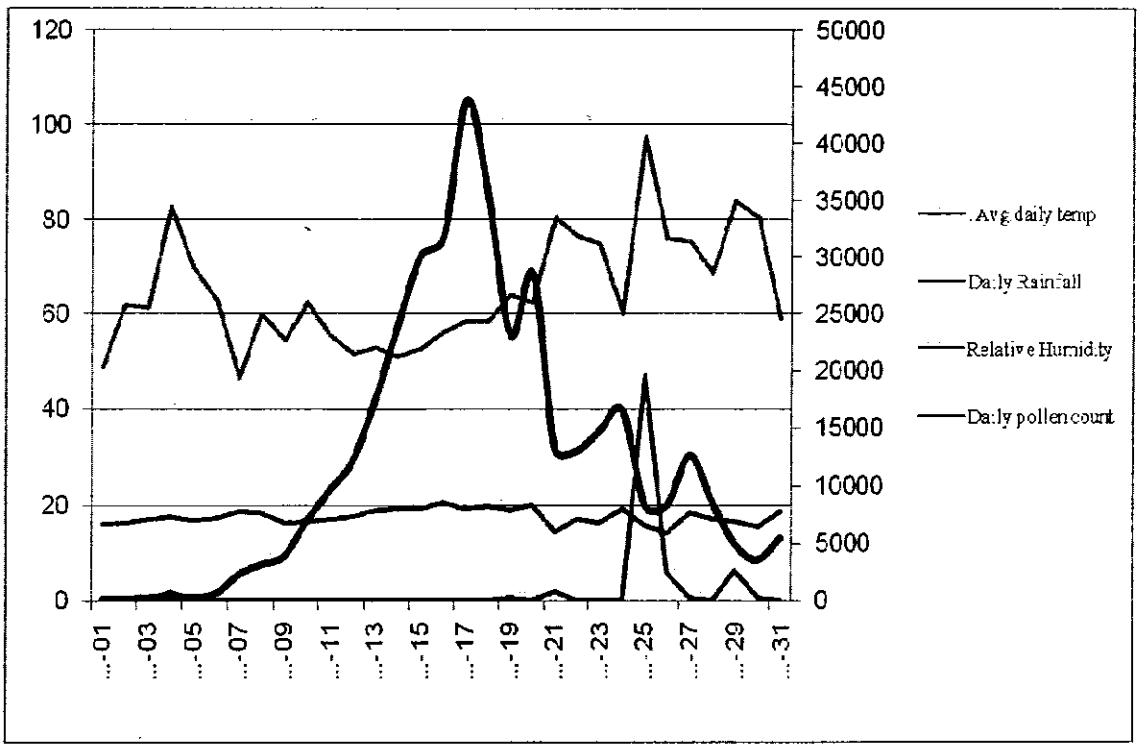
Figure 12: Relationship of TPC with average temperature, relative humidity and rainfall during February 2009.



March 2009:

In the first week of the March, pollen numbers were very low and then the TPC regularly increased in the second week with the rise in temperature and pollen numbers reached a maximum of 43,780 grains/m^3 at 19.45^{0}C on 17th march while at the end of the March pollen numbers progressively decreased with the drop in temperature and increase in rainfall. Rainfall was negligible in first three weeks of this month but the last week had heavier rainfall which decreased pollen grains into the atmosphere. In the beginning at high relative humidity pollen numbers were in smallest values and at the middle of the month, relative humidity decreased then pollen numbers reached to its peak but in the last eight days of the month pollen number gradually decreased with rising relative humidity.

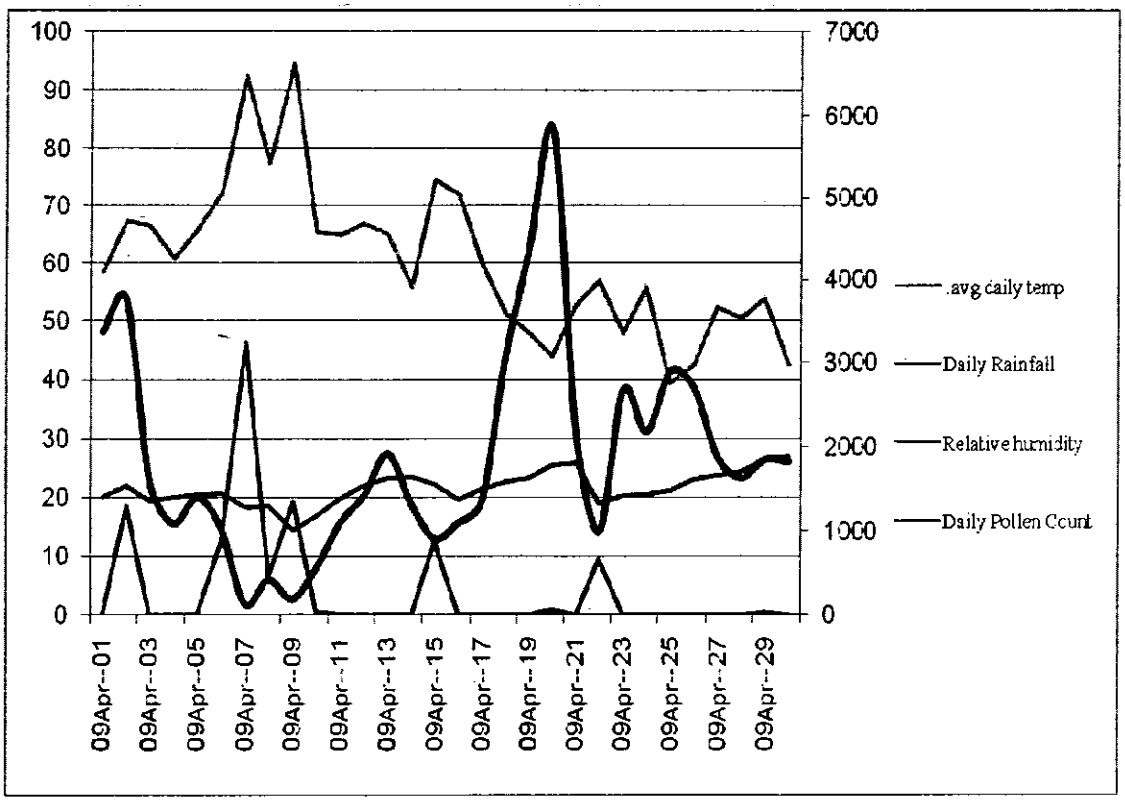
Figure 13: Relationship of TPC with average temperature, relative humidity and rainfall during March 2009.



April 2009:

In the first ten days at low temperature, less rainfall and average relative humidity fewer pollen numbers were present in the atmosphere but the pollen numbers increased with the increase in the above parameters and reached at its peak value i.e,5834/m^3 at 20^{th} of April. In the last ten days of this month pollen numbers showed insignificant values with the gradual increase and decrease in temperature and relative humidity at almost zero rainfall.

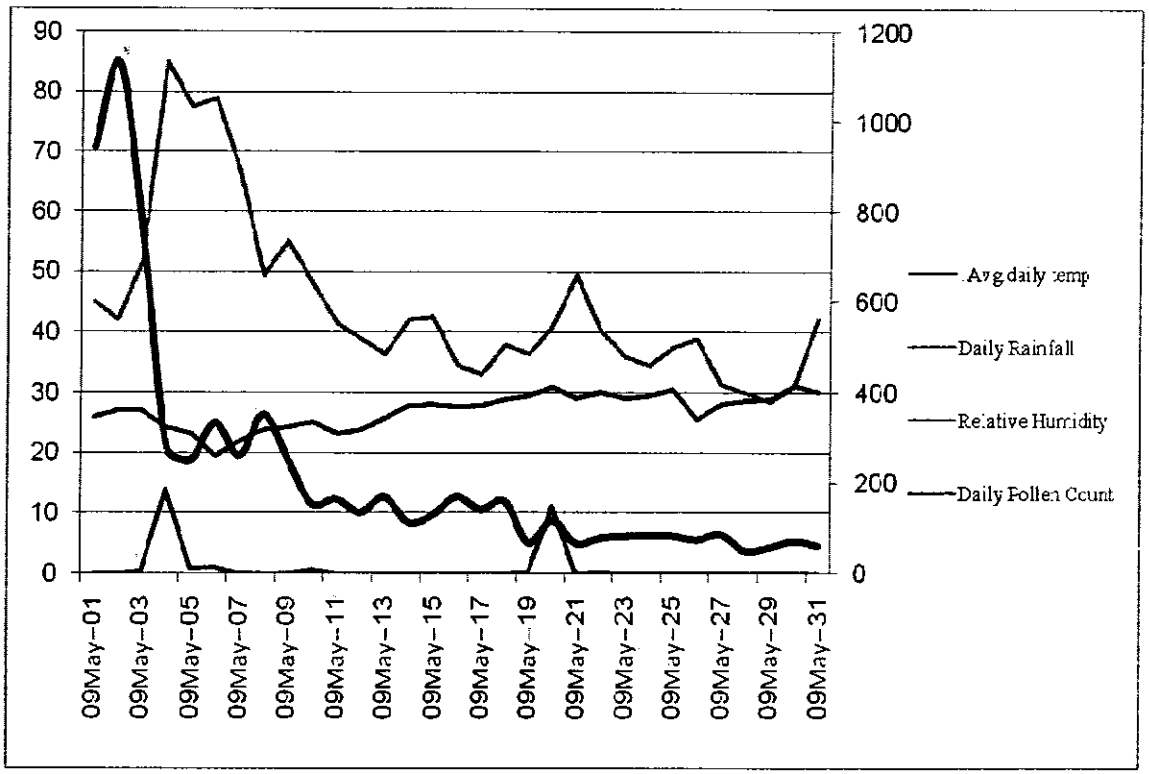
Figure 14: Relationship of TPC with average temperature, relative humidity and rainfall during April 2009.



May 2009:

On the 2nd of May maximum numbers of pollen count found to be 1132 grains/m^3 at 27^0C and it gradually decreased with the decrease in temperature. After first nine days pollen numbers gradually decreased with the gradual rise in temperature while rainfall and relative humidity showed minor variations during this month.

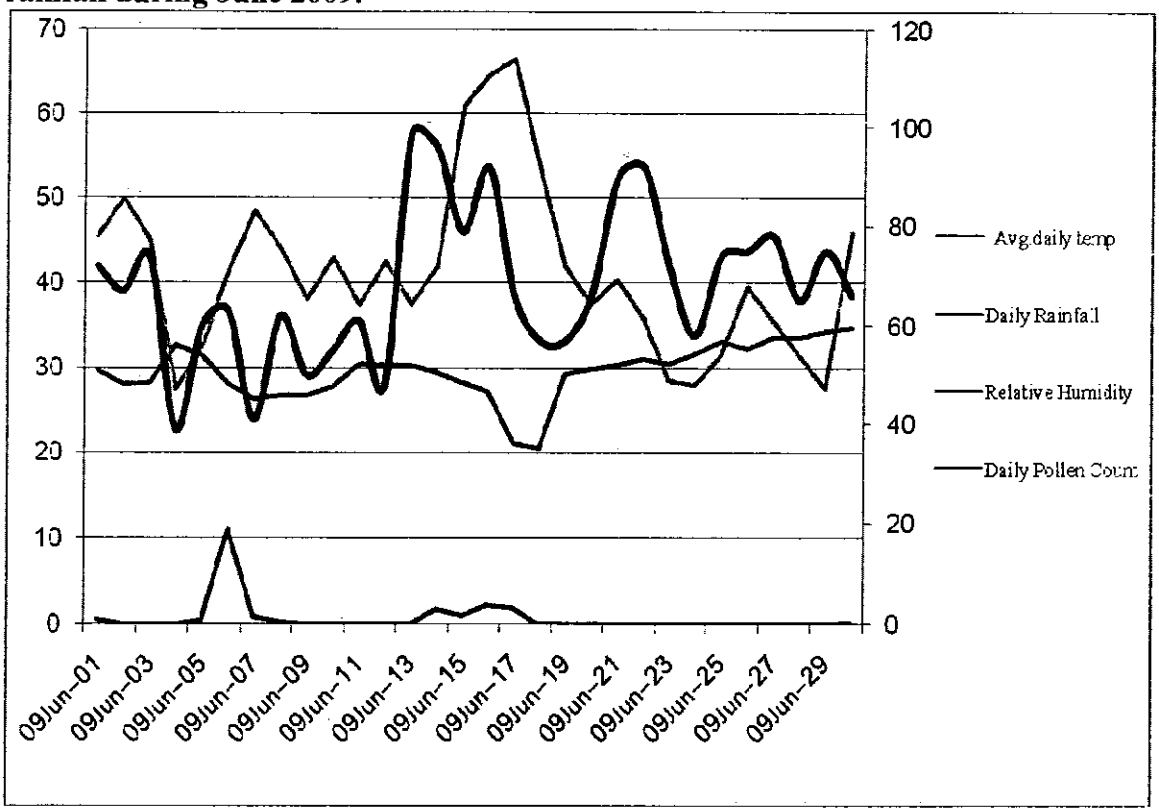
Figure 15: Relationship of TPC with average temperature, relative humidity and rainfall during May 2009.



June 2009:

The following figure shows the unbalanced relation between temperature and pollen numbers in the atmosphere. With the rise in temperature pollen number decreased and it reached the climax at low temperature and at the end of the month it gradually decreased with the rise in temperature. Pollen number was elevated at minimum rainfall but increased rainfall decreased the atmospheric pollen concentration. Relative humidity also showed the abnormal behavior. With the decrease of atmospheric humidity pollen number also decreased at start of month and at the middle of month pollen number augmented with the increase in relative humidity but at the end increased relative humidity decreased the pollen number.

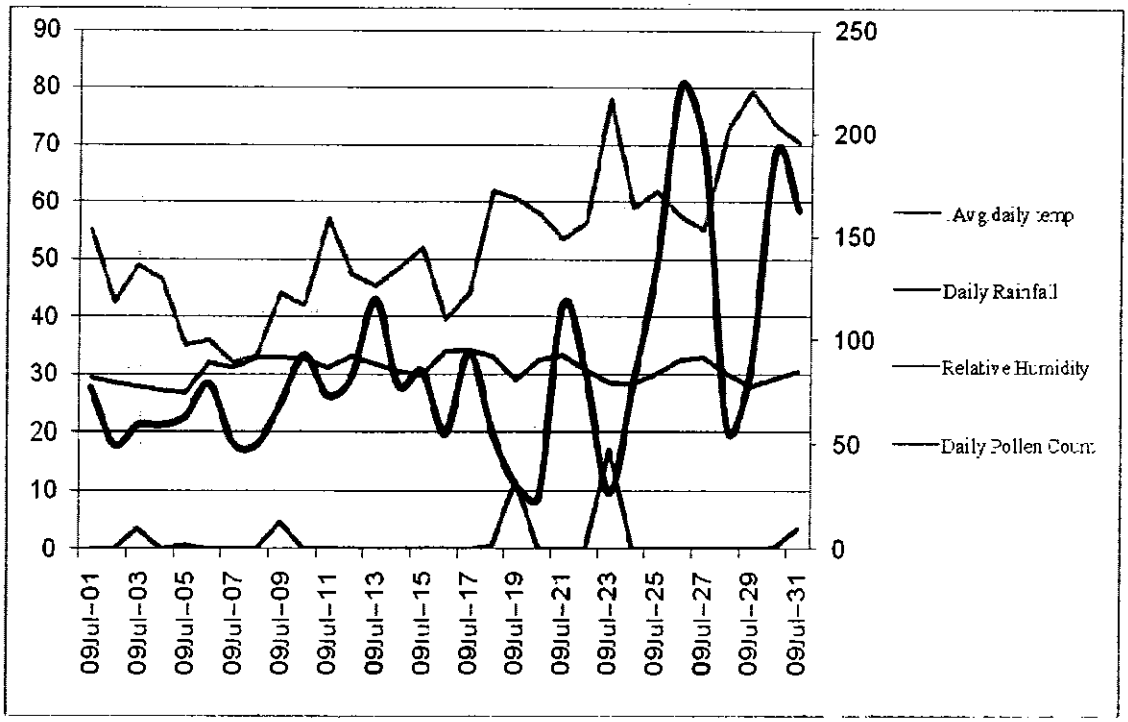
Figure 16: Relationship of TPC with average temperature, relative humidity and rainfall during June 2009.



July 2009:

In the month of July at average daily temperature around 30^0C pollen number remained very low 68 grains/m^3 and TPC reached its highest point for this month at 32^0C and subsequently decreased with fall in temperature. In the beginning of month pollen number was low with some amount of rainfall but it is interestingly to note that TPC decreased with increasing rainfall and in the last week of the month TPC was at its peak with no rainfall at all.

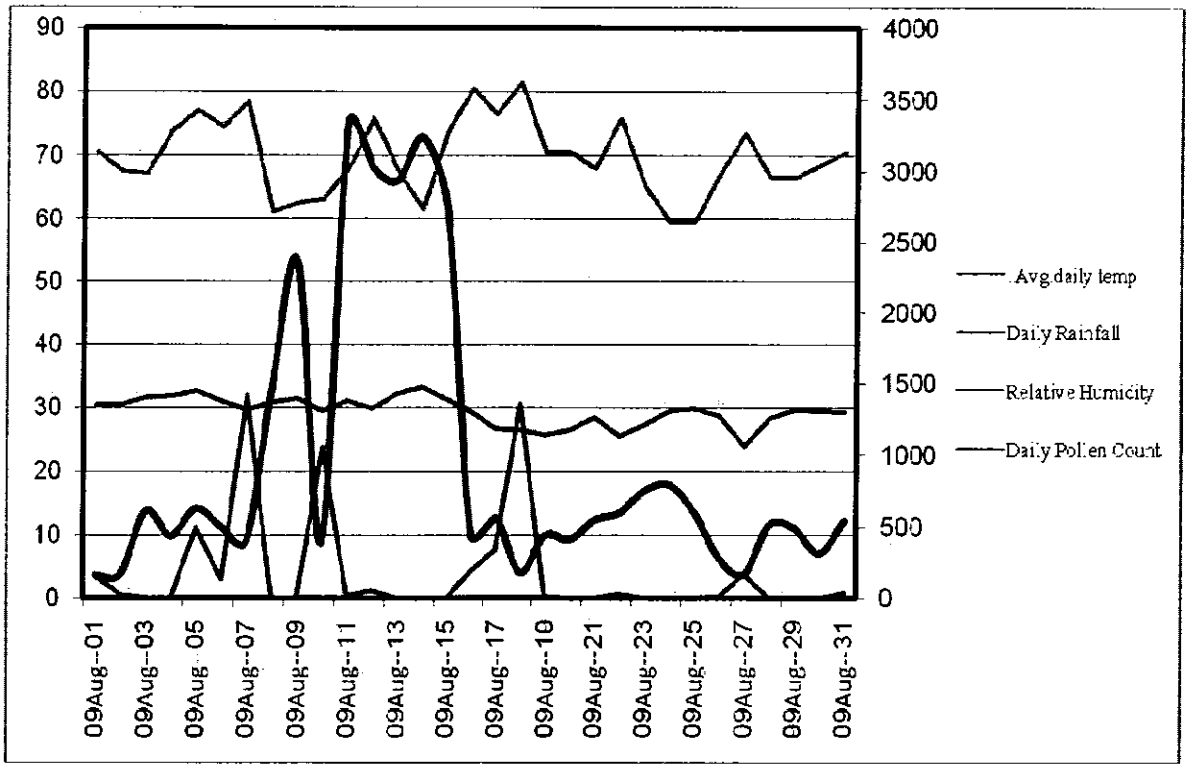
Figure 17: Relationship of TPC with average temperature, relative humidity and rainfall during July 2009.



August 2009:

Pollen count was as low as 4 grains/m^3 at the start of August which is the lowest for any month of the year but it slowly increased with rise in temperature. Pollen number was observed to be around 3300/m^3 as a maximum for August in the middle of the month at 31^{0}C average daily temperature and then it steadily decreased with a noticeable drop in temperature. As there was a bounty of rainfall in this month. Primarily pollen number increased with no rainfall but pollen number suddenly decreased with some rainfall and at the end of the month pollen number also decreased with the heavy rainfall. It is understood that increase in relative humidity decreases pollen concentration but during the mid of the pollen numbers was high with relative humidity of 72% towards end of the month pollen numbers abnormally decreased in atmosphere with the decrease in relative humidity.

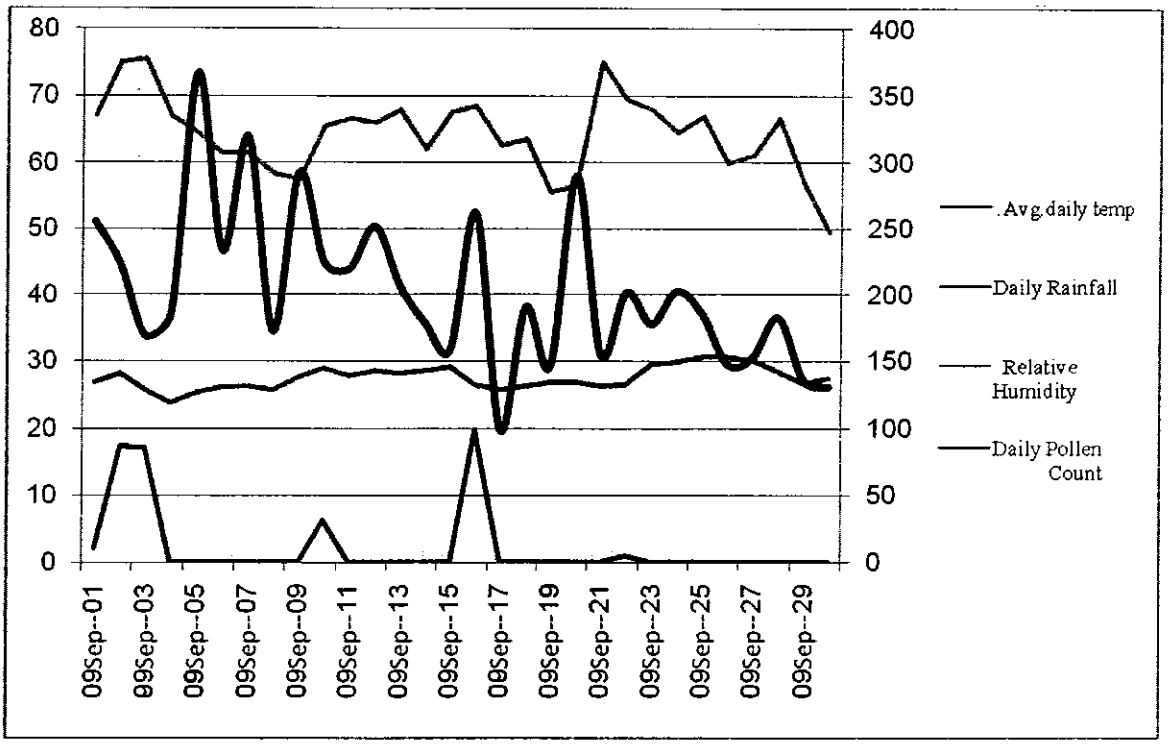
Figure 18: Relationship of TPC with average temperature, relative humidity and rainfall during August 2009.



September 2009:

In the first week of September, pollen numbers decreased due to rainfall and with the lower temperature and relative humidity as well. In the middle of this month pollen numbers decreased to about 100 grains/m^3 with the relative humidity 55% and temperature 27^0C in the presence of some rain. In the last week of the month pollen numbers further decreased with the increase in relative humidity and average temperature along with rainfall.

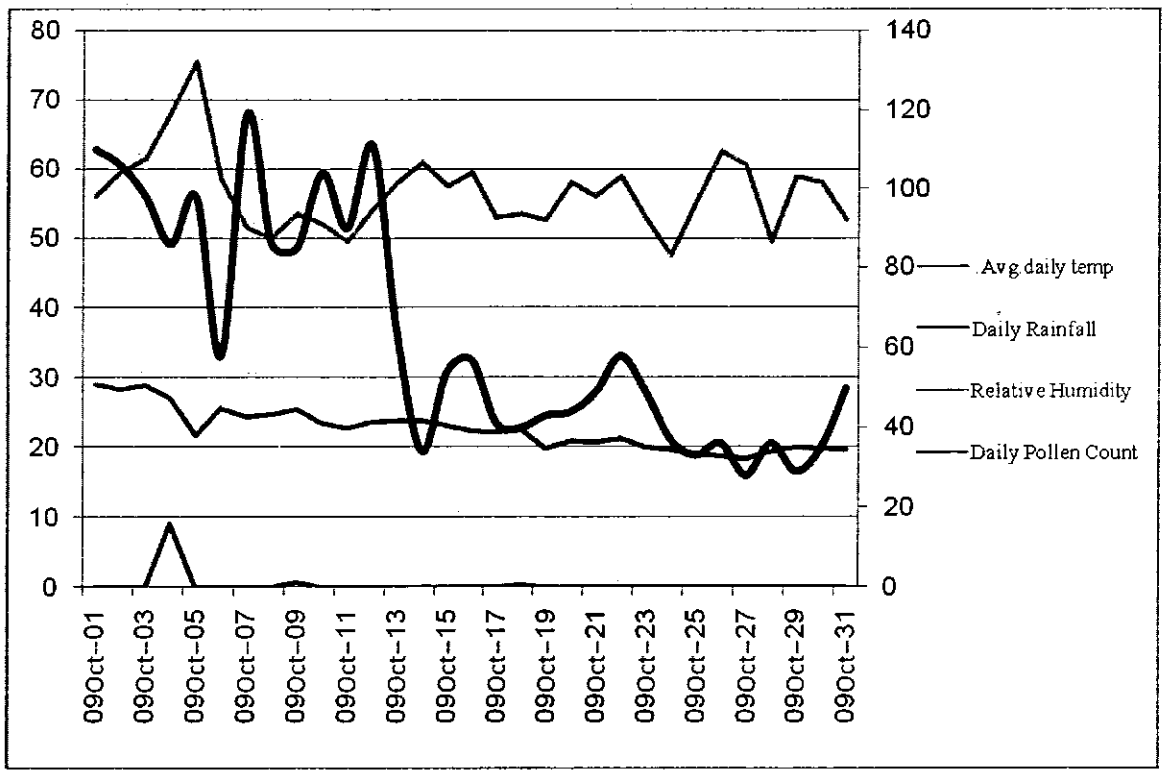
Figure 19: Relationship of TPC with average temperature, relative humidity and rainfall during September 2009.



October 2009:

During first week of October, pollen numbers initially decreased with the decrease in temperature, rainfall and low relative humidity. In mid of the month pollen numbers dropped to 35 grains/m^3 with the temperature about 22^0C and relative humidity about 60% at zero rainfall. This trend of low pollen number continued till the end of the month.

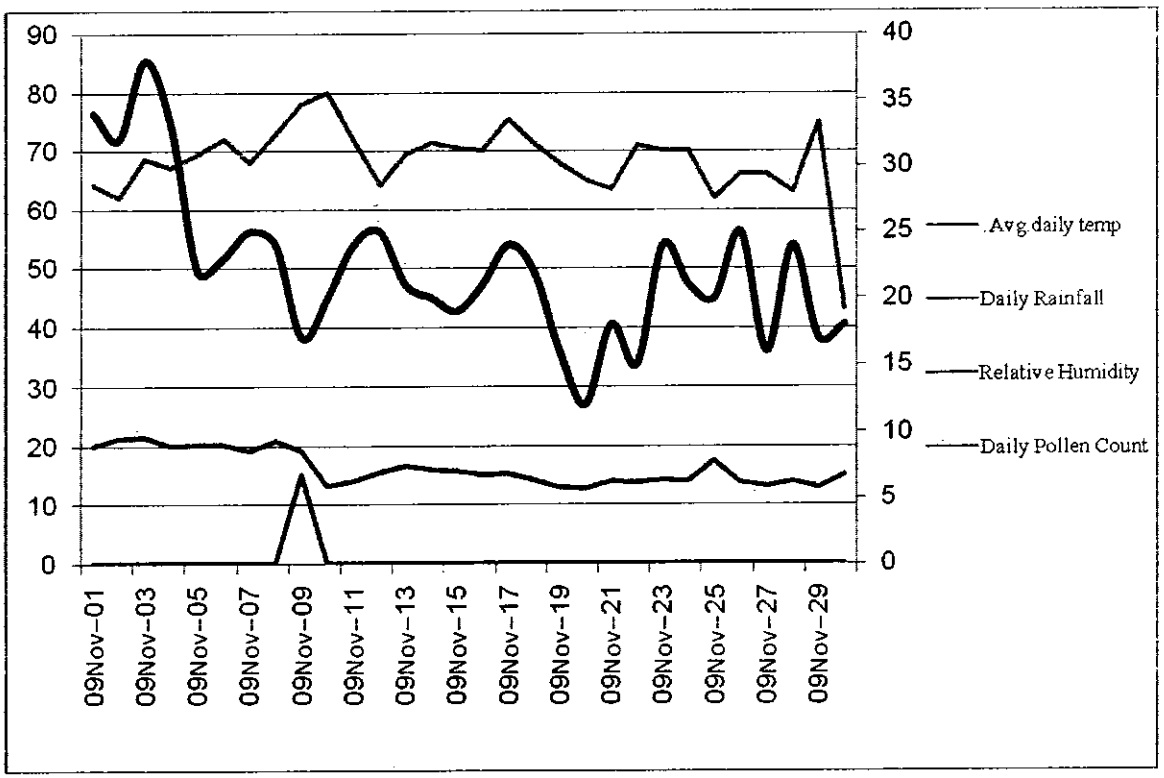
Figure 20: Relationship of TPC with average temperature, relative humidity and rainfall during October 2009.



November 2009:

It is observed from pollen data records for previous years that usually the TPC is very low during the month of November. Pollen numbers remained very low about 37 grains/m^3 in the beginning of the month with the relative humidity of 64%. In the middle of the month pollen numbers remained sharply about 19 grains/m^3 due to rain and decreased relative humidity while temperature starts to decrease. In the last week of this month pollen numbers remained low due to low temperature and relative humidity while rainfall was negligible.

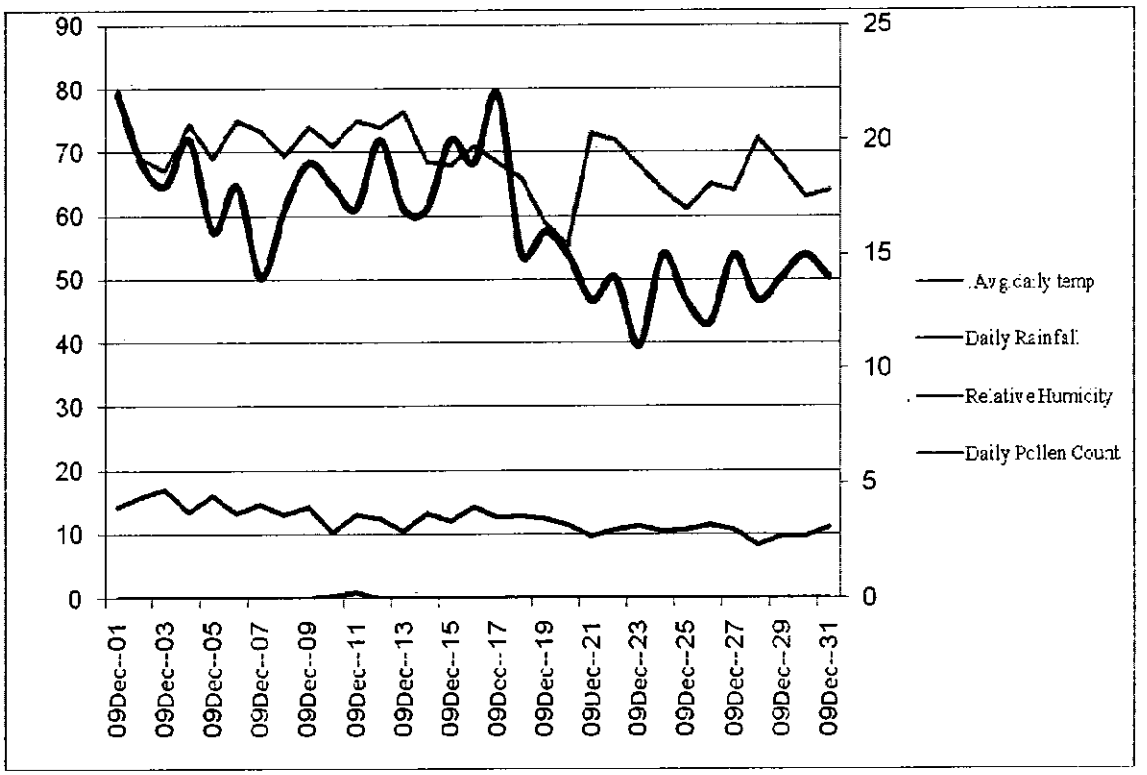
Figure 21: Relationship of TPC with average temperature, relative humidity and rainfall during November 2009.



December 2009:

Very few numbers of pollen grains were present into the atmosphere throughout this month. Pollen numbers started to decrease from the start of the month from relatively higher temperature and low rainfall and relative humidity of 68%. At the mid of the month pollen numbers slightly increased due to increased average temperature and decreased relative humidity with zero rainfall. At the last week of this month pollen numbers showed variations due to change in relative humidity and temperature at zero rainfall.

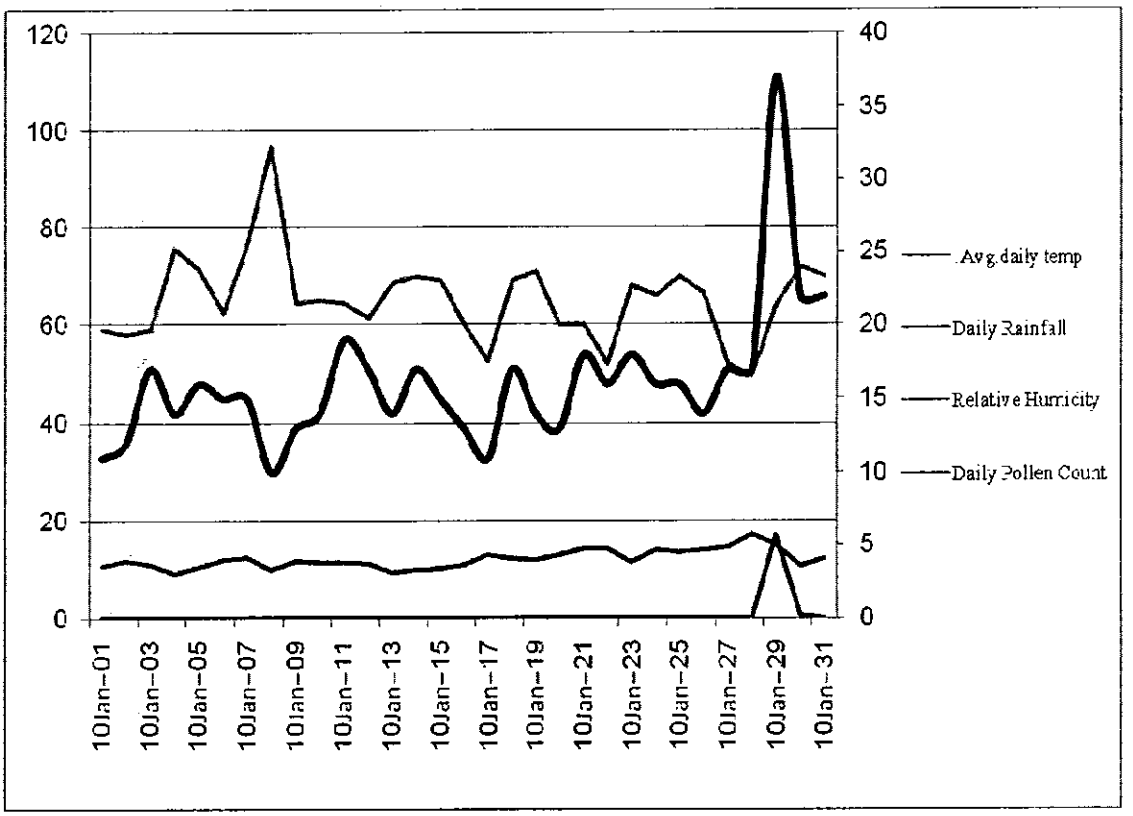
Figure 22: Relationship of TPC with average temperature, relative humidity and rainfall during December, 2009.



January 2010:

In the beginning of the month, pollen numbers shows clear variations based on rise and fall in relative humidity and average temperature at zero rainfall. At the middle of the month pollen numbers decreased due to low average temperature and increased relative humidity. Although in the last week of this month pollen number increased directly with the rainfall and relative humidity and indirectly with average temperature.

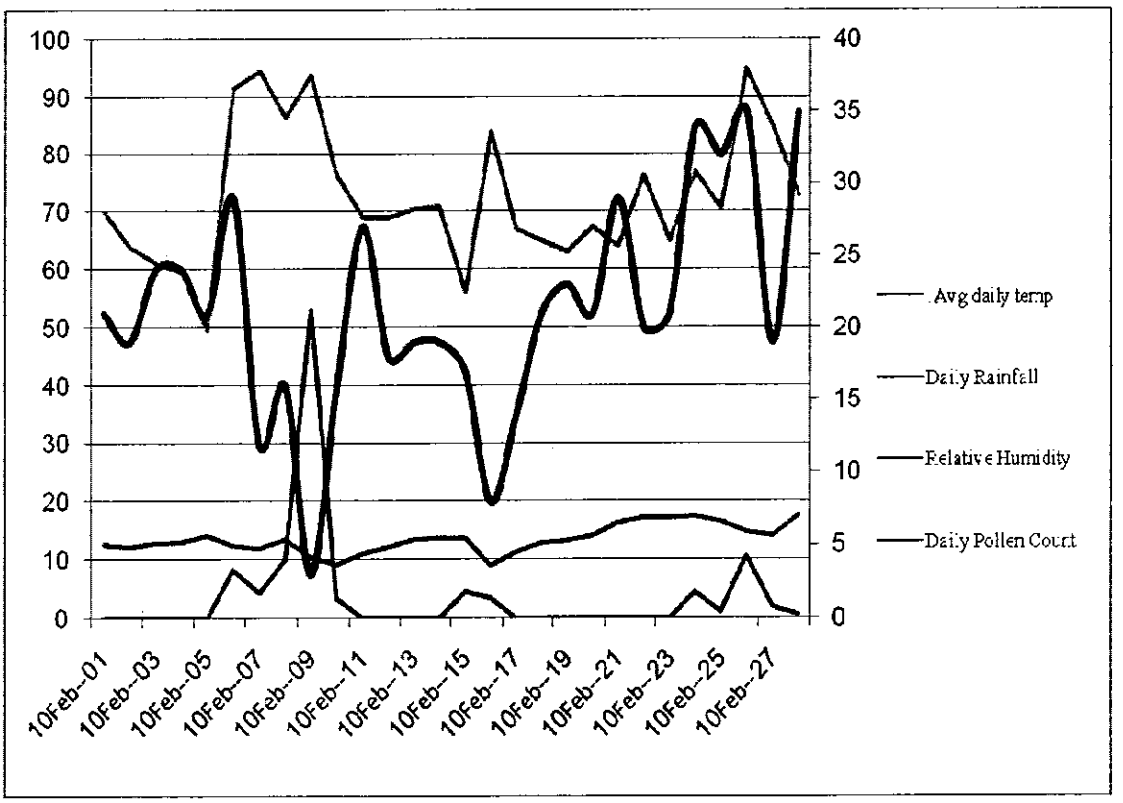
Figure 23: Relationship of TPC with average temperature, relative humidity and rainfall during January, 2010.



February 2010:

An abrupt fluctuation was observed for TPC due to heavier rainfall and change of temperature and relative humidity. Initially pollen numbers increased due to light rain and low relative humidity at about 12^0C. In the second week of this month, heavier rainfall decreased temperature and relative humidity due to which pollen numbers reached to its minimum. At the end of this month pollen numbers gradually increased due to rise in temperature with the decreased relative humidity and rainfall.

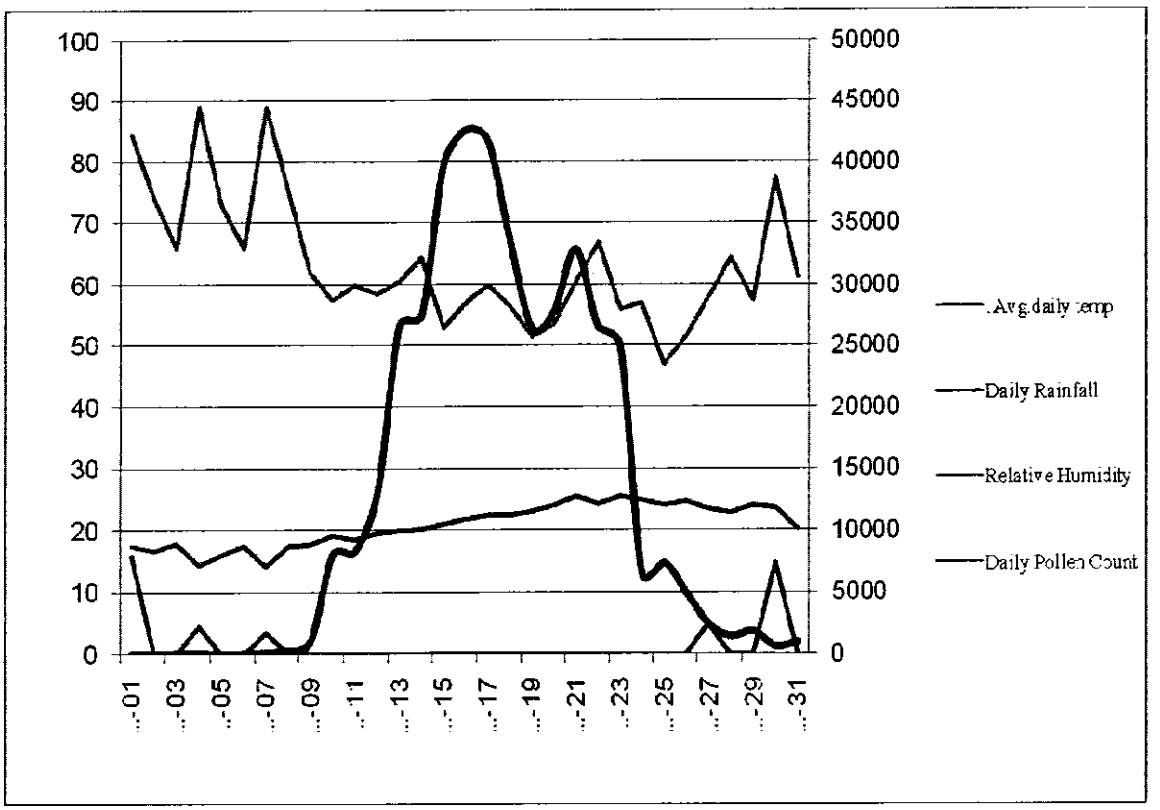
Figure 24: Relationship of TPC with average temperature, relative humidity and rainfall during February 2010.



March 2010:

In the beginning of March, pollen numbers were minimum mostly because of high rainfall and relative humidity. Then the pollen numbers increased due to high temperature and less relative humidity in the absence of rainfall. TPC reached to its peak in the middle of the month. This month showed the highest pollen numbers throughout the year. But at the end of the month pollen numbers gradually decreased due to heavy moisture produced by rain and higher relative humidity at the average daily temperature of 22^0C.

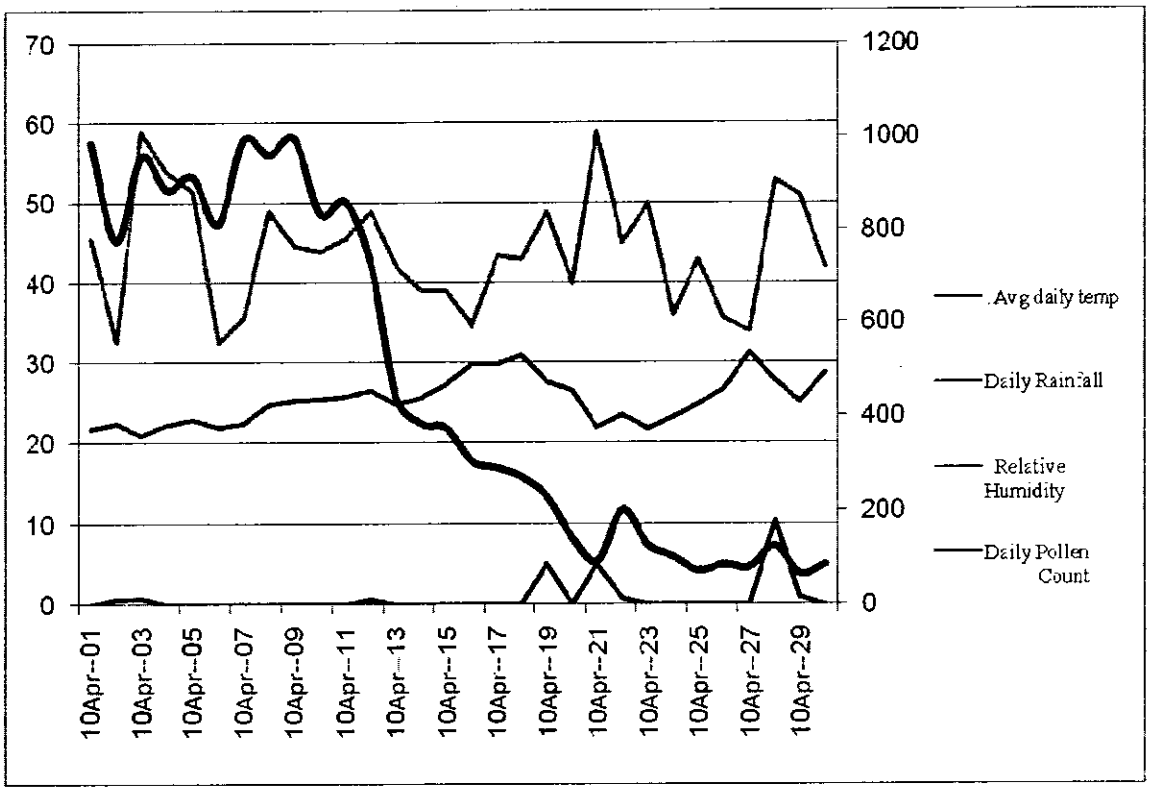
Figure 25: Relationship of TPC with average temperature, relative humidity and rainfall during March 2010.



April 2010:

Pollen numbers were relatively higher due to low relative humidity and zero rainfall while the temperature was optimum. In the middle of this month pollen numbers showed gradually decreasing behavior due to decreasing temperature and relative humidity while rainfall was negligible. At the last week of the month, pollen numbers showed fluctuations in decreasing manner with the decreasing temperature and minor rainfall while relative humidity also fluctuated.

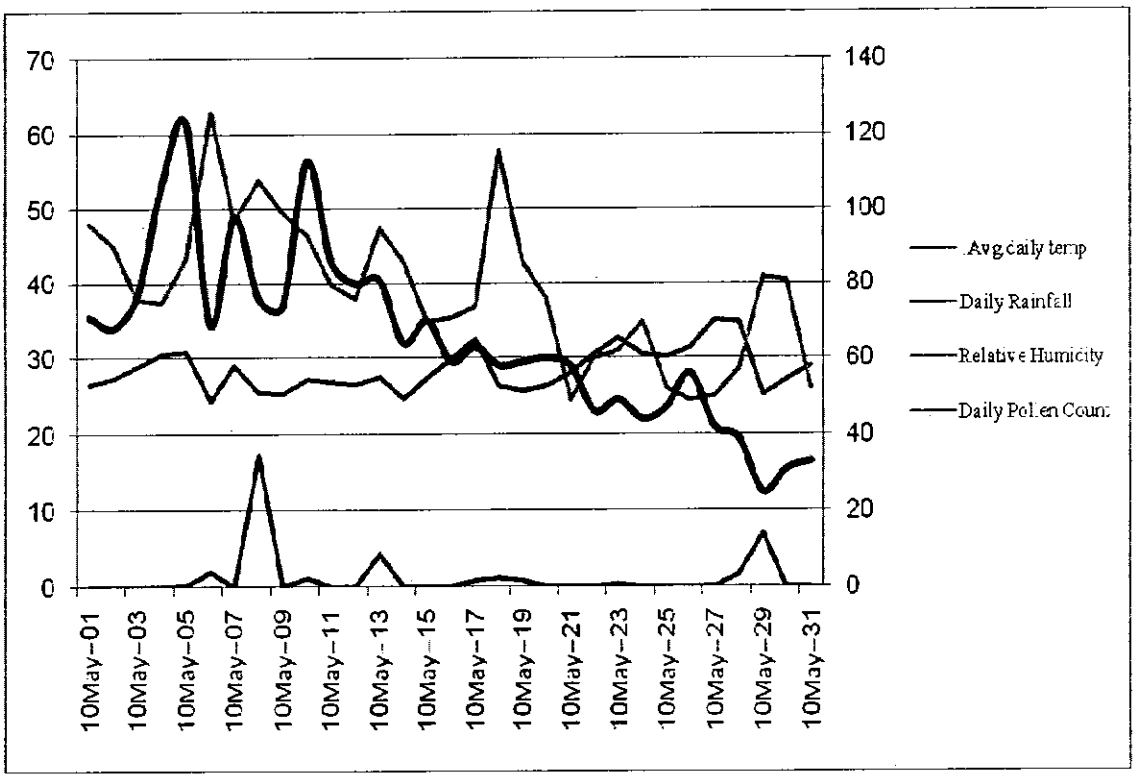
Figure 26: Relationship of TPC with average temperature, relative humidity and rainfall during April 2010.



May 2010:

With the start of the month, pollen numbers slowly increased with increasing temperature and decreasing relative humidity at zero rainfall. But then pollen numbers were gradually decreased with rising rainfall and relative humidity at low temperature. Pollen numbers showed the parallel decreasing behavior with increasing relative humidity even though temperature was increased to some extent. In the last three days of this month pollen numbers reached to its minimum level with decreasing temperature and increasing rainfall and relative humidity.

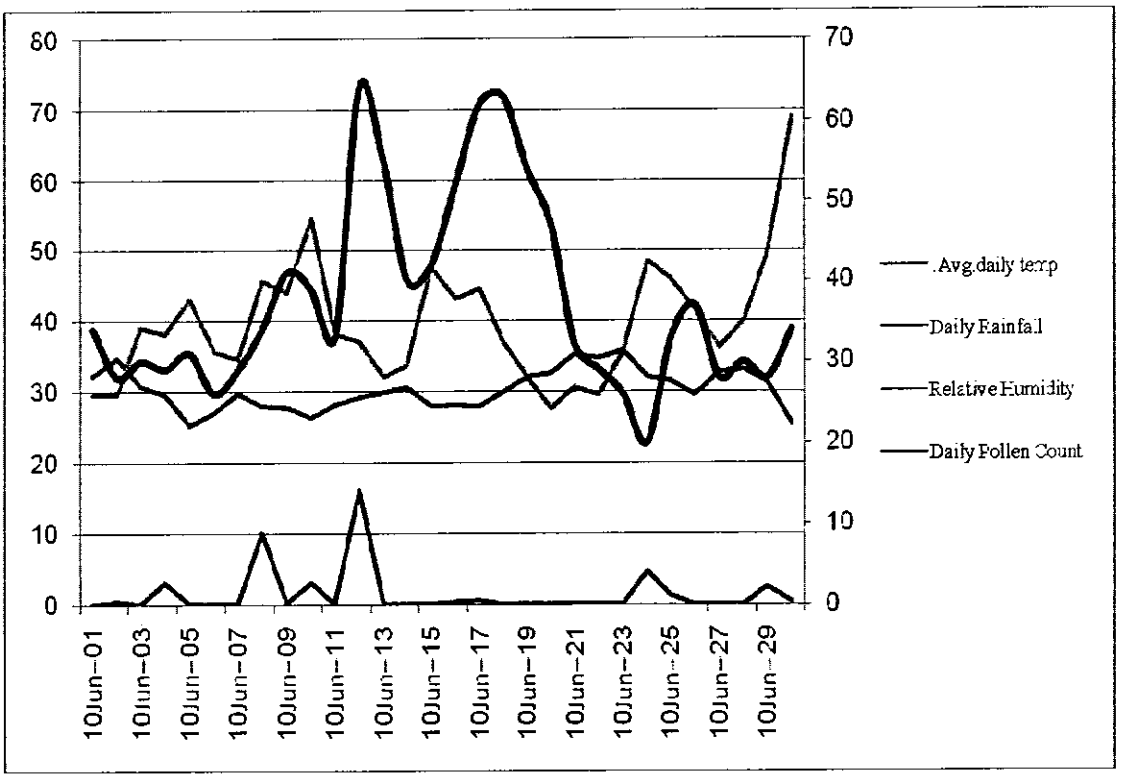
Figure 27: Relationship of TPC with average temperature, relative humidity and rainfall during May 2010.



June 2010:

In the first week of June pollen numbers decreased due to light precipitation and increased relative humidity at decreasing temperature. But at the mid of this month pollen numbers gradually increased and reached to its climax for the month due to increasing temperature and decreasing relative humidity at zero rainfall. In the last four days pollen numbers slowly decreased due to increasing relative humidity and decreasing average temperature.

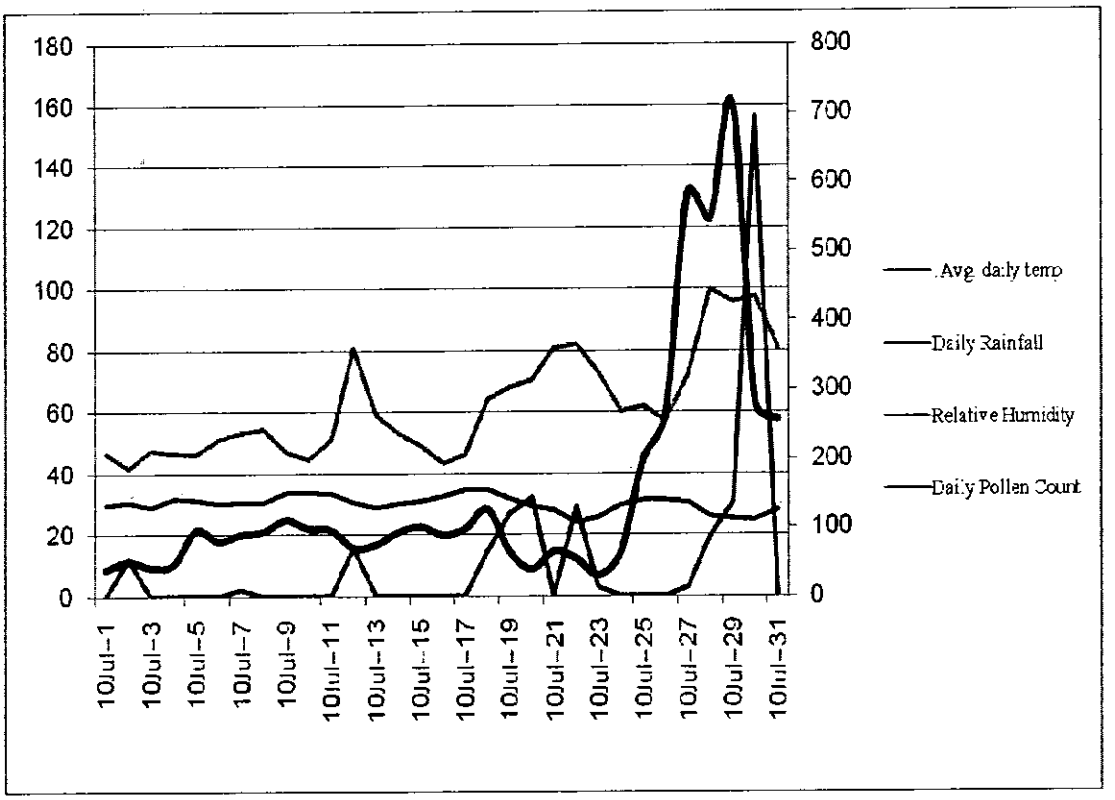
Figure 28: Relationship of TPC with average temperature, relative humidity and rainfall during June 2010.



July 2010:

The graph 30 shows the unexpected change in pollen numbers. Initially pollen numbers were few in numbers due to low temperature and high relative humidity. In the middle of this month TPC subsequently increased due to some amount of rain and relative humidity with increasing temperature. In the last week of July the pollen numbers reached to the 700 grain/m^3 and then immediately decreased to 200 grains/m^3 because of rainfall and increased relative humidity.

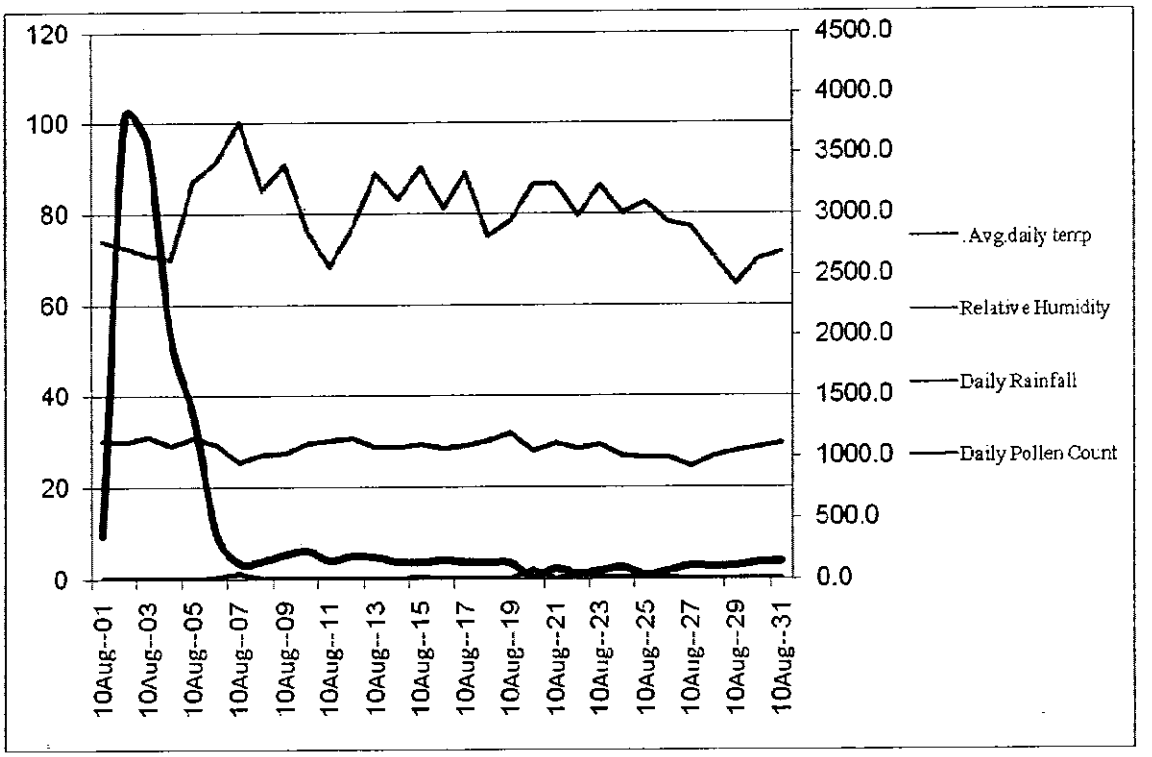
Figure 29: Relationship of TPC with average temperature, relative humidity and rainfall during July 2010.



August 2010:

Pollen numbers increased from few numbers and reached to about 3,806 on 2nd of the month along with the decrease in relative humidity and low temperature with some amount of rain. After that the pollen numbers subsequently decreased due to heavy rainfall with the fluctuated relative humidity and temperature as well.

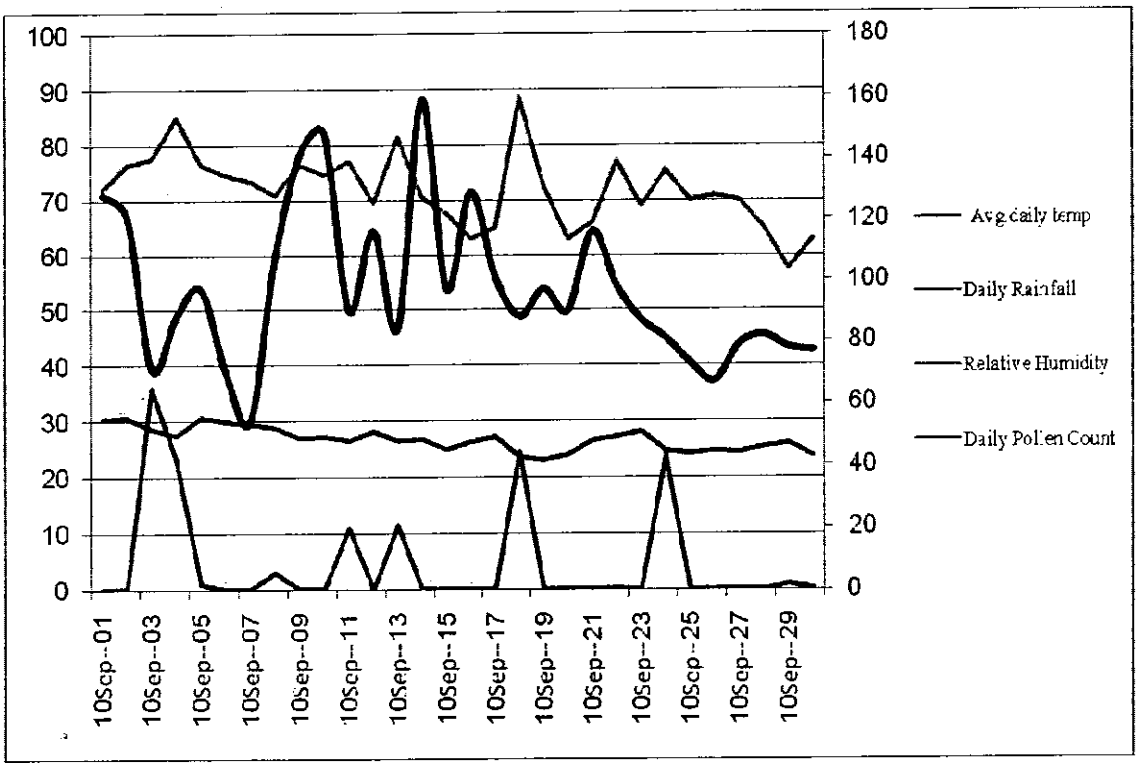
Figure 30: Relationship of TPC with average temperature, relative humidity and rainfall during August 2010.



September 2010:

During the first week of September pollen numbers subsequently decreased mostly due to heavy precipitation and increased relative humidity with the temperature about 29^0C. Afterwards the pollen numbers also showed variations due to fluctuating rain and relative humidity with the constant temperature. Pollen numbers were about 159 grains/m^3 in the middle of this month with 71% relative humidity, temperature about 27^0C with the small amount of rain. Importantly in the last week of this month pollen numbers subsequently increased with little rain.

Figure 31: Relationship of TPC with average temperature, relative humidity and rainfall during September 2010.



4.6 Comparison of pollen patients with pollen numbers (TPC) during 2009-2010:

In first part of the current chapter the data regarding patient number and their month wise frequency at various hospitals of Islamabad was studied to support the pollen allergy problem in the city. Here another simple relationship of total pollen count (during the years 2009-2010) can be made with the total pollen allergy patients appeared in the two major hospitals of Islamabad during same period (2009-2010). Data observations for TPC and pollen patient numbers lead us to a conclusion that the increase in TPC into the atmosphere has a direct impact on patient number and hence a higher risk factor in the development of allergic diseases (i.e. hay fever, eczema, asthma etc.) discussed as (Table 1&2). It has been observed that the airborne pollens usually induced the three allergic diseases i.e. Asthma, Allergic Rhinitis (Hay Fever)

and Atopic Dermatitis (Eczema). Of all these allergic patients, most of them visited to PIMS for nebulization on emergency basis during the pollen season, while a less also visited to NIH for allergy diagnosis and allergic vaccination mostly in the months of October, November and December (before the onset of pollen season). These data gathered from various sources/hospitals suggest that the prevalence of allergic diseases (hay fever, asthma and eczema) is on an increase over the last 9 years in the city (Islamabad and Rawalpindi). The cause of this increasing allergy (patients) trend is mostly due to favorable meteorological factors/variables (temperature, rain, relative humidity) required for pollen production and its dispersal.

Table 1: Comparison of pollen patients with pollen numbers during 2009

	Pollen Numbers (grains/m^3)	Pollen patients
March	3, 83,042	1883
April	56,865	877
August	30045	910

Table 2: Comparison of pollen patients with pollen numbers during 2010

	Pollen Numbers (grains/m^3)	Pollen patients
March	4, 09,162	2068
April	14,203	902
August	14690	975

Keeping in view these pollen allergies trends from hospital data and the month wise pollen spread, in some of the cases there was a clear picture available Figures (12-32) to establish a relationship between TPC and environmental variables. But on the other hand, there were several month's Figures (16,17,24) those do not show a clear

relationship for these meteorological variables and these co-relations were not successfully established through these figures. Keeping this problem in view, a multiple regression analysis was conducted in order to study these relationships in detail using available month wise data series, which was not possible to be studies with simple observation method.

4.7 Multiple Regression Analysis of TPC with Different Meteorological Parameters

As seen from the month wise Figures (12-32) of total pollen count (TPC) in relation to various climatic variables i.e. temperature, relative humidity and rain. A relationship was established between total pollen numbers observed specially in spring (highest pollen) season and above mentioned meteorological parameters, multiple regression technique was used according to the following equation,

$$Y = a_1 X_1 + a_2 X_2 + \cdots + a_K X_K + C$$

Along with the spring season pollen analysis by using these meteorological parameters, a prediction models for the years 2009 and 2010 was estimated. These models well predict the peak values Haroon and Rasul (2008).

4.8 Analysis of variance for the total pollen count, 2009

From January 2009 to September 2010 two extreme trends (peaks) were observed for total pollen count during the month of March (i.e. March, 2009 and March, 2010). For the month of March, 2009 the peak value for total pollen count was observed about 383042 grains/m^3 whereas, for the month of March, 2010 the observed value was 406192 grains/m^3. According to the predictive model for total pollen count the predicted values were found to be quite low as compared to the observed values,

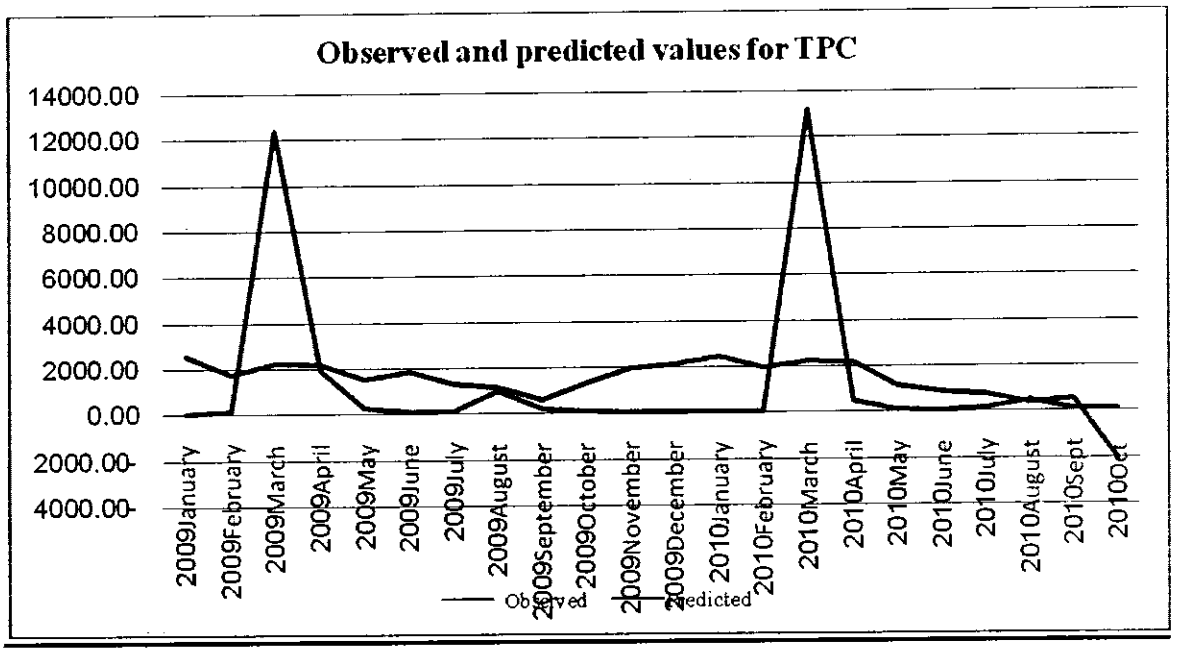
moreover these predicted values show a constant decreasing and subsequent increasing trend over the time. Especially the decreasing trend was observed for the late summer and fall to winter months, with an increasing trend for spring month, which is somehow in accordance with the observed values. While it is important to note that no significant regression ($P \leq 0.928411$) was found for total pollen count.

Table 3: ANOVA Result for TPC 2009 – 2010

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	21730586	5	4346117	0.259949	0.928411
Residual	267505450	16	16719091		
Total	289236036				

a. Predictors: TempMax, TempMin, Relative Humidity, Rainfall
b. Dependent Variable: TPC

Figure 33: Predictive Model Chart for TPC and Meteorological Parameters, 2009 - 2010



a. Predictors: TempMax, TempMin, Relative Humidity, Rainfall

b. Dependent Variable: TPC

4.9 Analysis of variance for Paper Mulberry pollen in March, 2009

The Paper Mulberry pollen abundance was studied for March 2009, with different meteorological parameters including Rainfall, Relative Humidity, Maximum Temperature and Minimum Temperature. The regression analysis was found highly significant ($P \leq 0.003557$). Observed values for pollen count of Paper Mulberry were found increasing from March 10th to March 20th, 2009. After March 20th the Paper Mulberry decreased suddenly, while the predictive model of Paper Mulberry for March 2009 is showing the similar increasing trends from March 6th to March 18th, 2009 as shown in table 4.

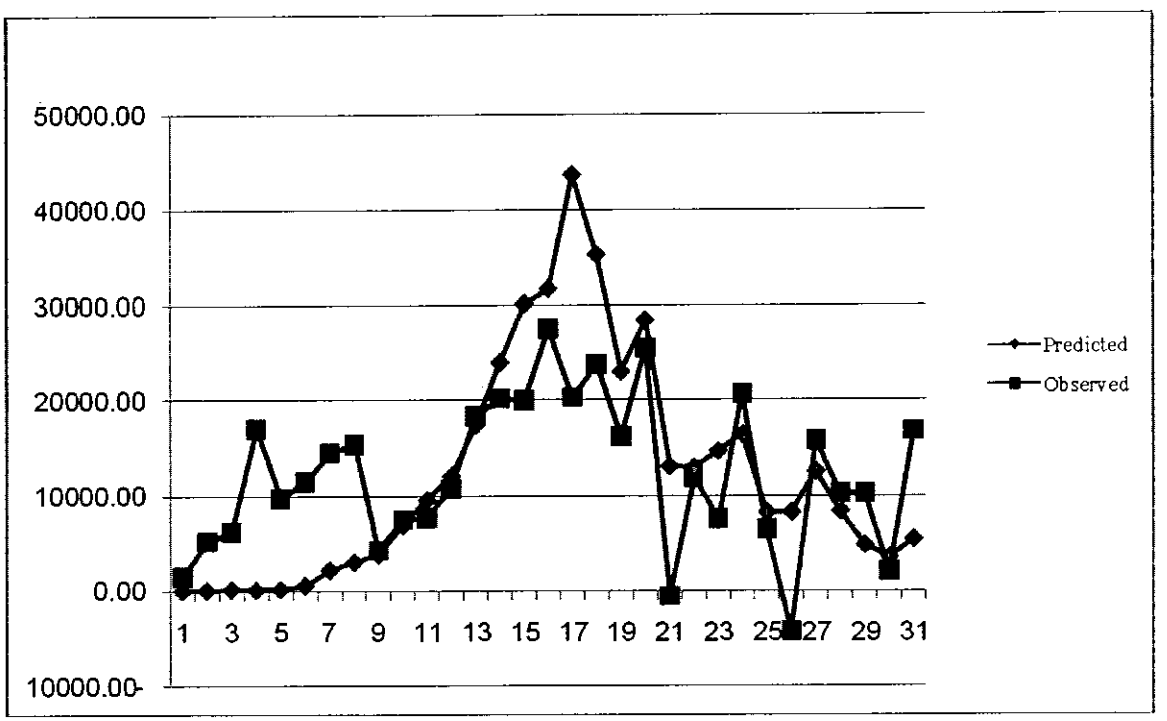
Table 4: ANOVA Result for PM pollen, March 2009

Model	Sum of Squares	df	Mean Square	F	Sig
Regression	1.839785E+09	4	459946227	5.113	0.003557
Residual	2.338732E+09	26	89951234		
Total	4.178517E+09				

a. Predictors: Temp Max, Temp Min, Relative Humidity, Rainfall

b. Dependent Variable: TPC

Figure 34: Predictive Model for PM, March 2009



a: Predictors: TempMax, TempMin, Relative Humidity, Rainfall

b: Dependent Variable: TPC

4.10 Analysis of variance for Paper Mulberry pollen in March, 2010

ANOVA for the Paper Mulberry pollens during March 2010, with different meteorological parameters including Rainfall, Relative Humidity, Maximum Temperature and Minimum Temperature(Average Temperature). The regression analysis in this case was also found significant ($P \leq 0.044$). Observed values for pollen count of Paper Mulberry were found increasing from March 8th to March 29th, 2010. While the prediction model of Paper Mulberry for March 2010 shows similar increasing trend from March 10th to March17th, 2010. Moreover, observed values showed highest number of pollens on 20th March, 2010 while predictive values showed highest number of pollens on 15thMarch, 2010.

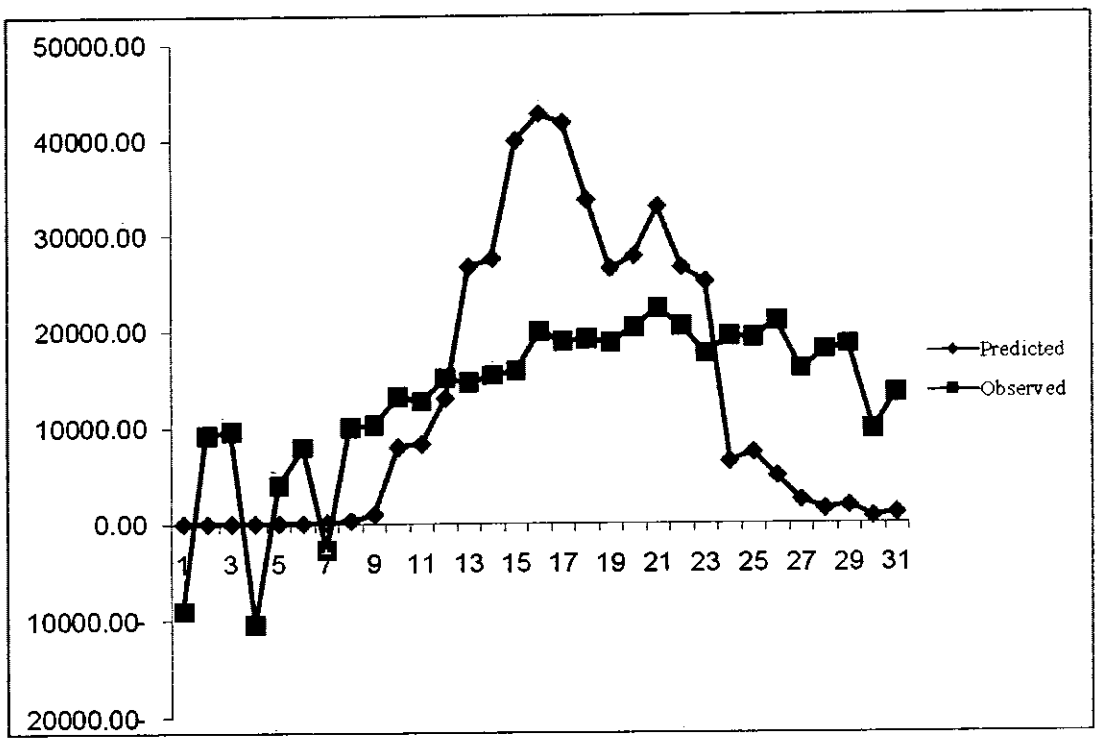
Table 5: ANOVA Result for PM pollen, March 2010.

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	2.041472E+09	4	510367987	2.841557	0.044403
Residual	4.669823E+09	26	179608558		
Total	6.711294E+09				

a. Predictors: TempMax, TempMin, Relative Humidity, Rainfall

b. Dependent Variable: TPC

Figure 35: Predictive model for PM, March 2010



a: Predictors: TempMax, TempMin, Relative Humidity, Rainfall

b: Dependent Variable: TPC

CHAPTER 5

DISCUSSION

Extremely high and low temperature greatly affects the plant physiology consequently affecting the reproductive processes to a great deal. It not only affects the pollen production rate but also has a great impact on the germination of pollen, therefore affecting the overall fertility. It is known that very high temperature adversely affect the pollen production while the optimum temperature enhances its production in most of the plant species (Koubouris, *et al.*, 2009).

In the present study, an effort is made to establish a co-relation of pollen numbers and environmental parameters minimum and maximum temperature (average temperature), relative humidity and rainfall. Pollen allergy patients were also observed in different hospitals of Islamabad namely in National Institute of health (NIH) and Pakistan Institute of Medical Sciences (PIMS). Generally a strong relationship is observed between average temperature and total pollen numbers in most of the cases (Figure 12-32). It is well established from the month wise TPC data that the average temperature normally increased the total pollen numbers in atmosphere but it was also observed that the optimum temperature more significantly induced an increased pollen production specially to its peak in spring season in the months of March and April. Maximum pollen number was 43,780 grains/m^3 at optimum average temperature (19.45^{0}C) combined with the moderate values for Relative Humidity (59%) and zero rainfall at 17th of March, 2009.

Similarly, the minimum pollen number (for the total atmospheric pollen) was recorded as low as 6 grains/m^3, at an average temperature of 10.75^{0}C combined with relative humidity at 53% and the highest rainfall of 100 mm on 14th February, 2009. Similar

trends for the maximum and minimum pollen numbers was reported by (Recio, *et al.*, 2010) as in our study the minimum pollen number is far lower than the usually low valued reported, but the heavy rainfall can be the important factor reducing the pollen number to this lower limit.

Nearly the same pattern for variations in total pollen numbers was observed during the following year i.e. 2010 as observed in 2009. Maximum pollen number was counted 41,800 (grains/m^3) at optimum average temperature 22.55^0, combined with relative humidity of 60% and zero rainfall at 17th March, 2010 while the minimum pollen number was observed at average temperature of 10.6^0C, combined with relative humidity of 53.1% and with the high rainfall (94 mm) at 9th February, 2010 (Haroon and Rasul, 2008).

During the two years analysis, It was observed that the maximum pollen numbers appeared in the month of March (2009-2010) at their optimum mean temperature of 21^0C, similarly the minimum pollen concentration was observed in the month of February (2009-2010) at optimum mean temperature of 10.7^0C (Hout, *et al.*, 2008).

It was observed that for 2009 the maximum number of TPC was at an average temperature of 17.5 ^{0}C while in 2010 TPC was maximum at average temperature 20.5^0C therefore this noticeable difference of 3^0C indicates that there must have been some other climatic factors/variables affecting the pollen production e.g. rainfall and relative humidity etc.

It also has been observed that the average temperature increases the pollen numbers in the atmosphere while relative humidity and rainfall decreases the pollen numbers (TPC), similar type of trends were reported on the effect of temperature, relative humidity and rainfall for the presence of ragweed pollen grains into the atmosphere (Barnes, *et al.*, 2001). This was also suggested that clear conditions, temperature and

relative humidity have minimal effects on daily pollen count however, under the condition of cold front, decreased pollen numbers into the atmosphere were observed. Similar trend was observed during the current study regarding TPC at lower temperatures and specially for Paper Mulberry pollen in relation to all these environmental variability (Barnes, *et al.*, 2001).

In addition to the month wise pollen variation charts (those provide daily TPC variation) trends, the regression analysis for the two years TPC pollen data with the environmental parameters was studied in order to obtain a better picture of pollen variation. Along with this regression analysis ANOVA results for Total Pollen Count were also analyzed for 2009-2010, that show two clear trends/peaks in March 2009 and March 2010 respectively (Fig. 33). Based on these trends/peaks a regression analysis was done for the estimation of the Paper Mulberry pollen with these environmental variables (Temperature, RH and Rain) during March 2009 and 2010. Although the ANOVA for TPC (2009-2010) was found insignificant ($P \leq 0.93$) whereas, ANOVA results for the Paper Mulberry pollens for the month of March, 2009 & March, 2010 which is the peak pollen season were found highly ($P \leq 0.004$) and ($P \leq 0.044$) respectively, signifying the relationship of the Paper Mulberry pollen production with the studied environmental variables specially during spring season i.e. March 2009 and 2010 (Fig. 34 & 35) similar observations are also reported for the spring season by Haroon and Rasul (2008).

During the current study, It was observed that 9952 pollen allergy patients appeared during the year 2009, whereas, 11607 patients were reported during the year 2010. Increase in the patient's number found mostly related to the increase in pollen numbers while, some other studies have also recognized the relationship between allergic symptoms and atmospheric pollen concentrations (Burr *et al.*, 2003; Cashel *et*

al., 2004; Goulet *et al.*, 1996; Kadocsa *et al.*, 1991; Sears *et al.*, 1989). High levels of pollens can therefore be a risk factor in the development of allergic rhinitis symptoms and some other such allergies. During the current research project, it was found that the pollen allergy is one of the major health problem in the Islamabad. A number of people have been reported with the pollen allergy symptoms especially in spring season when pollen concentration in the air was highest as a rule (Abbas, *et al.*, 2009). In Islamabad it is observed that the major cause of pollen allergy is the Paper Mulberry tree. Records from NIH and PIMS give us a clear picture of pollen allergy patients consulting each month with an indication that most of the pollen allergy patients were observed during the months of March, April, May, August, September, October and November (2009 and 2010). Most of the people consulted to the PIMS hospital physicians on emergency basis due to severe pollen allergy and hay fever during spring season. Some of the patients consulted to the NIH physicians during the months of September, October and November for the sake of vaccination.

Paper Mulberry no doubt is the major pollinating tree in Islamabad. However, some other trees and grasses are also responsible for the pollen allergy. According to Pakistan Meteorological Department, *Broussonetia papyrifera* pollen was the most common captured aero pollen in 2009-2010, Islamabad (Haroon and Rasool, 2008).

The study showed that two main types of plants producing pollens and playing a role in the genesis of seasonal pollen allergies are Broussonetia papyrifera and Cannabis sativa. Grass pollens and mould spores allergies are manifested perennially in susceptible individuals. As our results are specific to the city of Islamabad, Pakistan, therefore, further surveys need to be undertaken before generalization to a wider geographical region could be possible specially for the Paper Mulberry pollen of the related health/allergy problems (Abbas, et al., 200).

During peak pollen season (March), there were maximum pollen patients observed during the study of both years (2009-2010).

During peak pollen season of the two years (i.e. March), optimum average temperature was 19.45^{0}C and 22.55^{0}C respectively (2009-2010), whereas the relative humidity for the month of March of both years was 59% and 60% respectively. Under the influence of these meteorological factors the highest pollen count observed was 43,780 grains/m^3 and 41,800/m^3 respectively in the month of March (2009-2010) (Recio, et al., 2010). If we try to study in terms of the total pollen count for the whole month of March (31 days), it was about 3,83,042 grains/m^3 and 4,09,162 grains/m^3 respectively (2009-2010), This total count for the full month clearly indicates that the relative humidity was nearly equal in both years but there was a marked difference of 3.1^{0}C among the average temperatures for the two years, The year 2010 with higher average temperature produced more pollens compared to lower average temperature of 2009. Thus the optimum average temperature supported the highest pollen numbers in the air, Similarly it is found that the increase in spring pollen amount is stimulated by the rise in average temperature (Recio, *et al.*, 2010).

The second highest pollen concentration into the atmosphere was observed in the month of April during the spring season. Highest pollen numbers counted during the month of April (pollen observed for one day) were 5834 grains/m^3 and 996 grains/m^3 respectively in 2009-2010. Average temperature at these pollen numbers were 25.45^{0}C and 25.35^{0}C respectively. Similarly, relative humidity of both years was 44% and 45% respectively. It is interesting to note that the total pollen numbers of April of both years were 56,865 grains/m^3 and 14,203 grains/m^3 respectively which is far low than the last month i.e. March (Abbas, *et al.*, 2009).

The month of the August is the second pollen season of a year in Islamabad. Highest pollen number in the month of August (peak pollen values of a day) was 3336 grains/m^3 and 3579 grains/m^3 respectively (2009-2010). Average temperature during higher pollen numbers were 31.15^{0}C and 30.95^{0}C respectively (2009-2010). Relative humidity was 68% and 71% respectively (2009-2010). Total pollen numbers counted during the whole month of August were 30045 grains/m^3 and 14690 grains/m^3 respectively in (2009-2010). It is confirmed that the higher relative humidity decreased the pollen numbers into the atmosphere whereas Mandal, *et al.*, (2008) also suggested that relative humidity has a negative co-relation with pollen numbers into the atmosphere.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

Paper Mulberry has the highest pollen count in the spring (March to April), and is the main cause of allergic diseases like allergic rhinitis and eczema among the local residents of Islamabad. This time span of pollen grains is at its peak values and is very critical for allergy patients in the investigated region (Islamabad). Line graphs were established between selected meteorological parameters (Temperature, RH and Rain) and total pollen count throughout the study years (2009-2010) to show pollens trend due to abruptly changing climatic factors. Keeping in view these graphs, it is concluded that the Relative Humidity and precipitation showed negative relationship with the total pollen numbers into the atmosphere while temperature showed positive relationship with the atmospheric pollen numbers. In spite of this, relationship of pollen numbers with pollen patients was also established to study the affect of pollens on sensitized people of Islamabad. Meanwhile, pollen patients comparison charts were organized between the two local hospitals namely PIMS and NIH. According to these charts, high number of pollen allergy patients were observed in PIMS than in the NIH. We also applied the multiple regression technique in order to get clear picture of pollen numbers trend and establish predictive model for the two years data (March 2009-March 2010) which gave us the significant values for ANOVA.

Of all the allergenic species, Paper Mulberry is the major cause of pollen allergy in Islamabad. Paper Mulberry pollens comprises about 80% of all the allergenic pollens into the atmosphere of Islamabad. This tree has been found almost in all sectors of Islamabad. This exotic specie has high growth rate and extensive root network out-competing the native flora. Keeping in view the problem, Following are some of the

suggestions through which this plant can be completely eradicated. Paper Mulberry is Monoeceous plant. Only the male plant has a spike with pollens. This is important to note when ever the tree has been selected for eradication. . Firstly, this tree can be replaced by any other indigenous tree like Morus alba and Morus nigra. Secondly, proper preventive measures should be adopted by the allergy sensitized people before the onset of pollen season. Thirdly, pollen allergy awareness campaigns should be organized prior to start of pollen season.

REFERENCES:

Abbas, S., Raza, S. M and Khan M. J. (2009). Aerobiology and Pollen Allergy in Islamabd. *Journal of Pakistan Medical Research,* 48 (4): 79-82.

Barnes, K. C. (2006). Genetic Epidemiology of Health Disparities in Allergy and Clinical Immunology. *Journal of Allergy and Clinical Immunology,* 117 (2): 243-254.

Bhalla, P. L. and Singh, M. B. (2008). Biotechnology-Based Allergy Diagnosis and Vaccination. *Journal of Trends in Biotechnology*, 26 (3): 153-161.

Breton, M. C., Garneau, M., Fortier, I., Guay, F. and Louis, J. (2006). Relationship Between Climate, Pollen Concentrations of Ambrosia and Medical consultations for Allergic Rhinitis in Montreal, 1994-2002. *Journal of the Science of the total environment*, 370 (1): 39-50.

Cerceau-Larrival, M. T., Bocquel, C., Carbonnier-Jarreau, M.C. and Verhille, A.M. (1996). Pollen: Bio-Indicator of Pollution. *Journal of Aerosol Science*, 27 (1): 227-228.

Chehregani, A.,' Majde, A., Moin, M., Gholami, M., Ali Shariatzadeh, M. and Nassiri, H (2004). Increasing Allergy Potency of Zinnia pollen Grains in Polluted Areas. *Journal of Ecotoxicology and Environmental safety*, 58 (2): 267-272

Eugene, C. Ogden., Gilbert, S. Raynor, B. S. (2004). A New Sampler for Airborne Pollen: The rotoslide. *Journal of Allergy*, 40 (1): 1-11

GoP, 1998. Population and Housing Census of Pakistan, Population Census Organization, Staistics Division, Government of Pakistan. 354.

Haroon, M. A. and Rasul, G. (2008). Effect of Meteorological Parameters on Pollen Concentration in the Atmosphere of Islamabad. *Journal of Pakistan Meteorology,* 4 (8): 27-35

Van Hout, R. ., Chamecki, M., Brush, G., Katz, J. Parlange, M. B. (2008). The Influence of local Meteorological Conditions on the Circadian Rhythm of Corn (Zea Mays L.) Pollen Emission. *Journal of Agricultural and Forest Meteorology*, 148: 1078-1092.

Jato, V., Dopazo, A. and Aira, M. J. (2002). Influence of Precipitation and Temperature on Airborne Pollen Concentration in Santiago de Compostela. *Journal of Grana*, 41 (4): 232-241

Jose, L. A. M., Sanchez, J. M. B., Luguilde, D. N., Fernandez, C. D. L., Guardia, C. D. D. L. and Sanchez F. A. (2007). Forecasting Airborne Pollen concentration Time Series with Neural and Neuro-Fuzzy Models. *Journal of expert systems with applications,* 32 (1): 1218-1225.

Kawashima, S., Clot, B., Fujita T., Takahashi, Y., and Nakamura, K. (2007). An Algorithm and a Device for Counting Airborne Pollen Automatically using Laser Optics. *Journal of Atmospheric Environment*, 41 (36): 7987–7993.

Koubouris, G. C., Metzidakis., L. T. and Vasilakakis, M. D. (2009). Impact of Temperature on Olive (*Olea Europaea* L.) Pollen Performance in Relation to Relative Humidity and Genotype. *Journal of Environmental and Experimental Botany,* 67 (1): 209-214

Liang, K. L., Su, M. C., Shiao, J. Y., Wu, S. H., Li Y. H. and Jiang, R.S. (2010). Role of Pollen Allergy in Taiwanese Patients with Allergic Rhinitis. *Journal of the Formosan Medical Association,* 109 (12): 879-885.

Liu, Z-G., Song, J-J. and Kong, X-L. (2010). A study on Pollen Allergens in China. *Journal of Biomedical and Environmental Sciences,* 23 (4): 319-322.

Mandal, J., Chakaraborty, P., Roy I., Chatterjee, S and Gupta-Bhattacharya, S., (2008). Prevalence of Allergenic Pollen Grains in the Aerosol of the City of Calcutta, India: a two year study, *Journal of Aerobiologia,* 24 (3): 151-164.

Mitsumoto, K., Yabusaki, K. and Aoyagi, H. (2009). Classification of Pollen Species using Auto Fluorescence Image Analysis. *Journal of Bio-science and Bio-engineering,* 107 (1): 90-94.

Nava, G. A., Dalmago, G. A., Bergamaschi, H., Paniz, R., Santos, R. P. D. and Marodin, G. A. B. (2009). Effect of High Temperatures in the Pre-Blooming and

Blooming Periods on Ovule Formation, Pollen Grains and Yield of 'Granada' Peach. *Journal of Scientia Horticulturae,* 122 (1): 37-44.

Pacini, E. and Hesse M. (2004). Cytophysiology of Pollen Presentation and Dispersal. *Journal of Flora,* 199 (4): 273-285.

Pasken, R. and Pietrowicz, J.A. (2005). Using Dispersion and Mesoscale Meteorological Models to Forecast Pollen Concentrations. *Journal of Atmospheric Environment,* 39 (40): 7689-7701.

Ribeiro, H., Oliveira, M., Ribeiro, N., Cruz, A., Ferreira, A., Machado, H., Reis, A. and Abreu, I. (2009). Pollen Allergenic Potential Nature of Some Trees Species: a Multidisciplinary Approach using Aerobiological, Immunochemical and Hospital Admissions Data. *Journal of Environmental Research,* 109 (3): 328–333.

Recio, M., Docampo, S., Garcia-Sanvhez, J., Trigo, M.M., Melgar, M and Cabezudo B. (2010). Influence of Temperaturte, Rainfall and Wind Trends on Grass Pollination in Malaga (western Mediterranean coast). *Journal of Agricultural and Forest Meteorology,* 150 (7-8): 931-940.

William, R., Solomon., Ann-Arbor, M.D. and Mich (1984). Aerobiology of Pollinosis. *Journal of Allergy and clinical Immunology,* 74 (4 Pt 1): 449-461

Wolukau, J. N., Zhang, S. L., Xu, G. H. and Chen D. (2004). The Effect of Temperature, Polyamines and Polyamine Synthesis Inhibitor on in Vitro Pollen

Germination and Pollen Tube Growth of Prunus Mume. *Journal of Scientia Horticulturae,* 99 (3-4): 289-299.

Annexure 1:

The following tables 6 & 7 shows the pollen numbers in atmosphere produced by different species of plants and grasses in Islamabad territory, Pakistan. Peak pollen numbers of different plants and grasses in atmosphere exist in two seasons throughout the year. First season starts in early spring in the month of March and ends in April. Second season starts in first week of August and ends in last week of September while the grasses had highest pollen numbers in February and August throughout the year. From all these plants and grasses species, Paper Mulberry had the highest pollen count in atmosphere and causing different allergic and various respiratory problems to the local resident of the Islamabad territory and the surrounding areas.

Table 6: TPC during 2009, Islamabad

	Paper Mulberry	Acacia	Eucalyptus	Pines	Grasses	Cannabis	Dandelion	Alternaria
January	34	02	08	0	349	46	06	502
February	79	29	05	50	2804	136	04	558
March	381062	29	04	513	571	400	32	431
April	54780	21	62	51	214	295	44	1398
May	4814	11	52	132	287	540	43	967
June	355	06	51	104	145	462	65	861
July	232	01	08	03	75	983	68	1340
Aug	23969	01	02	02	252	4336	106	1377
Sep	404	01	00	00	224	4193	90	1216
Oct	246	00	00	00	83	941	37	661
Nov	61	00	00	00	30	221	38	320
Dec	48	00	00	00	12	107	33	308

Annexure 2:

Table 7: TPC during 2010, Islamabad

	Paper Mulberry	Acacia	Eucalyptus	Pines	Grasses	Cannabis	Dandelion	Alternaria
Jan	42	00	00	00	30	94	27	307
Feb	44	00	00	08	105	128	06	306
March	407614	39	00	156	415	469	10	459
April	13006	12	22	36	160	190	00	777
May	552	07	57	97	100	321	09	883
June	213	00	08	62	57	215	32	543
July	2954	00	01	08	77	637	46	905
Aug	12491	01	00	00	123	1129	37	909
Sep	477	00	00	00	221	1230	47	939