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Bioaerosol Detection, Identification and Impact Studies in Indoor and Outdoor Environments in Nacharam

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Abstract
Bioaerosols are particles which may be biological origin like microbes, plants, animals etc., or may be artificial like house dust, organic waste etc., Most of the bioaerosols host on humans and moist places. Size of bioaerosol particles varies from below 1 µm to 100 µm in aerodynamic diameter. Indoor bioaerosols may originate from outdoor air and indoor reservoirs. Major sources of bacteria and viruses are humans and pets-sneezing, coughing, dander and saliva. Fungi, many bacteria, protozoa, algae and green plants (pollen) are present outdoors that are induced indoors by natural or mechanical ventilation. Bioaerosols induce into human body by inhalation or by deposition on wounds. A possible reason of sick building syndrome (SBS) is the presence of Bioaerosols in the building Despite the defense mechanism of the body these Bioaerosols could cause damage to the body. Other common health effects of Bioaerosols are Viral: infections such as Common cold, Influenza, Measles, Bronchitis, Fungal – Histoplasmosis, Cocciodomycosis and Blastomycosis and Antigens: Allergic diseases of Hypersensitivity pneumonitis (HP)Allergic asthma, Rhynitis and Pergillosis. Control Strategies include -After identifying the airborne microorganisms the source can either be eliminated or its strength can be reduced. Preventive maintenance is one the most effective ways to control the microorganisms indoors Maintenance of air handling systems and Humidifiers using the re-circulated water should not be used. Steam should be used instead of cold water in humidifiers, heating and HVAC systems. Disinfectants and biocides should be used in the humidifier water reservoirs, which kill the microorganisms.

Keywords
Bioaerosols, Influenza, Measles, Bronchitis, Histoplasmosis, Cocciodomycosis

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1.INTRODUCTION

Air is a mixture of various components which includes myriad gases and several microbes. An average human being can live for two weeks without food, two days without water, but hardly for a minute without air. Air is very much essential for a human being to survive and this valuable air is being polluted day by day. Air pollution has become an area of concern as consists of various minute particle called microbes which may cause several problems in humans. The air without the pollutants released by the human, itself is very much polluted. Imagine the air we inhale after all the pollutants in the form of various emissions getting mixed up with it. Air we inhale may contain various pollutants, gases, and pollen, bacterial and fungal spores etc., which may be released by plants, fungal molds, bacteria and various other pollution emitters. This air we breathe may cause allergies in various people who are susceptible to the pollutants.

Aerobiology is a branch of biology that studies organic particles, such as bacteria, fungal spores, very small insects, pollen grains and viruses, which are passively transported by the air. In other terms it is explained as "microbiology of atmosphere". According to IUBS commission of aerobiology it has been regarded as transport of organisms and biological significant materials by the atmosphere. Aerobiologists have traditionally been involved in the measurement and reporting of airborne pollen and fungal spores as a service to allergy sufferers.

Aerobiology is study of biological particles present in the air, both outdoors (extramural) and indoor (intramural). Many aspects of our life are affected by biological particles that are carrot in the air and are deposited from it.

The term aerobiology was first coined by F.C. Meir's of U.S.A in 1930's it is a scientific discipline focused on the transport of organisms and biologically significant materials by the atmosphere. Because of the practical application in the diagnosis and treatment of the respiratory allergic disorders, aerobiological investigations have acquired new dimensions. This is very much evident by the spurt of research publications, review articles monographs, atlases, pollen/spore bulletins etc. in the recent times.

It is science and multidisciplinary approach focused on the transport of organisms and biologically significant materials. It is concerned with the source of an organism or material their release in the atmosphere, dispersion, deposition and impaction on animals and human systems. With the inception of International Biological Programmer (IBP) in 1964[1], the term has been further extended to include investigations of airborne materials of biological significance.

FUNGAL SPORES:

The spores of common air-borne fungi have thick melanized walls. The walls usually contain complex carbohydrates which are hydrophobic, and waxy. Hydrophobicity enables control of water loss, but the wall must also enable uptake of water prior to germination. Thus, spore survival in air is a fine balance between water loss and retention of metabolic activity. The cytoplasm of air-borne spores may be constituted of greater concentrations of compatible solutes such as glycerol which enable continued metabolism in conditions of reduced water availability. Use of glycerol in the cytoplasm enables loss of water from the cell without catastrophic consequences for water uptake in moist conditions.

Many molecules, including melanin reduce the penetration of radiation especially in the UV range. Melanin is contained in walls of spores, and so is the first barrier to UV. However, a range of other UV absorbing molecules are found in spores. The consequence is that energy is transformed usually to heat, which can be readily radiated from the spore in air.

Inhalation of spores in most cases has no effect on humans. The spore lodge on the moist surfaces of the lining of the airways and they are subsequently expelled in mucus. The remaining spores are neutralized by the immune responses. However, a few fungi evade the immune response and cause respiratory disease.

Airborne microbes (Fungi) are implicated in the causation of allergic diseases and infections in immune compromised patients. The fungi constitute an

independent group equal in rank to that of plants and animals. They differ from bacteria by having genetic material arranged on chromosomes, and membranes surrounding the nucleus. The fungi that produce spores and get airborne are called 'Airborne'. They cause a number of infections in tropical countries including ringworm, athlete's foot in human, and rust, smuts, and leaf, root, and stem rots in plants. *Aspergillus sp.* can invade the lungs and cause serious pneumonia in people with an impaired immune system. They are also established to cause Type I hypersensitive diseases with IgE mediated response. The common symptoms of hypersensitivity are bronchial asthma, allergic rhinitis and atopic dermatitis. Spores are the reproductive particles or seeds of fungi.

There are many different types, sizes and shapes.

Spores are unicellular or multicellular, reproductive or distributional cells developing into a number of different phases of the complex life cycles of fungi.Fungal spores can be readily classified by the Saccardian system, which relies on the number, shape and placement of spore cells to classify the fungi imperfecti. Most fungal spores in pollen preparation probably are phaeospores (dark spores) of the fungi imperfecti, rather thanascospores, basidiospores or spores of the lower fungi. However, repeating (asexual) spores of the basidiomycetesare very common in some sites. Wolf (1969) [5] demonstrated that dark fungal spores are more resistant to acetolysis then clear ones.

Examples of the important fungal spores in palynology include the forms *Helminthosporium* and *Alternaria* that in aero-allergy studies. More generally, dark, thick –walled fungal spores of the fungal imperfect are common in soil samples, such as those often studies in archaeological palynology. These same forms occur in abundance equal to that of terrestrial pollen when soil is washed into aquatic basins by watershed erosion, particularly after fires or intense human disturbance [2]

Fungal spores are also found in high numbers at certain times of the year. Many of these are not the source of human, animal or plant disease.

Pollution and cigarette smoke also play a role in affecting the immune system and possibly attributing to predisposing a person to having allergies.

There are about 80,000 named species and new species are being added at the rate of about 1500 species each year. Fungi can be classifying into two basic groups:

Molds: Fungi that grow in filamentous form

Yeast: these are characteristically single cells

Fungi in general and molds in particular, can cause disease in humans and animals in three ways:

Fungi can produce an actual infection in the host involving growth on or in the person or animal. It is quite uncommon for environmental molds to produce this sort disease unless one has a very severe reduction in the function of the immune system (e.g., is undergoing intensive therapy for cancer or is a high dose of corticosteroids for periods of time). Examples of fungal invasion are Aspergillosis, Histoplasmosis, Paracoccidiomycosis, Sporotrichosis, and Zygomycosis.

Fungi can produce toxins that make people or animals sick. Although some toxins can be inhaled, the toxin is most often introduced into the person or animal by ingestion of mold contamination foods. Some of the toxins are very powerful e.g. aflatoxins.

Fungi can produce allergic reaction in hypersensitive subjects e.g. eczema, allergic rhinitis, allergic asthma, atopic dermatitis etc.

2.REVIEW OF LITERATURE

Aerobiologists have traditionally been involved in the measurement and reporting of airborne pollen and fungal spores as a service to allergy sufferers [4]

In 2002 [12], algae and other small water-borne organisms were discovered to inhabit clouds. A large cloud has about as much water as a shallow lake of the same geographic size. An important medical application of aerobiology is the study of the transmission of airborne diseases. It is known that many bacteria and viruses can be transmitted by spread through the air, possibly within droplets. Aerobiology is a rapidly developing science, which also

involves interaction with engineering and meteorology.

Aerobiology work in nineteen hundred nineties has been reviewed by Spieksma (1991)[3,4], who worked extensively on house dust mites and allergy in Netherlands. In 1930s F.C. Meir[16] was the first person to coin the term aerobiology to describe a project that involved the study of life in the air [8]. Since then various definitions have been used viz. study of aerosolization, aerial transmission, and deposition of biological materials. They have defined it more precisely as the study of diseases that may be transmitted via the respiratory route. Despite various definitions in usage, the fact is it is relatively new science.

Outdoor aerobiology refers to the aerospora in the outside environment which may include various fungal and bacterial spore types, pollen grains, plant tissues and typical fragments etc., the rotorod sampler is used to collect the samples from the outdoor places. Outdoor Aerobiology is gaining importance as several people get allergenic to various kinds of spore types which are not visible to eye. Outdoor environment has much scope for the pollen and spore types to get transported from one place to another through wind currents.

Indoor aerobiology refers to the indoor allergens which cause severe allergies for the people who are susceptible to them. The indoor aerobiology has become a field of interest for many scientist and research fellows as the cases of indoor air borne allergies are increasing day by day. People think that indoor environment is much safe than the outdoor environment as there will be no pollution. But indoor environment is of the same danger as the outdoor environment as the spores and pollen can travel anywhere through winds.

The aerobiological investigations have been carried out extensively and in detail in the countries in United Kingdom, USA, New Zealand, Australia, and Canada. Since the beginning of early 1940's. In India, it is fairly a new branch and has attained importance and taken up by many scientists and enthusiastic workers from the beginning of 1960's[5]. Here we listed important contributors towards the field of Aerobiology.

The US/IBP Aerobiology program was initiated in 1964, which provided cohesive framework by placing Aerobiology in a system analysis, where each particle is considered with respect to its source, release and dispersion in the atmosphere, deposition and its impact on the object. This work culminated in1974 when the International Association for Aerobiology was formed under auspices of the International Union of Biological sciences. At present IAA is the biggest International Integration of Aerobiologists. It organizes the International conferences once in four years. It also publishes the International Aerobiology Newsletters twice in a year, which provides us with the information on the recent researches in Aerobiology from all over the world. Its first conference was organized in 1978 at Munich (Germany)[22]; second in 1982[7] at Seattle (USA) and third was held in Basel (Sweden) in 1986[6], fourth in Stockholm (Sweden) in 1990[18], fifth in Bangalore (India) in 1994[21] and very recently the 10thwas held in Sydney (Australia)in September 2014[26].

In 1952, Gregory and Hirst (1957) have conducted aerobiological investigations in Rotahmsted, Harpenden, U.K. They have trapped several spores like Alternaria, Ascospores, Cladosporium, Helminthosporium, Ganoderma, Uredospores etc. in huge concentrations during their investigations. Harvey et al (1969)[19] have performed airspora studies over Cardiff in UK and have exclusively studied the concentration of Chaetomium spores. Ogunlana (1975) [14] has conducted extensive investigations in Ibadan, Nigeria and found the most prevalent fungal genera in the air like Cladosporium, Curvularia, Fusarium, Aspergillus, Penicillium, Pithomyces, Aureobasidium, Geotrichum, Phoma, Nigrospora, Epicoccum, and Neurospora. Cadman (1991)[19] has conducted experiments in Johannesburg and Pretoria in South Africa in the year 1987-88[10] and found meteorological factors associated with the fungal spore concentration. In 1994[21], Joanna et al have conducted airspora studies in Durban, South Africa. They have identified nearly 15 types of fungal spores

and the most abundant being Cladosporium. Ismail et al (2002) [12] have conducted extensive investigations and isolated several genera (29) of fungi in the aeromicrobiota of western desert of Egypt. In Haifa, Israel Schlesinger et al (2006) [28] have conducted air sampling experiments before, during and after dust storms and found that the bacterial and fungal counts increased after the dust events. In Pakistan, Parveen et al (2012) [11] have studied the identification and quantification of airborne pollen from Hyderabad Sindh.

In India several such investigations were conducted on various atmospheric bio components and their impact has been studied for the last more than 6 to 7 decades. In one of the earlier investigations in India Baruah (1961) [5] has studied the airspora of cowshed in Gauhati, Assam. In the same year (1961) [5] Sreeramulu has assessed the concentration of fungus sopores in the air inside cattle shed at Waltair in Andhra Pradesh. In 1981[7] Vittal and Krishnamoorthi conducted experiments in an agricultural field in Madras. In a recent survey conducted in the suburb of Kolkata, Chakrabarti et al (2012) [11] have conducted experiments for 5 consecutive years and compared with meteorological parameters.

In Hyderabad the research is also carried out in Kapra Lake, Safilguda Lake and Tank bund in which showed a high concentration of spores of *Alternaria,Aspergillus* etc., and this work was carried out by Ramachander Rao and his students in 2009[2,28].

Research is carried out in Hyderabad on "Clinical approach to indoor and outdoor mold spores in causing allergy" and had found 37 types of spores of Aspergillus, Alternaria, Torula, Trichomes, Nigrosporaetc., this work was carried by Ramachander Rao and his students in 2001[9].

In 2007 Aliya Asif Ali [2,28] as conducted aerobiological experiments in a semi indoor environment and isolated 32 different genera of fungi.

Venkata Sai Krishna (2008) attempted an analysis of fungal bioaerosol in a semi indoor type vegetable market in Malkajgiri Hyderabad and isolated 25 different fungal spore types. In 2009[23] several investigators have conducted experiments in different geographical locations. Kedarinath (2009) [23] studied airborne pollen concentration in an indoor environment. Srikanth Reddy (2009) [23] studied pollen bioaerosols in Malkajgiri. Vinay Kumar attempted pollen bioaerosols analysis of Sufilguda area. Nikhil (2009)[23] estimated the airborne pollen concentration of the Hussain Sagar (Tank Bund) lake.

Phaninder (2009)[23] has studied the aeromycoflora of Kapra lake area and its relevance to public area, where as pollen analysis was made by Rohith (2009)[23]. Vijay Krishna Kulkarni (2009) [23] has isolated fungal aerosols from the atmosphere of popular Hussain Sagar (Tank Bund) lake.

In 2012, research was carried out in patients house on "Viable aero plankton indoors and their role in causing allergy" by Ramachander Rao[11] and his students, and found that overall spore concentration was far higher in patients house as compared to the controls house. In a very recent study, Habeeb Ahmed and Abdul Razzak (2013) [26] have isolated a different fungal spore types from the houses of allergic patients.

3. MATERIALS AND METHODS

To trap the air borne fungal spores, the following method is employed

Rotorodair sampler

Apparatus used for trapping the air borne particles or air, is known as "air sampler". Different kinds of samplers have been employed in various countries. Even in India aerobiologists are using different kinds of samplers for trapping the components of air spora in various parts of the country. According to Gregory (1961), in any aerobiological work, the apparatus employed to catch the air borne particle is important as each has its own virtues and limitations. The choice of sampler depends upon its efficiency in catching the aero-spora components and also on the components that we want to investigate thoroughly. In the present investigation the "Rotorod Sampler" method was used.

ROTOROD SAMPLER

Figure1: Rotorod Sampler

DESCRIPTION:

Perkins (1957) developed a battery operated Rotorod sampler sampling at constant rotational speed since the efficiency of stationary impactor sampler is low and highly variable, the rotating impactor has been advantageously used. The device relies upon the high efficiency with which the small air borne particles are deposited on narrow oriented arms at right angles to high velocity winds. A battery-operated small motor with constant speed is used to whirl sticky coated brass rotates about its axis at a constant speed. It is been developed into a cheap, portable, highly efficient sampler with great sensitivity. It is well fitted for use in the field and is relatively independent of the external wind speed. In the Rotorod sampler, instead of moving spores impacting surface in current of air, the surface is rotated so that is strikes the spores. The volume of air swept can be calculated from the frontal area of the rod, the diameter through which it is turned and the number of revolutions for which it is run. The sampler does not require vacuum system. It is very suitable for field sampling field and number of rods can be carried. The collecting arms of this model are made up of brass having 0.159cm cross sectional area. It is in square shape and slightly bent inwards. The vertical arms are 6cm long and 4cm wide from the axis. According to Gregory (1951), this width should give more than 60-70% efficiency of deposition for 20um diameter spores at wind speed about 4m.p.h. The model employs miniature D.C motor with controlled speed of the type used for recorders. With the rods n proper position, the motor gives about 23,000rpm.

3.1 SAMPLING RATE:

Since the sampler was originally intended for the direct observation of under spores on rods. No mounting was necessary. The use of glycerin, gelatin or petroleum jelly has been recommended now. The Rotorod sampler has also been widely used for a wide variety

of air borne particles. After setting the jelly or Vaseline, the edges of cello tape were trimmed back to the width of rods with sharp razor blade (the alternative would be to apply the transparent cello tape, trim and then coat with adhesive). The cello tape was cut into four equal parts (each of 105cm length) before the application of the adhesive. After exposure to the air, these were mounted beneath a cover glass with a suitable mounting medium like glycerin jelly.

3.2 COLLECTION EFFECIENCY:

The model has been tested for efficiency and it shows 85% efficiency. The sampling efficiency for particles greater than 15µm is 100%. Wind speed has little effect in the efficiency drag and load on the motor. The Rotorod tested by carter (1965) showed 60-90% efficiency. The Rotorod sampler is used for short period sampling up to 2 hours. The sampler is volumetric and highly efficient. The efficiency is not affected at even high wind speed.

MOUNTANT MEDIUM USED AND ITS PREPERATION:

Glycerin jelly was used as the mutant, which has the best optical properties in visual examination. The composition and preparation of this are as follows

Table1: Composition of Glycerine jelly

INGREDIENTS	QUANTITY
Gelatin	40gm
Glycerin	120gm
Distilled water	140ml
Phenol crystals	0.5gm

Measured amount of glycerin and distilled water were mixed in a beaker and heated in a water bath for 2-3 hours. While heating this mixture, gelatin was added slowly by stirring with a glass rod, to avoid clumping. After complete dissolution of gelatin, phenol crystals were added as preservative and metabolic inhibitor, after cooling, it transforms into cake of glycerin jelly. The jelly can be melted by keeping in the hot water bath, whenever required, for mounting the slides permanently.

3.3METHOD OF SAMPLING:

The air sampler experiments were conducted by operating the Rotorod sampler in the patient's house. The Rotorod sampler was kept at variable height of 2-4 feet from the ground level. The transparent cello tape fixed on the two arms coated with white petroleum jelly acts as adhesive, which permits the particles from the air to stick on the surface of the tape. The tape was changed after each sampling. The slides were prepared as described earlier and mounting was done with the help of mounting medium.

SCANNING:

The scanning of slides was done regularly after the preparation of slides. The conversion factor of the sampler is 5. For example, if the total number of fungal spores' types are 20 for the total catch, the n the total number of fungal spores/m^3 of air= 5*20=100/m^3 of air. Assuming the taping efficiency to be 75% with the help of conversion factor, we can easily estimate the fungal spore concentration per meter cube of air. The constant factor is irrespective of locality, season and weather. All the time described in the work is given in Indian Standard Time (IST).

3.4 Composition of the catches and identification:

The deposition on the arm of the cellophane tape exposed varied from humus, dust particles, fungal spores, Hyphal fragments and microscopic plant parts etc.The identification of the fungal spores and other types was based upon the morphological character and visual identification by comparison with reference slides prepared.

4. RESULT:

The outdoor samples were collected from Industrial area of Nacharam.18 samples were collected from here and all the samples were collected in the morning time. Meteorological data was collected on all the days of sampling at this place (Table 1a). Airsamples were collected April through September 2018. Overall concentration of 9115 spores /$m^3$of air was recorded at this site, of which the maximum concentration was observed in the month of September (3775/$m^3$of air), followed by August (1685/m^3) and June (1235/m^3) (Table 1b). The concentration of Alternaria (14.2%) and Cladosporium (9.1%) was huge followed by hyphal fragments, insect scales and plant trichomes (Table 1b

& Fig 1a). The percentage contribution of individual spore types revealed Deuteromycotina (58) to be dominant followed by others (29), Ascomycotina (8) and Basidiomycotina (5) (Fig 1b).

Table-1a: Date and time of sample collection along with weather conditions at **S1**

S.NO	Date of Sample collected	Time of Sample collected	Temperature(C)	Humidity (%)	Rainfall	Sky condition	Air condition
1	20-04-2018	10:00 AM	33	31	No	Clear	Calm
2	24-04-2018	10:20 AM	37	34	No	Clear	Windy
3	14-05-2018	12:20 PM	39	36	No	Clear	Windy
4	19-05-2018	11:30 AM	38	29	No	Clear	Windy
5	22-05-2018	09:33 AM	29	35	No	Clear	Windy
6	26-05-2018	10:01 AM	32	31	No	Clear	Calm
7	19-06-2018	09:10 AM	28	39	No	Clear	Windy
8	22-06-2018	09:35 AM	30	40	No	Clear	Calm
9	26-06-2018	09:06 AM	27	36	No	Clear	Windy
10	30-06-2018	09:15 AM	29	33	No	Clear	Calm
11	05-07-2018	09:05 AM	28	43	No	Clear	Windy
12	08-07-2018	09:15 AM	29	54	No	Clear	Windy
13	16-08-2018	09:20 AM	30	55	No	Cloudy	Windy
14	23-08-2018	09:15 AM	28	61	Yes	Cloudy	Windy
15	28-08-2018	09:30 AM	27	78	Yes	Cloudy	Windy
16	01-09-2018	10:05 AM	30	54	Yes	Cloudy	Windy
17	13-09-2018	9.25AM	29	41	No	Clear	Windy
18	16-09-2018	11.27	36	45	No	Clear	Windy

Figure1A: Graphical representation of fungal spore concentration in **S1** from April to September 2018.

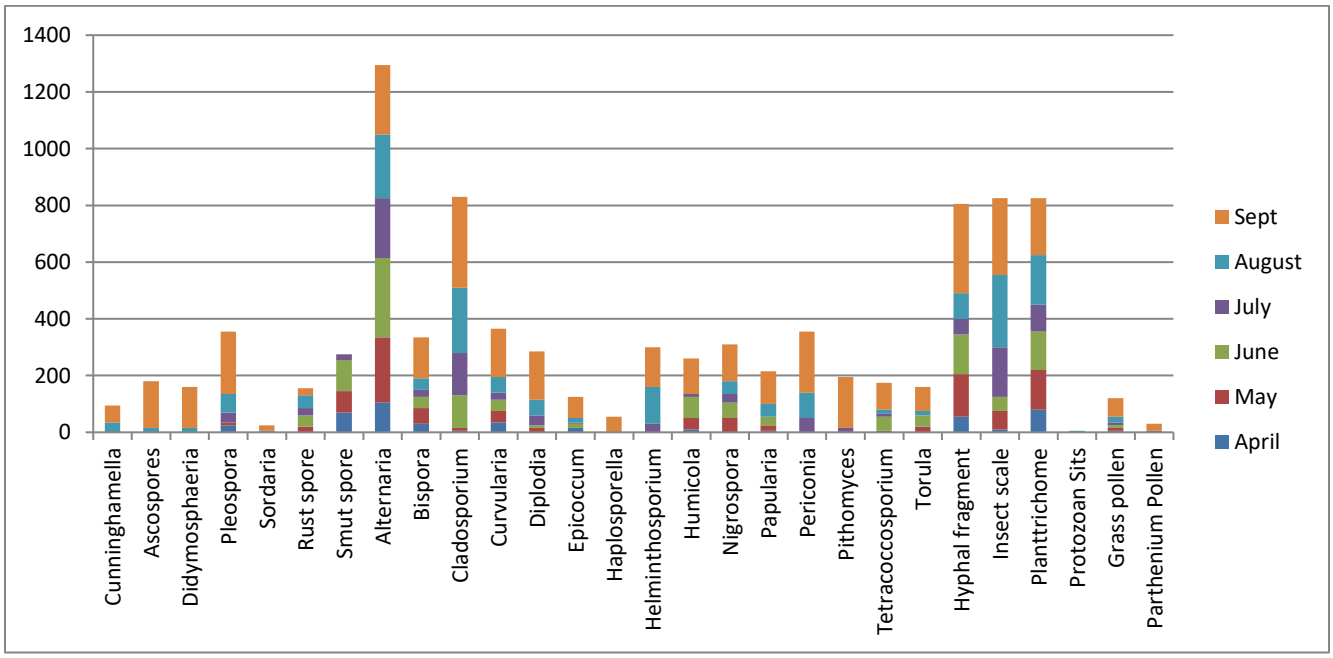
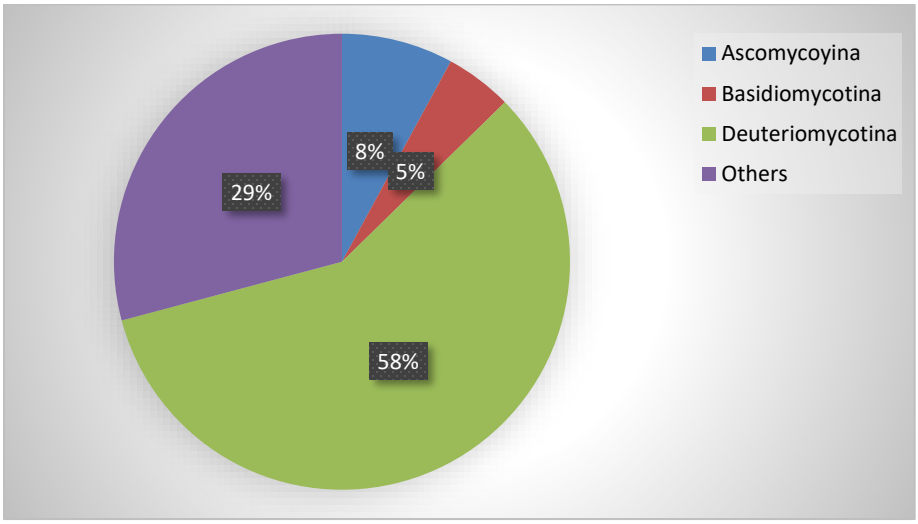


Figure 1B: Percentage contribution of fungal and other groups at **S1** from April to September 2018.



CONCLUSION:

This study revealed that a great variety of fungal spores constitute the airborne fungal spores in the major junctions of Hyderabad. The results revealed that there is huge concentration of Alternaria, Cladosporium and Helminthosporium etc. In the present investigation there is a possibility that the increase in the concentration of bio aerosols might be causing the problem to the people during daytime. Water is usually resulting in continuous wetness of the soil, which might be acting like a support for the growth of fungi in outdoor environment. The fungal spores released by excessively grown fungi may get circulated in environment which might be making the people to get expose to such fungal spores. This might have resulted in skin allergy.

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