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# Aerobiology

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## RESEARCH ARTICLE

**ASSESSMENT OF BIODETERIOGENS OF INTRAMURAL ENVIRONMENT OF KANHERI CAVES****Satnam Singh Sohal<sup>1</sup>, Satish Bhalerao<sup>2</sup> and Hile Vijay<sup>3</sup>**<sup>1</sup>Department of Botany, Maharshi Dayanand College, Mumbai.<sup>2</sup>Department of Botany, Wilson College, Mumbai.<sup>3</sup>Department of Botany, Bhavan's Hazarimal Somani College, Mumbai.**ABSTRACT**

Kanheri caves are a group of rock cut monuments situated at Borivali National park surrounded by deep green forest. Kanheri caves represents Buddhist caves 475 m above sea level. Due to close proximity to urban settlement, these caves are well exposed to anthropogenic activities, pollution and weathering due to biological growth such as fungi, lichens, Algae and Bacteria. The present study focused on assessment of biodeteriogens of intramural Environment of Kanheri caves. The study was carried out for six months from March to Aug 2013. For Petri plate fungal culture Rose Bengal streptomycin media was used. Sampling was carried out once a month. Glycerin jelly coated Slides were exposed every 15 days and observed under the microscope. Fungal spores contributed maximum, Pollen grains and algal hyphae were also encountered during the sampling but not quantified. Miscellaneous bioparticulate matter such as plant parts like trichomes, insect parts were also observed.

**Keywords :** Caves, Fungal spores, Pollen grains, bioparticulate matter

**INTRODUCTION**

Kanheri caves are a group of rock cut monuments situated at Borivali National park surrounded by deep green forest. Kanheri caves represents Buddhist caves 475 m above sea level. Due to close proximity to urban settlement, these caves are well exposed to anthropogenic activities, pollution and weathering due to biological growth such as fungi, lichens, Algae and Bacteria. The airborne particulate matter normally contains a variable percentage of biological particles that may be viable (able to germinate and/or to develop) or not viable. The viable biological particulate is constituted of microorganisms (viruses, bacteria, fungi and their spores), spores of bryophytes and pteridophytes, lichen propagules, algal cells, pollen grains, protozoan cysts.

In the recent years, the aerobiology has a new field of application: the conservation of cultural heritage. Many organisms and microorganisms able to damage works of art (biodeteriogens) are diffused by air, such as bacteria, fungi, algae, lichens, bryophytes and pteridophytes. The spores or vegetative forms of these biodeteriogens can reach the materials surfaces by deposition and contaminate them. If the cells are sufficiently viable, the environmental conditions (climate and microclimate) and the substrate characteristics are favourable; the biological contaminants can develop and colonize the surfaces. Anthropogenic activities and littering inside the caves also contributes to growth of biodeteriogens.

**MATERIAL AND METHODS****A) Gravity slide sampling:**

Glycerine jelly coated micro slides were exposed, by using locally fabricated Durham's spore sampler due to its economy and simplicity, in spite of its limitations. The exposures were done at a height of 2 meters, for duration of twice a month consecutive for six month inside the caves. The Glycerine jelly had the following constituents:

**\*Corresponding Author**Email: [drsatisbhalerao@yahoo.com](mailto:drsatisbhalerao@yahoo.com)

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Glycerine	-150gm
Gelatin	-50gm
Distilled Water	-150ml
Phenol Crystals	-5gm
And a small trace of saffranin	

For the preparation of glycerine jelly, the desired quantity of gelatin was mixed with distilled water taken in a beaker. It was boiled in a water bath. Glycerine was added to this after about 30 minutes and constantly stirred with a glass rod. The boiling was continued for another 1<sup>1/2</sup>hrs, till the mixture became homogenous and translucent without any air bubbles. After removing the beaker from the water bath phenol was added to the mixture and stirred again. Phenol acts as a preservative as well as metabolic inhibitor. A few drops of conc. saffranin were added to the mixture at this stage and the medium was once again mixed thoroughly. The mixture was then poured into sterilized glass vials, covered to avoid contamination, and was stored at room temperature.

For exposure of the slide, a small piece of glycerine jelly was placed over the micro slide and gently heated over the gas flame till it melted. A thin smear of it was made by using another slide drawn over it an angle. Each of these slides after exposure for 24 hrs. was replaced by fresh glycerine jelly coated slide. The exposed slides were placed horizontally in slide boxes and brought to the laboratory for microscopical examination.

A cover slip (18mm X 18mm) was placed over the exposed slide after placing a drop of molten glycerine jelly. The edges of the cover slip were sealed with DPX and after proper labeling the slide was kept horizontally for at least an hour. Scanning of these exposed slides was regularly carried out under high power (10X45) of the Research microscope. A constant quadrature of exposed area of 3.24cm<sup>2</sup> was thoroughly screened for the air-borne micro bio particles.

The pollen grains caught on the exposed slides were identified by morphological features. Reference slides, standard references and illustrations (Nair, 1996) were used for comparative studies leading to their correct identification. Pollen grains were identified on the basis of their size, shape, the type and distribution of apertures and ornamentation pattern

of exine. For the identification of fungal spores, their distinct morphological features were marked out with the help of referenced slides and standard illustrations and reference books (Alexopoulos 1962, Barnett and Hunter 1972, Tilak 1989, 1998).

The number of pollen grains, fungal spores and other biological particles were counted accordingly and the results were calculated and arrived at, to give the number per cm<sup>2</sup> from the constant exposed area of 3.24cm<sup>2</sup> per slide. The month wise average % contribution of individual spore group to the monthly total air spora was tabulated. This method brought out the qualitative analysis of air-borne microbial-components.

### B) Petri plate Culture Method:

Petri plates containing Rose Bengal Streptomycin (RBS) Agar medium were exposed once a month for 10 minutes at a height of 2 meters from ground level at the Indoor environment of caves. The RBS Agar medium consisting of the following ingredients was prepared as follows:

Rose Bengal Dye	-0.05gm
Bacto-Peptone	-2.00gm
Bacto-Agar	-20.00gm
Glucose	-10.00gm
Magnesium Sulphate	-0.50gm
Potassium Dihydrogen Phosphate	-0.50gm
Distilled water	-1000ml

All the above ingredients were mixed in a beaker by adding Distilled water and were boiled in a water bath. It was continuously stirred with a glass rod. Later on it was sterilized by autoclaving at a pressure of 15 lbs. for 20 minutes. Soon after cooling the medium to about 45°C in an incubator, streptomycin sulphate 40 units and crystalline penicillin 20 units were added and stirred under sterile environment. The medium was then poured into 10 cm diameter petridishes, each containing 20 ml medium covered with Petri lid and taped immediately, under aseptic conditions. After cooling and solidifying for about 2 hours, the petri-plates were then stored at room temperature for 3 days. These were then examined for the growth of any contaminants and the selected petri-plates were then taken to the sites for exposure. After exposure for 10 minutes each Petri-plate was immediately covered with lid and taped. These were then taken to the laboratory and incubated at 28°C-





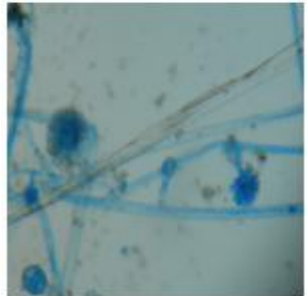
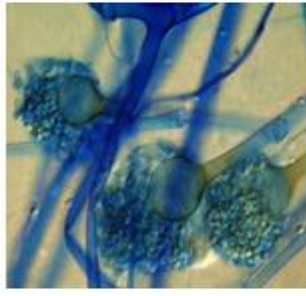
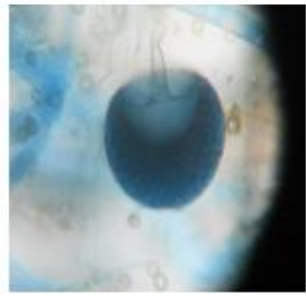

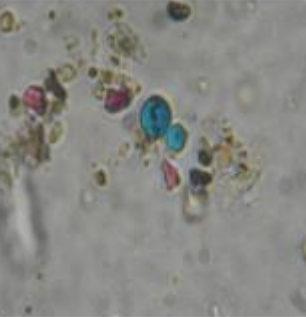


30°C in an inverted position for 7 days. The fungal colonies developed were identified at the generic level from their characteristic branching of conidiophores, morphology of spores and sporulation. These were compared with the reference slides and standard illustrations.

This method had the advantage over the gravity slide sampling in that while the latter method could not identify the small rounded spores to their genera due to similarities in their morphology. With the Petri-plate method these spores germinated to develop into colonies. These colonies showed distinct conidiophores or branching characteristic of the various genera of fungi producing small rounded

spores, along with colonies of other genera having spores of distinct morphological identities.

**RESULTS & DISCUSSION:**

Overall in the present study fungal spores were recorded more in the intramural environment. The common fungal spore trapped during the present study from the intramural environment of the caves includes the following: *Absidia sp.*, *Alternaria alternata*, *Aspergillus flavus*, *Basidiospores*, *Chaetomium globosum*, *Cladosporium sp.*, *Cunninghamella sp.*, *Curvularia sp.*, *Dreschlera sp.*, *Fusarium sp.*, *Penicillium sp.*, *Rhizopus sp.*, *Trichoderma sp.* Pollen grains of grasses were also observed in addition to plant parts, insect parts, Trichomes, etc.

		
<b><i>Alternaria alternata</i></b>	<b><i>Alternaria alternata</i></b>	<b><i>Aspergillus flavus</i></b>
		
<b><i>Absidia sp.</i></b>	<b><i>Rhizopus sp.</i></b>	<b><i>Dreschlera sp.</i></b>
		
<b><i>Grass Pollens</i></b>	<b><i>Lichens</i></b>	<b><i>Insect parts</i></b>



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## RESEARCH ARTICLE

## PREVALENCE OF AIRBORNE FUNGAL DIVERSITY IN THE KITCHEN ENVIRONMENT OF JABALPUR CITY

Verma KS and Koshta R\*

Aeroallergens, Immunology and Angiosperm's Diversity Laboratory  
Rani Durgawati University, Jabalpur (M.P.)-India

### ABSTRACT

The present approach aims to screen out the prevalence of airborne fungal spores of intramural and extramural environments of kitchen in Jabalpur city. The air sampling was conducted using Anderson two stage Sampler (Anderson, 1958;1966) containing SDA plates. Different kitchen environments of Jabalpur city were surveyed every month for a period of one year (January 2012-December 2012). During the study period a total of 33 fungal species belonging to 16 genera were observed. *Aspergillus* species were recorded the maximum (9) followed by 4 species of *Penicillium*. *Aspergillus niger* was found to be the most dominant of all fungal types in both indoor and outdoor environment, followed by *Penicillium chrysogenum*, *Cladosporium* spp. and *Curvularia lunata*. The maximum fungal load was found in the month of March. The more number of fungi were found as a tendency of attraction towards the moisture availability and nutrition present in the kitchen environment.

**Keywords:** Airborne fungi, Kitchen, Jabalpur, Intramural, Extramural.

### INTRODUCTION

Fungi are eukaryotic microorganism that colonizes dead organic material in outdoor and indoor environment. The species that are able to colonize indoor environment can utilize nutritional source available in indoor materials and moisture is the most important factor controlling fungal growth (Burge, 1992). Mold grows at room temperature, so mold constantly thrive and related allergy flourishes in house. The airborne fungal spores will find an appropriate place to live and grow in kitchen because it contains adequate food and moisture (Strachan, 1988 and Platt et al. 1989).

#### \*Corresponding Author

Email: [ruchi.koshta26@gmail.com](mailto:ruchi.koshta26@gmail.com)

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Examination and characterization of fungal distribution in air of a particular region can be helpful in identifying associations between domestic fungal species profiles and clinical diagnosis and prevention of seasonal allergic diseases (Wu et al. 2000).

The main objective of this study is to determine the qualitative and quantitative aspects of indoor and outdoor fungal load of Kitchen environment of Jabalpur and identification and preservation of fungal isolates and also to determine the relationship between indoor and outdoor aerofungi of study area.

### MATERIAL AND METHODS

Air sampling was carried out fortnightly using Anderson two stage sampler (Anderson, 1958) during the period from January 2012 to December 2012. Present study was conducted for the qualitative and quantitative evaluation of different fungal colonies in indoor and outdoor environment of selected residential sites. Sample were collected from different



residential areas and situated in different locations and environments of Jabalpur (M.P.). Air sample were collected from indoor kitchen area and outdoor environment of the house. Sabourands Dextrose Agar Medium (Chowadhry, 2000) was used for isolation of fungi. The culture plates were incubated in inverted position at 28°C for 3 to 5 days depending upon the growth of colonies. Colonies were counted and identified. The total number of colony forming units (CFU) per plates was calculated. The pure culture was maintained at 4 °C and identified with help of standard literature (Clements and Shear, 1954; Barnett 1960; Ellis, 1971; Subramaniam, 1971; Dennis and Cramer, 1978; Tilak, 1989 and Schubert et al. 2007).

The slide culture technique (Johnson, 1946) is used to observe morphological characteristics of molds without disturbing the arrangement of spores and conidial ontogeny over a period of time in a given area of the preparation.

**RESULT AND DISCUSSION**

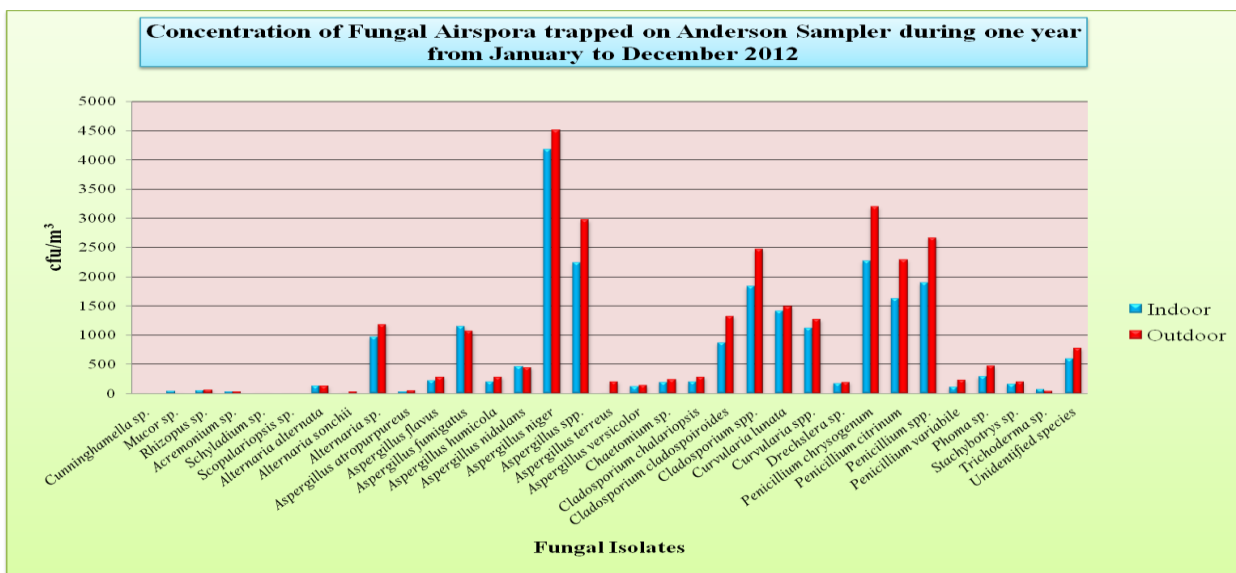
Fungi are found worldwide and reproduce rapidly. The distribution of aeroallergens changes from

country to country and even within regions of the same country. Indoor fungi are a mixture of those which have entered from outdoors and those which readily grow and multiply indoors. *Aspergillus* and *Penicillium* are less common outdoors and are usually considered the major indoor fungi (Burge, 1985 and Licorish et al. 1985).

Great concern has been expressed about potential health hazards to humans, with a special focus on allergenic or toxigenic fungi and their association with air quality (Horner et al. 1995). As a result of these investigation altogether 33 types of fungal cultures were isolated using Anderson two stage sampler belonging to 6 different genera, out of 6, 3 belonging to Zygomycotina, and 3 belonging to Ascomycotina, 27 belonging to Deuteromycotina. Verma et al. (2009) also found 20 different type of fungal spores during the survey of kitchen environment from which 3 type of fungal isolates were identified in Zygomycetes, 3 from Ascomycetes, and 14 in Deuteromycetes. During the study period a total of 33 fungal species belonging to 16 genera were observed. *Aspergillus* species were recorded the maximum (9) followed by 4 species of *Penicillium*.

**Refer Appendix -1. For Table 1: Concentration of fungal airspora of different Kitchen of Jabalpur (Jan. to Dec. 2012)**

**Fig. 1:** Concentration of Fungal Airspora trapped on Anderson Sampler during one year from January to December 2012



Fairs et al. (2010) also found that *Aspergillus* /*Penicillium*-type (Asp/Pen-type) spores were common indoors and exceeded outdoor levels, with the highest concentrations detected in properties over 90 years old (P=.006) and terraced properties (P=.003).

In the present study *Aspergillusniger* was found to be most dominant in all fungal forms and occurred with 16.829%, followed by *Penicilliumchrysogenum* 10.599%, *Cladosporium* spp. 8.35%, and *Curvularialunata* 5.635% was prevalent in both indoor and outdoor environment. Similarly in another study, the genus *Aspergillus* showed the highest concentration in winter and another peak concentration in summer (Su et al. 2001) and Mushtaq et al. (2011) also found that most frequently isolated fungal genera were *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium* in study area.

During this study highest numbers of fungal colonies were recorded in month of March 2012. This result suggested that month of March is suitable for growth of these fungal spores in both indoor and outdoor environment. It has also been reported by Ebner et al. (1989) that *Aspergillus* concentrations also increased substantially in March and August.

## CONCLUSION

This study concludes that there is rich fungal biodiversity in the environment. Indoor air quality is essential for indoor survival as majority time spent indoors. In this present studies it was found that fungal level of kitchen can vary to large extent, due to both environmental and anthropogenic reasons. Monthly variation also recorded due to slight differences in meteorological parameters in different months. It will be helpful in establishing correlation between fungal allergen in air and kitchen environment, thus achieving effective management of allergic disorder. Lifestyle differences and indoor allergen exposure increases, due to high temperature existing indoor and humidity have been suggested to be the potential cause of fungal allergy. Thus increasing the ventilation rate by means of mechanical or natural system can play a key role in improving the kitchen air quality.

## ACKNOWLEDGEMENT

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## RESEARCH ARTICLE

**AEROMYCOLOGICAL SURVEY IN DAIRY FARM NEAR BHEDAGHAT,  
JABALPUR****Verma Karuna S and Sahu Manju Lata**

Aeroallergens, Immunology and Angiosperm Diversity Lab, R.D. University, Jabalpur-01 (M.P.) India.

**ABSTRACT**

Aeromycoflora of indoor and outdoor environments of a Dairy farm, Bhedaghat at Jabalpur were studied by Anderson Two Stage Air Sampler (Anderson 1958; 1966) for one year, from January – December, 2012. The indoor air showed higher number of spores than the outdoor air. Out of total 2080.2CFU/m<sup>3</sup> fungal colonies were recorded the incidence of spores was significantly higher in indoor air (1370.7 CFU/m<sup>3</sup>) than outdoor air (709.5 CFU/m<sup>3</sup>). Out of the total fungal counts, 4 spore types belonged to *Phycomycotina*, 4 spore types belonged to *Ascomycotina* and 25 spore types to *Deuteromycotina*. *Aspergillus* was the most dominant spore type with 26.90% of occurrence in the indoor and 22.26% of occurrence in the outdoor air. *Cladosporium* was the most dominant spore type with 44.92% of occurrence in the indoor and 16.23% of occurrence in the outdoor air. Other dominant fungal spore types present in air were *Penicillium*, *Curvularia*, *Fusarium*, *Rhizopus* and *Mucor*. Fungal colonies were recorded throughout the year but highest in the month of March. Aeromycological survey showed that dairy workers were exposed to large quantities of fungal spores in their working environments, which is a potential risk factor as causative agent to different types of health problems.

**Keywords :** Aeromycological, aeromycoflora, spores, indoor air, outdoor air, dairy Farm

**INTRODUCTION**

In India a large number of people are occupationally involved with different types of cattle sheds. In these sheds, a wide range of fungal growth substrates like moldy livestock foods, moldy hay, bedding of animals and their excreta are present, which could provide a huge airborne fungal spores load making these places unhygienic for the workers. Consistently more respiratory symptoms and impaired levels of respiratory function among the dairy farmers were reported by many researchers (Dalphin et. al., 1998 a,b; Wasteel et. al, 2000).

The Indian cowsheds are generally places with high humidity where raw and decomposing cow-dung, straw, livestock foods and other materials provide suitable substrates for the growth of fungi (Adhikari et al., 1999). Fungal spores are universal atmospheric components both indoors and outdoors although their number and types vary with time of day, season, geographical location and local spore source which are variable. Fungi from a wide variety of genera have a great capacity to colonize much kind of substrates and develop in extreme environmental conditions (Comtois, 1990). Many fungi reported from air were potential to create health hazard to both humans and animals (Burr et al., 2007).

Dairy workers are very close to the dairy environment they may suffer from some allergic disorder or disease. A large no. of people work in cattle shed around the world, pulmonary function and higher frequency of respiratory symptoms have been

**\*Corresponding Author**Email: [karunaverma@gmail.com](mailto:karunaverma@gmail.com)

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reported in dairy farmers (Adhikari et al., 2004). Higher frequencies of air borne fungal spores were recorded by many workers from occupational environments (Vittal and Glory 1985; Lacey and Crook 1988 and Singh and Singh, 1996) including a few studies from dairy barns (Hanhela et al., 1995 and Kullman et al. 1998).

## MATERIAL AND METHODS

The present study was carried out in indoor and outdoor of a Dairy cattle shed in Bhedaghat, Jabalpur during January- December 2012. Aeromycoflora was monitored from indoor and outdoor of dairy cattle shed via Anderson Two Stage air (Andersen, 1958; 1966) sampler fortnightly over the year January-December 2012. Samples for fungi were collected using SDA (Sabourauds Dextrose Agar) medium with streptomycin. The Petri plates were exposed for 10 minutes at 1.5m height above the ground level. After the exposure plates were incubated at 25 + °C for 3-5 days. After exposure fungal colonies were counted for individual species and CFU/m<sup>3</sup> (colony forming unit per cubic meter m<sup>3</sup>). Microscopic slides stained with lactophenol and cotton blue were prepared from each CFU and observed microscopically.

## RESULTS & DISCUSSION:

The aeromycological survey indicates the concentration and variation of fungi prevailing at indoor and outdoor of cattle shed of dairy farm. The present study revealed that total 31 fungal species belonging to 3 genera, 4 spore types belonged to phycomycotina, 2 spore types to ascomycotina and 25 spore types to deuteromycotina were isolated in indoor and outdoor air of cattle shed.

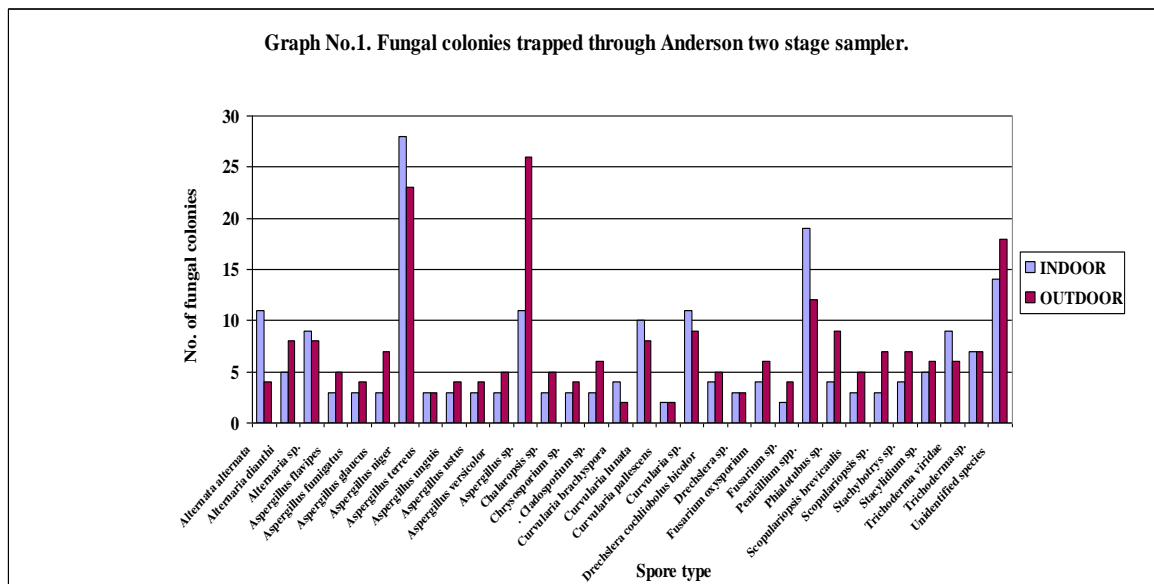
The total concentration fungal spores isolated in indoor air are 1370.7 CFU/m<sup>3</sup> while it was 709.5 CFU/m<sup>3</sup> in outdoor air. The dominant fungi were isolated from indoor *Aspergillusniger* (26.90%), *Penicilliumnotatum* (17.09%), *Cladosporiumherbarum* (16.23%), *Aspergillusfumigatus* (9.75%), *Rhizopusstolonifer* (6.94%), *Fusariumsolani* (6.72%), *Curvularialunata* (6.65%) and *Trichodermaviridae* (5.81%) followed by other species are given in table-1.a

**Table-1:** List of viable fungal spores in indoor and outdoor of dairy farm

S.No.	Fungal isolates	Total		Total %
		Indoor	Outdoor	
	<b>Zygomycotina</b>			
1	<i>Mucor Sp.</i>	6	6	9.20
2	<i>Mucormucedo</i>	8	3	6.91
3	<i>Rhizopusnigricans</i>	8	5	8.92
4	<i>Rhizopusstolonifer</i>	13	4	10.67
	<b>Ascomycotina</b>			
5	<i>Candida sp.</i>	4	2	3.95
6	<i>Chetomium sp.</i>	6	3	5.81
	<b>Deuteromycotina</b>			
7	<i>Alternaria sp.</i>	10	3	8.20
8	<i>Alternariaalternata</i>	12	4	10.30
9	<i>Alternariacitri</i>	5	3	5.27
10	<i>Alternariasolani</i>	12	3	9.47
11	<i>Aspergillus sp.</i>	15	5	12.96
12	<i>Aspergillusflavus</i>	11	7	12.96
13	<i>Aspergillusfumigatus</i>	19	11	20.28
14	<i>Aspergillusnidulans</i>	7	1	4.33
15	<i>Aspergillusniger</i>	53	23	49.16
16	<i>Aspergillusterrus</i>	10	2	7.17
17	<i>Aspergillusustus</i>	9	1	5.45
18	<i>Aspergillusversicolor</i>	9	8	12.69
19	<i>Cladosporium sp.</i>	10	5	10.66
20	<i>C.cladosporoides</i>	6	12	14.74
21	<i>C.herbarum</i>	31	46	61.15
22	<i>Curvularia spp.</i>	7		3.95
23	<i>Curvularialunata</i>	13	5	11.96
24	<i>Dreschlera</i>	6	3	6.08
25	<i>Fusariumsolani</i>	14	5	11.64
26	<i>Nigrospora</i>	6	1	3.86
27	<i>Penicilliumnotatum</i>	32	8	25.20
28	<i>P.rysogenum</i>	12	9	14.55
29	<i>Phoma</i>	9	4	8.35
30	<i>Trichodermaviridae</i>	11	2	7.93
31	<i>Trichoderma sp.</i>	9	2	6.67
32	<b>Unknown Spores</b>	8	5	9.27
	<b>Grand Total</b>	<b>391</b>	<b>201</b>	<b>399.36</b>

The outdoor air sampling of dairy farm, showed dominant fungal spores is *Cladosporiumherbarum* (44.92%), *Aspergillusniger* (22.26%), *Aspergillus fumigatus* (10.535), *Cladosporiumclado- sporoids* (11.85%), *Aspergillusflavus* (7.17%), *Mucormucedo* (6.11%), *Curvularialunata* (5.31%) and *Fusariumsolani* (4.92%). Few fungal spore types remained unidentified and placed in the group of "Unknown spores".





In the present investigation *Aspergillusniger* (26.90%) and *Penicilliumnotatum* (17.09) showed highest contribution in indoor air of dairy cattle shed (Verma, 1998; Adhikari, 2004 and Reddi, 2004) while *Cladosporium* most abundant in outdoor (Reddi, 2004). Other researchers (Lugauskas, 2004; Karwowska, 2005; Abd-Elall, 2009 and Matkovic, 2009) reported the dominance of *Aspergillus* and *Penicillium* in indoor of cattle houses but higher concentration of *Phoma* uniquely observed in cattle shed. *Phoma* spores can be recommended for the primary skin prick testing in dairy farmers of this area while evaluating the mold allergen sensitivity.

In the present study it is seen that *Aspergillusniger* and *A. fumigatus* are distributed throughout the year. This is seemed to be the serious condition where these two organisms are the principal etiological agent of invasive aspergillosis (Tome et. al., 2000 and Pini et. al., 20004). Aspergillosis has shown to bring about the mycosis and other allergic disease in cattle where infection was primarily respiratory spreading to the lungs (Shreeramulu, 1961). The mycotoxins released from *Aspergillus sp.* are considered as established biological occupational carcinogenic capable of causing liver and lung cancer. *A. fumigatus* is also a great hazard to animals and its toxin cause respiratory disease in animals (Zhang et. al., 1997).

The Deuteromycotina was most abundant in indoor than the outdoor air; Zygomycotina forming next abundant group in indoor air than outdoor air. The Ascomycotina was much less in Dairy. Dairy indoor environment showed a fairly large number of forms some of which are allergenic because fungi exist as saprophytes on specialized substrate such as Keratinized animal tissue and dung.

This study also provided the information regarding the density and monthly distribution of indoor and outdoor aero fungi in cattle shed. During these twelve months Jan-Dec. 2012, highest number of fungal colonies were recorded in March month i.e. 335.35 CFU/m<sup>3</sup> and lowest number of fungal colonies in June month i.e. 81.19 CFU/m<sup>3</sup> data showing climatic condition of March month is more suitable for growth of these fungal spores.

**CONCLUSION:**

In view of present result and discussion, it is concluded that indoor airspora in Dairy farms is rich in percentage contribution than the outdoor airspora. Wet and humid environment provide suitable condition for growth of fungi consequently increasing the airborne spores load. As above mention that cattle houses are considered occupational environments with high levels of exposure to fungi. Activities in these indoor places such as cleaning and feeding animals increase occupational risk of exposure to



airborne microorganisms. Although atmospheric sampling and preliminary data suggests that fungal spores causing some allergenic disease so it is necessary to aware people from such allergies so identification of airborne fungal pathogens and their effects on Dairy workers and animals health will thus prevent and reduce the number of disease. Such investigation have brought out utility of inter disciplinary approach of Aeromycology.

### Acknowledgement

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## RESEARCH ARTICLE

**ISOLATION AND IDENTIFICATION OF ASPERGILLUS SPS. FROM REGULARLY USED CONDIMENTS AND SPICES****Patil Jasmin J and Ansari Samreen**

Botany research lab, C.H.M College, Ulhasnagar

**ABSTRACT**

The mycoflora associated with spices not only deteriorate the quality of spices and condiments but also increase the chance of consuming toxic elements through harmful fungi like Aspergilli. *Aspergillus* species associated with 12 different spices such as Clove, Turmeric, Cardamom, Fennel, Bishop's weed, Cumin seeds, Coriander seeds, Cinnamon, Sesame seeds, Mustard seed, Black pepper and Capsicum were studied by using Petridish method, following Direct Plate Method as suggested by International Standard for Seed Testing (ISTA). In the present study, it was found that all the spices and condiments are heavily infected by fungi. Numbers of *Aspergillus* species were recorded along with *Alternaria*, *Penicillium*, *Fusarium*, *Curvularia*, *Rhizopus*, *Mucor*, *Cladosporium* from the sample of 12 spices during the period of investigation. *Aspergillus flavus* (17.74%), *Aspergillus niger* (17.12%), *Aspergillus versicolor* (14.51%), *Aspergillus fumigatus* (11.29%) were found associated in very high concentration as major contaminants in the spice samples. Total eleven species of *Aspergillus* were found associated with different spices. Two species were associated with Mustard seeds and Clove; three species were associated with *Curcuma*, four with Cardamom and Black pepper, five with Cumin, sesame seeds and with Bishop's weed, six with Cinnamon and Fennel, seven with Coriander seeds. Capsicum showed highest fungal infestation with nine different species of *Aspergillus*.

**Keywords :** *Cladosporium*, Spices, Fungi, *Aspergillus*, Infestations, etc.

**INTRODUCTION**

Spices and herbs are valued for their distinctive flavors, colors and aromas and are among the most versatile and widely used ingredient in food preparation and processing throughout the world (Ayres et al., 1980). They are widely used as raw materials for pharmaceutical preparations (Galenic products) and as a supplement for dietetic products, especially for "self medications" in public (Weiser et al., 1971). Spices themselves have little or no nutritional value as mere collections of carbohydrates, fats and proteins of potential interest; it is the fact that spices contain no calories. Most species owe much of their flavoring properties to volatile oils, but in some cases the flavor is due to fixed oil. These include the

alcohols, esters, terpenes, phenols and their derivatives, organic acids, alkaloids and resins.

Since spices harbor many kinds of organisms, including those associated with food spoilage. Depending on chemical nature, different organic substances harbor different microorganisms. These may spoil the quality of the substrate by discoloration, formation of foul odour, change of chemical makeup or production of toxic substances.

Spices are consumed daily in India and hence microbiological examination of spices is important from the point of view of their quality which can be affected by contaminating microorganisms during storage. Contamination of various toxigenic moulds in spices is also known, but little data is available on spices from central India. Hence it was thought necessary to study the seed mycoflora especially of *Aspergillus* species from the spices. The present investigation deals with the *Aspergillus* species associated with twelve different spices such as Clove, *Curcuma*, Cardamom, fennel, Cumin seeds, Coriander

**\*Corresponding Author**Email: [jasmin131185@yahoo.com](mailto:jasmin131185@yahoo.com)

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seeds, Cinnamom, Sesame seeds, Mustard seeds, Black pepper, Bishop's weed and Capsicum.

Field fungi capable of attacking and infecting the growing product may cause varying degrees of decomposition and damage. The damage from invading fungi and molds may be manifested as leaf spot diseases, dry rot, decomposed and discolored tissue of stems and roots, or decay in seeds and fruits. Storage fungi (which can grow under limited moisture conditions) may cause moldiness in some products stored under conditions of temperature and relative humidity favorable to their growth.

Pockets of moist product can arise in a dried and otherwise normal product through roof leaks, insect activity, and moisture translocation when temperature gradients develop within the product mass. These pockets can promote the rapid growth of molds in the stored product. Moldiness can range in appearance from mycelium-matted leafy spices and surface mold on cassia bark, to internal molds in nutmegs and capsicum pods.

## MATERIAL AND METHODS

Different samples of the spices were collected in the sterile polythene bags from two different kitchens of different localities in the Mumbra city. The samples were brought to the laboratory for further study. Measured quantity of samples i.e. five grams were plated on sterilized Malt extract medium and Rose

Bengal Streptomycin media in Petri dishes. The media used were sterilized by autoclaving at 120°C for 20 minutes and also following Direct Plate Method as suggested by ISTA (1966). Measured amount of each samples of the same quantity were placed on moist sterilized blotting paper in pertridish. After plating the samples the plates were incubated at room temperature and observed regularly after 3 days. The fungal growth appearing over the surface of seeds or on the media adjacent to plated seeds was picked up and transferred to the fresh culture media for pure isolates. The fungi were examined under microscope by preparing the slides in lacto phenol cotton blue mounting media. The fungal colonies were identified using standard methodologies. *Aspergillus* species were identified with the help of standard published literature.

## RESULTS & DISCUSSION:

During the present survey, a total of 11 *Aspergillus* species were recorded along with *Alternaria*, *Penicillium*, *Fusarium*, *Curvularia*, *Rhizopus*, *Mucor* and *Chaetomium* from the samples of all the spices studied. *Aspergillus* spp. was found to be associated with all the spices in all the months and every time during the period of investigations. *Aspergillus flavus*, *A.fumigatus* and *A.niger* were found associated in very high concentration with every spice in every season of the year.

**Table 1.** Seasonal distribution of *Aspergillus* species associated with different spices

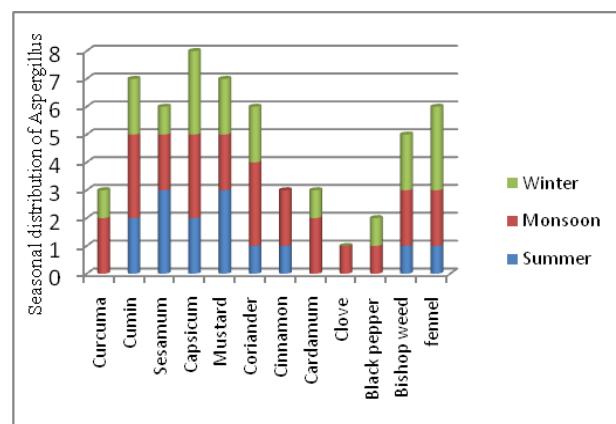
Names of spices	Names of spices	Summer	Monsoon	winter
(Botanical name)	(Common name)			
<i>Curcuma longa</i>	Curcuma (Haldi)	-	++	+
<i>Cumin cyminum</i>	Cumin seeds (Zira)	++	+++	++
<i>Sesamum indicum</i>	Sesame seeds (Til)	+++	++	+
<i>Capsicum annum</i>	Capsicum (Mirch)	++	+++	+++
<i>Brassica compestris</i>	Mustard (Sarson)	+++	++	++
<i>Coriandrum sativum</i>	Coriander (Dhania)	+	+++	++
<i>Cinnamonum beylanicum</i>	Cinnamon (Dalchini)	+	++	-
<i>Elettaria cardamomum</i>	Cardamom (Elaichi)	-	++	+
<i>Eugenia caryophyllata</i>	Clove (loung)	-	+	-
<i>Piper nigrum</i>	pepper (Kali Mirch)	-	+	+
<i>Carum opticum</i>	Bishop's weed (Ajwain)	+	++	++
<i>Foeniculum vulgare</i>	Fennel seeds (Sonff)	+	++	+++

(- No infestation of *Aspergillus*, + *Aspergillus* infestation, ++ High infestation, +++ Severe infestation)



**Table 2.** Percent of individual *Aspergillus* species associated with spices

Sr. No.	Names of Fungi	No. of spices	Percentage
1	<i>Aspergillus niger</i>	11	17.74%
2	<i>Aspergillus flavus</i>	11	17.74%
3	<i>Aspergillus fumigates</i>	7	11.29%
4	<i>Aspergillus ochraceus</i>	3	04.83%
5	<i>Aspergillus candidus</i>	6	09.96%
6	<i>Aspergillus tamari</i>	2	03.22%
7	<i>Aspergillus terreus</i>	3	04.83%
8	<i>Aspergillus sydowi</i>	6	09.67%
9	<i>Aspergillus versicolor</i>	9	14.51%
10	<i>Aspergillus repens</i>	2	03.22%
11	<i>Aspergillus solani</i>	2	03.22%

**Fig 1:** % Contribution of individual *Aspergillus* species associated with spices (Site-A: Indoor & Site-B: Outdoor), Fig 2. Seasonal distribution of *Aspergillus* spp. on various spices.

Results are tabulated in Table 1-2 along with graphs Fig1.- % Contribution of individual *Aspergillus* species associated with spices (Site-A: Indoor & Site-B: Outdoor), Fig 2.-Seasonal distribution of *Aspergillus* sp. on various spices. Capsicum showed highest fungal infection among the spices studied. About 15.51% of *Aspergillus* species found associated with this spice. *Aspergillus* species also have been reported to be most frequent occurrence on seeds of some spices and condiments. Other than *Aspergillus* species *Alternaria* and *Penicillium* were found to be associated in high concentration. The rhizome of *Curcuma longa* also showed fungal contamination of about 5.17% of *Aspergillus* species. Three species of *Aspergillus* found to be associated during the present study. Clove showed fungal infestation especially of *Aspergillus* genus about 3.44%. Two species of this genus found to be associated. The fungus showed its appearance

during monsoon season. Four species of *Aspergillus* found to be associated with Cardamom. They found predominant in monsoon and winter seasons. Black pepper showed about 8.62% of fungal contamination of *Aspergillus*. About 5 spp. were found to be associated. The moisture content also affects the presence of fungal spores in spices. Sesame seeds showed fungal infestation in winter, summer as well as in monsoon i.e. fungal contamination present throughout the year of about 8.62% of *Aspergillus* found to be associated with this spice. Mustard seeds showed high concentration of fungal infection, out of which *Aspergillus* genus showed poor degree of contamination of about 3.44%. Bishop's weed also showed 8.62% of fungal infection especially of genus *Aspergillus*, of about seven different spp. of *Aspergillus* found to be associated with this spice.



**CONCLUSION:**

During the study period, it was found that all the spices and condiments are heavily infected by fungus. All the spices showed a heavy contamination of fungi; however, the extent of infestation varied with the spices. Such variation may be assigned to the difference in their physiochemical qualities and chemical content. The fungal infestation of species of *Aspergillus* and *Penicillium* along with spices is of significance since they are known to produce toxic metabolites to cause various changes in seed constituents and reduction in germination power. Although the spices found in India may be pure in the sense that they may not be grossly adulterated with foreign matter, they are far from pure microbiologically. In view of these observations the question of health hazard due to the consumption of these spices deserves careful attention.

**Acknowledgement:**

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## RESEARCH ARTICLE

## AEROBIOLOGICAL ASSESSMENT OF THE INTRAMURAL ENVIRONMENT OF MAHARSHI DAYANAND COLLEGE LIBRARY

Sohal Satnam Singh<sup>1</sup>, Bhalerao Satish<sup>2\*</sup> and Deshbhratar Shantaj<sup>3</sup>

<sup>1</sup>Department of Botany, MaharshiDayanand College, Mumbai.

<sup>2</sup>Department of Botany, Wilson College, Mumbai.

<sup>3</sup>Zoology Department, Bhavan's Hazarimal Somani College, Mumbai.

### ABSTRACT

Aerobiology deals with the study of the movement and dispersal of living as well as non-living material through the atmosphere. Indoor or intramural environment as of library provides congenial conditions such as low temperature and high humidity as compared to outside environment. The location of Maharshi Dayanand college library is such that it is subjected to heavy air pollution with varied species and quantity of microorganisms. The present study from Nov 2010 to Oct 2011 aimed at qualitative & quantitative analysis of intramural environment of the library. In the intramural environment of library total 19446. Pollen and fungal spore types were recorded of which 966 (4.97%) were pollen grains and 18480 (95.03%) were fungal spores. The percentage of pollen grains recorded in the intramural environment of library is very low. Total 5 species of *Aspergillus* were recorded: i. e, *Aspergillus flavus*, *Aspergillus fumigatus*, *A. nidulans*, *A. niger* and *Aspergillus oryzae*. Throughout the period of study, *Aspergillus sp.* spores were recorded with highest percentage (27.71%) Among the fungal spores, *Cladosporium spores* were recorded the second largest in percentage (26.70%) Among the pollen grains, Grasses recorded the highest percentage of (1.44 %) with a total of 280 pollen grains throughout the year in the intramural environment of the library. The peak season for Grass pollens was from Dec 2010 to June 2011 with maximum in October 2011. Large no of Dust mites were also recorded from carpets, book covers and dust. Miscellaneous type like hyphal filaments, Algal filaments and plant cell fragments were also recorded throughout the year. Meteorological parameters were recorded and fungal growth was correlated with variation in temperature and %age humidity. The fungal growth was maximum during the months of Aug to Oct which showed high humidity in the atmosphere. The concentration of pollen grains was also correlated with wind speed.

**Keywords :** Airborne bioparticles, Intramural Environment, Library, Allergy

### INTRODUCTION

Aerobiology deals with the study of the movement and dispersal of living as well as non-living material through the atmosphere. Indoor environment as of library provides congenial conditions such as low temperature and high humidity as compared to outside environment. The library particularly provides a rich stock of substrate for the microorganisms to

thrive on. The bindery glue, cloth covering in addition to paper support fungal growth. Fungal spores are amongst the most common airborne particles present in air. The library is also subjected to the growth of the fungi which can result in a variety of adverse health effects, which includes infectious diseases like skin irritation, reddening of eyes, respiratory allergies etc. Fungal spores count directly influence the manifestation of allergic symptoms in sensitive individuals.

#### \*Corresponding Author

Email: [drsatishbhalerao@yahoo.com](mailto:drsatishbhalerao@yahoo.com)

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### MATERIAL AND METHODS

#### A) Selection of Site:

Initially a detailed survey of Library atmosphere was undertaken to select the site, keeping in mind the





objectives of the study. This resulted in the selection of site as MaharshiDayanand College Library.

### B) Floristic surveys:

Regular periodic visits were made to the Library, vicinities and surrounding area to study and record the flowering period of the Angiosperm species and collection of fungi samples for culturing. The polliniferous material of these species were brought to the laboratory for preparing reference slides of confirmed pollen types, so as to correctly identify the trapped air-borne pollen types. The reference slides were prepared by using the same type of glycerin jelly as in gravity slide sampling. This made the comparison and identification of trapped pollen grains easier.

### C) Gravity slide sampling:

Glycerine jelly coated micro slides were exposed, by using locally fabricated Durham's spore sampler due to its economy and simplicity, inspite of its limitations. The exposure were done at a height of 2 metres, daily for a duration of 7 consecutive days a month inside the library and surrounding area for two years. The Glycerine jelly had the following constituents:

Glycerine	-150gm
Gelatin	-50gm
Distilled Water	-150ml
Phenol Crystals	-5gm
and a small trace of saffranin	

### D) Petri plate Culture Method:

Petri plates containing Rose Bengal Streptomycin (RBS) Agar medium were exposed once a month for 10 minutes at a height of 2 meters from ground level at the Indoor and outdoor environment of library. Three exposures/trappings were done in a day at 8.00hrs, 12.00hrs and 16.00hrs, once a month for one year.

The RBS Agar medium consisting of the following ingredients was prepared as follows:

Rose Bengal Dye	-00.05gm
Bacto-Peptone	-02.00gm
Bacto-Agar	-20.00gm
Glucose	-10.00gm
Magnesium Sulphate	-00.50gm
Potassium Dihydrogen Phosphate	-00.50gm
Distilled water	-1000ml

### E) Volumetric sampling using Tilak Air sampler:

The standard Tilak Air sampler (Tilak and Kulkarni, 1970) was employed for continuous volumetric sampling of air for 8 days a month for one year, i.e. from 1<sup>st</sup> November 2010 to 31<sup>st</sup> October 2011, at the intramural environment of MaharshiDayanand College library.

### F) Calculations to obtain conversion factor:

Calculated conversion factor for Tilak Air sampler is = 14

The volume of air sampled per minute = 5litre/min

The number of spores, thus scanned, multiplied by conversion factor would give the number of pollen/fungal spores in m<sup>3</sup> of air.

For example, 10 spores X 14=140 spores in m<sup>3</sup> of air.

Thus the data provided in the tables are after using the conversion factor=14.

### RESULTS:

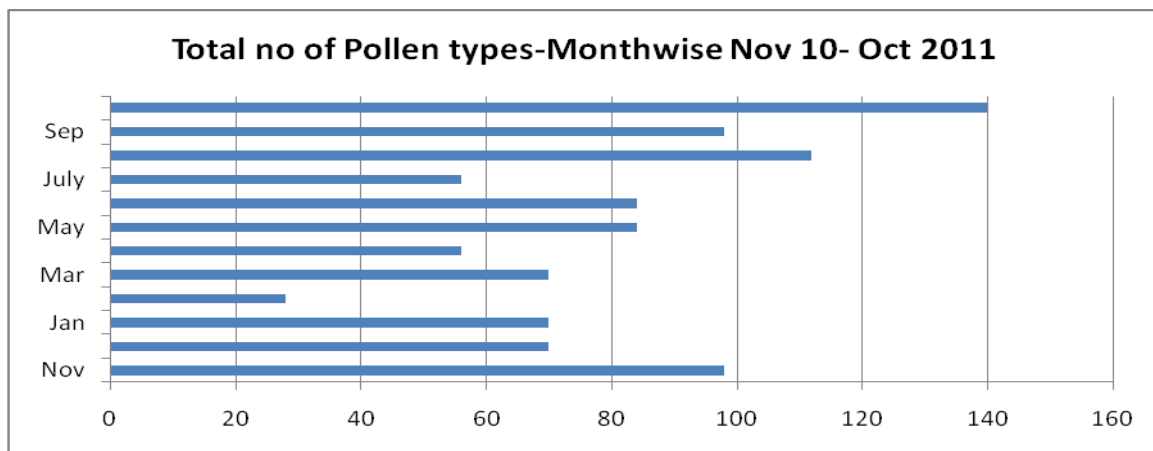
In the intramural environment of library total 19446. Total 20 pollen and 17 fungal spore types were recorded during this study. Pollen and fungal spore types were recorded of which 966 (4.97%) were pollen grains and 18480 (95.03%) were fungal spores. The percentage of pollen grains recorded in the intramural environment of library is very low. Fungal spores were recorded highest during the month of September. Total 5 species of *Aspergillus* were recorded i: e, *Aspergillusflavus*, *Aspergillusfumigatus*, *A.nidulans*, *A.niger* and *Aspergillusoryzae*. Throughout the period of study, *Aspergillus sp.* spores were recorded with highest percentage (27.71%) Among the fungal spores, *Cladosporium spores* were recorded the second largest in percentage (26.70%) Among the pollen grains, Grasses recorded the highest percentage of (1.44 %) with a total of 280 pollen grains throughout the year in the intramural environment of the library. The peak season for Grass pollens was from Dec 2010 to June 2011 with maximum in October 2011. Large no of Dust mites were also recorded from carpets, book covers and dust. Miscellaneous type like hyphal filaments, Algal filaments and plant cell fragments were also recorded throughout the year. Meteorological parameters were recorded and fungal growth was correlated with variation in temperature and %age humidity. The fungal growth was maximum



during the months of Aug to Oct which showed high pollen grains was also correlated with wind speed. humidity in the atmosphere. The concentration of

**Table 1:** Monthly average concentration of air-borne pollen grains and other types trapped by using Tilak Air sampler and their % age Contribution-Indoor Environment

Sr. No.	Pollen type	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Total	%age
1	<i>Acacia auriculiformis</i>						14						28	42	0.216
2	<i>Amaranthus/ Chenopodium type</i>	28	14	14	-	-	-	14	28	28	28	14	14	182	0.936
3	<i>Bougainvillea spectabilis</i>	14	14	-	-	-	-	14	14	-	14	14	-	84	0.432
4	<i>Carica papaya</i>														
5	<i>Cassia siamea</i>												28	28	0.144
6	<i>Clerodendroninerve</i>					14								14	0.72
7	<i>Cocosnucifera</i>														
8	<i>Cyperusrotundus</i>														
9	<i>Delonixregia</i>														
10	Grasses	28	28	28	14	14	28	28	14		14	28	56	280	1.44
11	<i>Hibiscus rosasinensis</i>	14	-	-	-	-	-	-	-	14	14	28	-	70	0.36
12	<i>Lagerstroemia speciosa</i>														
13	<i>Lantana camara</i>														
14	<i>Moringaoleifera</i>														
15	<i>Neriumindicum</i>														
16	<i>Peltophorumpterocarpum</i>														
17	<i>Ricinuscommunis</i>														
18	<i>Samaneasaman</i>														
19	<i>Syzygiumcumini</i>														
20	<i>Tridexprocumbens</i>														
21	Unidentified Pollen	14	14	28	14	42	14	28	28	14	42	14	14	266	1.367
		98	70	70	28	70	56	84	84	56	112	98	140	966	4.97

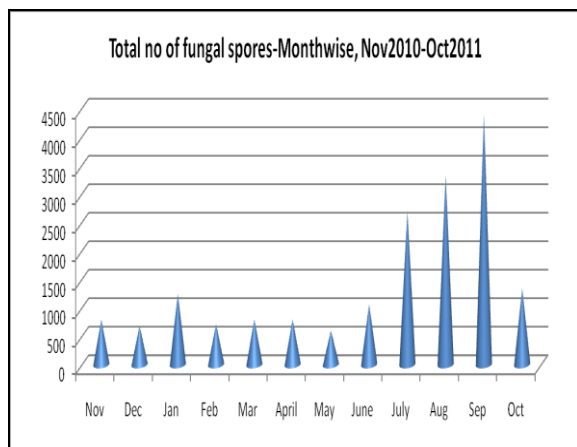


**Fig. 1 :** Total number of pollen types Monthwise (Nov 10 to Oct- 2011)

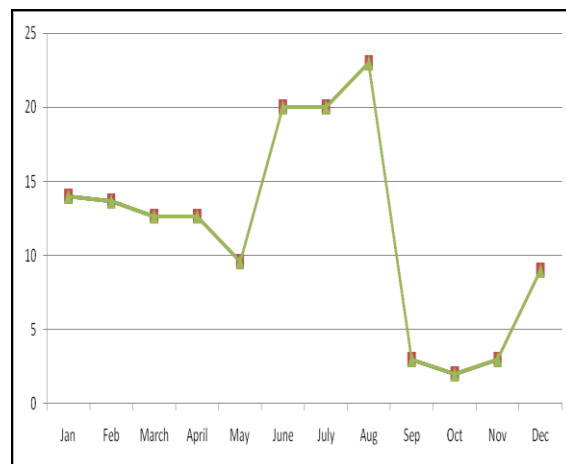


**Table-2:** Monthly average concentration of air-borne fungal spores and other types trapped by using Tilak Air sampler and their % age Contribution-Indoor Environment.

Sr. No.	Fungal spores	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Total	%age
1	<i>Absidia sp.</i>	14	-	-	-	-	-	-	14	42	70	98	14	252	1.295
2	<i>Alternariaal ternata</i>	70	42	70	56	70	154	70	196	294	518	686	126	2352	12.095
3	<i>Aspergillusflavus</i>													0	
4	<i>Aspergillus fumigatus</i>													0	
5	<i>Aspergillusnidulans</i>	224	196	350	336	196	266	196	350	658	1050	1274	294	5390	27.71
6	<i>Aspergillusniger</i>													0	
7	<i>Aspergillusoryzae</i>													0	
8	<i>Basidiospores</i>	-	-	-	-	-	-	-	-	14	28	98	28	168	0.864
9	<i>Chaetomium globosum</i>	70	154	280	70	126	-	-	-	70	56	-	-	826	4.247
10	<i>Cladosporiumsp</i>	308	182	266	98	238	210	196	322	1064	924	1036	350	5194	26.70
11	<i>Cunningha mella sp.</i>	-	14	98	14	-	-	-	-	-	-	-	-	126	0.648
12	<i>Curvularia sp.</i>	28	70	14	-	14	14	14	28	42	56	308	70	658	3.383
13	<i>Dreschlera sp.</i>	14	-	-	-	-	-	-	-	14	14	126	224	392	2.016
14	<i>Fusariumsp</i>	42	-	56	-	-	-	-	-	14	14	42	98	266	1.367
15	<i>Penicillium spp.</i>	-	-	-	-	-	-	-	-	14	28	42	-	84	0.432
16	<i>Rhizopus spp.</i>	-	-	14	56	70	28	56	98	308	378	448	126	1582	8.135
17	<i>Trichoderma sp.</i>	-	-	28	28	42	56	56	28	98	154	168	-	658	3.383
18	Unidentified sp.	28	28	70	56	42	70	14	28	70	42	70	14	532	2.735
		798	686	1246	714	798	798	602	1064	2702	3332	4396	1344	18480	95.03



**Fig. 2:** Total number of fungal spore- month wise Nov. 2010 to Oct. 2011



**Fig. 3:** Petri plate Culture Method: Month wise average total fungal colonies developed on Petri Plates-(Indoor Environment).

**CONCLUSION:**

Airborne Pollen and fungal spores which are allergenic to inhabitants in the intramural environment especially libraries contributes a great

threat. The present study concludes presence of significant concentration of fungal spores generated from old papers, books and air conditioning vents. Significant amount of pollens were also accounted for.



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## RESEARCH ARTICLE

## AEROBIOLOGICAL INVESTIGATIONS OVER JOWAR CROP FIELD AT RAHURI

Gadekar SS

Department of Botany, Nowrosjee Wadia College, Pune -411001

## ABSTRACT

Aerobiological investigations have been carried out at Rahuri for four consecutive seasons over Jowar crop. Tilak volumetric air sampler was used to estimate different aerobiocomponents both qualitatively and quantitatively with respect to meteorological parameters. Analysis of airspora after air sampling revealed 63 spore types including 34 from Deuteromycotina, 18 from Ascomycotina, 4 from Basidiomycotina, 2 from Phycomycotina and 5 other types. Percentage contribution of these spore groups to the total airspora revealed that, Deuteromycotina (60.37%) as a dominant group followed by Ascomycotina (18.96%), Basidiomycotina (16.63%), other types (2.54%) and Phycomycotina (1.5%). Average percentage contribution of each spore type to the total airspora of four consecutive seasons revealed that *Cladosporium* (15.44%) as a dominant type followed by *Curvularia* (7.65%), *Alternaria* (7.32%), Smut spores (6.49%), *Cercospora* (4.94%), *Helminthosporium* (4.13%) etc. Pathogenic spore types like Rust, Smut, *Alternaria*, *Curvularia* and *Helminthosporium* have been observed in sufficiently higher concentrations followed by respective disease incidence. Seasonal variations and diurnal periodicity studies revealed typical rhythm and pattern of spore incidence.

**Keywords :** Aerobiocomponents, Air sampling, diseases, Meteorology.

## INTRODUCTION

The fungal spores present in the atmosphere have been found to be responsible for the causation of various diseases over many important crop plants. Severe diseases result into maximum losses of these crops in terms of quality as well quantity of the crop yield affecting economy of the farmers.

Jowar is the major staple food and fodder crop in Maharashtra and other states of India. It is affected by various airborne diseases. Hence, this topic has been selected for the crop protection. Louis Pasteur (1861) in his germ theory of diseases demonstrated that the air was a carrier of many common germs. Ehrenberg (1872) first published information on the microorganisms collected from the atmospheric dust. These aerobiological investigations carried out in Maharashtra through the school of Aerobiology by Prof. Tilak, who was honoured as father of Indian Aerobiology at Magadh University, Bodha Gaya.

**\*Corresponding Author**Email: [vspvishu118@gmail.com](mailto:vspvishu118@gmail.com)

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## MATERIAL AND METHODS

Material is the atmospheric biocomponents over the environment of Jowar crop field at Rahuri dist. Ahmednagar. Air sampling was carried out using continuous volumetric Tilak air sampler for four consecutive seasons comprising two rabi and two kharif seasons.

Tilak air sampler (1970) was kept at a constant height of 1.5m above the ground level, sampling the air at the rate of 5l /min which deposits the airspora over the cellophane tape, fixed over the drum by impingement process. Cellophane tape loaded airspora have been replaced weekly. It is cut into 16 equal parts and mounted over the clean glass slides in melted glycerine jelly. Slides have been scanned under 45x10x combination of binocular research microscope for qualitative and quantitative estimation of airspora. Data of meteorological parameters have been daily recorded for its relevance on spore incidence.

## RESULTS &amp; DISCUSSION:

Monthwise percentage contribution of each spore group to total airspora for the first and Rabi seasons revealed *Deuteromycotina* as dominant (66 and 69%)



in all the months. (fig. 1a and 1b). While concentration of deuteromycotina is as follows:

1<sup>st</sup> Rabi (N-66, D-66, J-63 and F-50),

2<sup>nd</sup> Rabi (N-66, D-66, J-69 and F-53)

Fig 1a and 1b.

1<sup>st</sup> Kharif (N-63, D-64, J-58 and F-58), 2<sup>nd</sup> Kharif (N-57, D-57, J-54 and F-59) fig 2a and 2b.

Monthwise percentage contribution of each spore group to the total airspora for the first and second Kharifs revealed incidence of high airspora in all the groups may be due to high rainfall, high relative humidity and moderate temperature, as prevalent meteorological factors as compared to Rabi seasons. However first Kharif season revealed higher percentage contribution during September (P4, A21, B15 and D58) and during second Kharif it was recorded unevenly i.e., P in July (1.95), A in August (26.96), B in October (17.79) and D in October (58.75), which may be due to more rainfall in the respective months (fig. 2a and 2b).

Variation in percentage contribution of different spore types during two Kharif and two Rabi seasons revealed *Cladosporium* (20%, 19%, 11% and 11%) as dominant spore type during all the four seasons. And it was more during both the Rabi seasons (20% and 19%) as compared to both Kharif seasons. (11 and 11%) (Table 1). It may be due to variation in the environmental parameters during Kharif and Rabi seasons respectively.

*Alternaria*, *Curvularia*, Basidiospores and *Cladosporium* revealed highest monthwise percentage contribution during August as compared to the other months of the second Kharif season (fig.3). Diurnal periodicity curves of *Curvularia* and *Alternaria* revealed peak points at 14-16 hours representing day spora group (fig 4a & 4b). Month-wise variation in some of the prominent spore types have been recorded during two Rabi seasons and two Kharif seasons.

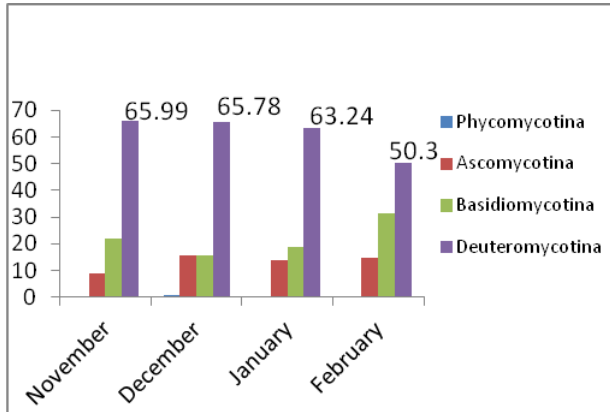
**Table 1:** Variation in percentage contribution of different spore types during 2 Kharif and 2 Rabi seasons.

Sr. No.	Dates	Total No. of spores obtained	Dominant spore type	Lowest spore type
1	1 <sup>st</sup> Rabi (1st Nov to 20th Feb)	46	1. <i>Cladosporium</i> (19.59%)(21644)	1. <i>Trichoconis</i> 0.01%)(14)
			2. Smut spores (12.52%)(13832)	
2	2 <sup>nd</sup> Rabi (6th Nov to 25th Feb)	54	1. <i>Cladosporium</i> (18.79%)(30660)	1. <i>Apiorhynchostoma</i> (0.02%)(28)
			2. <i>Curvularia</i> (8.67%)(14154)	
3	1 <sup>st</sup> Kharif (10 <sup>th</sup> July to 20 <sup>th</sup> Oct)	63	1. <i>Cladosporium</i> (10.59%) (32984)	1. <i>Diplodia</i> (0.14%) (308)
			2. <i>Curvularia</i> (7.26%) (15680)	
4	2 <sup>nd</sup> Kharif (1st July to 10th Oct)	63	1. <i>Cladosporium</i> (10.59%)(23492)	1. <i>Corynespora</i> (0.16%)
			2. <i>Alternaria</i> (6.48%) (14350)	

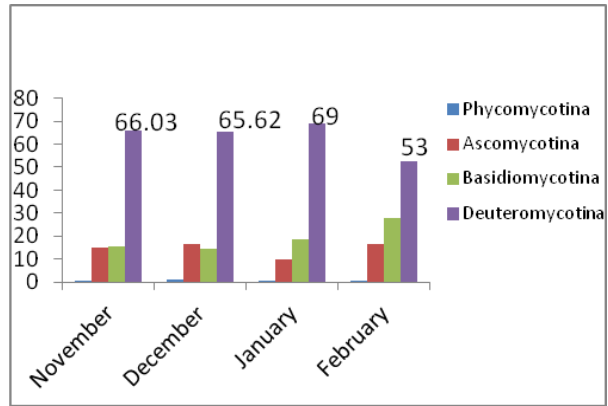
**Table 2:** Seasonal variations of average percentage contribution of each class to the total airspora.

CLASS	R1	R2	K1	K2
Phycomycotina	0.55	0.76	2.3	1.5
Ascomycotina	13.72	14.61	19.7	25
Basidiomycotina	22.54	19.75	15.34	15
Deuteromycotina	63	65	62.15	58

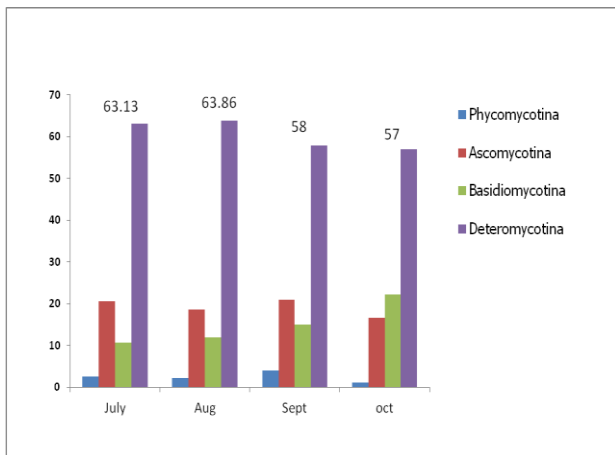




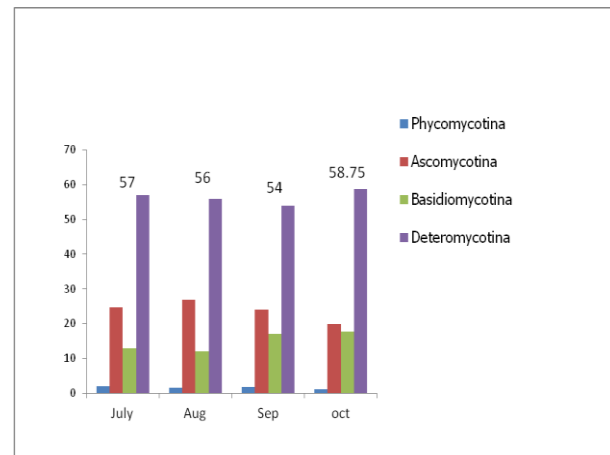
**Fig. 1a.** Monthwise Average percent age contribution of each spore group to the total airspora for the 1st Rabi season.



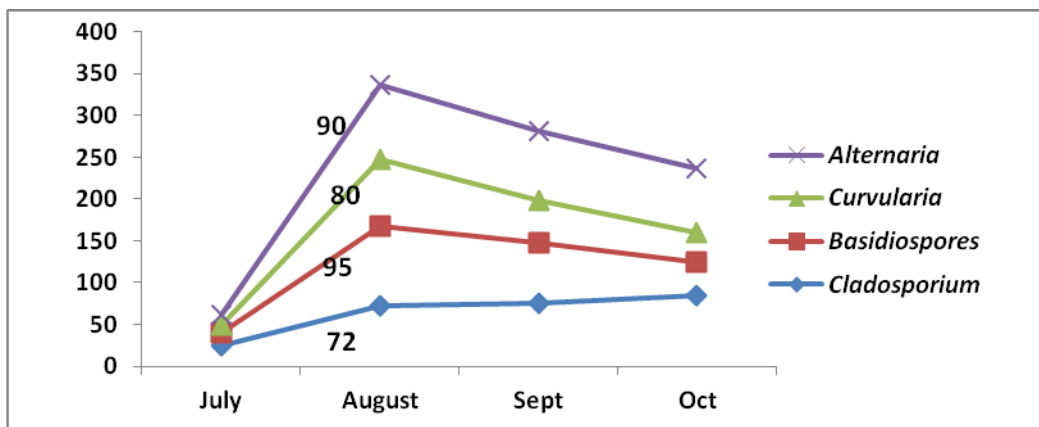
**Fig. 1b.** Monthwise Average percent age contribution of each spore group to the total airspora for the 2nd Rabi season.



**Fig 2a.** Monthwise average percentage contribution of each spore group to the total airspora for the 1st Kharif season

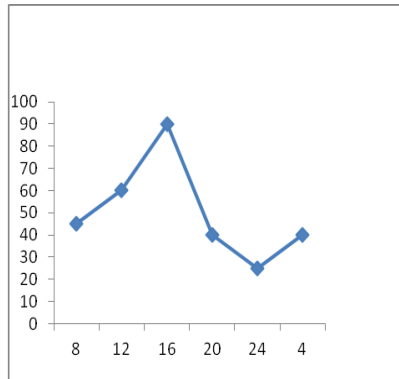


**Fig2b.** Monthwise average percentage contribution of each spore group to the total airspora for the 2nd Kharif season

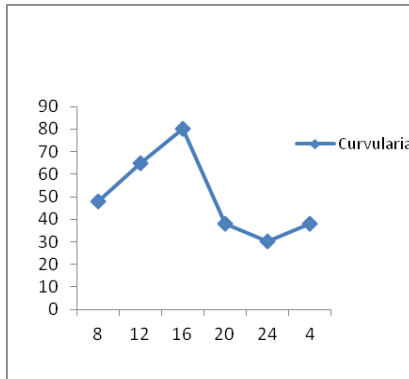


**Fig.3.** Relevance of metrological parameters with the spore load during second kharif. Meterological Relevance to Some Dominant Spore

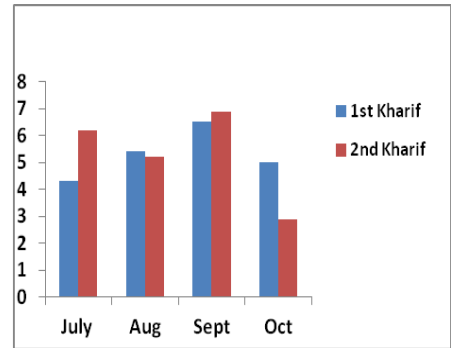




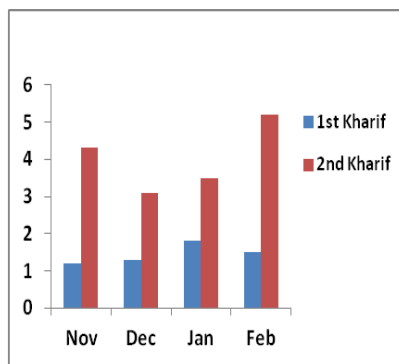
**Fig. 4a.** Dirunal periodicity curve of Alternaria



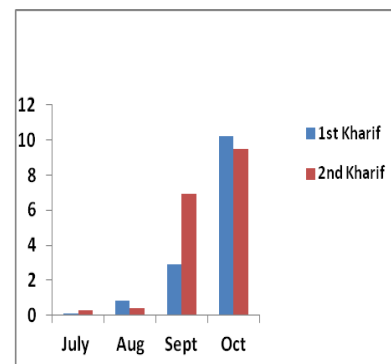
**Fig. 4b.** Dirunal periodicity curve of Curvularia



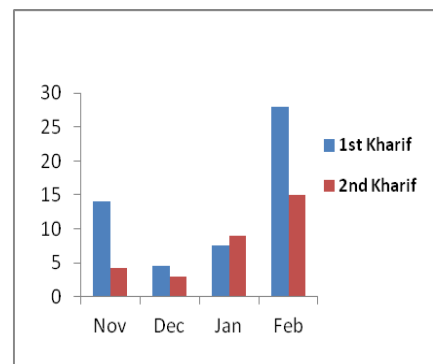
**Fig. 5a.** Monthwise average percentage contribution of Rust to the total airspora (1st & 2nd Kharif)



**Fig. 5b.** Monthwise average percentage contribution of Rust to the total airspora (1st & 2nd Rabi)



**Fig. 6a.** Monthwise average percentage contribution of smut to the total airspora (1st & 2nd Kharif)



**Fig. 6b.** Monthwise average percentage contribution of smut to the total airspora (1st & 2nd Rabi)

Prominent Pathogenic spore types like rust and smuts revealed specific pattern of month-wise distribution during four consecutive seasons i.e. R1, R2, K1 and K2. Rust spores revealed highest average percentage contribution during September of both the kharif seasons (K1 and K2) as compared to other months while during first and second rabi seasons (R1 and R2) average percentage contribution was higher during second rabi as compared to first rabi and was highest during second rabi. (fig.5a and 5b)

Smut spores revealed rhythmic variations and particular pattern of monthwise distribution during four consecutive seasons. It was found highest during both the kharif seasons in the month of October as compared to other months of both the kharifs. It was comparatively lower in both the rabi seasons in all the months. Within the rabi seasons it was found highest during the first rabi season in the month of February as compared to all the months of both the rabi

seasons. However, average percentage contribution of smut spores was scanty during July and August of both the kharif seasons, it slightly increased in the September of both the kharif seasons and highest during October of both the kharif seasons.

When the crop was at emergence and grain formation stage similar was the case during both the rabi seasons executing higher percentage contribution during February after the emergence stage of the crop (fig 6a and 6b).

Seasonal variation of average percentage contribution of each class to the total air spora during four consecutive seasons (R1, R2, K1 and K2) revealed interesting rhythm Deuteromycotina revealed highest percentage contribution in all the four seasons. Phycomycotina and Ascomycotina revealed progressive increase from R1, R2, K1 and K2. While Basidiomycotina and Deuteromycotina revealed





progressive decrease in average percentage contribution from R1, K1 and K2. While R2 revealed highest percentage contribution to all four consecutive seasons. (Table 2)

### CONCLUSION:

Aerobiological investigations over jowar crop field at Rahuri during four consecutive seasons revealed interesting findings in general and pathogenic aerospora and exhibited rhythmic monthly and seasonal variations in relevance to meteorological parameters and growth stages of jowar crop. These studies and extensive data for a long period will help significantly in formulation of useful and appropriate model for disease forecasting system for the protection of jowar crop from airborne diseases in our country.

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## RESEARCH ARTICLE

## ASSESSMENT OF AIR MICROFLORA IN SELECTED GARDENS IN DAHISAR

Lad Devdatta and Shetti Paramjeet

Department of Zoology, Wilson College, Chowpatty, Mumbai 400 007

## ABSTRACT

The present investigation aims at assessment of the microflora present in the air of some selected gardens in Dahisar. The assessment of microflora of all the four selected sites was carried out using the Koch sedimentation method. The study revealed that the site 1 i.e. Riddhi Siddhi garden had 3913.41 cfu/m<sup>3</sup> microbial load at the morning time which reduces to 1410.23 cfu/m<sup>3</sup> in the evening. Similarly site 2 i.e. BMC garden has 21611.90 cfu/m<sup>3</sup> microbial load which is the highest in the present research study observed during the morning time and it too reduced to 6416.58 cfu/m<sup>3</sup>. At site 3 and 4 the same observations have been noted where at site 3 i.e. Ever United garden, the morning microbial load was 10435.76 cfu/m<sup>3</sup> which then reduced to 3149.50 cfu/m<sup>3</sup> in the evening. Devyani garden which is the 4<sup>th</sup> site has shown the microbial load reduction by almost 5 fold, as the morning readings were 12184.45 cfu/m<sup>3</sup> and the evening readings noted were 2725.99 cfu/m<sup>3</sup>. This study reveals that it is necessary to supervise the air quality of all the four sites periodically and take appropriate measures that would protect it from further excessive microbial pollution in the future..

**Keywords :** Air Microflora, Dahisar Gardens, Koch sedimentation method, microbial pollution

## INTRODUCTION

Air contains various biological elements such as seeds, mould spores, yeast, bacteria, viruses, insect eggs, small worms and protozoan cysts. (Krzysztofik, *et al.* 1997) Air basically serves as a temporary habitat for a variety of microorganisms. Microorganisms drift by attaching to dust particles of mineral and organic origin, by attaching to vegetal and animal remains, or by getting immersed in water, such substances that carry these organisms are termed as bioaerosols. (Krzysztofik, 1992) Bioaerosols consist of all airborne particles of biological origin, i.e. bacteria, fungi, fungal spores, viruses, and pollen, and their fragments, including various antigens. Particle sizes of bio-aerosol range from aerodynamic diameters of 0.5 to 100 µm. The number of microbes in the air varies according to atmospheric conditions. Nevalainen *et al.* (1991); Cox *et al.* (1995); Grinshpun *et al.* (2005).

The surrounding air is the best dispersal medium for pathogenic bacteria and viruses which may cause numerous diseases of the respiratory system (Bugajny *et al.* (2005)). Mould fungi present in the air can cause numerous mycoses, allergies and toxic reactions in humans. Since the number of respiratory allergies is on rise due to the microflora in atmospheric air, the regular study and monitoring of the outdoor air microflora is becoming of utmost importance. (D'Amato *et al.* (2000).

The present research study aims on assessment of the microflora present in the air of some selected gardens in the Dahisar. Dahisar is the last suburban area of the northern part of Mumbai city. It is one of the main areas of the Mumbai city with many upcoming residential buildings. The Dahisar area contains many small and big gardens which are frequently visited by the large number of people from the nearby residential areas in the morning and evening for the purpose of recreation.

People visiting these gardens are frequently exposed to the microflora present in the garden and so the microflora assessment and testing is being intended through the present research study.

**\*Corresponding Author**Email: [devdatta.lad@gmail.com](mailto:devdatta.lad@gmail.com)

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## MATERIAL AND METHODS

**Study Area:** Dahisar is the last suburban region of the Mumbai city. It is subjected to extreme anthropological activities as the area is an upcoming hub of various big and small residential complexes. The region also contains many small and big gardens which are visited by the people on a regular basis. The study area consists of 4 different gardens situated in the Dahisar region. The first being the Riddhi Siddhi garden (Site 1), second the BMC garden (Site 2), third the Ever United garden (Site 3) and the fourth Devyani garden (Site 4).

**Assessment:** The assessment of microflora of all the four selected sites was carried out using the Koch sedimentation method. According to the Koch sedimentation method, open Petri dishes containing Nutrient Brothagar (Himedia) medium were exposed at all the four selected gardens for 5 minutes, 150 cm above ground level during morning & evening. The plates after sampling were transported to the laboratory, where they were incubated for 48 hours at 37°C.

After incubation the total number of growing colonies was counted. Each colony represents a single colony forming unites (CFU). The results were recalculated per cubic meter of air (CFU/m<sup>3</sup>). The colony forming unites (CFU) weredetermined using the Omelianski's formula.

$$\text{CFU/m}^3 = a \cdot 10000/p \cdot t \cdot 0.2$$

where:

- a - the number of colonies on the Petri plate.
  - p - the surface area of the Petri plate.
  - t - the time of Petri plate exposure.
- (Krzysztofik B 1992)

## RESULTS & DISCUSSION:

The results obtained from the present research study clearly indicate that the concentration of the microflora at all the four study sites is extremely high during the morning time while it reduces drastically until evening.

The site 1 i.e. Riddhi Siddhi garden has 3913.41 cfu/m<sup>3</sup> microbial load at the morning time which reduces to 1410.23 cfu/m<sup>3</sup> in the evening. Similarly site 2 i.e. BMC garden has 21611.90 cfu/m<sup>3</sup> microbial load which is the highest in the present research study

observed during the morning and it too has reduced to 6416.58cfu/m<sup>3</sup>.

At site 3 and 4 the same observations have been noted where at site 3 i.e. Ever United garden the morning microbial load is 10435.76 cfu/m<sup>3</sup> which has reduced to 3149.50 cfu/m<sup>3</sup> in the evening. Devyani garden which the 4<sup>th</sup> site has shown the microbial load reduction by almost 5 fold, as the morning readings were 12184.45 cfu/m<sup>3</sup> and the evening results analyzed were 2725.99 cfu/m<sup>3</sup>.

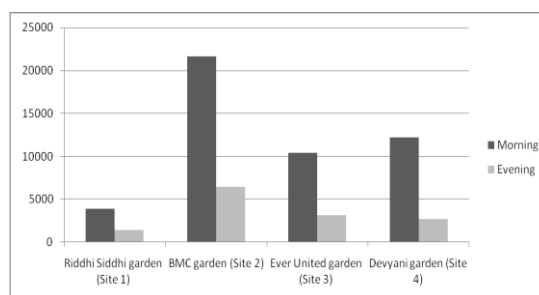
This clearly indicates that the people visiting these gardens during morning time are extremely exposed to the risk of high microbial contamination.

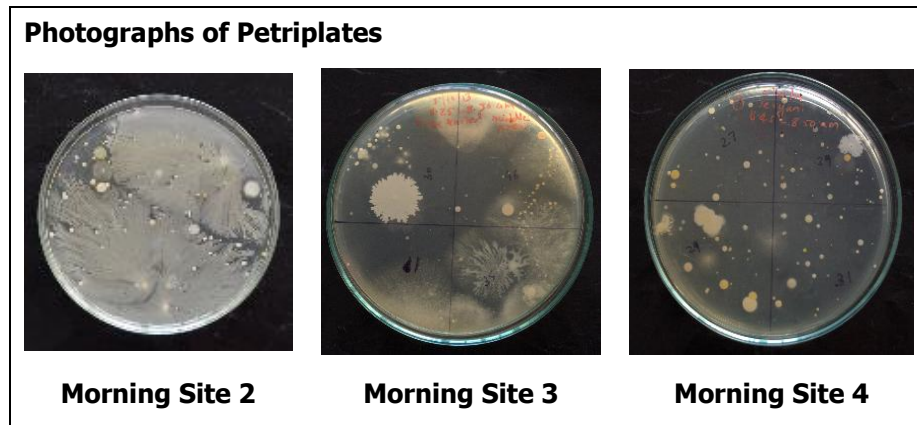
The workers in these gardens have a daily activity to burn the dry dead plant material of the garden in the evening so as to reduce the dry plant waste load and also to restrict the mosquito population. Due to this burning activity the microbial load must also be going down in the evening.

Air, an essential element of the natural environment is being contaminated by a growing number of different pollutants. Rapid urbanization in the surrounding areas of the study site must have led to a menacing concentration of the air pollutant emission.

**Table 1:** Concentration of microflora in the air of four different gardens of Dahisar region in Mumbai City (cfu/m<sup>3</sup>).

Study Area	Sampling Time	
	Morning	Evening
Riddhi Siddhi garden (Site 1)	3913.41	1410.23
BMC garden (Site 2)	21611.90	6416.58
Ever United garden (Site 3)	10435.76	3149.50
Devyani garden (Site 4)	12184.45	2725.99





The atmosphere always absorb substantial amounts of harmful contaminants such as different powders, organic compounds, non – organic compounds of nitrogen, sulphur, coal and other compounds as well as various microorganisms.(Marta Małecka-Adamowicz *et al.*(2010); M. Stryjakowska-Sekulska *et al.*(2007); Vinita Katiyar(2013);F.O. Ekhaiseet *al.*(2010)).

But the air's ability to self-clean has become very limited at all the four study sites and hence it has become necessary to supervise its quality periodically and take measures that would protect it from further excessive microbial air pollution in the future.

#### Acknowledgement:

The authors of the paper are thankful to the HOD of the Zoology Department of Wilson College, Prof. S. S. Parkar for the permission to use the Zoology Laboratory to carry out the desired research work. We are also thankful to our Lab Assistant Ms. Sheela Lad for her timely help with materials during the Laboratory analysis. We will always be grateful to Prof. NaseerJahagirdar from the Microbiology Department of Wilson College for his helping hand towards the identification of the microflora. And special thanks to Mr. JayeshShembavnekar, M.Sc. I Zoology student, for clicking good photographs of the petri plates.

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## RESEARCH ARTICLE

## VARIATION IN RATE OF POLLEN FERTILITY IN SOME MEMBERS OF FAMILY RUBIACEAE

Dassharma Kakoli, Ravnang Pratik\* and Qureshi Nazia

Department of Botany, Bhavan's College, Andheri (W).

### ABSTRACT

Aerobiology is a scientific and multidisciplinary approach focus on the transport of organisms and biologically significant materials (Edmonds 1973). It deals with the study of air spora such as algal filament, fungal, bryophyte and pteridophyte spores and pollen grains of gymnosperms and angiosperms and other microorganisms (S. T. Tilak 1982). Since air currents play significant role in the dispersal and pollination of pollen grains, the palynological studies can be interestingly associated with aerobiology. The pollen grain which is a highly reduced male gametophyte in flowering plants is one of the efficient research tools for studying the effect of various chemicals on its metabolism since it carries the male genetic material. Sucrose is reported as the best source of carbohydrate for pollen germination and pollen tube growth [Adams (1916), Brink (1924), Hrebetova and Tupy (1964)]. The Rubiaceae family is world's fourth largest flowering plant family with around 11,000 species, including coffee (coffea), quinine (cinchona), and beautiful tropical ornamentals such as *Gardenia*. Around 400 species of this family have become rare and endangered. Therefore, the members of Rubiaceae family are an important collection for conservation, research, horticulture and education. In the present study, three members of Rubiaceae family were used as experimental models to study their pollen physiology. The pollen grains of plant species, *Hamelia patens*, *Mussaenda sps.*, *Gardenia thunbergia*, were studied for pollen germination and pollen tube growth. The pollen grains of the above plant species were treated with 10%, 15% and 20% sucrose concentrations. Effect of Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) on the pollen grains of above species was also examined. *Hamelia patens* showed best results followed by *Mussaenda sps* followed by *Gardenia thunbergia*. Interestingly both *Hamelia patens* and *Gardenia thunbergia* showed best efficiency in 20% sucrose concentration while *Mussaenda sps* was most efficient in 10% sucrose concentration. The results obtained lead to the conclusion that Brewbaker and Kwack's medium gives more satisfactory results as compared to the results obtained using only sucrose as a growth medium.

**Keywords** : Palynology, *Hamelia patens*, *Mussaenda sps.*, *Gardenia thunbergia*, Brewbaker and Kwack's medium, Rate of fertility

### INTRODUCTION

Aerobiology is a scientific and multidisciplinary approach focused on the transport of organisms. Since air currents play significant role in the dispersal and pollination of pollen grains, the palynological studies can be interestingly associated with aerobiology.

The pollen grain is a highly reduced male gametophyte in flowering plants, generally shed off from plants after the dehiscence of the anther which carries the male genetic components transmitted in sexual reproduction of all higher plants and thus, can be used as a very efficient research tool for studying the effects of various chemicals on its metabolism. Maheshwari, (1963) and several other scientists demonstrated the role of pollen in sexual reproduction. Besides embryologists and morphologists, pollen grains have attracted the attention of physiologists, bio-chemists, cell biologists,

#### \*Corresponding Author

Email: [pratikr865@gmail.com](mailto:pratikr865@gmail.com)

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geneticists and to a large extent the plants breeder. The pollen grains are also being effectively used in genetic engineering. The use of pollen grain has already been extended in many more areas of research.

Rubiaceae family is world's fourth largest flowering plant group with around 11,000 species. Around 400 species of this family have become rare and endangered. Therefore, the members of Rubiaceae family are an important source for conservation, research, horticulture and education. In the present study, three members of Rubiaceae family were used as experimental models to study their pollen physiology. The pollen grains of plant species, *Hamelia patens*, *Mussaenda* spp. and *Gardenia thunbergia* were studied for pollen germination and pollen tube growth. *Hamelia patens* is a dependable and useful perennial shrub with a long blooming season. It is extremely drought tolerant and thrives in any soil as long as it is well-drained. The stems and leaves have been used for tanning and a concoction reportedly is used for various medicinal purposes. It is also rich in active phytochemicals such as alkaloids and flavonoids. Propagation is done by seed and half-ripen fruit cuttings. *Hamelia patens* is a heat tolerant species that has potential value as a resource efficient landscape plant. *Mussaenda* is an ornamental shrub suited to tropical and subtropical climates with a bright future both as landscape plants and in floral decorations. The most distinctive feature of *Mussaenda* is that the floral display is primarily derived from the calyx. *Mussaenda* seed is significant for hybridization while vegetative methods are used for commercial propagation. Alternative means of propagation include grafting and layering. *Gardenia* is an evergreen perennial shrub with medium growth rate showing whorled phyllotaxy. The waxy, highly fragrant white flowers of *Gardenia* have great horticultural potential due to its strong aroma and aesthetic value. Large species number and the great morphological, anatomical and biological variation make Rubiaceae a very interesting family for study. Considering the economic importance and horticultural significance of family Rubiaceae, the above mentioned three members of the same family were considered for the study. In the present work, the comparative account of the above three plant

species is done with respect to the variation in their rate of pollen fertility

## MATERIAL AND METHODS

Two distinct parameters: pollen germination and pollen tube growth was studied from the dissected anthers of three members, *Hamelia patens*, *Mussaenda* spp. and *Gardenia thunbergia* of family Rubiaceae using acetocarmine as a stain. Since sucrose has already been concluded as the best energy supplier for pollen germination and pollen tube growth, it was taken as a basic medium for germination pollen grains (Tupy 1960); Hrebetova and Tupy (1964). 10%, 15% & 20% sucrose concentration was taken for the preparation of basic growth medium for pollen to find the optimum concentration required for pollen fertility rate. Most efficient concentration of sucrose as a basic culture medium was noted at *H. patens* (20%), *Mussaenda* spp. (10%) and *Gardenia thunbergia* (20%) respectively. After standardization Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) was used separately used for the three plants with their respective optimized sucrose solution. Pollen grains were counted randomly at 40x magnification to calculate the percentage of pollen germination. After calibrating the ocular micrometer, the pollen tubes were also measured at the same magnification.

Composition of Brewbaker and Kwack's medium is as follows:

Content	Composition of medium (mg/l)
Sucrose	100,000 (10%)
H <sub>3</sub> BO <sub>3</sub>	100 (0.01%)
Ca(NO <sub>3</sub> ) <sub>2</sub> .2H <sub>2</sub> O	300 (0.03%)
MgSO <sub>4</sub> .7 H <sub>2</sub> O	200 (0.02%)
pH	7.3

The observations clearly indicated better results with Brewbaker and Kwack's medium as compared to only sucrose as growth medium.



**RESULTS & DISCUSSION:**

Interestingly both *Hamelia patens* and *Gardenia thunbergia* showed best efficiency in 20% sucrose concentration in both the parameters (rate of pollen germination and rate of pollen tube growth) while in *Mussaenda sps* best positive result in both the parameters was observed in 10% sucrose concentration. Highest germination in *Hamelia patens* was recorded as 55.5%(in only sucrose) and 66.66%(in BK media), whereas pollen germination in *Gardenia* was found to be 31.25% (in only Sucrose) and 38.45% (in BK medium) in the same concentration. Highest pollen germination recorded in *Mussaenda sps* was 57.14% (in sucrose only) and 60% (in BK medium). Highest pollen tube length observed in *Hamelia patens* was 168.75 μ (in only sucrose) and 281.25μ (with BK media) whereas in *Gardenia thunbergia* maximum pollen tube length was found to be 71.25μ (in only sucrose) and 86.25μ (in BK medium). In *Mussaenda sps.* maximum pollen tube length noted was 116.25μ (in only sucrose) and 157.5μ (in BK medium). Incidentally in *Gardenia thunbergia*; neither pollen germination nor growth of pollen tube was noticed in Brewbaker and Kwack's medium with standardize 10% sucrose concentration. However minimum efficacy in both parameters was observed in *Gardenia thunbergia* as compared to both *Hamelia patens* and *Mussaenda sps*.

Sucrose is the best source of carbohydrate for pollen germination and pollen tube growth (Tupy

1960); Hrebetova and Tupy (1964). Thus significant tube growth in adequate sugar solution, particularly in Sucrose have been reported by Brink (1924); and Gollmick (1942). Highest rate of germination and better tube growth was observed on sucrose medium as compared to other sugar solutions i.e. glucose and fructose, which indicates the higher rate of respiration of a particular plant (Hrebetova and Tupy (1961). The germination of pollen of cabbage was maximum in sucrose, while longest pollen tube was observed in raffinose (Chiang 1974). Hrebetova and Tupy (1961,1964) have suggested that in some species pollen germination and tube growth showed considerable increase with many other sugars as well. Balasubrahmanyam (1959) found that 6% sucrose was the best medium for guava pollen germination. The germination of pollen of apple was observed in 10% sucrose Tupy (1960). Arromov (1956) noticed best germination of grape pollen in 10 - 20% sucrose medium. Chiteley and Naik (1971) noticed 20% sucrose as best medium for germination in *Sesbania grandiflora*. *Dolichos lablab* showed 29% germination in 25% sucrose. The presence of sucrose, glucose and fructose in pollen was also reported by O'Kelly (1955), Kessler (1960); which are utilized in respiration. Repeated studies on extracts of pollen by researchers have revealed the presence of endogenous sucrose in the pollen. It has been also noticed that 2 celled pollen grains need 10-20% sucrose, while 3 celled pollen need as high as 50% for germination. Various types of sugars were used from time to time by many researchers (Bishop (1949) - Lactose).

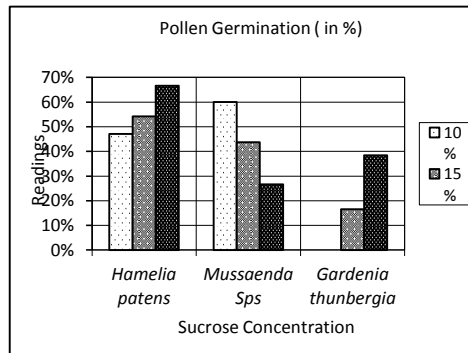
**Table 1. Pollen germination**

Plant name	Sucrose conc.	Basic Sucrose medium	Brewbaker & Kwack's medium
<i>Hamelia patens</i>	10%	37.5%	47.05%
	15%	42.85%	54.16%
	20%	55.5%	66.66%
<i>Mussaenda sps.</i>	10%	57.14%	60%
	15%	42.85%	43.75%
	20%	16.66%	26.66%
<i>Gardenia thunbergia</i>	10%	--	--
	15%	12.5%	16.5%
	20%	31.25%	38.45%

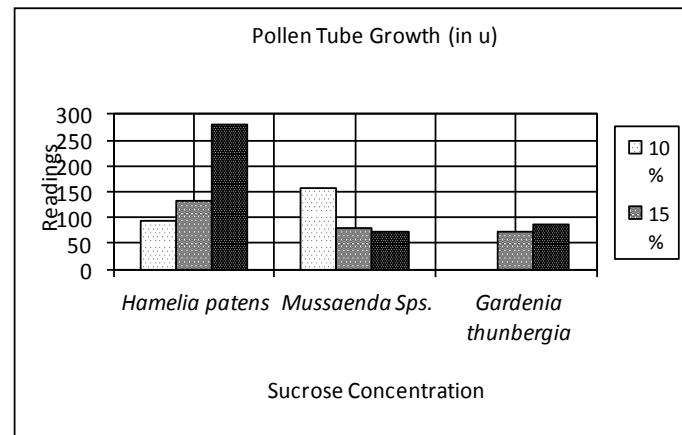
**Table 2. Pollen tube growth (in μ)**

Plant name	Sucrose Conc.	Basic Sucrose medium	Brewbaker & Kwack's medium
<i>Hamelia patens</i>	10%	75	93.75
	15%	108.75	131.25
	20%	168.75	281.25
<i>Mussaenda sps.</i>	10%	116.25	157.5
	15%	78.75	78.75
	20%	56.25	71.25
<i>Gardenia thunbergia</i>	10%	--	--
	15%	67.5	71.25
	20%	71.25	86.25





**Fig 1. Pollen germination**



**Fig 2. Pollen tube growth**

O'Kelley, (1955) have also studied the pollen germination in many plants. When pollen of *Benincosa hispida* and *Lagenaria vulgaris* were supplemented with dextrose and galactose, Vasil (1960) demonstrated that pollen germination was poor. On studying the effect of glucose, sucrose and fructose on apple pollen, Vasil (1960) reported better germination in sucrose.

All the above mentioned reports have clearly suggested maximum potential of sucrose as a carbohydrate source for energy. Interestingly, it was observed that amongst all the three cultivars, *Hamelia patens* showed best result in both pollen germination as well as pollen tube length formation. Therefore it was found that the results were substantially favourable in *Hamelia patens* in contrast to the *Mussaenda sps* and *Gardenia thunbergia*.

Pollen grains are very specialized and complex plant cells. Thus pollen germination and growth of pollen tube are important research materials for morphological, physiology, biotechnological, ecological, evolutionary, biochemical, molecular and biological studies [Dane, F., Olgun, G. and Ozlem, D. (2004)]. During *in vitro* pollen germination and tube growth not only the effect of enzyme activity but also the effect of boron, calcium, light, hormones and other factors were studied for different plants [Prajapati and Jain, (2010)]. Satisfactory pollen germination requires sugar, especially sucrose with other substances [Patel, V.A., Patel, D. and Jain, B.K. (1997)]. In pollen tube culture, externally supplied sugars play a vital role in

controlling osmotic pressure of the medium and simultaneously increase the growth rate of pollen tubes [Baloch, M.J., Lakho, A.R., Bhutto, H. and Solangi, M.Y. (2001)]. It was found that boric acid has pronounced stimulatory effect on germination and pollen tube growth [Taylor, L.P. and Hepler, P.K. (1997) and Feijó, J.A., Malho, R. and Obermeyer, G. (1995)].  $Ca^{2+}$  plays a key role in the regulation of pollen tube growth [Steer, M.W. and Steer, J.M. (1989)]. According to Bendnarska, K. (1989), calcium is an inorganic substance with notable effect on pollen tube growth and an essential requirement of pollen tube growth. Dickinson, D.B. (1967) has also reported that calcium controls the permeability of pollen tube membrane. Thus supplementation of calcium in the medium leads to development of straight and rigid pollen tube with vigorous growth. A positive correlation between rate of pollen tube growth and quality of the resulting progeny was also explained [Delph-Lynda, F., Weining, C. and Suttivan, K. (1998)]. According to Brewbaker and Kwack [Brewbaker, J. L. and Kwack, B.H. (1963)] magnesium ions enhance the effect of calcium ions resulting in vigorous growth of pollen tube.

In the present study, it was also observed that the results obtained with Brewbaker and Kwack's medium showed more satisfactory results as compared to the results obtained using only sucrose as a growth medium.





**CONCLUSION:**

Considering overall results, it can be concluded that pollen physiology with respect to pollen germination and pollen tube length is showing marked diversity in all the three members. Since the germination of pollen is directly responsible for next generation of the plant, *Hamelia patens* showing best performance should be extensively cultivated. Simultaneously, it has also been noticed that *Hamelia patens* showed longer tube length in pollen which corroborate the probable answer to vigorous rate of the resultant progeny in this member. Since *Hamelia patens* is of great horticultural significance as well as in herbal and various other industries it can be concluded that the member *Hamelia patens* can be an important source in enhancing the economy of the country.

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## RESEARCH ARTICLE

## AIR DISPERSION OF VIABLE ALGAE IN THE EXTRAMURAL ENVIRONMENT OF PUNE

**Patil VS and Patil SR**

Science Research Center, Department of Botany, Yashwantrao Mohite College, Bharati Vidyapeeth University, Pune-38.

### ABSTRACT

Air dispersion of viable algae in the extramural environment of Pune have been studied for six months, fortnightly from October 2011 to March 2012 by impaction culture method using BBM medium. As per the existing record of 24 algal aeroallergens, we found 15 genera and 21 species at Pune. These belong to Cyanophyceae 11 Genera & 21 species, Chlorophyceae 2 genera and Bacillariophyceae 2 genera i.e. *Anabaena* (6 Sp.), *Phormidium* (4 Sp.) and *Calothrix* (3 Sp.) etc. recorded causing allergy in sensitive victims. Out of totally recorded 228 airborne algal genera we found 40 algal genera and 29 species of which 3 have been found to be new record for aerobiology in India. These are *Camptylonema* Sp., *Dichothrix* Sp. and *Psedoanabaena* Sp. During this study 528 colony unit have been selected randomly, which revealed maximum percentage contribution of *Chroococcus* Sp. (14.9%) as dominant genus followed by *Chlorella* and *Chlamydomonas* (9.96%) each to the total aerophycoflora. Hence, it has been proved that these three unicellular algal forms have been found to be dominant as compared to colonial and filamentous forms of algae. Site wise dispersion and distribution of aeroalgae revealed that maximum 12 genera have been recorded at site no.5 followed by site no.6 (11 genera) and minimum at site no. 3 (3 genera). Site wise frequency studies revealed highest count of *Anabaena* (188 out of 528 regularly at all the six sites) followed by *Chlorella* (178 out of 528) and *Phormidium* (170 out of 528). Only *Anabaena* was found at all the six sites constantly, while *Gloeocapsa* (at Site no.6), *Scytonema* (Site no. 5) and *Aulsoria* (Site no.5) each at single site only and absent at remaining five sites.

Keywords : Extramural Environment, Viable Algae,

### INTRODUCTION

Marvelous contribution of various scientists consequently resulted in the development of aerophycology as a new branch of science. Prominent among them are Parshwanath (1979), Singh (1981), Tilak (1983), Santra (1987), Sabia Anis (1989), Sharma (1990), Ramchandra Rao (1996), Jadhav (2006), Quazi (2010), Tarar (2010) etc. Hence this investigation has been undertaken to elaborate studies on airborne algae at Pune.

Pune is a mega city having 160 km distance from Mumbai located towards the southern direction at the Latitude 18°32' N, Longitude 72° 51' E and Altitude 560m (1840 ft) above sea level. (Map: 1). As a source of airborne algae there are many water resources in and around Pune which contribute to the airborne algae.

Environmental record of meteorological parameters of Pune during study period (from October 2011 to March 2012) have been mentioned below maximum temperature ranging from 28.5° -34° C, minimum temperature ranging from 11°-23.9° C, rainfall (7.2-10 mm) has been recorded only in the month of October 2011, relative humidity ranges from 90-95% and wind velocity 3.2-28.8 km/h in the direction of West-East.

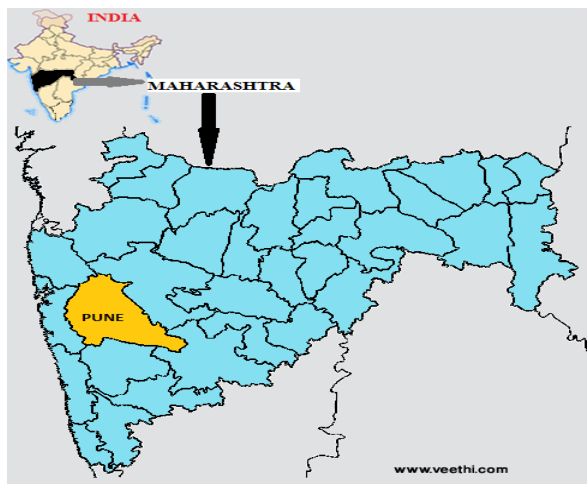
#### \*Corresponding Author

Email: [vspvishu118@gmail.com](mailto:vspvishu118@gmail.com)

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Map: 1. Highlighting the of Pune

## MATERIAL AND METHODS

Six various sites have been selected from different parts of Pune representing different localities and environments. Air sampling was carried out fortnightly, by riding the two wheeler Activa scooter at the speed of 40-55 km/hr over the roads (Site no. 1 to 6) using petriplate exposure method (Tilak and Anis1989). The Agarised Bold's Basal Medium (BBM)and Chu No-10 have been used in the culture plates from October 2011 to March 2012 during six months season, for culturing the aeroalgae.

Site wise exposed petriplates have been well labeled, sealed and incubated in illuminated culture racks with 40 watt fluorescent tube lights, giving a light intensity of 2000 to 2500 Luxcontinuously for 24 hrs, in a A/C culture room at  $25 \pm 1^{\circ}\text{C}$  temperature for 15 days. The cultures had been frequently enriched with sterilized 2 ml. liquid BBMand Chu No-10 respectively for the enhancement of growth of algae, besides avoiding drying.Petri plates have been observed regularly for the growth of algae and random samples picked up for identification.

The slides were prepared by mounting little algal material in 50% glycerin, sealed with transparent nail paintand observed under the binocular research microscopeusing different magnifications.The algal genera and species have been identified on the basis of their morphological characters using authentic literature and reference slides. Sub-cultures have been maintained after isolation.

## RESULTS & DISCUSSION:

Air sampling was carried out fortnightly for six months; exposing 72 culture plates, randomly selecting 528 colony units have been evaluated. Cyanophyceae members are dominant than the Chlorophyceae and Bacillariophyceae. The investigations at six sites revealed highest percentage contribution of *Chroococcus* (14.9%) followed by *Chlamydomonas* 9.96% at Site no. 4 (Mutha River side) and *Chlorella*9.96% at Site no. 2 (Pashan Lake) (Table. 3 and Fig. 2). These two sites have been located near water resources. (Table no. 1). Hence it has been proved that these three unicellular algal forms are dominant as compared to colonial and filamentous forms of algae.The unicellular as well as small colonial forms are dominant (Fig.1). Fortyaeroalgal genera have been encountered out of 228 (total record) during this study, including 3 genera as new records for aerobiology in India. These are *Camptylonema* Sp., *Dichothrix* Sp. and *Psedoanabaena* Sp. (Table no. 2). Fortyaeroalgal genera have been encountered out of 228 (total record) during this study, including 3 genera as new records for aerobiology in India. These are *Camptylonema* Sp., *Dichothrix* Sp. and *Psedoanabaena* Sp. (Table no. 2).

Table No: 1 Sites selected for the air sampling from October 2011 to March 2012 at Pune.

Sites no.	Name of the sites
Site no. 01	Nalstope- Karve road- Warje road
Site no. 02	Paud- Pashan- Bavdhan road
Site no. 03	Aaditya Birla Hospital road
Site no. 04	J.M - F.C road
Site no. 05	Sus – University road
Site no. 06	Katraj bypass highway-Sinhagad road

Site wise highest frequency have been recorded for *Anabaena* (188 out of 528) followed by *Chlorella* (178 out of 528) and *Phormidium* (170 out of 528).Only *Anabaena* was found at all the six sites constantly, *Chlorella* and *Phormidium* at 5 sites each,while *Gloeocapsa* (at Site no.6), *Scytonema* (Site no. 5) and *Aulsoria* (Site no.5). Each of them was found only at single site and absent at remaining five sites (Table. 4).



**Table no. 2:** Incidence of class wise genera and species at six different sites from Oct. 2011 to Mar. 2012.

Sr. No.	Name of the algae	Sr. No.	Name of the algae	Sr. No.	Name of the algae	Sr. No.	Name of the algae
<b>Cyanophyceae</b>				<b>Chlorophyceae</b>			
1.	<i>Anabaena</i> sp.	21.	<i>C.droryphoum</i>	41	<i>Nodularia</i> sp.	1	<i>Chlorella</i> sp.
2.	<i>A.laxa</i>	22	<i>C.stagnale</i>	42	<i>Nostoc</i> sp.	2	<i>Chlorococcum</i> sp.
3.	<i>A.fragile</i>	23	<i>Dichothrix</i> sp.	43	<i>N. prolofica</i>	3	<i>Cosmarium</i> sp.
4.	<i>A. Orientalis</i>	24	<i>Gloeocapsa</i> sp.	44	<i>N. maculiforme</i>	4	<i>Chlamydomonas</i> sp.
5.	<i>A.sheria</i>	25	<i>G.fusco-lutea</i>	45	<i>Oscillatoria</i> sp.	5	<i>Oedogonium</i> sp.
6.	<i>A. variabilis</i>	26	<i>Gloeococcus</i> sp.	46	<i>O. subbrevis</i>	6	<i>Spirogyra</i> sp.
7.	<i>Anabaenopsis</i> sp.	27	<i>Gloeotrichia</i> sp.	47	<i>Phormidium</i> sp.	7	<i>Vaucheria</i> sp.
8.	<i>Aphanocapsa</i> sp.	28	<i>Hapalosiphon</i> sp.	48	<i>P. laminosum</i>		
9.	<i>A.roseana</i>	29	<i>H.welwitchii</i>	49	<i>P. tenue</i>	<b>Bacillariophyceae</b>	
10.	<i>Aulsoria</i> sp.	30	<i>Lyngbya</i> sp.	50	<i>P. foveolarum</i>	1	<i>Navicula</i> sp.
11.	<i>Botridiopsis</i> sp.	31	<i>L.kashyapii</i>	51	<i>P. jenkelianum</i>	2	<i>Nitzschia</i> sp.
12.	<i>Calothrix</i> sp.	32	<i>L.lachneri</i>	52	<i>Plectonema</i> sp.	3	<i>Pinnularia</i> sp.
13.	<i>C.thermalis</i>	33	<i>Mastigocladus</i> sp.	53	<i>Pseudoanabaena</i> sp.		
14.	<i>C.bharadwaji</i>	34	<i>Microcheatae</i> sp.	54	<i>Rivularia</i> sp.		
15.	<i>C.jawonica</i>	35	<i>M. tenera</i>	55	<i>Scytonema</i> sp.		
16.	<i>Camptylonema</i> sp.	36	<i>Microcoleus</i> sp.	56	<i>Spirulina</i> sp.		
17.	<i>C. indicum</i>	37	<i>Microcystis</i> sp.	57	<i>Stigonema</i> sp.		
18.	<i>Chroococcus</i> sp.	38	<i>M. flose-aque</i>	58	<i>Westiellopsis</i> sp.		
19.	<i>C.dispersus</i>	39	<i>M. elabens</i>	59	<i>W.prolifica</i>		
20.	<i>Cylindrospermum</i> sp.	40	<i>M. pulverea</i>	60	<i>Xenococcus</i> sp.		

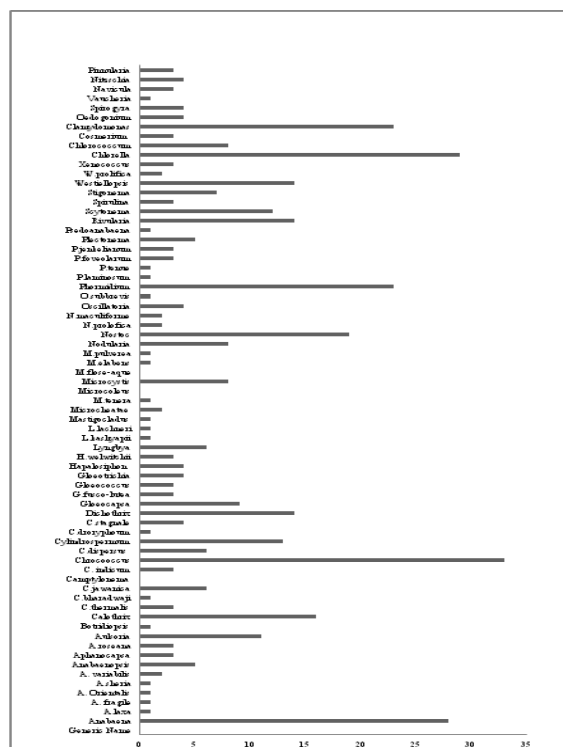
**Table.3** Percentage contribution of dominant aeroalgal types of different six sites of Pune.

Sr.No.	Genus name	Site. No. 1	Site. No. 2	Site. No. 3	Site. No. 4	Site. No. 5	Site. No. 6
1	<i>Anabaena</i>	9.1	6.1	9.7	7.38	7.8	4.6
2	<i>Anabenopsis</i>	4	2.4	0	0	0	0
3	<i>Aulsoria</i>	2.2	2.4	0	0	1.4	6.6
4	<i>Calothrix</i>	0	0	5.8	5	7.6	5.3
5	<i>Chlorella</i>	6.1	9.96	7.5	10	0	9.3
6	<i>Chlorococcum</i>	6	3.4	2	0	0	1.3
7	<i>Chroococcus</i>	12.1	9.3	0	14.9	0	9.2
8	<i>Chlamydomonas</i>	4.7	2.7	5.7	9.96	3.2	5.3
9	<i>Cylindrospermum</i>	0.5	2.2	4	3.1	6.6	5.1
10	<i>Dichothrix</i>	2.2	5.12	4	3.1	6.4	2.8
11	<i>Gloeocapsa</i>	1.7	3.2	4	1.1	0	4
12	<i>Hapalosiphon</i>	2.7	1	4	0	1.6	1.4
13	<i>Nodularia</i>	0	3	0	0	3.4	2.6
14	<i>Nostoc</i>	7.1	4.9	4	5.4	2.2	5.3
15	<i>Phormidium</i>	7.6	9.3	4	8.4	9.4	5.3
16	<i>Rivularia</i>	1	5.3	2	3.07	3.4	4
17	<i>Scytonema</i>	3	2	4	4.6	5	1.3
18	<i>Westiellopsis</i>	3.2	0.2	4	4	4.8	2.6

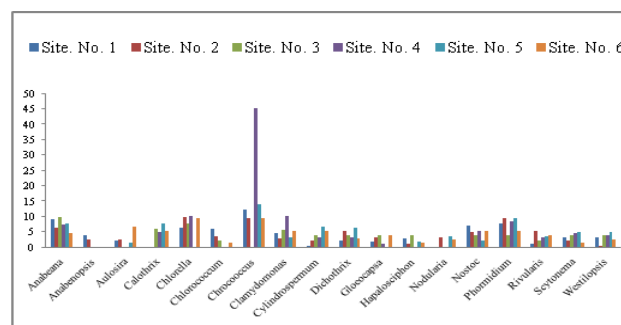


**Table 4:** Frequency of dominant aeroalgal types of different six sites

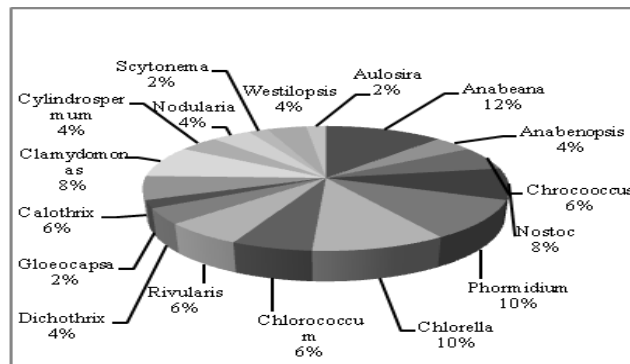
Sr. no	Genus name	Site. No. 1	Site. No. 2	Site. No. 3	Site. No. 4	Site. No. 5	Site. No. 6	Out of 528	Incidence for Sites
1.	<i>Anabaena</i>	37	25	40	19	39	28	188	6
2.	<i>Anabenopsis</i>	16	0	0	39	0	0	55	2
3.	<i>Aulosira</i>	0	0	0	0	40	0	40	1
4.	<i>Calothrix</i>	0	0	24	13	38	0	75	3
5.	<i>Chlorella</i>	25	40	31	26	0	56	178	5
6.	<i>Chlorococcum</i>	24	14	0	0	0	56	94	3
7.	<i>Chroococcus</i>	49	38	0	0	70	0	157	3
8.	<i>Chlamydomonas</i>	19	0	0	26	16	32	93	4
9.	<i>Cylindrospermum</i>	0	0	0	0	33	32	65	2
10.	<i>Dichothrix</i>	0	21	0	0	32	0	53	2
11.	<i>Gloeocapsa</i>	0	0	0	0	0	24	24	1
12.	<i>Nodularia</i>	0	0	0	0	17	16	33	2
13.	<i>Nostoc</i>	29	20	0	14	0	32	95	4
14.	<i>Phormidium</i>	31	38	0	22	47	32	170	5
15.	<i>Rivularis</i>	0	22	0	0	17	24	63	3
16.	<i>Scytonema</i>	0	0	0	0	25	0	25	1
17.	<i>Westiellopsis</i>	0	0	0	0	24	16	40	2



**Fig. 1:** Frequency against occurrence of aerophycal genera reported in the atmosphere of Pune city



**Fig. 2:** Site wise comparative aeroalgal genera occurrence in the environment of Pune

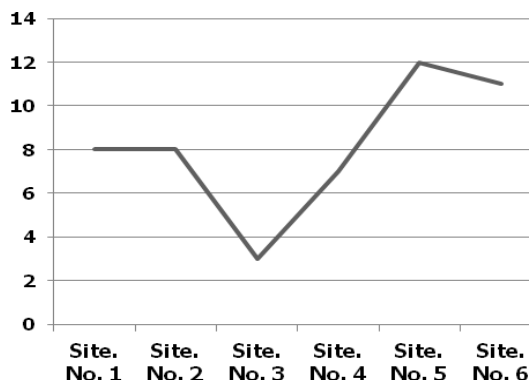


**Fig. 3:** Frequency of dominant and viable aeroalgae in the environment of Pune.



**Table 5:** Total algal genera count for the selected sites of Pune.

Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Algal genera	8	8	3	7	12	11

**Fig 4:** Site wise aeroalgal incidence in the environment of Pune.

The highest incidence was revealed by *Anabaena* 12% followed by *Phormidium* and *Chlorella* 10% each. Lowest incidence was revealed by *Gloeocapsa*, *Scytonema* and *Aulsoria* 2% each. (Graph.3). It has been found that the unicellular, colonial and unbranched filamentous forms like *Anabaena* and *Phormidium* are common.

## CONCLUSION:

The environmental conditions and the natural water resources also majorly contributed to the aeroalgal dispersion and viability showing site wise variation. Aeroalgal members have been encountered from Cyanophyceae, Chlorophyceae and Bacillariophyceae. Out of three classes Cyanophyceae members shows highest count. Most of coccooid unicellular form like *Chlorella* Sp., colonial form like *Chroococcus* Sp., unicellular flagellate form like *Chlamydomonas* Sp. and unbranched filamentous form like *Anabaena* Sp. and *Phormidium* Sp. are viable and very common in dispersion. Environment of Pune shows an aeroalgal biopollutants, which may cause

allergy in sensitive victims. Thus aeroalgal flora is rich and viable in Pune.

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## RESEARCH ARTICLE

## AN OVERVIEW OF AEROBIOLOGY OVER GROUNDNUT CROP AT PATAN (M.S)

Shinde Manjusha <sup>1\*</sup> and Mahajan Minakshi <sup>2</sup>

<sup>1</sup>Balasaheb Desai College, Patan, Dist-Satara, Maharashtra, India.

<sup>2</sup>Fergusson College, Pune

### ABSTRACT

An aerobiological investigations have been carried out over groundnut crop field for two consecutive seasons from (July 2011 to November 2011) kharif and from (January 2012 to May 2012) summer, using Tilak air sampler. Comparative study of these two seasons revealed abundant total airspora during kharif (rainy) (447594) as compared to summer season (178430) comprising 95 spore types which exhibited monthly variation during both the seasons. Observations of Kharif season revealed that the total spore load was maximum during October 2011 (248122) as compared to the total of remaining three months (165396) which may be due to heavy rain fall during October 2011, other congenial conditions and incidence of 24 types of ascospores released due to rains. Thus ascospores acted as bio indicator of heavy rainfall. Highest spore count has been recorded on 13 th October 2011 (88270) which was due to 655,2mm rainfall, 250C temperature and 65% RH on preceding day 12th October 2011 and 25 mm rainfall on same day, which was responsible for release of ascospores. Thus environmental parameters have played significant role on the spore load; this was coincided with maximum incidence of foliar diseases like Tikka disease and Rust of Groundnut and higher percentage contribution of pathogens of these diseases. Deuteromycotina has been found to be dominant class (58.19%) followed by Ascomycotina (18.81%), Basidiomycotina (10.89%), other types (9.19%), Phycomycotina (2.26%) and Myxomycotina (0.62%). Dominant spore types in descending order have been found to be *Cladosporium* (15.53%), *Aspergillus* (9.19%) and *Nigrospora* (6.23%) and least in occurrence have been found to be *Ceratosprium* (0.004%) and others in between. These investigations may be helpful in laying down some basic principles required for disease forecasting system of important diseases of groundnut to save crop losses and yield of crops due to diseases at Patan.

**Keywords :** Aerobiology, Groundnut, Environmental parameters, Diseases, Patan

### INTRODUCTION

Patan is located in the vicinity of Koyana Dam and is earthquake prone area situated at 17022'30" North latitude and 73054'10" Longitude and above 2200-feet from main sea level having heavy rainfall around 3000 mm per annum. Gregory (1952) proposed the term Aerospora to describe airborne pollen grains and fungal spores. Agarwal (1969), elaborated significant role of fungal spores on plant diseases. Vegetation as well as biogeographical zone determines the fungal spores of respective area. The concentration of fungal

spores is determined by time, day, weather parameters and seasons. Aerobiological studies have been selected due to its lack in this region, for qualitative and quantitative estimation of general and pathogenic airspora over an oil seed and fodder cash crop like groundnut as extramural investigation have been carried out from 11th July 2011 to 18 th November 2011 and summer season from 1 st January 2012 to 18 th May 2012 over groundnut crop fields. (Table.1)

Groundnut (*Arachis hypogaea* L.) belongs to family Fabaceae is an important oil seed cash crop in tropical and subtropical countries of the world. India is major country in groundnut production 4.19 million hector of land under groundnut production in India. Gujarat, Rajasthan, Maharashtra are major groundnut producing states in India. Groundnut crop is affected

### \*Corresponding Author

Email: [sman\\_2050@yahoo.com](mailto:sman_2050@yahoo.com)

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by some airborne fungal diseases. The present investigation deals with study of concentration of aerospora over groundnut field at Patan.

Many scientists studied diseases of groundnut thoroughly. (Chahal et al.1971), Shanta.P, (1960), Quazi, ((1985), Sulaiman and Agashe (1965), Woodroof (1933), Mahajan and Pande (2002), Kadam.et al (2008) Mali and Gaikwad (2011) and focused aerobiological investigations over groundnut crop and its diseases. Aerospora of groundnut with various parameters has been studied by many Kalkar and Patil (1997), Mallaiah (1989), Aher et al. (2002), Arsule and Pande (2011), Jadhav et al (2010).

Crop is affected by many pathogens which are mainly uneven in distribution irrespective of vegetation cover and climatic conditions. The Tikka disease and Rust disease are caused by *Cercospora arachidicola* (Hori) and *Cercospora personatum* (Berk and Curt) Deighton, and *Puccinia arachidis speg* respectively. Rust disease was first reported by Chahal and Chahan (1971).The pathogen attacks the leaves of crop, other important but less frequent diseases of groundnut are leaf spot caused by *Leptosphaerulina crassica* (sechet) Jackson and bell, *Alternaria alternata* (FY) Keissler and *Myrothesium rode* ex. The disease is spread only through the air by outbreak of epiphytotics.

## MATERIAL AND METHODS

Aerobiological investigation has been carried out over groundnut (variety S.B.11) field at two different sites at Kaloli and at Meshtewadi of Patan. Sampling was carried by using continuous volumetric Tilak Air Sampler, installed in groundnut field at 1.5 meter height from Ground at Patan. Slides have been prepared and scanned. Identification was done by

using authentic literature, photographs, reference slides etc. Daily meteorological data has been maintained.

## RESULTS & DISCUSSION:

During both the seasons, total 95 bio components including various types of fungal spores of saprophytic fungi, pathogenic fungi, and other biocomponents like pollen grains, plant parts, fungal hyphae, algal filaments, mites, protozoan cyst, insect parts and other unidentified fungal spores were observed. Two types from Myxomycotina, four types from Phycomycotina, 25 types from Ascomycotina, 5 types from Basidiomycotina, and 51 spore types Deuteromycotina types while 8 different types of biocomponents have been recorded over groundnut crop field. Table .3. Class wise percentage contribution during both seasons revealed the group Deuteromycotina has been found to be dominant with 54.80% and 66.70%. Followed by Ascomycotina with 23.40% and 7.29%. Basidiomycotina with 11.34% and 9.76%, other types 7.48% and 13.49%. Phycomycotina 2.28% and 2.21% and Myxomycotina 0.60% and 0.52% spore contribution to the total air spora of two seasons have been encountered respectively.

Percentage contribution of airspora was more during Kharif (71.49%) as compared to summer (28.50%) as there was no single spore free day, during both the seasons. Maximum spore load has been encountered during October 2011 as Compare to other months. According to seasonal variation 36.95% spore load was recovered in south west monsoon that is (July to August 2011)and 74.04% spore load was observed in retreating monsoon (September to November 2011).

**Table 1-seasonwise duration of groundnut crop and sampling**

Sr. No	Season	Variety	Date of Sampling	Sowing Date	Harvesting Date	Date Sampling Stopped	Days	Place
1	Kharif	S.B.11	11 July 2011	18 July2011	10 Nov 2011	18 Nov 2011	128	Kaloli
2	Summer	S.B.11	1 Jan 2012	8Jan 2012	20 May 2012	28 May 2012	118	Meahtewadi





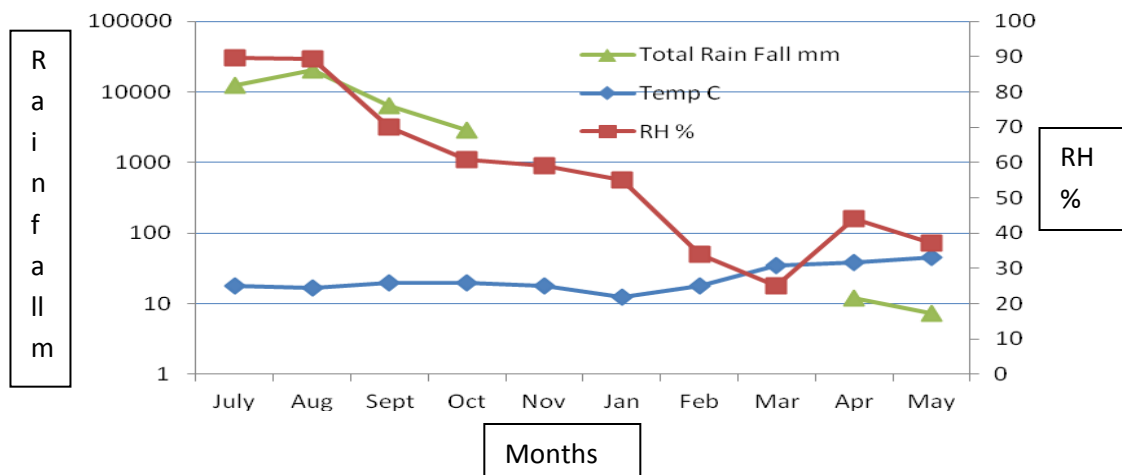


Fig. 1: Meteorological Data

Table 2 : Monthly concentration of airborne pathogenic spores over ground nut crop field during 1<sup>st</sup> and 2<sup>nd</sup> season in relation to meteorological parameters

Spore	Parameters										
	Ave.	July	Aug	Sept	Oct	Nov	Jan	Feb	Mar	Apr	May
Temp <sup>o</sup> <sub>C</sub>		25.14	24.6	26	26	25.13	22	25	30.8	31.8	33.12
RH %		89.54%	89.48%	70%	60,8%	59.13%	55%	34%	25%	44.11%	37.2%
Total rainfall		12218 mm	20412 mm	6350 mm	2872 mm	-	-	-	-	12 mm	7.2 mm
<i>Cercospora</i>	-	630	2282	4676	21882	616	98	280	336	182	616
<i>Alternaria</i>	-	784	896	1288	8218	1764	1652	3150	1120	252	560
<i>Puccinia</i>	-	2128	2072	2268	4872	1610	2324	2756	518	210	-

During summer season 57.20% spores have been encountered in cold season (Jan and Feb) and rest 42.79% in hot months of summer (Mar to May) 2012).(Graph 1).

Some spores have been found to occur constantly during both the seasons include *Cladosporium*, *Alternaria*, *Aspergillus*, *Nigrospora*, *Periconia*, *Curvularia*. Among all the spore types, *Cladosporium*, (12.13%and 24.04%), followed by *Aspergillus* (8.52%and 10.86%). (table 3), *Nigrospora*, *Alternaria* found abundant while *Drechslera* was dominant in summer season. Our finding correlates with A Janaki Bai and Subba Reddi (1981) there is high incidence of

airsports in this region because of congenial environmental conditions like rainfall, Temperature, relative humidity and abundance of substratum from thick forest litter around. Observation of *Pringsheimia* revealed interesting findings during kharif season its percentage contribution has been 7.75% (34692) only, while during summer crop season it is 0.18% (336) only. This indicates that it belongs to wet airspora group hence found in prominently more percentage during kharif season having congenial conditions for *Pringsheimia* (average temp.25.20c, average RH-74%,Total rain fall 4441mm).During summer season due to unfavourable condition (31 mm rainfall, 33.10c and RH 44%).(Table.2).



**Table 3:** Reveals season wise comparative concentration and percentage contribution of each spore group and spore type during two kharif season over groundnut crop fields from 11 July 2011 to Nov 2011 and 1 January 2012 to 18 May 2012

Sr. No.	Name of Spore	Kaloli	%contribution/m3	Meshtewadi	%contribution/m3	Total	%
	<b>Myxomycetes</b>						
1	<i>Physarum</i>	1526	0.3409	770	0.431542	2296	0.36676
2	<i>Stemonitis</i>	1470	0.3284	168	0.094155	1638	0.26165
			0		0	0	0
	<b>Total</b>	<b>2996</b>	<b>0.6694</b>	<b>938</b>	<b>0.525696</b>	<b>3934</b>	<b>0.62841</b>
	<b>phycomycetes</b>						
1	<i>Albugo</i>	3402	0.7601	1918	1.074931	5320	0.84981
2	<i>Circinella</i>	966	0.2158	406	0.22754	1372	0.21916
3	<i>Cunnighamella</i>	4172	0.9321	630	0.35308	4802	0.76706
4	<i>Sclerospora</i>	1708	0.3816	994	0.557081	2702	0.43161
						0	0
	<b>Total</b>	<b>10248</b>	<b>2.2896</b>	<b>3948</b>	<b>2.212632</b>	<b>14196</b>	<b>2.26764</b>
	<b>Ascomycetes</b>						
1	<i>Ascospores</i>	1498	0.3347	1302	0.729698	2800	0.44727
2	<i>Bitrimonospora</i>	1848	0.4129	1400	0.784621	3248	0.51883
3	<i>Calospora</i>	630	0.1408	476	0.266771	1106	0.17667
4	<i>Cheatomium</i>	2702	0.6037	364	0.204002	3066	0.48976
5	<i>Claviceps</i>	4144	0.9258	56	0.031385	4200	0.6709
6	<i>Cucurbit aria</i>	42	0.0094	588	0.329541	630	0.10064
7	<i>Dydimosphaeria</i>	4410	0.9853	1190	0.666928	5600	0.89453
8	<i>Hypoxyylon</i>	3164	0.7069	574	0.321695	3738	0.5971
9	<i>Hysterium</i>	7042	1.5733	826	0.462927	7868	1.25682
10	<i>Leptosphaeria</i>	21056	4.7043	1624	0.910161	22680	3.62286
11	<i>Lophiostoma</i>	168	0.0375	14	0.007846	182	0.02907
12	<i>Masarina</i>	0	0	112	0.06277	112	0.01789
13	<i>Melanospora</i>	420	0.0938	56	0.031385	476	0.07604
14	<i>Metasphaeria</i>	5712	1.2762	252	0.141232	5964	0.95268
15	<i>Otthia</i>	84	0.0188	126	0.070616	210	0.03355
16	<i>Parodiella</i>	434	0.097	42	0.023539	476	0.07604
17	<i>Passaraniella</i>	336	0.0751	70	0.039231	406	0.06485
18	<i>Pleospora</i>	10430	2.3302	2632	1.475088	13062	2.0865
19	<i>Pringsheimia</i>	34692	7.7508	336	0.188309	35028	5.59531
20	<i>Rosellina</i>	518	0.1157	182	0.102001	700	0.11182
21	<i>Sordaria</i>	4186	0.9352	28	0.015692	4214	0.67314
22	<i>Sporormia</i>	420	0.0938	84	0.047077	504	0.08051
23	<i>Teichospora</i>	476	0.1063	490	0.274617	966	0.15431
24	<i>Valsaria</i>	196	0.0438	196	0.109847	392	0.06262
25	<i>xylaria</i>	168	0.0375	0	0	168	0.02684
			0		0	0	0
	<b>Total</b>	<b>104776</b>	<b>23.409</b>	<b>13020</b>	<b>7.296979</b>	<b>117796</b>	<b>18.8165</b>



Table 3: Continued

Sr. No.	Name of Spore	Kaloli	%contribution/m3	Meshtewadi	%contribution/m3	Total	%
	<b>Basidiomycetes</b>						
1	<i>Basidiospores colour</i>	6146	1.3731	4242	2.377403	10388	1.65936
2	<i>Basidiospores hyaline</i>	14588	3.2592	2842	1.592781	17430	2.78424
3	<i>Ganoderma</i>	2184	0.4879	112	0.06277	2296	0.36676
4	<i>Rust spore</i>	12950	2.8932	5628	3.154178	18578	2.96762
5	<i>Smut spore</i>	14896	3.328	4606	2.581404	19502	3.11522
6	<i>Urospores</i>	0	0	0	0	0	0
			0		0	0	0
	<b>Total</b>	<b>50764</b>	<b>11.342</b>	<b>17430</b>	<b>9.768537</b>	<b>68194</b>	<b>10.8932</b>
	<b>Deuteromycetes</b>						
1	<i>Alternaria</i>	12950	2.8932	6734	3.774029	19684	3.14429
2	<i>Aspergillus</i>	38164	8.5265	19390	10.86701	57554	9.19358
3	<i>Beltrania</i>	336	0.0751	420	0.235386	756	0.12076
4	<i>Beltraniella</i>	224	0.05	126	0.070616	350	0.05591
5	<i>Bipolaris</i>	84	0.0188	0	0	84	0.01342
6	<i>Bispora</i>	3780	0.8445	2352	1.318164	6132	0.97952
7	<i>Botriodiplodia</i>	28	0.0063	70	0.039231	98	0.01565
8	<i>Ceratosporium</i>	28	0.0063	0	0	28	0.00447
9	<i>Cercospora</i>	30086	6.7217	1512	0.847391	31598	5.04741
10	<i>Cladosporium</i>	54334	12.139	42910	24.04865	97244	15.5336
11	<i>Clasterosporium</i>	238	0.0532	126	0.070616	364	0.05814
12	<i>Cordana</i>	686	0.1533	686	0.384464	1372	0.21916
13	<i>Coryneospore</i>	1638	0.366	350	0.196155	1988	0.31756
14	<i>Curvularia</i>	7742	1.7297	3598	2.016477	11340	1.81143
15	<i>Darluka</i>	448	0.1001	448	0.251079	896	0.14313
16	<i>Deightonella</i>	126	0.0282	182	0.102001	308	0.0492
17	<i>Diplocladiella</i>	42	0.0094	0	0	42	0.00671
18	<i>Diplodia</i>	350	0.0782	476	0.266771	826	0.13194
19	<i>Drechslera</i>	1120	0.2502	1274	0.714005	2394	0.38241
20	<i>Epicoccum</i>	7350	1.6421	1652	0.925853	9002	1.43796
21	<i>Exosporium</i>	1218	0.2721	0	0	1218	0.19456
22	<i>Fusariella</i>	1442	0.3222	252	0.141232	1694	0.2706
23	<i>Fusarium</i>	2436	0.5442	168	0.094155	2604	0.41596
24	<i>Gilmanigella</i>	0	0	126	0.070616	126	0.02013
25	<i>Haplosporella</i>	462	0.1032	294	0.16477	756	0.12076
26	<i>Helicomycetes</i>	182	0.0407	294	0.16477	476	0.07604
27	<i>Helminthosporium</i>	2674	0.5974	1232	0.690467	3906	0.62394
28	<i>Heterosporium</i>	672	0.1501	392	0.219694	1064	0.16996
29	<i>Hirdunaria</i>	126	0.0282	28	0.015692	154	0.0246
30	<i>Lacellina</i>	3052	0.6819	518	0.29031	3570	0.57027



Table 3 : Continued

Sr. No.	Name of Spore	Kaloli	%contribution/m3	Meshtewadi	%contribution/m3	Total	%
31	<i>Menoniella</i>	854	0.1908	0	0	854	0.13642
32	<i>Nigrospora</i>	30856	6.8937	8176	4.582189	39032	6.2349
33	<i>Odium</i>	322	0.0719	686	0.384464	1008	0.16102
34	<i>Penicillium</i>	1330	0.2971	4844	2.71479	6174	0.98622
35	<i>Periconia</i>	8162	1.8235	7280	4.080031	15442	2.46668
36	<i>Pestilotia</i>	1540	0.3441	686	0.384464	2226	0.35558
37	<i>Pithomyces</i>	11256	2.5148	1820	1.020008	13076	2.08874
38	<i>Pseudotorula</i>	2338	0.5223	1386	0.776775	3724	0.59487
39	<i>Pyricularia</i>	1036	0.2315	0	0	1036	0.16549
40	<i>Sirodesmium</i>	112	0.025	294	0.16477	406	0.06485
41	<i>Spegazzinia</i>	1050	0.2346	1260	0.706159	2310	0.369
42	<i>Spermospora</i>	2114	0.4723	0	0	2114	0.33769
43	<i>Spicaria</i>	434	0.097	70	0.039231	504	0.08051
44	<i>Sporidesmium</i>	84	0.0188	238	0.133386	322	0.05144
45	<i>Stemophillium</i>	56	0.0125	84	0.047077	140	0.02236
46	<i>Stigmina</i>	784	0.1752	168	0.094155	952	0.15207
47	<i>Tetracoccosporium</i>	630	0.1408	1302	0.729698	1932	0.30861
48	<i>Tetraploea</i>	462	0.1032	826	0.462927	1288	0.20574
49	<i>Torula</i>	9030	2.0175	3290	1.84386	12320	1.96798
50	<i>Tricoconis</i>	280	0.0626	462	0.258925	742	0.11853
51	<i>Tryblidium</i>	574	0.1282	532	0.298156	1106	0.17667
			0		0	0	0
	<b>Total</b>	<b>245322</b>	<b>54.809</b>	<b>119014</b>	<b>66.70067</b>	<b>364336</b>	<b>58.1984</b>
	<b>Other type</b>						
1	Fungal hypae	15414	3.4437	4788	2.683405	20202	3.22703
2	Insect parts	4200	0.9384	4676	2.620636	8876	1.41784
3	Pollen grain	1638	0.366	5040	2.824637	6678	1.06673
4	Protozoan cyst	1806	0.4035	1036	0.58062	2842	0.45398
5	Algal filaments	952	0.2127	532	0.298156	1484	0.23705
6	Mites	84	0.0188	28	0.015692	112	0.01789
7	Plant part	6958	1.5545	5012	2.808945	11970	1.91207
8	unidentified	2436	0.5442	2968	1.663397	5404	0.86323
			0		0	0	0
	<b>Total</b>	<b>33488</b>	<b>7.4818</b>	<b>24080</b>	<b>13.49549</b>	<b>57568</b>	<b>9.19581</b>
	<b>Grand total</b>	<b>447594</b>	<b>100</b>	<b>178430</b>	<b>100</b>	<b>626024</b>	<b>100</b>



**CONCLUSION:**

It may be concluded that kharif airspora and pathogenic airspora has been found to be abundant may be because of favourable meteorological parameters like heavy rainfall, high humidity and moderate temperature while quite less during summer crop season due to hot condition with high temp., less humidity, accompanied by minimum scanty total rainfall. In kharif season, the Tikka disease caused by *Cercospora arachidicola* and rust by *Puccinia arachidis* were prominent whereas the traces of diseases were not encountered during summer season of groundnut field.

Thus meteorological parameters have been found to play detrimental role in incidence of airspora and due to heavy rainfall wet airspora has been found abundantly as compare to dry air spora apart from constant air spora.

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## RESEARCH ARTICLE

## INDOOR AIR QUALITY ASSESSMENT BY INVESTIGATION OF AIRBORNE FUNGI

Gayathri N, Kamtekar A and Bagkar P\*

Department of Biotechnology, Bhavan's College, Munshi Nagar, Andheri (West), Mumbai 400 058

### ABSTRACT

Airborne particles are a major cause of respiratory ailments such as allergies, asthma, and pathogenic infections of the respiratory tract in man. Continued exposure to indoor airborne mold and/or mycotoxin causes many multi-system adverse human health effects. Students and teachers spend a major portion of the day in their educational institutions. The present study is aimed at isolating and identifying fungal flora in the dust samples of various indoor environments in Bhavan's College, Andheri (West), Mumbai. The samples collected were inoculated onto Potato Dextrose Agar (PDA) and Sabouraud's agar (SAB) and incubated at room temperature for seven days. Factors such as temperature, relative humidity of the sampling site and the time of exposure were noted. Macroscopic and microscopic analyses of the colonies were carried out. The level of contamination of each site was expressed in percentage of the total number of fungal species obtained from the study. The observations indicate that the indoor fungal concentrations are highly variable as the samples collected from the chosen sampling sites harbor fungi of diverse species. Future investigations are needed to further examine the effects of these exposures on the related health problems and to devise methods to improve the indoor air quality.

**Keywords :** Air borne fungi, air quality, fungal flora, Bhavan's college

### INTRODUCTION

Indoor air is almost never free from allergens, bacteria, dust and fungal spores which originate due to various natural and anthropogenic activities (Al-Doory *et al.*, 1980; Lacey, 1981). Qualitative assessments of indoor fungi are valuable for assisting the evaluations of air quality and health hazards. Quantitative evaluation of fungal concentrations and exposure thresholds aid in evaluating and monitoring the impact of fungi on human health (Shelton *et al.*, 2002).

Fungal spores, which are ubiquitous, are associated with adverse and diverse health effects ranging from allergies to asthma (Chapman, 2006; Kurup *et al.*, 2000). More than 80 genes of fungi have been associated with respiratory tract ailments (Horner *et al.*, 1995). Organic dust in the air includes live or dead micro-organisms and allergens in addition to other antigens. Individuals may get sensitized to such bio-aerosols due to repeated continuous exposure.

College is a locale where there is an incessant stream of students and staff who spend more than half of their waking hours in the premises. In spite of regular and continuous movement of people, the surroundings is always kept clean and neat. Bhavan's College is situated in the Bharatiya Vidya Bhavan's Campus at Munshi Nagar, Andheri (West). The campus of the college, covering a vast area of 42 acres, is one of the biggest in Mumbai. The college is surrounded by

### \*Corresponding Author

Email: [dryeragi@reddifmail.com](mailto:dryeragi@reddifmail.com)

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a wide variety of trees, ornamental plants and shrubs. The objective of the present study was to assess the indoor air quality in terms of aeromycoflora in selected areas of the college. The outcome of the study would be a comprehensive resource that can be used for comparative purposes in future studies monitoring indoor air quality in the premises.

## MATERIAL AND METHODS

### Sampling Location

Samples were collected from six different collection sites - all within the campus of Bharatiya Vidya Bhavan, Andheri (W), Mumbai. The sample sites were chosen keeping in mind the wide variation in their characteristics. The sites were labeled as S1 to S6 as follows: Biotech classroom (S1), Library (S2), Canteen (S3), Biotechnology lab work place (S4), Biotechnology lab preparation room (S5), Botanical garden (S6). S1 is a confined entity which is well ventilated. S2 is repository of books and research journals which are prone to biodeterioration. S3 is a crowded site of day-long activity relating to food. S4 and S5 are aseptically maintained whereas S6 has a lot of greenery. The sampling conditions were 75-80% relative humidity and a temperature of 27-30°C.

### Sampling methods

Dust and air samples were collected from the above mentioned locations. Dust samples were collected using sterile cotton swabs. The collected dust samples were immediately transferred to 10 ml of sterile saline, from which 0.1ml was surface spread on Potato Dextrose Agar (PDA) and Sabouraud Agar (SAB) plates individually. Air samples from all the above mentioned sampling locations were collected using Gravity Sedimentation and Solid Impaction

methods to isolate fungal spores present in the air. Sterile Petri-plates containing PDA and SAB media were horizontally placed and kept open for 5 minutes for Gravity sedimentation. Hi-Air air sampler system, at a suction rate of 280 L/min, was used to collect the air samples for 3 minutes by solid impaction technique. The agar plates and strips were incubated at room temperature for 7 days and observed regularly for growth of organisms. The fungal growth on the plates was examined macroscopically for color and appearance of the colony and microscopically using lacto-phenol cotton blue. The isolates were maintained on agar slants for future studies. The number of colonies recorded is expressed as colony forming units (CFU) and calculated as follows:

$$\text{CFU/m}^3 = \frac{\text{Number of colonies obtained}}{40 \times \text{sampling time in minutes}}$$

$$\text{Relative Distribution} = \frac{\text{No of colonies of genera}}{\text{Total no. of colonies of all genera}} \times 100$$

## RESULTS & DISCUSSION:

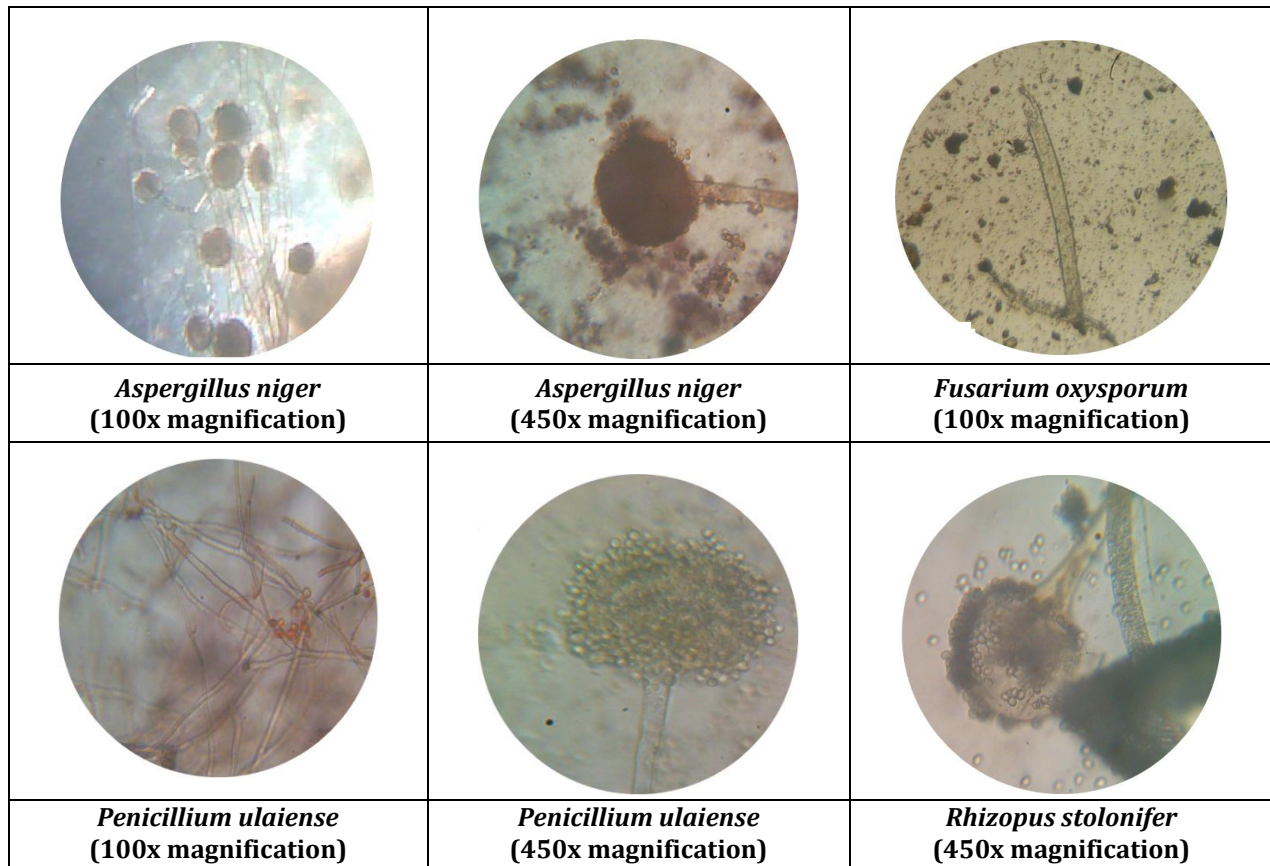
The plates and strips were observed for seven days for growth of fungal colonies. Visible fungal colonies were observed in all the media 72 hrs of incubation at room temperature. The fungal species obtained in the study were *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium ulaiense*, *Alternaria sp.* and *Rhizopus stolonifer* (Table 1, Figure 1). The fungal diversity as determined by both the methods are represented in figures 2 and 3 and the relative distribution of the observed fungal genera is shown in figure 4.

**Table 1: Fungal diversity in the six sampling sites**

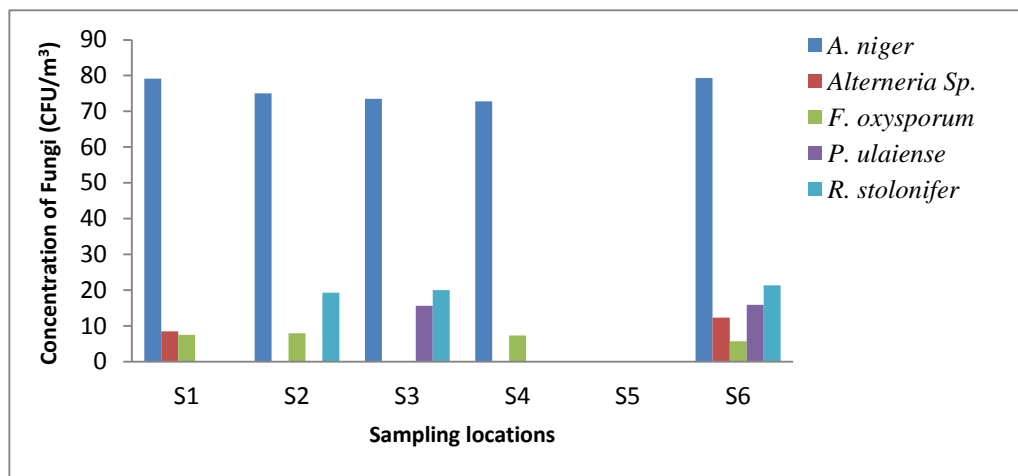
Sr. No.	Sampling Sites	Observed Fungal Species				
		<i>Aspergillus niger</i>	<i>Alternaria sp.</i>	<i>Fusarium oxysporum</i>	<i>Penicillium ulaiense</i>	<i>Rhizopus stolonifer</i>
1.	S1	+	-	-	-	-
2.	S2	+	-	-	-	-
3.	S3	+	-	-	+	+
4.	S4	-	-	-	-	-
5.	S5	+	-	-	-	-
6.	S6	+	+	+	+	+

+ indicates presence and - indicates absence of fungi.





**Figure 1: Photomicrographs of the observed Aeromycoflora**



**Figure 2: Prevalence of air borne fungi as determined by Solid Impaction method**

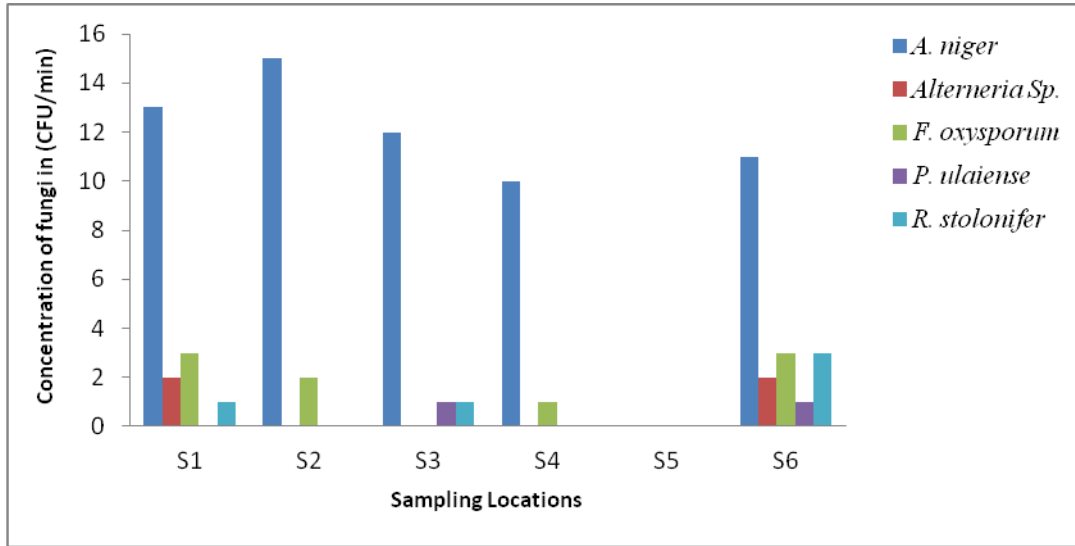
The ascomycete fungi are the most abundant type in the present study, *Aspergillus niger* being the most abundant fungal species observed in five of the six sampling sites.

Indoor air quality assessment, monitoring and maintenance are important because populations

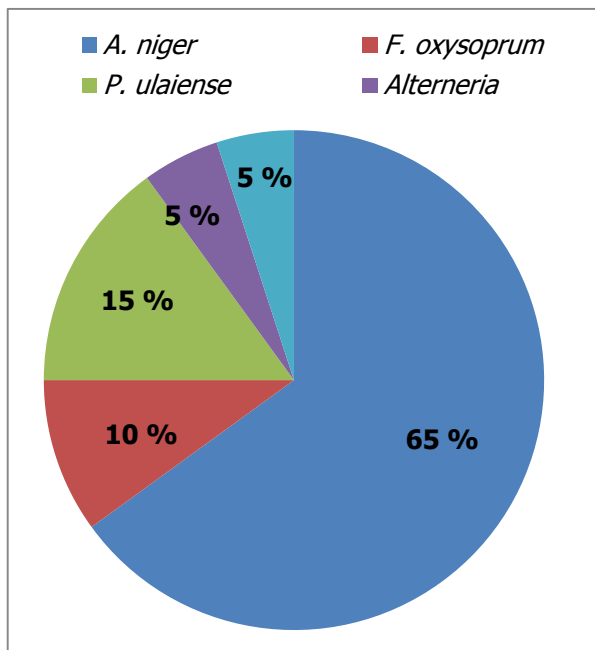
spend a substantial amount of time within the confines of buildings. Sampling requires that measured volume of air containing a representative fraction of the same kind of particles as in ambient air is collected (Burge, 1992). The samples for the study were taken from six different sites which were diverse in nature.







**Figure 3: Prevalence of air borne fungi as determined by Gravity Sedimentation method**



**Figure 4: Relative distribution frequency of air-borne fungi.**

The Biotechnology classroom is on the first floor of the new building and has very good ventilation whereas the Botanical garden is highly frequented by people throughout the day. This is clearly reflected in the prevalence of elevated spore levels in the latter. Age and type of Building are important predictors of indoor fungal spore concentrations (Fairs *et al.*, 2010)

and increased temperature has been associated with elevated indoor fungal spore levels (Ren *et al.*, 2001). High prevalence of mycoflora in the canteen and botanical garden is observed in this study. The Biotechnology Lab and the preparation rooms are aseptically maintained using disinfectants and these locations show the presence of no or minimal fungal spores. Where no indoor source of fungal spores is evident, indoor concentrations of individual spore types typically reflect outdoor concentrations and seasonal patterns. (Shelton *et al.*, 2002).

Based on the health hazards they may cause, the fungi isolated in this study can be classified as A and B. *Aspergillus* and *Fusarium* are grouped under Class A which includes fungi or their metabolites that are highly hazardous to health and hence require immediate attention if present in dwellings. *Alternaria* and *Penicillium*, on the other hand, are included under Class B, which may cause allergic reactions to occupants if present indoor over a long period. *A. niger* causes black mold disease in certain fruits and vegetables and some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins (Abarca *et al.*, 1994). Inhalation of large amounts of spores can be deadly due to serious lung disease aspergillosis. *F. oxysporum*, a soil inhabitant that degrades lignin and complex carbohydrates (Christakopoulos *et al.*, 1996; Rodriguez *et al.*, 1996),



are also beneficial plant endophytes. In the current study, this species is noted only in the botanical garden. *Alternaria* species and *Penicillium ulaiense* are known plant pathogens and common allergens in humans growing indoors. The former readily cause opportunistic infections in immune-compromised people. *Rhizopus stolonifer*, commonly found on bread surfaces from where it takes its nutrients, is a plant pathogen.

### CONCLUSION:

Aerobiological sampling attempts to both identify and quantify allergenic particles in the air. The present study has clearly demonstrated the prevalence of *A. niger*. Only one of the six sampling sites in the study, the botanical garden, shows the presence of five different varieties of fungal spores. Persistent dampness may be the reason for this. It was seen that the vegetative forms and spores of all the isolated fungi cannot withstand 1:200 dilution of local disinfectants and hence can be effectively used against fungal contaminants. Seasonal variations of the air-mycoflora is planned to be studied.

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## RESEARCH ARTICLE

## AEROMYCOLOGICAL STUDY OF APMC FRUIT MARKET OF VASHI

Baviskar RN<sup>1</sup> and Suryawanshi NS<sup>2</sup><sup>1</sup>Department of Botany, ICLES' M. J. College, Vashi, Navi Mumbai.<sup>2</sup>Research Laboratory, Department of Botany, K. V. Pendharkar College, Dombivli (E)-421203 (MS) India.

## ABSTRACT

A present survey of aeromycoflora of APMC fruit market of Vashi, Navi Mumbai was conducted during two successive seasons from August to October, 2011 and August to October, 2012. During this period Apple (*Pyrus malus* L.) are abundant in the market of Vashi. The aeromycological study was carried out by using gravity slide as well as petriplate exposure method with a view to correlating the decay of Apples in the market. Twenty five microfungi were trapped from the air over the fruit market and the *Penicillium expansum* and *Aspergillus sp.* were found to be dominant.

**Keywords :** Apple, APMC fruit market, aeromycoflora.

## INTRODUCTION

Present investigation deals with the study of aeromycoflora of APMC fruit Market, Vashi. Keeping in this view attempt was made to investigate the aeromycoflora on apple in Vashi fruit market and observed twenty five fungi viz. *Sphaeropsis pyriputrescens*, *Venturiainaequalis*, *Botrytis cinerea*, *Alternaria alternata*, *Mucor piriformis*, *Aspergillus fumigatus*, *A. flavus*, *A. tenuis*, *A. niger*, *Phytophthora cactorum*, *Phytophthora parasitica*, *Sclerotinia fructigena*, *Rhizopus nigricans*, *Rhizopus stolonifer*, *Rhizopus sarrhizus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium digitatum*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium solitum*, *Penicillium commune*, *Penicillium regulosum*, *Penicillium expansum* and *Curvularia lunata*. Above twenty five fungi were pathogenic as well as non-pathogenic. The pathogenic fungi viz. *Penicillium expansum* and *Aspergillus niger* were dominant and serious on apple fruits. It is very severe and causing blue mold and *Aspergillus* rot respectively.

The wide variety of biological particles present in the atmosphere, there is a very significant number of fungal spores. The biopollutants of the atmosphere are causing serious diseases of crops in the vegetable and fruit markets. These agricultural commodities are being attacked in their post harvest conditions viz. in packaging, transit, trans-shipment and storage. Many workers investigated the occurrence of aeromycoflora in the different crop field and their correlation with the different diseases of fruits viz (Papaya, banana, citrus and pineapple), cereals (rice, jawar, wheat and bajara), sugarcane etc. (Tilak and Kulkarni, 1980; Sharma and Bhattacharjee, 2001; Medhi and Sharma, 2010) studied the aeromycoflora in the fruit markets. Apples in the markets of Vashi were reported to be decayed due to the invasion of certain microbes. In view of the above reports major vegetable and fruit markets of Vashi, Navi Mumbai was surveyed from aeromycological point of view. Chenulu and Thakur (1968) reported that *Aspergillus niger* and *Rhizopus oryzae* were considered to be responsible to cause major diseases in various fruits in Delhi market. Aeromycoflora were largely determined by topography, meteorological parameters, vegetation and biotic factors including human activities. The study of fungal aerospora of market may have some implications on the health of people working in the

**\*Corresponding Author**

Email: [baviskar\\_ramesh@yahoo.co.in](mailto:baviskar_ramesh@yahoo.co.in)

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market, customers, sellers, etc. Keeping in view of the above, an attempt was made to investigate the occurrence of aeromycoflora and the incidence of diseases of these useful fruit. Among the various pathogens *Aspergillus niger* and *Penicillium expansum* is an important post harvest disease of apple and it's responsible for most losses that occur in most commercial store rooms (Spottset *al.* 1999) found to be dominant in the store houses of local and central fruit markets of various places of Maharashtra, particularly in Mumbai and Navi Mumbai (APMC Market, Vashi) in packing boxes noted different damages of apple.

## MATERIAL AND METHODS

The consecutive survey was carried out from August to October, 2011 and August to October, 2012. In the APMC fruit Market of Vashi. Air samplings in the fruit market of apple at two weeks intervals using Gravity slide and Petriplate exposure methods using Czapek'sDox Agar Medium. Petriplate were exposed to the air in fruit market at different time intervals such as 0, 5, 10 and 15 minutes and at different heights i.e. 0 levels (ground level), 500cm, 1000cm and 2000cm above ground level for trapping aeromycoflora. These agar plates were incubated at (28 ±1) °C for 7 days. After seven days colony character, culture pattern were studied and identified different aeromycoflora using literatures. Total twenty five fungi were found in APMC fruit market Vashi at different height and time interval were considering the study of aeromycoflora. (Sreeramulu, 1959; Asanet *al.*, 2002; Uddin, 2004).

## RESULTS & DISCUSSION:

A total of twenty five mycoflora were trapped and observed from the air of APMC fruit market Vashi. There are twenty five fungi were noted viz. *Sphaeropsispyriputrescens*, *Venturiainaequalis*, *Botrytis cinerea*, *Alternaria alternata*, *Mucor piriformis*, *Aspergillus fumigatus*, *A. flavus*, *A. tenuis*, *A. niger*, *Phytophthora cactorum*, *Phytophthora parasitica*, *Sclerotina fructigena*, *Rhizopus nigricans*, *Rhizopus stolonifer*, *Rhizopus arrhizus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium digitatum*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium solitum*,

*Penicillium commune*, *Penicillium regulosum*, *Penicillium expansum* and *Curvularia alunata* using gravity slide and agar plate exposing method. Agar plates were exposed at 0, 5, 10, 15 minutes at different levels and accordingly the mycoflora were trapped.

**Table 1:** Frequency of occurrence of mycoflora at different height (cm) in the market of apple.

Fungi	Height (cm)			
	Ground level(0)	500	1000	2000
<i>Sphaeropsispyriputrescens</i>	+++	++	+	-
<i>Venturiainaequalis</i>	+++	++	+	+
<i>Botrytis cinerea</i>	+++	++	+	-
<i>Alternaria alternata</i>	++++	+++	++	+
<i>Mucorpiriformis</i>	+++	++	+	-
<i>Aspergillusfumigates</i>	++++	+++	++	+
<i>Aspergillus flavus</i>	++++	+++	++	+
<i>Aspergillustenuis</i>	++++	++	++	+
<i>Aspergillus niger</i>	++++	+++	++	+
<i>Phytophthoracactorum</i>	+++	++	++	+
<i>Phytophthoraparasitica</i>	+++	++	++	+
<i>Sclerotinafructigena</i>	+++	++	++	+
<i>Rhizopusnigricans</i>	+++	++	+	-
<i>Rhizopus. Stolonifer</i>	++++	+++	++	-
<i>Rhizopusarrhizus</i>	+++	++	+	-
<i>Penicilliumchrysogenum</i>	+++	++	++	+
<i>Penicilliumcitrinum</i>	+++	++	++	+
<i>Penicillium digitatum</i>	++++	+++	++	+
<i>Penicilliumfuniculosum</i>	++++	+++	++	+
<i>Penicillium italicum</i>	+++	++	++	+
<i>Penicilliumexpansum</i>	++++	+++	++	+
<i>Penicilliumsolitum</i>	+++	+++	++	+
<i>Penicillium commune</i>	+++	++	++	+
<i>Penicilliumregulosum</i>	+++	++	+	+
<i>Curvularia lunata</i>	++++	+++	+	+

N.B. = +: 25 per cent frequency of occurrence of fungal species; ++ : 50 per cent frequency of occurrence of fungal species; +++ : 75 per cent frequency of occurrence of fungal species; ++++ : 100 per cent frequency of occurrence of fungal species.

The fungal spores settled down on agar plate at different level and at different time intervals shown in Table 1 and Table 2. *Sphaeropsis pyriputrescens*, *Botrytis cinerea*, *Rhizopus nigricans*, *R. stolonifer*, *Rhizopus arrhizus*, *Mucor piriformis*, were not found at the height of 2000cm.



Table 2: Frequency of occurrence of mycoflora at different periods of exposure in the fruit market of apple

Fungi	Different Period of exposure (in minutes)			
	0	5	10	15
<i>Sphaeropsisriputrescens</i>	-	+	++	+++
<i>Venturiainaequalis</i>	-	+	++	++
<i>Botrytis cinerea</i>	-	+	+++	+++
<i>Alternaria alternate</i>	-	++	+++	++++
<i>Mucorpiriformis</i>	-	+	++	++
<i>Aspergillus fumigatus</i>	-	++	++	++++
<i>Aspergillus flavus</i>	-	++	+++	++++
<i>Aspergillus tenuis</i>	-	+	+++	+++
<i>Aspergillus niger</i>	-	++	+++	++++
<i>Phytophthora cactorum</i>	-	+	++	+++
<i>Phytophthora parasitica</i>	-	+	++	+++
<i>Sclerotinia fructigena</i>	-	+	++	+++
<i>Rhizopus nigricans</i>	-	+	++	+++
<i>Rhizopus. Stolonifer</i>	-	+	++	++++
<i>Rhizopusarrhizus</i>	-	+	++	+++
<i>Penicilliumchrysogenum</i>	-	+	++	+++
<i>Penicilliumcitrinum</i>	-	+	++	+++
<i>Penicillium digitatum</i>	-	+	+++	++++
<i>Penicilliumfuniculosum</i>	-	+	+++	++++
<i>Penicillium italicum</i>	-	+	++	+++
<i>Penicilliumexpansum</i>	-	++	+++	++++
<i>Penicilliumsolutum</i>	-	+	++	+++
<i>Penicillium commune</i>	-	+	++	+++
<i>Penicilliumregulosum</i>	-	+	++	+++
<i>Curvularia lunata</i>	-	+	++	+++

N.B. = +: 25 per cent frequency of occurrence of fungal species; ++ : 50 per cent frequency of occurrence of fungal species; +++ : 75 per cent frequency of occurrence of fungal species; ++++ : 100 per cent frequency of occurrence of fungal species.

The most dominant aeromycoflora on agar plate were observed in Vashi fruit market. *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *A. niger*, *P. funiculosum*, *P. digitatum*, *Rhizopusstolonifer* and *Penicillium expansum*. *Aspergillusniger* and *Penicillium expansum* were found serious on apple and were recorded at different height. Most of aeromycoflora *Sphaeropsis pyriputrescens*, *Venturia*

*inaequalis*, *Botrytis cinerea*, *Alternaria alternate*, *Mucorpiriformis*, *Aspergillus fumigatus*, *A. flavus*, *A. tenuis*, *A. niger*, *Phytophthora cactorum*, *Phytophthora parasitica*, *Sclerotinia fructigena*, *Rhizopus nigricans*, *Rhizopus stolonifer*, *Rhizopusarrhizus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium digitatum*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium solutum*, *Penicillium commune*, *Penicillium regulosum*, *Penicillium expansum* and *Curvularia lunata* were observed at ground level and followed by 500, 1000 and 2000cm. similarly aeromycoflora occurrence at different time period. The maximum number of fungi were noted at 15 minutes time intervals and followed by 10, 5 and 0. Mycoflora were not settled on agar plate as compared to 15 minutes. Similar reports were illustrated by Lim *et al.* (1980) and Padmanavan *et al.* (1953).

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## RESEARCH ARTICLE

## INTRAMURAL AEROMICROBIOTA OF LIBRARY AT PUNE, MAHARASHTRA, INDIA.

**Jogdand SB and Ingole AC\***

Science Research Centre, Dept. of Botany, BVDU, Y. M. College, Erandawne, Pune-411038.

### ABSTRACT

Intramural aerobiological investigations have been carried out using Rotorod air sampler for a period of six months from 1<sup>st</sup> January 2013 to 30<sup>th</sup> June 2013 in the college library. Totally Sixty three aerobiocomponents have been encountered including eight other types and fifty fivespore types belonging to Phycomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina have been encountered. Half yearly findings revealed Aspergilli(23%) to be dominant followed by *Cladosporium* (22%) and *Nigrospora*(18%). Fourteen types of ascospores which were absent from January to May 2013 have been reported in the month of June due to rain fall(48 mm) responsible for their release, higher RH(82%) and moderate temperature(19°C). They act as bioindicators for the rain fall, increase in RH and decrease in temperature. Thus environmental parameters play determinantal role in the aerospora. Class wise percentage contribution of library aerospora in the order of dominance have revealed Deuteromycotina (63.39 -24,055/m<sup>3</sup>) followed by other types (21.975-8342/m<sup>3</sup>), Basidiomycotina(8.99-3415/m<sup>3</sup>),Ascomycotina (5.281-1890/m<sup>3</sup>) and Phycomycotina (0.355-135/m<sup>3</sup>). However, Myxomycotina members have not been found during the study period. The smut spores have been obtained during the study and the probable source has been found to be the smut disease of *Cynodon* grass around the library. The prominent health hazards among the library personnel and users have been noticed so far and damage to the library material have been worked out.

**Keywords** Intramural environment, Aerospora, Air sampling, Biodeterioration, Allergy, Bioterriogens and Allergens.

### INTRODUCTION

People spend their most of the day time in indoor environment. Assessment of indoor air quality is of chief and immense importance, during this six months study. Aeromycology of the intramural environment constitute one of the major aspects mainly because of the dominance of fungal spores in the aerospora (Tilak, 1991). The number of fungal spore types and their diversity vary with time of day, weather, seasons,

Geographical location and the presence of local sources. The highest number of airborne spores was found in temperate and tropical region and the least in desert. (Lacey, 1981). There is impact of aerobiocomponents on plants, animals and human beings (Agarwal et al., 1969).

Since fungal spores have been identified as a major cause of bio deterioration of all kinds of stored products hence cannot be over looked throughout the world, leading to both qualitative and quantitative loss. Library materials like cellulose form the basic constituent of papers, glue in bounded books and leather as a bonding materials, all forming an ideal substrate for growth, sporulation and proliferation of fungi. Inhalation of mold spores dispersed from moldy books during handling was a common process. The

#### \*Corresponding Author

Email: [jogdand.sudam@gmail.com](mailto:jogdand.sudam@gmail.com)

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occurrence of dermatitis, respiratory and cardiac diseases along with allergic manifestations in library handlers and workers is a major problem. Therefore this study on air borne fungi has being conducted in the YMC library of Pune having 160 km distance from Mumbai located toward the southern direction at the Latitude 18°32' N, Longitude 72° 51' E and at Altitude 560m (1840 ft) above sea level.

## MATERIAL AND METHODS

The site selected was library of Yashwantrao Mohite College B.V.D.U., Erandawne, Pune. Material for the experiment is the books, racks, papers, periodicals and intramural aeromicrobiota grown over them which is studied by air sampling method using Rotorod air sampler of Perkins (1951) and modified by Harrington (1957). It consists of battery operated motor which rotates at 2300rpm. The two brass rods (6.2cm long) fixed on horizontal arm connected to motor at 8cm distance from each other oriented at right angles to dashing air at high velocity. Petroleum jelly coated two cello fane tapes were fixed on the two arms of the sampler.

Air sampling was carried out by Rotorod air sampler which have been operated daily for half an hour (between 2pm and 2.30 pm), in the YMC library at 1 meter height from ground level, from 1<sup>st</sup> January

2013 to 30<sup>th</sup> June 2013. The two strips of loaded (deposited) cello fane tapes from Rotorod sampler were mounted on a clean slide using melted glycerin jelly in the laboratory. The total number of spore/m<sup>3</sup> of air at that particular site and height of that time was obtained by multiplying each spore types by its conversion factor (5).

## RESULT

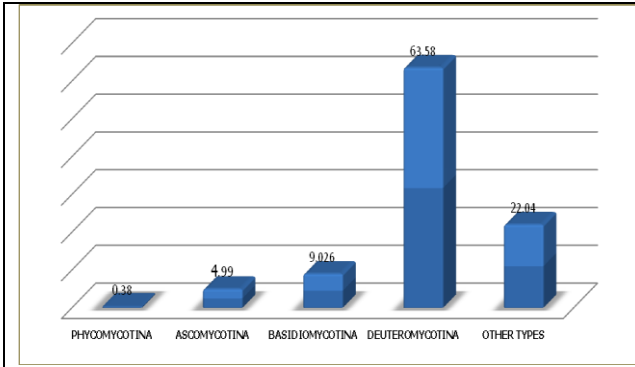
Qualitative and quantitative analysis of intramural aeromicrobiota revealed 63 types of airborne biocomponents amounting to 37,962 spore/m<sup>3</sup>. The totally 55 fungal spore types have been reported amounting to 29,620 spore/m<sup>3</sup> and 8 other types (8,342/m<sup>3</sup>).

Out of 55 types of fungal spores in the order of dominance, 37 belonged to Deuteromycotina (63.58%-24,055 spore/m<sup>3</sup>), 3 to Basidiomycotina (9.025%-3,415 spore/m<sup>3</sup>), 14 to Ascomycotina (4.995%-1,890 spore/m<sup>3</sup>) and 01 type belonged to Phycmycotina (0.357%-135 spore/m<sup>3</sup>). Eight other types (22.05%-8,342/m<sup>3</sup>) have been reported in the order of dominance including cellulose fibers (6.264%), fungal hyphae (5.167%), epidermal hairs (4.876%), insect wings (2.59%), insect scales (1.995%), Pollen grains (0.965%), algal filaments (0.172%) and unidentified types.

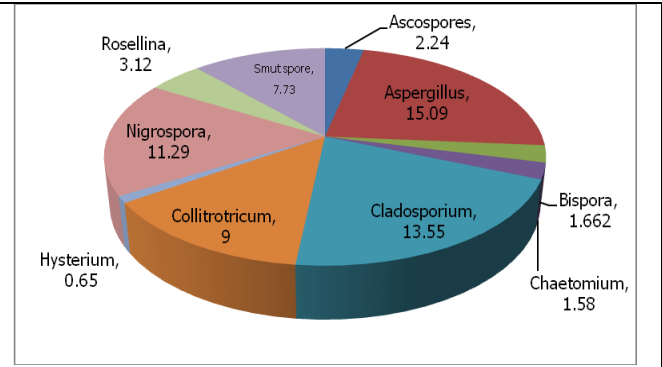
**Table 1:** Average percentage contribution of some of the dominant fungal spores to the total aerospora during six months (i.e. January to June 2013) from library of YMC.

Sr. No.	Types of spores	January	February	March	April	may	June	Total	Average %
1.	Ascospores	0	0	0	0	0	2.24	2.24	2.24
2.	Aspergillus	14.1	16	14	12.04	15.4	19	90.54	15.09
3.	Bispora	3.7	2.03	1.4	0.78	1.09	0.97	9.97	1.61667
4.	Chaetomium	2.7	0.3	0	0	0	1.73	4.73	1.576667
5.	Cladosporium	16.3	15	14.46	14	4.54	17	81.3	13.55
6.	Collitroticum	9	0	0	0	0	0	9	9
7.	Hysterium	0	0	0	0	0	0.65	0.65	0.65
8.	Nigrospora	8.69	14.01	10	10	11	14	67.7	11.28333
9.	Rosellina	0	0	0	0	0	3.12	3.12	3.12
10.	Smut spore	10	8.21	7.6	8.1	6.18	6.3	46.39	7.731667



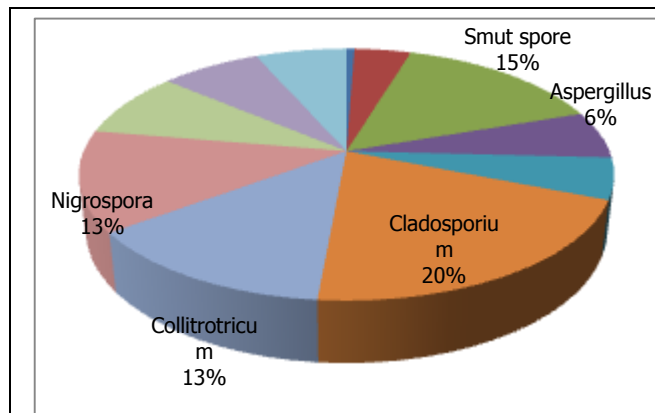


**Fig 1:** Average class wise percentage contribution of aerospora to the total aerospora during `study period (six months i.e. January to June 2013)

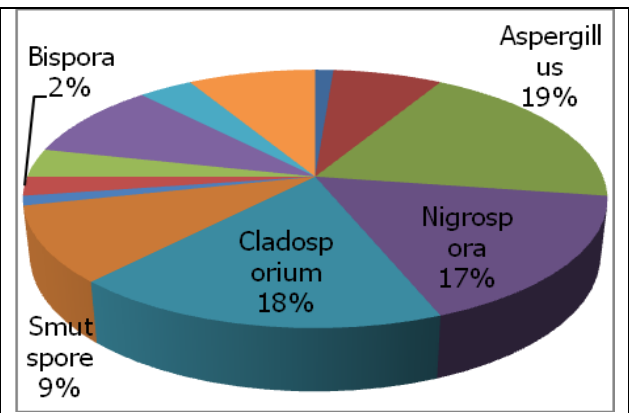


**Fig. 2:** Average percentage contribution of some of the dominant fungal spores to the total aerospora during six months (i.e. January to June 2013) from library of YMC.

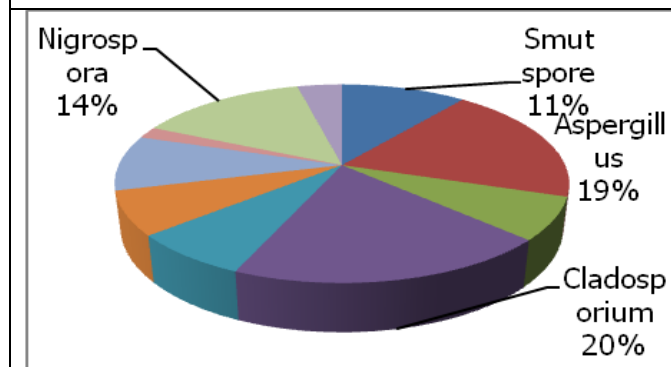
**Fig. 2 to 7:** Month wise quantitative analysis of intramural environmental aerospora during six months (i.e. January to June 2013) from YMC library.



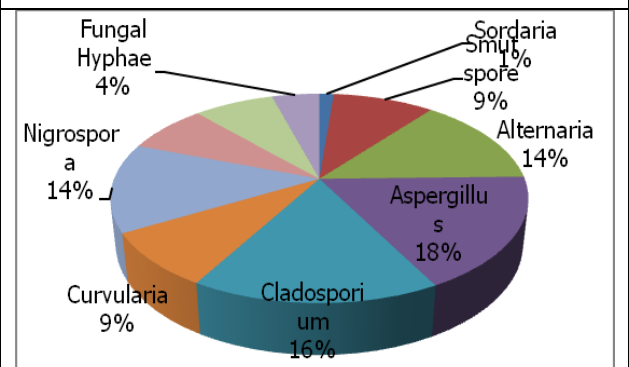
**Fig. 3:** Percentage contribution of aerospora in January 2013.



**Fig. 4:** Percentage contribution of aerospora in February 2013.



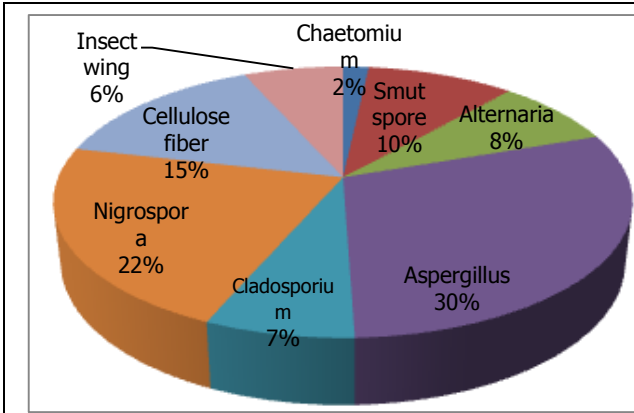
**Fig 5:** Percentage contribution of aerospora in March 2013.



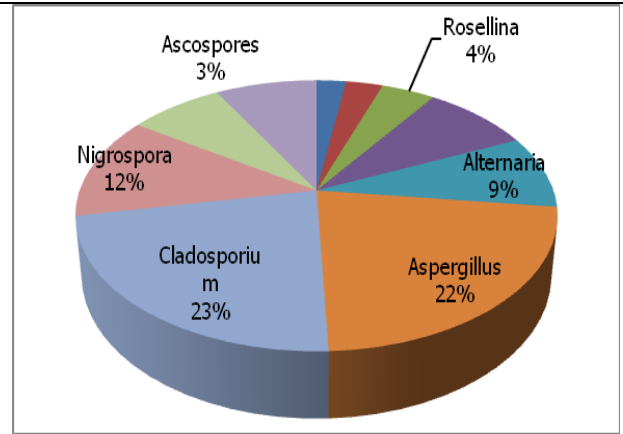
**Fig 6:** Percentage contribution of aerospora in April 2013





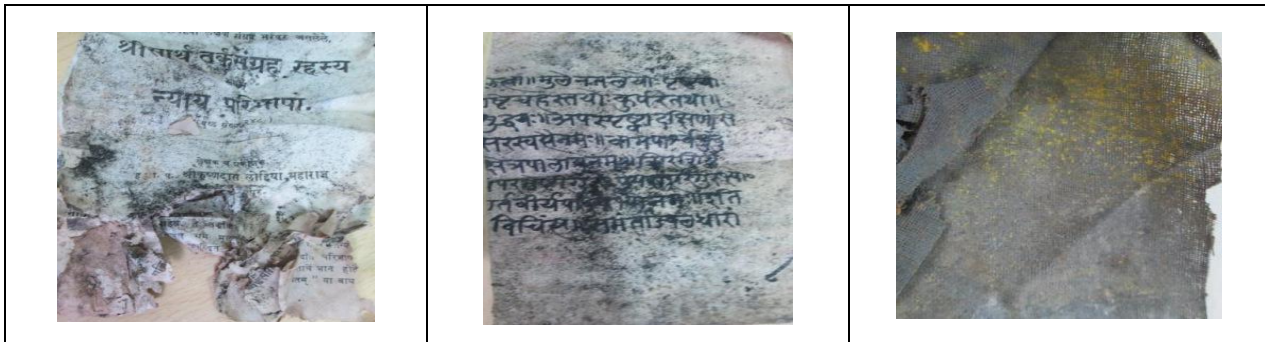


**Fig 7:** Percentage contribution of aerospora in May 2013.



**Fig 8:** Percentage contribution of aerospora in June 2013.

**Fig 9 :** photographs of deteriorated books pages and bonding materials:



Average Percentage contribution of fungal spores in the order of dominance have been recorded during six months such as *Aspergillus* spp (15.09%), *Cladosporium* (13.55%), *Nigrospora* (11.29%), *Smut spore* (7.76%) while other spore types revealed less percentage contribution. Fourteen ascospore types have been recorded only in June after the rain fall (48mm) in the order of dominance such as *Rosellinia*, unidentified *ascospores*, *Chaetomium*, *Didymosphaeria*, *Sordaria*, *Leptosphaeria* etc. These spores acted as bioindicator of rain fall.

**DISCUSSION:**

The present aerobiological investigation was undertaken to study the intramural environmental aeromicrobiota from library located at Yashwantrao Mohite College, Pune. The study was carried out to find out cause of biodeterioration of stacked books,

news papers, journals, periodicals, paper materials and wooden book racks, due to airborne microbes, which cause health hazards among the students, readers, workers and book handlers due to inhalation of airborne microbes from the book materials.

In present study *Aspergillus* spp (15.09%), *Cladosporium* (13.55%), *smut spores* (7.76%) were dominant. According to Takahashi (1997), Sen and Asan (2001) and El-Morsy (2006) *Cladosporium* spores dominate the aerospora in hot climates. These results coincide with our findings. According to Shaheen (1992) the abundance of *Cladosporium* throughout the year may be attributed to the structural features of the spores such as small size, thin exine and smooth wall which favour and facilitated the transport of aerospora.

The impact of environmental parameters on aerospora during January 2013 revealed increase



representing 50 fungal spore types at average temperature 21.3°C and RH 67.6%. Decrease in temperature accompanied by rise in RH have been found responsible for the increase in aerospora. While during April 2013 rise in average temperature (29.5 °C) and fall in RH (44.5 %) have been found responsible for decrease of aerospora representing 33 fungal spore types. Thus environmental parameters proved profound impact on the aerospora clearly. The smut spores have been obtained during the study and the probable source has been found to be the smut disease of Cynodon grass around the library.

Fourteen types of ascospores which were absent from January to May 2013 because of absence of rains have been reported in the month of June only due to rain fall (48 mm), higher RH (82%) and moderate temperature (19°C). They act as bioindicator for the rain fall, increase in RH and decrease in temperature. Thus environmental parameters play determinantal role in the release of ascospores and total aerospora. The various biodeteriogens obtained during the study have been found to cause biodeterioration of books, papers as evidenced from the damaged papers shown below, binding materials, threads and fabrics etc. especially during rainy season when these goods were stored uncared in damp places, leading to health disorders among the visitors. (Fig 9.)

## CONCLUSION:

Findings revealed 63 types of aerobiocomponents causing biodeterioration of library materials as evidenced by damaged valuable ancient literature major biodeteriogens recorded are *Cladosporium*, *Aspergillus*, *Chaetomium*, *Alternaria*, *curvularia* etc. The visitors have been found suffering from various health disorders like itching, sneezing, cough, fever etc. due to these airborne microbes leading to allergic manifestations.

Hence, this work is significant for protection of valuable ancient literature, management of biodeteriogens and health conservation of visitors.

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## RESEARCH ARTICLE

## COMPARATIVE STUDY OF AEROMYCOFLORA OF TWO PUBLIC LIBRARIES

Yeragi SS, Yeragi PS and Prabhu VN

K. J. Somaiya College of Science &amp; Commerce, Vidyavihar, Mumbai – 400077.

## ABSTRACT

A systematic aero mycological survey was conducted in Mumbai Marathi Granthalay at Mulund and G. K. Khandekar Granthalay Mulund, Suburb of Mumbai. Both the libraries are associated with public reading facilities; hence many readers visit to these libraries. This investigation was carried out for a period of one year from June 2011 to May 2012. For trapping the fungal spores petri plate method was adopted. The result showed incidence of varieties of mycoflora in the environments of both the libraries. Total fifteen fungal spore types have been identified during the period of investigation.

In Mumbai Marathi Library twelve fungal genera were identified. The most dominant genera were *Aspergillus* (25.38%), followed by *Cladosporium* (14.59%), *Penicillium* (13.73%), *Curvularia* (8.12%), and others.

In Khandekar library, total ten fungal genera were isolated. The most dominant genera were *Aspergillus* (22.51%), followed by *Cladosporium* (15.72%), *Penicillium* (12.34%), *Alternaria* (16.35%), etc. In both the cases September was the month of highest incidence. The month of September was dominated by *Cladosporium* spp in both the library. It was observed that both the library were at ground floor and directly exposure to the atmosphere. It is but natural, that the fungal spores get easy to enter in the premises of library. Mumbai Marathi Library having more humidity hence fungal spores were dominating and deteriorating the books heavily than other library. Pre-monsoon is the least infested period in both the libraries.

**Keywords** : Library, Aeromycoflora, Mulund

## INTRODUCTION

Now-a-days, public related libraries are the direct exposure to the atmosphere hence huge number of mycoflora easily gets way into it affecting the health of workers as well as deterioration of books. In both the libraries, there is a 5-10% scrap of books every year due to infestation of fungal flora.

During monsoon, humid condition of indoor is more, which enhance the luxurious growth of biofouling fungi. Mumbai Marathi Library is more exposed to fungal spores because of leakages in the building than G. K. Library. Both the Libraries are on ground floor due to which the mycoflora easily introduced in the libraries. To stop this major loss of books, the college libraries are setup under air-conditioned. In Air-conditioned environment it is notified that the loss of books is least compare to open Library. In public libraries, low cost books are affected heavily to mycofloral infestation.

The study of airborne fungal flora is known as aeromycology. The intramural study of fungal spore is of immense importance due to its role in the field of



human allergy, plant diseases and also due to microbial deterioration of the materials like library books, textiles, printed surfaces etc. such type of work is carried out by Agarwal, (1974), Tilak (1976).

Cellulose, a main constituent of paper is susceptible to degradation by many species of fungi and bacteria. Other components of paper is glue or casein also serves as substrate for fungi. Under favourable conditions, the paper may be stained or discolored by the product of microbial metabolism and ultimately complete destruction of paper. Our Indian, books are more susceptible for such activities hence every year major loss is there. The study of airborne fungal spores inside the libraries have been carried out in many parts of our countries, Vinod (2012), in Mukherjee (1973), Tilak (1984) etc.

The atmosphere of library is very suitable for easy growth of fungi due to low light, intensity, humidity and lack of cleanliness. All such factors, prove helpful in the qualitative and quantitative increase of fungal flora inside the library. Pesticide, cleanliness and fully air-condition could stop the loss of books by deterioration.

## MATERIAL AND METHODS

The present investigation was carried out for one year from June 2011 to May 2012. Air sampling was carried out by petriplate method using Rose-Bongol Agar media (RBS). The plates were exposed for 15 minutes at a place and 5 feet above the ground, thrice in a week. Identification was mainly based on their morphological character.

## RESULTS & DISCUSSION:

In the present study altogether fifteen fungal types of colonies have been noticed (Table.1) of the maximum fungal spores were observed in M.M. Library. The common dominant fungal spores were *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria*, *Rhizopus*, in both the libraries during the survey period. July-August were the peak months and highest incidence of fungal flora for both the library.

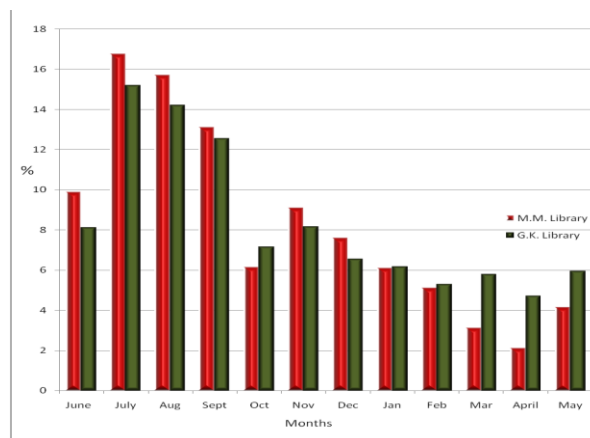
In M.M. Library, a total twelve fungal types have been recorded during the study period. The dominant fungal spores were *Aspergillus Spp* (25.38%) followed by speaks of *Cladosporium Spp* (14.59%), *Penicillium*

*Spp*(13.73%), sterile hypae (6.02%) unidentified (6.03%) and others ranged between 2-3% only.

**Table 1:** Percentage composition of fungal colonies in the libraries for the period of one year.

Sr. No.	Fungal type	M.M. Library %	G.K. Library %
1.	<i>Aspergillus Spp</i>	25.38	22.51
2.	<i>Alternaria Spp</i>	--	16.35
3.	<i>Penicillium Spp</i>	13.73	12.34
4.	<i>Cladosporium Spp</i>	14.59	15.72
5.	<i>Rhizopus Spp</i>	8.13	5.42
6.	<i>Bipolaris Spp</i>	3.18	--
7.	<i>Cunninghamella Spp</i>	2.17	--
8.	<i>Curvularia Spp</i>	8.12	6.47
9.	<i>Epicoccum Spp</i>	2.15	1.21
10.	<i>Fusarium Spp</i>	2.11	--
11.	<i>Trichoderma Spp</i>	3.14	2.29
12.	<i>Tricothesium Spp</i>	2.16	1.51
13.	<i>Apophysomysis Spp</i>	3.09	3.01
14.	Sterile hyphae Spp	6.02	8.12
15.	Unidentified Spp	6.03	5.11

**Comments:** requires thorough editing by English faculty, then accepted for publication.



**Fig.1:** Mean month wise percentage contribution of fungal colonies in both libraries.

In G.K. Library total ten fungal colonies types have been recorded during investigation time. In this also the species of *Aspergillus* (22.51%) *Alternaria Spp* (16.35%), *Cladosporium Spp* (15.72%), *Curvularia Spp* (6.47%) sterile hypae (8.12%) unidentified (5.11%) and remaining ranged between 1-5% only.



The result was also tested bio-statistically using the null hypothesis ( $H_0$ ), t-test has been used between the data of two libraries. It has been found that the calculated value is less than table value.

### CONCLUSION:

In the present investigation, noticed that the fungal growth were maximum in open library than closed. Leakages make the walls in wet condition accelerating easy sedimentation of fungal spores. The books should be also kept in closed cupboards. It is also noticed that air-conditioned library unfavore for settlement of mycoflora. Allergenic *Aspergillus*, *cladosporium*, *Penicillium* are well known (Tilak 1976) so in the light of collected data cleanliness and repair is suggested to minimizes the harmful effect of fungi for the heath of library staff and readers.

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## RESEARCH ARTICLE

## INVESTIGATION ON AIRBORNE MOULDS IN GOVERNMENT HOSPITAL WARDS OF WARUD CITY, AMRAVATI DISTRICT

Jadhao Rajusing<sup>1</sup>, Ghaware Ashish<sup>1</sup>, Chavhan Arvind<sup>1</sup>  
and Pawar Santosh<sup>2\*</sup>

<sup>1</sup>Department of Zoology Shri Shivaji Science College, Amravati – 444603, India

<sup>2</sup>Department of Zoology, Govt. Vidarbha Institute of Science and Humanities, Amravati, India.

### ABSTRACT

The airborne culturable moulds and spor types in the indoor environment of Govt. hospital wards of Warud were monitored over one year period from August 2011 – November 2012, using a two stage Andersen air sampler and Burkard personal slide sampler. Among culturable moulds *Cladosporium herbarum* and *Penicillium citrinum* were most frequently recorded. Aspergilli/Penicilli among airborne conidial types was most frequently recorded. The percentage of occurrence of *mycoflora* was higher in General Ward in comparison to Post Surgical Ward of hospital.

**Keywords :** Airborne moulds, *Cladosporium herbarum*, *Penicillium citrinum*, *mycoflora*. Warud.

### INTRODUCTION

Environment plays an important role in the precipitation of allergic symptoms. With rapid industrialization and urbanization the air is becoming polluted with different bio aerosols and this has increased the risk of allergic disorders. Many surveys have been conducted to elucidate the magnitude of the problem. Since the 1960's the prevalence of asthma has been increasing and this increased prevalence is associated with a high degree of sensitization to inhalant allergens. (Pandey & Mishra, 2011) Many airborne fungal spores are important as allergens, human and plant pathogens and in the bio-deterioration of stored materials as well as spoilage of food stuffs.

The vast and diverse geographical region essentially demands the preparation of a list and calendar of allergenic fungal spores, since the allergens differ to a certain extent from region to region. India being located between 7 0 N to 63.6 0N

and 67 0 E to 98 0 E, is bestowed with a wide range of biomass. Such diversity in the vegetation contributes an enormous variation in the quality and quantity of airborne fungal spores from different parts of India. Accordingly, the incidence and symptomatology of allergic patients from different parts of India is extremely variable. Despite this, no comprehensive survey of mould spores in hospital wards of Warud city is available. Hospitalized patients have a potential risk for nosocomial infections (Martins-Diniz et al., 2005). Airborne fungi are one of the main causes of fungal infections in this group. The objective of this study was to investigate the concentration and species of airborne fungi in hospital wards of Warud city of Amravati District. Hence, for the first time a qualitative and quantitative survey of atmospheric fungal spores of hospital wards of Warud city of Amravati District was undertaken and the results of which are described in this communication.

### MATERIAL AND METHODS

Air sampling of hospital wards was carried out by using together an Andersen sampler and a Burkard Personal Volumetric sampler. Three samples approximately at ten days interval in a month were

#### \*Corresponding Author

Email: [sspawar@yahoo.com](mailto:sspawar@yahoo.com)

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taken from General ward and Post-surgical wards of hospital from August 2012 to July 2013.

**1. Andersen Sampler:** Two stage Andersen's sampler was used. Petridishes containing Streptomycin Rose Bengal Agar medium (Martin, 1950) were used for exposure in the sampler and the duration of sampling was 2 minutes. Sampling was done at 2 feet above the ground.

After exposure the Petridishes were brought back to the laboratory in a pre-sterilized polythene bags and incubated at 25±20C temperature. A total of 18 petriplates were exposed every month in each ward from August 2012 to July 2013. The different species of fungi appearing on isolation plates were subcultured and identified.

The fungal isolates were characterised and identified according to the manual of Barnett and Hunter (Barnett and Hunter, 1972). A complete record of data regarding the total number of colonies per plate and the total number of colonies of each species in a petriplate was recorded. After entry of monthly data the average fungal colony counts have been converted in to number values using the conversion factor (15.87). Per cent concentration of each fungal spore was calculated.

**2. Burkard Volumetric Personal Air Sampler:** Sampling was also carried out with a Burkard Volumetric Personal Air sampler. The sampler with a suction rate of 10 L/min. was operated for 5 minutes.

The air borne particles (*mycoflora*) were deposited on micro slides smeared with cotton blue stained glycerine jelly. The sets of exposed slides were brought back to the laboratory in a sterilized covered Petridishes. Each slide was warmed gently over flame to remove the moisture. The dust particles, soot particles and insect parts were removed by sterilized dissecting needles and forceps using a hand lens. The slide was then mounted with a cover glass. Only fungal spores under the cover glass were taken in to account for the counts. Spore types were identified up to the generic level with microscopic methods, according to Barron (1972). As spores of *Aspergillus* and *Penicillium* could not be distinguished on the basis of morphology therefore they were grouped collectively under the category 'Asp/Pen'. The counts are

expressed as number per cubic meter of air by using conversion factor (71.43).

**3. Statistical analysis:** The data were analysed by Pearson's test for correlation.

## RESULTS

### 1. Andersen Sampler:

During studies on indoor air of hospital wards a total of 26 species belonging to 10 genera were isolated from both the wards i.e. General ward and Post-surgical ward. The percentage of occurrence of *mycoflora* was higher in General ward (51.93 %) in comparison to Post surgical ward (48.06 %). The highest number of Colony Forming Unit (CFU) was recorded for *Cladosporium herbarum* (3681.84 CFU/m<sup>3</sup>) followed by *Penicillium citrinum* (3062.91 CFU/m<sup>3</sup>), *Aspergillus niger* (2126.58 CFU/m<sup>3</sup>) and *Aspergillus flavus* (1047.42 CFU/m<sup>3</sup>) (Table-1). Highest per cent concentration of the spores was recorded in the month of January (17.21%) followed by February (16.84%), March (14.75%) and December (13.81%). While least per cent concentration of spores was recorded in the month of July (1.41%). All the 26 species were isolated from both the wards. The spores of *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *C. Oxysporum* and *Penicillium citrinum* were recorded throughout theyear. *Cladosporium herbarum* was numerically the most abundant in both the wards. Its contribution was highest in the Post-surgical ward and least in General ward.

### 2. Burkard Volumetric Personal Air Sampler:

The total aeromycoflora encountered in the atmosphere over the year was 68916 fungal spores/m<sup>3</sup> Distributed amongst 10 types. 'Asp /Pen' (52.43%) constituted the predominant spore type. *Cladosporium* (27.97%) was other numerically significant type recorded in annual calendar. The highest number of fungal spores were present in December (8641.82 spores/m<sup>3</sup>) and lowest in April (3356.74 spores/m<sup>3</sup>). The spores of 'Asp/Pen' and *Cladosporium* were recorded throughout the year.

Most frequently trapped spores were *Alternaria* and *Curvularia* sp. Highest number of spores were recorded from General ward in comparison to Post surgical wards. Highest no. of spores was recorded.



**Table 1.** Fungal colonies calendar showing the annual total for fungal colonies from August 2012 to July 2013.

Fungal spores	No. of CFU / m <sup>3</sup>												Total
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mar	Jun	Jul	
Absidia sp.	-	-	-	-	-	47.61	63.48	79.35	-	-	-	-	190.44
Alternaria alternate	63.48	-	-	47.61	-	-	31.74	-	15.87	63.48	47.61	47.61	317.4
A. carthami	-	-	-	-	-	47.61	-	-	-	-	-	-	47.61
A. dianthi	-	-	-	-	-	79.35	95.22	79.35	-	-	-	-	253.92
A.dianthicola	-	-	-	-	-	-	111.09	79.35	-	-	-	-	190.44
A. geophila	-	-	-	-	-	63.48	63.48	-	-	-	-	-	126.96
A. humicola	-	-	-	-	-	95.22	79.35	63.48	-	-	-	-	238.05
A. radicina	-	-	-	-	111.09	95.22	63.48	-	-	-	-	-	285.66
A. sonchi	-	-	-	-	126.96	111.09	-	79.35	-	-	-	-	317.4
A. tenuissima	-	-	-	-	126.96	95.22	-	79.35	-	-	-	-	301.53
Alternaria sp.	-	-	-	-	-	-	79.35	-	95.22	111.09	-	-	285.66
Aspergillus candidus	-	-	-	190.44	126.96	-	-	-	-	-	-	-	317.4
A. flavus	-	-	-	158.7	174.57	190.44	158.7	238.05	126.96	-	-	-	1047.42
A. koningi	-	-	-	-	31.74	-	47.61	-	63.48	-	-	-	142.83
A. niger	79.35	63.48	174.57	158.7	190.44	285.66	317.4	396.75	238.05	95.22	126.96	-	2126.58
A. sydowi	-	-	111.09	-	396.75	317.4	238.05	285.66	190.44	-	-	-	1539.39
Chaetomium cristatum	-	-	-	-	-	63.48	31.74	-	-	-	-	-	95.22
C. globosum	-	-	-	-	-	63.48	47.61	31.74	-	-	-	-	142.83
Cladosporium herbarum	-	634.8	507.84	396.75	428.49	396.75	317.4	285.66	238.05	-	476.1	-	3681.84
C. oxysporum	-	126.96	158.7	190.44	111.09	126.96	79.35	95.22	-	-	-	79.35	968.07
Curvularia lunata	-	-	-	-	-	126.96	79.35	47.61	31.74	-	-	-	285.66
C. pallescens	-	-	-	-	63.48	79.35	63.48	47.61	-	-	-	-	253.92
Drechslera australiensis	-	-	-	-	47.61	31.74	-	-	-	-	-	-	79.35
Fusarium oxysporum	-	-	-	-	-	47.61	-	-	-	-	-	-	47.61
Penicillium citrinum	111.09	126.9	158.7	-	317.4	396.75	634.8	714.15	396.75	95.22	-	111.09	3062.91
Syncephalastrum	-	-	-	-	126.96	111.09	95.22	-	95.22	-	-	-	428.4

Spore types identified from Burkard sampler from August 2012 to July 2013. in the month of December followed by October, November and January. Least number of spores was recorded in the month of April.

### 3. Statistical analysis:

Results of statistical analysis suggest that the *mycoflora* of general ward might be very positively associated with *mycoflora* of post surgical ward. The results showed that, the fungi load were significantly not different for the wards studied.

## DISCUSSION AND CONCLUSION

A combination of two techniques, viz. Andersen sampler and Burkard volumetric air sampler has been used in the present investigation to get a fairly complete picture of the air *mycoflora* of the hospital wards. In fact, such a combination for sampling indoors was suggested by earlier worker 5 who emphasized the need of using two trapping methods during aerobiological surveys, one for the microscopic

assessment of the total air spora and the other for the identification of predominant types in culture. Twenty six species of fungi, belonging to Deuteromycetes, were isolated and identified from the General and Post- surgical wards of hospital by Andersen sampler which included many potential allergens. Species of *Aspergillus*, *Alternaria*, *Cladosporium* and *Penicillium* were most frequently isolated from indoor air of hospital wards.

The presence of aspergilli in the hospital environment is a major extrinsic risk factor for the occurrence of nosocomial invasive aspergillosis (*Martins-Diniz et al., 2005; Martin, 1950; Barnett and Hunter, 1972; Barron, 1972; Tilak, 1982; Dutkiewicz and Augustowska, 2006; Sessa et al., 1996*). The number and types of fungus spores in indoor air seem to depend on air exchange with the outside and the presence of indoor spore sources like patients, visitors etc (Lacey, 1981; Caretta, 1992; Wu et al., 2005; Sandra et al., 2009). *Cladosporium herbarum* was recovered in large number from the Post-surgical





ward. *Cladosporium* grow well indoors in fiberglass insulation or highly humid surfaces (Ghani, 1997). Many reports indicated that this genus is the predominant fungus and one of the most common isolates cultured from inside the hospitals which are in keeping with our results. The predominance of *Aspergillus* and *Penicillium* in the air of indoor environments of hospital wards was established by earlier workers (Ghani, 1997; Ekhaise et al., 2008; Hedayati and Mohamad, 1999; Pakshir et al., 2007).

Predominance of *Cladosporium*, *Aspergillus* and *Penicillium* has also been confirmed by Burkard sampler data. High frequency of *Cladosporium* in General ward of hospital was revealed by Burkard sampler. Spores of *Beltrania* and *Ulocladium* found on Burkard trap slides could not be recovered on the plates of Andersen sampler. In both the methods i.e. cultural (Andersen sampler) and noncultural (Burkard sampler) the isolated genera were same because of this may be the indoor air depends upon the outside ventilation. The highest per cent concentrations of spores were recorded from General ward in comparison to Post surgical wards. The reason of this may be, because of restricted entry of visitors, more disinfected area of the hospital and closed and controlled condition of the ward.

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## RESEARCH ARTICLE

# ISOLATION AND IDENTIFICATION OF AEROMYCOFLORA FROM BHAVAN'S COLLEGE CAMPUS, ANDHERI

Parameswaran U and Vaidya VA

Department of Botany, Bhavan's College, Andheri (West), Mumbai 400058.

## ABSTRACT

Aeromycoflora are fungi from air with potential to cause diseases in plants, animals and human beings. In the present studies, air samples from Bhavan's College Campus, Andheri were analyzed and fungi were isolated and identified prevalent during monsoon. Fungi were isolated by plate exposure method and purified by serial dilution method. Fungi obtained were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Aspergillus ustus*, *Candida albicans*, *Epicoccum sp.*, *Fusarium equisetii*, *Helminthosporium sp.*, *Mucor mucedo*, *Rhizopus stolonifer* and *Penicillium notatum*. Most of the fungi obtained showed luxuriant growth probably due to high humidity and suitable temperature for their growth.

**Keywords** Aeromycoflora, Monsoon, Fungi.

## INTRODUCTION

Bhavan's College, Andheri has a huge campus with a few educational institutes, a botanical garden, a lake and a large number of ornamental plants. More than 30% world population is known to suffer from allergic ailments such as bronchial asthma, allergic rhinitis and atopic dermatitis. Major causal agents are pollen grains, fungal spores, dust mites, plant fragrances and food. Clinically important fungal allergens are different species of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, *Alternaria alternata*, *Cladosporium cladosporoides*, *Ganoderma leucidum*, *Mucor mucedo*, *Fusarium solani*, *Curvularia lunata*, *Neurospora sitophila*, *Scopulariopsis brancaulistoo*. Air carries a large number of bioparticles (biopollutants) and chemicals which poses burden for asthma, bronchitis, etc., of humans. The bioparticles include fungal spores etc. These are causative agents of respiratory disorders like asthma etc.

Rapid industrialization and urbanization though has resulted in booming the economy of the country but it has also contributed significantly in enhancing problems of patients suffering from respiratory disorders as quality of the air is deteriorated due to addition of large number of pollutants in the air. Air is not the natural environment for their growth and multiplication of air mycoflora but it acts as a good medium for their dispersal from one place to another. Several factors like humidity, temperature, sunlight and suspension of organic and inorganic material affect the distribution of microbes in the air. In addition to this, many physical, chemical and biological factors bring about changes in composition of aeromycoflora of the area.

In the present work, aeromycoflora were studied in the laboratory of Department of Botany.

## MATERIAL AND METHODS

For isolation of mycoflora from the air, sterile Petri plates containing 20 ml of Potato Dextrose Agar medium (200 gm potato, 20 gm dextrose, 20 gm agar agar and 1000 cc distilled water) were exposed to air at different locations of Laboratory of Botany

### \*Corresponding Author

Email: [ushap15@gmail.com](mailto:ushap15@gmail.com)

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Department, Bhavan's College for 24 to 48 hours and incubated at 28° C temperature for four days. Results were recorded for cultural and morphological characters of fungi isolated (Table 1). Cultures were purified and preserved. Percent frequency in terms of CFUs (Colony Forming Units) was calculated (Table 2) by using following formula:

$$\% \text{ Frequency} = \frac{\text{No. of observation in which colony appeared}}{\text{Total No. of observations recorded}} \times 100$$

Percentage contribution for each organism was also calculated (Table 3) by using the formula:

$$\% \text{ Contribution} = \frac{\text{Total no. of colony one species}}{\text{Total No. of colonies of all the species}} \times 100$$






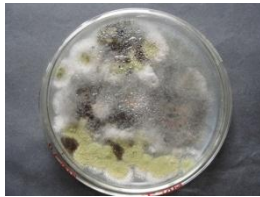


## RESULT



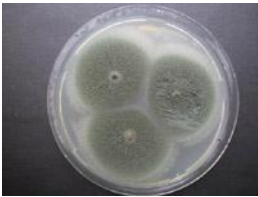
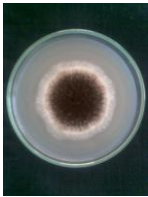
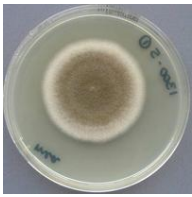

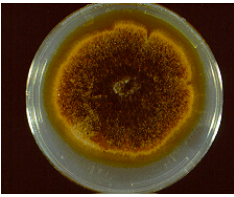

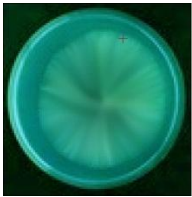



Among the total number of fungi collected from airspora, *Aspergillus niger* was maximum in terms of CFUs (Colony Forming Units) and frequently isolated. There were five species of *Aspergillus* followed by *Rhizopus stolonifer*, *Candida albicans*, *Mucor mucedo*, *Epicoccum sp.*, *Helminthosporium sp.*, *Fusarium equisetii* and *Penicillium notatum*.

**Table 1:** Various species of fungi isolated from the indoor environment

Sr. No.	Class of Fungi	Name of the Fungi	Morphological Characters
1	Deuteromycetes	<i>Aspergillus niger</i>	Colonies spread rapidly, mycelium white to dark brown to black conidial heads, Conidiophores erect with a vesicle, sterigmata and chains of round conidia.
		<i>Aspergillus flavus</i>	Colonies yellow turning to yellow green. Having phialides borne directly on the vesicle, Conidia globose or subglobose.
		<i>Aspergillus fumigatus</i>	Smoky green colonies, velvety, Young heads bluish green, Conidiophores smooth, short, often greenish, Vesicles flask shaped phialides borne directly on vesicles, closely packed, lower ones deflected upwards. Conidia small, globose, smooth.
		<i>Aspergillus nidulans</i>	Colonies light green smooth velvety. Conidial heads columnar short brown with distinct foot cell. Sterigmata biseriata conidia globose.
		<i>Epicoccum sp.</i>	Colonies yellowish brown. Sporodochia present, sporulation sparse. Blastoconidia formed singly or densely compacted slightly pigmented. Conidia globose to pyriform.
		<i>Fusarium equisetii</i>	Colony peach colored, conidiogenous cells hyaline, enteroblastic, mono or polyphialidic. Macroconidia abundant, typically falcate with foot cell, tapering at both the ends, 4 septate.
		<i>Candida albicans</i>	Colonies slimy yeast like, white to creamy. Budding cells present on pseudomycelium made up of pseudo septa resulting in budding cells which are ovoid.
		<i>Helminthosporium sp.</i>	Olive brown, proliferating cottony colony with beaded appearance, septate. 6 - Celled conidia, very tapering at the tip.
2	Ascomycetes	<i>Aspergillus ustus</i>	Colonies brownish yellow becoming purplish grey. Reverse yellow conidiophores short conidia globose.
		<i>Penicillium notatum</i>	Profusely branched, septate, hyaline, greenish yellow. Long with broom like branching, flask shaped sterigmata, globose conidia in chains.
3	Phycomycetes	<i>Mucor mucedo</i>	Colonies browning grey, sporangiophore long, terminating into a globose sporangium with round spores. Columella globose
		<i>Rhizopus stolonifer</i>	Colonies spread rapidly with white fluffy mycelium; rhizoids are dark brown, sporangiophore long, sporangia globose, shining white turning black at maturity.


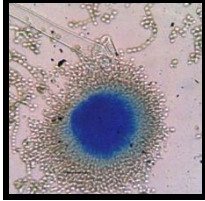


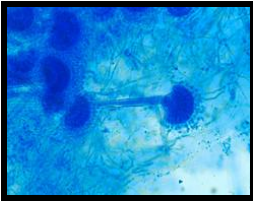
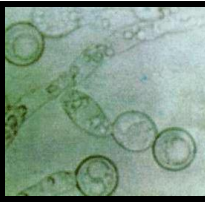
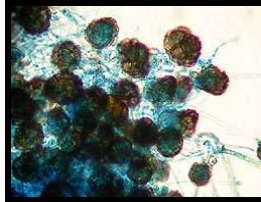
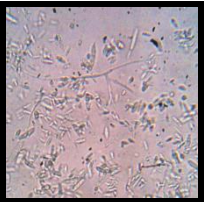

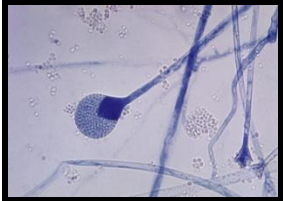




			
			
<b>Fungal colonies on exposed Petri plates</b>			

			
<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus nidulans</i>	<i>Aspergillus niger</i>
			
<i>Aspergillus ustus</i>	<i>Candida albicans</i>	<i>Epicoccum sp.</i>	<i>Fusarium equisetii</i>
			
<i>Helminthosporium sp.</i>	<i>Mucor mucedo</i>	<i>Penicillium notatum</i>	<i>Rhizopus stolonifer</i>

**Cultural characteristics of pure cultures of isolated fungi**



			
<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus nidulans</i>	<i>Aspergillus niger</i>
			
<i>Aspergillus ustus</i>	<i>Candida albicans</i>	<i>Epicoccum sp.</i>	<i>Fusarium equisetii</i>
			
<i>Helminthosporium sp.</i>	<i>Mucor mucedo</i>	<i>Penicillium notatum</i>	<i>Rhizopus stolonifer</i>

**Microscopic Characteristics of the isolated fungi**

Table 2: Airborne CFUs recorded			Table 3: Percent contribution of airborne fungi		
Sr. No.	Organisms	% Frequency	Sr. No.	Organisms	% Contribution
1	<i>Aspergillus flavus</i>	75	1	<i>Aspergillus flavus</i>	12.24
2	<i>Aspergillus fumigatus</i>	12.5	2	<i>Aspergillus fumigatus</i>	1.02
3	<i>Aspergillus nidulans</i>	62.5	3	<i>Aspergillus nidulans</i>	8.16
4	<i>Aspergillus niger</i>	100	4	<i>Aspergillus niger</i>	19.38
5	<i>Aspergillus ustus</i>	50	5	<i>Aspergillus ustus</i>	4.08
6	<i>Candida albicans</i>	87.5	6	<i>Candida albicans</i>	18.36
7	<i>Epicoccum sp.</i>	50	7	<i>Epicoccum sp.</i>	4.08
8	<i>Fusarium equisetii</i>	25	8	<i>Fusarium equisetii</i>	2.04
9	<i>Helminthosporium sp.</i>	37.5	9	<i>Helminthosporium sp.</i>	3.06
10	<i>Mucor mucedo</i>	62.5	10	<i>Mucor mucedo</i>	7.14
11	<i>Penicillium notatum</i>	25	11	<i>Penicillium notatum</i>	2.04
12	<i>Rhizopus stolonifer</i>	87.5	12	<i>Rhizopus stolonifer</i>	18.36



**DISCUSSION:**

Prevalence of fungi in monsoon can be attributed to high relative humidity and optimum temperature during rainy season but since the campus in Bhavan's College has a wide flora and associated with many microbes which can also prove to be antagonists against many fungi which are fatal to useful plants. *Rhizopus stolonifer* has been reported to cause respiratory allergy in some patient. Verma and Chile (1992) also reported greater variety of aeromycoflora during May to October. This is generally attributed to favorable conditions for growth during this period. Singh and Siddiqui (2004) have done similar work in polluted and unpolluted air zones and reported that survival of air borne spores would depend on several factors like wind velocity, distances from source, time in air, relative humidity, gaseous composition of the air, sunshine and species itself. Although aeromycoflora was dominated by saprobes, the plant pathogenic and human allergic fungi were also encountered. The studies indicate that the incidence of air borne fungal spores of clinical significance show greater variation in response to the environmental conditions. *Aspergillus* species are the opportunist organisms and generally harmless in their normal environment but becomes pathogenic in compromised hosts having lower resistance. Several species of *Aspergillus* are known to cause Aspergillosis. *Penicillium*, *Cladosporium* and *Curvularia* are also considered as respiratory allergens and mycotoxin producing fungi. The prevalence of these fungi in study site explored potential risk of allergy among residing people. Sawane (2010) had done a survey of airborne *Penicillium* in different atmospheres of Nagpur and concluded its high percentage in the air as potential

risk factor for allergic disorders to people residing nearby.

**CONCLUSION:**

Thus it is concluded from the present studies that the study of aeromycoflora with special reference to species of *Aspergillus* responsible for causing Aspergillosis as well as allergic disorders will definitely in preventing various diseases. Some fungi like *Fusarium* species can also be utilized as microbial weapons in the form of antagonists which will protect the flora of Bhavan's College campus against pathogenic fungi. Various fungi obtained will be preserved for academic curriculum of different classes so also in research. Microorganisms like fungi can also be used as markers or biological indicators to help in forecasting of weather conditions

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## RESEARCH ARTICLE

## AEROALGAL SAMPLING OF A CINEMA HALL

Pandkar JT

Department of Botany, Fergusson College, Pune-4

## ABSTRACT

Aero-algal sampling, in an air cooled cinema hall, with approximate capacity of 1200 persons was attempted for the first time, to study impact of aero-algal forms on public health & also effect of air cooler on the occurrence of these forms. Sampling was done using rotarod air sampler with duration of 30 minutes exposure for each sampling. Out of 3 samples obtained (3 slides & 3 cultures), total 13 aero-algal forms belonging to cyanophyta & chlorophyta were identified. Forms such as *Trentipholia* (46.15%) was found to be dominant followed by *Phormidium truncicola*, *Phormidium muscorum*, *Phormidium luridum*, *Merismopedia punctata*, *Chroococcus minutes*, *Microcystis stagnalis* & *Pleurococcus* each representing (7.69%) respectively. Forms such as *Microcystis* & *Phormidium* reported to be allergenic to human being were also encountered. Further studies in this aspect are necessary, which will help to find out some remedial solution to minimise exposure to such health hazards..

**Keywords** : Aero-algae, Cinema Hall, Allergy, Public Health

## INTRODUCTION

Aero-algal studies have been done from various different aspects which can fall into either of the following broad two categories: - Extra mural or Intra mural studies.

Enrenberg (1844) identified 13 algal genera from dust samples collected at sea. Brown et.al (1963, 1964) reported the dispersal of various aero-algal forms from across the island of Oahu, Hawaii and the heterogeneity observed among various aero-algal forms. Floger et.al (1976) reported fresh water diatoms from a dust storm. McGraw (1976) during his studies of Taylor, Dry Valley, Victoria Land and Antarctica, concluded that algae are frequently picked up from soil by strong wind current. Holand (1973, 1977) reported over 40 genera of algae collected from house dust samples. Ehrensman and Hatch (1975) at California studied the effect of relative humidity on the

survival of air borne unicellular algae. Carson and Brown (1976) correlated terrestrial and air-borne algae with meteorological conditions on the island of Hawaii. Lustgraft (1979) reported algae from indoor air and mattress dust. Roy Ocotta and Carrera (1993) reported algae from the indoor and outdoor atmosphere of waste water treatment plant. Sheno and Ramlingam (1976) reported the rain as a source for some aquatic and terrestrial algae to become air-borne. Chanda and Pandey (1982) reported several algal forms growing on the walls of buildings at Calcutta. RamchanderRao and Jadhav (1996) using foot wear dust, food dust, leaf dust, bed dust, nasal secretion and spider webs reported 22 algal genera, Pandkar (2011, 2012) reported presence of allergenic algae at human breathing level and Fan dust sampling as an effective mode of aero sampling and so on.

All the above studies indicate towards the presence of aero-algal forms from different sources and conditions, it's mode of dispersal and allergenic forms. But studies with respect to its direct impact on public health have not been much emphasised. Hence in the present studies, the effect of aero-algal forms on the public health (visiting a cinema hall) was considered.

**\*Corresponding Author**

Email: [jogita\\_pandkar@yahoo.co.in](mailto:jogita_pandkar@yahoo.co.in)

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## MATERIAL AND METHODS

Panchsheel cinema hall is situated in the busy locality of Sitabuldi market, Nagpur, Maharashtra. It is a big air cooled hall with approximate capacity of 1200 and running three shows every day. From three different spots, three samples were collected from May- June respectively. (Table- II).

Sampling was done using rotarod air sampler (Herrington, 1959), with the exposure of 30 minutes for each sampling. Two parallel celloclaps were mounted across two bars of the sampler. A thin layer of petroleum jelly was spread on the non- sticky surface. Operational time of the sampler was noted and one of the cellophane strips was mounted on a clean glass slide in glycerine jelly and another strip was dropped in a sterile test tube containing B.G.11 culture medium. Entire mounting and culturing procedures were carried out on the sampling sites, to avoid contamination during transit. Cultures were allowed to grow under natural conditions.

Algal growth was observed after 3-4 weeks. Slides were prepared using iodine stain and glycerine as mounting medium. Slides were scanned for algal identification. The algal genera were identified visually on the basis of their morphological characters by comparing them with the standard literature available (Fritsch (1935s, 1945) and Desikachary (1959).

## RESULTS & DISCUSSION:

Out of 3 samples obtained (3 slides & 3 cultures), total 13 aero-algal forms belonging to cyanophyta & chlorophyta were identified. Forms such as *Trentipholia* (46.15%) was found to be dominant followed by *Phormidium truncicola*, *Phormidium muscorum*, *Phormidium luridum*, *Merismopedia punctata*, *Chroococcus minutes*, *Microcystis stagnalis* & *Pleurococcus* each representing (7.69%) respectively. (Table-I).

*Trentipholia* was found to be dominant form, represented in all the three samples collected. Forms such as *Pleurococcus*, *Phormidium mucosum*, *Phormidium luridum* and *Merismopedia punctata* were observed from spote-I (culture) only. Similarly *Phormidium truncicola* from spote-II (slide), *Chroococcus minutes* from spote-II (culture) and *Microcystis stagnalis* from spot-III (culture) were observed. (Table-II)

From the above observation it is clear that maximum no of aero-algal forms 62.5% were encountered from spot-I i.e. Inside of entrance to the hall, where less air is been circulated as compare to the other two spots. Spot- II (one of the cinema hall seat) and Spot-III (on the stage near the cinema screen) show 37.5% and 25% of total aero-algal spora respectively (Table-II).

**Table-1:** Total aero-algal forms encounter from a cinema hall

SR. NO	PARTICULAR	SLIDES	CULTURES	TOTAL
1	Number of samples	3	3	3
2	Duration of sampling (Minutes)	90	90	90
3	Total algal forms recorded	7	6	13
4	Cyanophyta	1	5	6
5	Cocoidcyanophyta	----	3	3
6	Filamentous cyanophyta	1	2	3
7	Chlorophyta	6	1	7
8	<i>Trentipholia</i>	6	----	6
9	<i>Pleurococcus</i>	----	1	1
10	<i>Phormidium truncicola</i>	1	----	1
11	<i>Phormidium mucosum</i>	----	1	1
12	<i>Phormidium luridum</i>	----	1	1
13	<i>Merismopediapunctata</i>	----	1	1
14	<i>Chroococcusminutes</i>	----	1	1
15	<i>Microcystisstagnalis</i>	----	1	1

**Table-2 :** Summary of aero-algal forms encounter from different sampling spots from a cinema hall.





FORM	SPOTE-I		SPOTE-II		SPOTE-III		TOTAL
	SLIDE	CULTURE	SLIDE	CULTURE	SLIDE	CULTURE	
<i>Trentipholia</i>	01	-----	03	-----	02	-----	06
<i>Pleurococcus</i>	-----	01	-----	-----	-----	-----	01
<i>Phormidium mucosum</i>	-----	01	-----	-----	-----	-----	01
<i>Phormidium truncicola</i>	-----	-----	01	-----	-----	-----	01
<i>Phormidium luridium</i>	-----	01	-----	-----	-----	-----	01
<i>Merismopediapunctata</i>	-----	01	-----	-----	-----	-----	01
<i>Chroococcus minutus</i>	-----	-----	-----	01	-----	-----	01
<i>Microcystis stagnalis</i>	-----	-----	-----	-----	-----	01	01

**SPOT-I** Inside of entrance to the hall; **SPOT-II** One of the cinema hall seat; **SPOT-III** On the stage near the cinema screen.

## CONCLUSION:

It has been observed that the people ranging from age-group, from few months- near about eighty years were the frequent visitors to various cinema halls for their entertainment. They remain in this hall for at least three hours per show. During this period they are exposed to allergy causing algal forms, present in air. Hence there is a need to study the presence and listing of such aero-algal forms and to find out ways which will help in minimising the aero-algal counts.

## Acknowledgement:

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## RESEARCH ARTICLE

## STUDY OF FUNGAL SPORES IN LIBRARY ENVIRONMENT

Sonawane MD\*, Kotwal SG and Gaikwad KN

P. G. Department of Botany, K.T.H.M. COLLEGE, Nashik, MS.-422002.

## ABSTRACT

A Survey for air borne fungal spores in indoor and outdoor environment of Library building of K.T.H.M. College, Nashik, was carried out by using Perkin's Rotorod Air Sampler from November 2008 to April 2009. The sampler was operated twice a week in the stack room, student library and reading hall. During the investigation, total 31 air borne components were trapped, out of which 26 were fungal spores' types and 5 other types. The most dominant fungal spore types were *Aspergillus* 8.21 % followed by *Cladosporium* 7.04 %, *Nigrospora* 7.0 %, *Alternaria* 5.57 %, *Biospora* 4.49 %, *Helminthosporium* 3.81 %. Besides fungal spores hyphal fragments, pollen grains, insect scales, plant parts and unidentified spores were also recorded. Most of the fungal spores are well known for bio-degradation of paper and are potential agents of allergic respiratory disorders, skin irritation etc. among the students and employees of the library.

**Keywords :** Library, Allergy, Bio-degradation.

## INTRODUCTION

Aeromycology is the study of airborne fungal flora. Fungi grow on organic material like fruits, vegetables, wood, clothes, leather, paper etc. So, it is an important indoor pollutant. It deteriorates books, archives and paintings on papers, etc. Cellulosic material acts as a suitable substrate for the growth of various fungi (Singh et-al, 1990).

It is also well known fact that people spend most of their time indoors. Fungi acts as a pathogen causing Respiratory tract infections like rhinitis, sinusitis, bronchitis; skin infections, ear infections (otomycosis) and eye infections (oculomycosis). Airborne fungi are a potential source of allergic disorders (Agashe S.N. & Anand P. 1982). Fungi and pollen grains trigger allergic reactions causing bronchial asthma, eczema, itching and watering of eyes etc.

Books in the libraries provide a very good substrate for fungi since binding glue, cloth covering and paper supports its growth. Inhalation of fungal spores dispersed from mouldy books during handling is a common practice in library. The occurrences of allergic disorders among library workers are well documented. Vaidya K.K. and Murdhankar S.M. in 1990 performed their experiments in ambient air inside central library in thesis and binding section at university of Poona. Tilak recorded fungi on papers and books belonging to species *Alternaria*, *Fusarium*, *Cladosporium*, *Chaetomium* and *Nigrospora* etc.

So, we carried out a survey of airborne fungal spores inside K.T.H.M. College library with "Rotorod Air Sampler."

## MATERIAL AND METHODS

Air sampling was carried out by using Rotorod Air Sampler of Parkins (1957) modified by Harrington (1959). This is very suitable for short period sampling up to 2 hrs. and its efficiency is largely independent of wind speed. The Sampler was operated inside the K.T.H.M. College Nashik, twice in a week for half an hour.

**\*Corresponding Author**

Email: [dr.mdsonawane@yahoo.in](mailto:dr.mdsonawane@yahoo.in)

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Simultaneously Petri-plates containing potato-dextrose-agar medium and streptopenicillin were also exposed in the Library for twenty minutes. The Sampling was carried out for a period of 6 months (From November 2008 to April 2009). The method of sampling, slide preparation and the scanning was done as method described earlier (Tilak and Srinivasulu). The different spore types were identified by comparison with the fungal collection from affected books, comparison with standard slides and photographs, characteristics and by visual identification.

**RESULT**

During the period of present investigation air monitoring was carried out in the indoor air of library. Total 31 air borne components were identified up to generic level and classified under their respective groups. Out of 31 components 26 are fungal spores and

5 other types. Out of 26 fungal spores 5 spores belong to Phycomycotina, 7 belong to Ascomycotina, 2 to Basidiomycotina, 12 to Deuteromycotina and 5 to other types which include hyphal fragment, insect scale, pollen grain, plant part and unidentified spores. In order of dominance Deuteromycotina stood first with highest concentration (49.57%) to the total aerospora followed by other types (21.11%), Ascomycotina (12.27%), Phycomycotina (10.85%) and Basidiomycotina (6.16%).

**DISCUSSION:**

A total of 31 types of isolates 8 types were having paper deteriorating activity. These are *Caldosporium* (7.04%), *Penicillium* (4.16%), *Aspergillus* (8.21%), *Curvularia* (2.05%), *Alternaria* (5.57%), *Rhizopus* (2.58%), *Chaetomium* (1.40%) *Insects and scales* (5.57%).

**Table 1:** Total Concentration and Percentage contribution of Each Airborne Component Inside The Library

Sr. No.	Spore Type	Total No Of Spores / m3 of air	Percentage contribution to air - spore	Sr. No.	Spore Type	Total No Of Spores / m3 of air	Percentage contribution to air - spore
<b>[A]</b>	<b>PHYCOMYCOTINA.</b>			<b>[D]</b>	<b>DEUTEROMYCOTINA</b>		
1.	<i>Albugo</i>	34	2.05	15.	<i>Alternaria</i>	95	5.57
2.	<i>Circinella</i>	26	1.49	16.	<i>Aspergillus</i>	140	8.21
3.	<i>Cunninghamella</i>	55	3.23	17.	<i>Bispora</i>	75	4.49
4.	<i>Mucor</i>	28	1.62	18.	<i>Caldosporium</i>	120	7.04
5.	<i>Rhizopus</i>	42	2.58	19.	<i>Curvularia</i>	35	2.05
<b>[B]</b>	<b>ASCOMYCOTINA</b>			20.	<i>Epicoccum</i>	30	1.76
6.	<i>Bitrimonospora</i>	16	0.93	21.	<i>Exosporium</i>	18	0.58
7.	<i>Chaetomium</i>	24	1.40	22.	<i>Heiminthosporium</i>	65	3.81
8.	<i>Hysterium</i>	40	2.35	23.	<i>Nigrospora</i>	98	5.74
9.	<i>Melanospora</i>	23	1.34	24.	<i>Penicillium</i>	71	4.16
10.	<i>Pleospora</i>	37	2.17	25.	<i>Pithomyces</i>	48	2.81
11.	<i>Teichospora</i>	45	2.64	26.	<i>Torula</i>	50	2.94
12.	<i>Xylaria</i>	25	1.47	<b>[E]</b>	<b>OTHER TYPE</b>		
<b>[C]</b>	<b>BASIDIOMYCOTIN A</b>			27.	Hyphal Fragment	65	3.81
13.	<i>Puccinia</i>	30	1.76	28.	Insect scale.	95	5.57
14.	<i>Smuts</i>	75	4.40	29.	Pollen grain.	90	5.27
				30.	Plant part.	60	3.51
				31.	Unidentified spore.	50	2.94



The spores of *Aspergillus* are in highest concentration (140/m<sup>3</sup>). They occurred regularly throughout the period of investigation. It is reported that *Aspergillus* is allergic, biodeteriorating and also responsible for Aspergillosis from both outdoor and indoor environment (Shivpuri and Agarwal, 1969). *Alternaria*, *Aspergillus*, *Bispora*, *Caldosporium*, *Helminthosporium*, *Nigrospora*, *Penicillium*, Insect scales, Hyphal Fragments, Pollen grains occurred in the air throughout the period of investigation.

The result of exposed petriplates also showed same spore types *Caldosporium*, *Penicillium*, *Aspergillus*, *Alternaria* were found associated with the deteriorated book samples during cultural studies.

The occurrence of hyphal fragments (3.81%) in the air of library is suggestive of deteriorated material of books inside the library. The insect scales, whole insects and insect parts shows major contribution (5.57%) to total airspora of library. These may be coming from affected books may probably be helpful in colonization of fungi.

At the same time fungal spores like *Aspergillus*, *Caldosporium*, *Penicillium*, *Nigrospora* are potential agents of allergic respiratory disorders and skin diseases. So to minimize the harmful effects of fungal spores for the of library staff, students, readers and precious collection of books and literature certain corrective measures (Bank 1974) can be definitely reduce frequency of their occurrence like, installation of exhaust fans, use of vacuum cleaner to remove dust, disinfection of shelves with fungicides, discarding damaged books and start planning for regular cleaning and preventive process.

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**RESEARCH ARTICLE****PRELIMINARY SURVEY OF OTHER AEROBIOCOMPONENTS AT PUNE  
MAHARASHTRA, INDIA****Pawar SG and Ingole AC**

Science research centre, Department of Botany, Yashwantrao Mohite College, B. V. D. U. Pune.

**ABSTRACT:**

Survey of aerobiocomponents except fungal spores have been carried in the intramural environment of printing press using Tilak air sampler and library using Rotorod air sampler from January 2013 to May 2013 simultaneously. Some interesting findings have been recorded. Quantitative estimation revealed 06 other types of aerobiocomponents except fungal spores in the order of dominance i.e. fungal hyphae (30%), cellulose fibres (24%), epidermal hair (23%), insect scales/wings (17%), pollen grains (05%) and algal filament (01%).

Monthly percentage contributions of these other biocomponents in the intramural air of Pune revealed highest catches of fungal hyphae during January (33%), February (29%), April (31%), May(32%) while March represented second rank in the order of dominance i.e. after insect scale/wings (27%). Followed by cellulose fibres highest in April (26%), February (25%), January and May (24% each) and March (22%). Algal components have been recorded least i.e. 1% during January, April and May each and zero per cent during February and March 2013.

While Rotorod air sampling in college library revealed cellulose fibres (28%), epidermal hair (25%), insect scale/wings (22%), fungal hyphae (20%), pollen grains (0.4%) and algal filament (0.1%). In order of dominance during study period. Monthly percentage contribution of cellulose fibres is the highest during May (39%), April (33%), March (30%) February (30%) and ranked second during January (25%) after insect scales/ wings (28%) and least incidence was of algal filaments i.e. during January (0.2%), February (0.1%), May (0.1%) and zero per cent during March and April. Other types were found in between. These aerobiocomponents have been found to be common allergens and particularly higher catches and fungal hyphae and insect parts usually coincide with higher wind velocities or storms.

**Key words:** Aeromicrobiocomponents, Air Sampling, Allergy, Allergens, college library, Printing press.

**INTRODUCTION**

Aerobiologists so far focused their attention on investigations of fungal spores, pollen grains or mites as allergens. Krishnamurthi and Vittal (1983), Tilak and Quazi (1985), Tilak and Rao (1987), Tilak and Jogdand (1987). However, little work has been done on hyphal fragments (Tilak and Bhalke 1981) and other types which have been also proved to be

potential allergens. Hence this topic has been selected for the study to explore, qualitative and quantitative analysis of role of other types of aerospora in allergic manifestation in relation to meteorological parameters and workers, handlers, students in the library and printing press, where cellulose material is present in the form of papers, binding materials, glue, gum, wooden racks etc. These materials have been found oftenly affected by cellulytic and other fungi in addition to termites, mites and other types of aerospora damaging the valuable ancient rare printed literature causing severe loss of such literature which will be never found thereafter

**\*Corresponding Author**Email: [arunaingole434@gmail.com](mailto:arunaingole434@gmail.com)

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## MATERIALS AND METHODS

Material for the experiment is the intramural aeromicrobiota which is studied by air sampling method using electrically operated volumetric continuous Tilak air sampler was set in the printing press at 1 meter height from ground level, from 1<sup>st</sup> January 2013 to 31<sup>st</sup> May 2013 in the B. V. D. U., Erandawne, Pune. Petroleum jelly coated cello fane tape was fixed around the drum in the center for every week in the beginning. Starting point was coincided with inlet orifice tube of the sampler and the sampler was started in the evening continuously for seven days. At the end of the week 14 slides were prepared after cutting loaded (deposited) cello tape with a blade in 14 segments each represented twelve hours aerospores, using melted glycerin jelly in the laboratory. These slides have been scanned under Japanese Nikon steriobinocular research microscope using 10x X 40x.

While other material for the experiment is the intramural aeromicrobiota which is studied by air sampling method using Rotorod air sampler. The Rotorod air sampler was fully described by Perkins (1957) and modified by Harrington (1959). It consists of battery operated motor which rotates at 2300rpm coated sticky tape on brass rods around its axis at a constant high speed. The rods have been oriented at right angles to high velocity of air dashing on the rods. Petroleum jelly coated cello fane tape was fixed on the two arms of the sampler. Rotorod air sampler which have been operated daily for half an hour (between

2pm and 2.30 pm), in the YMC library at 1 meter height from ground level, from 1<sup>st</sup> January 2013 to 31<sup>st</sup> May 2013. The two strips of loaded (deposited) cello fane tape from Rotorod sampler was mounted on a clean slide using melted glycerin jelly in the laboratory. The total number of spore/m<sup>3</sup> of air at that particular site and height of that time was obtained by multiplying each spore types by its conversion factor (5). Identification of aerobiocomponents by using authentic literature, reference slides and expertise.

## RESULTS AND DISCUSSION

Preliminary survey of other aerobiocomponents except fungal spores at Pune, during study period (1<sup>st</sup> January to 31<sup>st</sup> May 2013) revealed incidence and varying percentage contribution from printing press and library of six types i.e. algal filaments (0.62% and 0.7 %), cellulose fibres (24.49 % and 28.36%), epidermal hairs (23.11% and 25.26%), fungal hyphae (30.41% and 19.40%), Insect wings and scales (16.71% and 22.30%), and pollen grains (4.63% and 3.91%). The study in the printing press revealed highest percentage contribution of fungal hyphae (30.41%) as compared to remaining components and was also higher than that of library (19.4%) followed by cellulose fibres in the press (24.49%) which was less than that of library (28.36%). While lowest percentage contribution was recorded in the case of algal filaments in the press (0.62%) as compared to remaining five types and also less than that of library (0.74%).

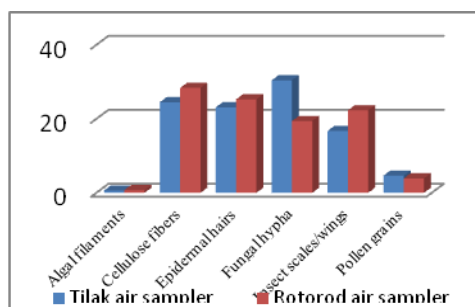


Fig. 1: Comparative analysis of other aerobiocomponents during study period (from 1st January to 31st May 2013)

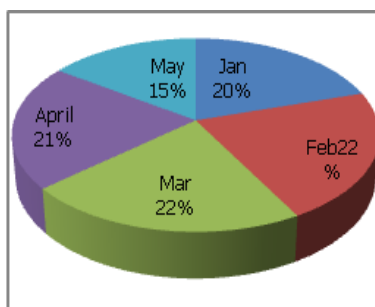


Fig 1. monthly percentage contribution of aerobiocomponents from printing press during study period (i.e. January to May 2013).

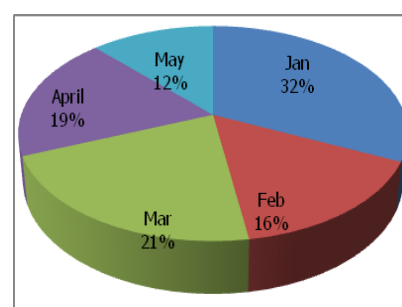


Fig 2. monthly percentage contribution of aerobiocomponents from library during study period (January to May 2013)



Monthly percentage contributions of these other biocomponents in the intramural air of Pune revealed highest catches of fungal hyphae during January (33%), February (29%), April (31%), May (32%) while March represented second rank in the order of dominance i.e. after insect scale/ wings (27%). Followed by cellulose fibres highest in April (26%) February (25%) January and May (24% each) and March (22%). Algal components have been recorded least i.e. 1% during January, April and May each and zero per cent during February and March 2013. While Rotorod air sampling in college library revealed cellulose fibres (28%), epidermal hair (25%), insect scale/ wings (22%), fungal hyphae (20%), pollen grains (04%) and algal filament (01%). In order of dominance during study period.

However, increase load of fungal hyphae and insect scales and wings have been found to act as bioindicators of high wind velocity (wind air) and dry weather. They have been found to cause severe allergy in sensitive victims. Abundant occurrence of hyphal fragment in the aerospora was found to be mainly due to dry weather conditions and strong wind current, spores catches on windy days were usually dominated by hyphal fragments similar findings were recorded by Patil (1985). Also Tilak and Bhalke (1981) reported high concentration of hyphal fragment on windy days.

In present study at college library have been revealed the highest percentage contribution of hyphal fragments (33%) in month of January while Harvey (1970) at Cardiff observed the highest concentration of hyphal fragment in August, Talde (1969) reported 12.2% at Parbhani, Wankede (1983) reported 0.52%, Arun et al (2001) reported concentration of hyphal fragment (5.5%), Swapna et al (2012) reported other types (4.26%), Lalchand et al (2011) reported other types (3.4%), M. Saibaba (1984), Pollen grains (other biocomponents) and fungal spores are among the most abundant airborne bioparticles (Agashe et al. 2002). etc. All the six components have been reported persistently during five months of the study period but varied in percentage contribution in printing press as well as library.

## CONCLUSION

Aerobiocomponents have been found to be common allergens and particularly higher catches and fungal hyphae and insect parts usually coincide with higher wind velocities or storms.

## Acknowledgements:

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## RESEARCH ARTICLE

## AEROMYCOLOGICAL SURVEY OF PIMPRI-CHINCHWAD AREA, PUNE

Sinha Arundhati\*, Cholke Pravin B and Pable Priyanka

Prof. Ramkrishna More Arts, Commerce and Science College, Akurdi, Pune-44.

**ABSTRACT:**

Airborne particles of biological origin are mainly consisting of fungal spores, pollen, bacteria, viruses, algal filaments, epidermal hairs, plant fragments etc. Fungi are among the most important aero-allergens. Fungal spores constitute a significant fraction of air-borne particles. Extramural aerobiological research includes aeromicrobial survey at various places like College campus, Bus stand, Railway station, Garbage depot, crop fields etc.

The present study was carried out for a period of two years (2010-2012) to identify culturable fungi in urban environment and to study the variation in their concentration at four different sites. In the vegetable markets of metropolitan cities, rotten vegetables and fruits, gunny bags, paper bags, packing materials, straw, discarded leaves and stems forms the main substrates for the growth of airborne fungi. Hence petriplate exposure experiments were conducted in Pimpri vegetable market nearby slum for the afore-said period.

Volumetric information on the culturable molds present in the air of different sites was collected by exposing petriplates at four different sites in study area. Not all genera recorded on the cellotape were found growing on culture plates but only 26 culturable genera were recorded. The genera such as *Aspergillus*, *Penicillium*, and *Trichoderma* were precisely identified by their cultures. The maximum contributor of the aerospora in 2010-2012 was *Cladosporium* sp.1 with 8.79% contribution followed by *Aspergillus* sp.1 (6.15%) and *Helminthosporium* (5.79%). In second year, *Cladosporium* with 6.58% contribution tops the rank and it was followed by *Helminthosporium* (5.38), *Aspergillus* sp.1.(4.90) and *Alternaria* sp. 1with 4.47% contribution.

**Key words:** Aerospora, fungal spores, Pimpri-Chinchwad.

**INTRODUCTION**

Aerobiology is a scientific and multidisciplinary approach focused on the biodiversity of biologically significant materials. Airborne particles of biological origin are mainly consisting of fungal spores, pollen, bacteria, viruses, algal filaments, epidermal hairs, plant fragments etc. They occur in varying concentration in the atmosphere depending on climatic factors, location (Indoor or Outdoor), altitude and proximity to large or small waterbodies. When dispersed in air they are known as aerosols.

Extramural aerobiological research includes aeromicrobial survey at various places like College campus, Bus stand, Railway station, Garbage depot, crop fields etc. The present study was carried out to identify culturable fungi in urban environment and to study the variation in their concentration at four different sites. In the vegetable markets of metropolitan cities, rotten vegetables and fruits, gunny bags, paper bags, packing materials, straw, discarded leaves and stems forms the main substrates for the growth of airborne fungi.

A residential area having closely aggregated houses and a site in industrial area having food processing industries in the vicinity was also selected for trapping culturable fungi from the air.

**\*Corresponding Author**Email: [arundhatisinha74@gmail.com](mailto:arundhatisinha74@gmail.com)

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## MATERIALS AND METHODS

As a part of extramural study, Petriplate exposure methods was used to know the status of culturable airborne fungi at different experimental sites in the study area.

Petriplates containing Potato Dextrose Agar as a culture medium (P.D.A.) were exposed at four different sites. The sites were Sector A., Sector B, Sector C and Sector D.

Petriplates containing Potato Dextrose Agar as a culture medium were exposed 1 m above the ground level once in a month for 15 minutes. The petriplates after exposure were incubated at laboratory temperature for 5-7 days till sporulation. The fungal forms were identified and isolated to obtain pure cultures. The fungal colonies were counted. Identification of fungal colonies up to generic level was done on the basis of colony characteristics, growth pattern and morphology of fungal spores and was subsequently confirmed with the help of relevant literature (Gilman 1957, Barnett 1991, Ellis 1971 and Subramanian 1971).

At the time of petriplate exposure, about 30 ml of sterilized medium was poured quickly under aseptic conditions in each petriplate (Size- Lid O.D.x height mm. 100 x 15 and Base O.D.x Total height mm 94 x 17.). Petriplates containing medium were covered with lid.

Occurrence of culturable fungal colonies was correlated with meteorological factors such as rainfall, relative humidity and temperature. Meteorological data for the period of study was collected from Meteorological Department, Simla office, Pune.

## RESULTS AND DISCUSSION

As a part of extramural aerobiological study, volumetric information on the culturable molds present in the air of different sites was collected by exposing petriplate at four different sites in study area. Not all genera recorded on the cellotape were found growing on culture plates but only 26 culturable genera were recorded. The genera such as *Aspergillus*, *Penicillium*, and *Trichoderma* were precisely identified

by their cultures which otherwise would have remained ignored or grouped under *Aspergilli*.

**A group wise list of fungal taxa identified from the exposed petriplates at different sites has been mentioned in alphabetical order**

### *Zygomycotina*

1. *Cunninghamella sp.*
2. *Mucor sp.*
3. *Rhizopus sp-1*
4. *Rhizopus sp-2*
5. *Rhizopus sp-3*

### *Ascomycotina*

6. *Chaetomium*

### *Deuteromycotina*

7. *Alternaria sp- 1*
8. *Alternaria sp- 2*
9. *Alternaria sp- 3*
10. *Aspergillus sp- 1*
11. *Aspergillus sp- 2*
12. *Aspergillus sp- 3*
13. *Aspergillus sp- 4*
14. *Aspergillus sp- 5*
15. *Cercospora sp.*
16. *Chlamydomyces sp.*
17. *Cladosporium sp-1*
18. *Cladosporium sp -2*
19. *Curvularia sp -1*
20. *Curvularia sp -2*
21. *Drechslera sp -1*
22. *Drechslera sp -2*
23. *Epicoccum sp*
24. *Fusarium sp -1*
25. *Fusarium sp -2*
26. *Fusarium sp -3*
27. *Gleotrichum sp.*
28. *Helminthosporium sp*
29. *Heterosporium sp.*
30. *Humicola sp*
31. *Memnoniella sp.*
32. *Nigrospora sp.*
33. *Paecilomyces sp.*
34. *Papularia sp.*



Site wise 35. total colony count during petriplate exposure at four different sites in 2010-12 revealed that 36. highest colony count (680) was recorded at Sector A with 36.17% contribution followed by Sector B (545 colonies) with 28.98% contribution, Sector C (435 colonies) with 23.13% contribution. The least colony count (220) was recorded at Sector D with 11.17% contribution.

The maximum contributor of the aerospora in 2010-12 was *Cladosporium* sp.1 with 8.79% contribution followed by *Aspergillus* sp.1 (6.15%) and *Helminthosporium* (5.79%). In second year, *Cladosporium* with 6.58% contribution tops the rank and it was followed by *Helminthosporium* (5.38), *Aspergillus* sp.1 (4.90) and *Alternaria* sp. 1 with 4.47% contribution. *Humicola* (0.21%) and *Chlamydomyces* (0.30%) contribution registered as the lowest contributor of aerospora in respective years.

Maximum incidence of *Cladosporium* during monsoon was encountered at Sector B however its incidence during winter and summer season was maximum at Sector A. Maximum incidence of *Aspergillus* during all season was recorded at Sector A. Dominance of *Curvularia* during monsoon and winter was observed at Sector B, whereas during summer season its higher concentration was recorded at Sector A.

However *Aspergillus* sp.1 exhibited somewhat equal distribution in all seasons.

## CONCLUSION

In both the years of investigation, the maximum fungal forms were observed at Sector A and minimum at Sector D.

Biocomponents like fungal spores and pollen grains may initiate allergic response to susceptible individuals. Allergic people have an altered capacity to react to potential allergens, being hypersensitive to them, causing several types of eye, skin and respiratory disorders. Airborne infections and the resulting diseases threaten the lives and productivity of human beings, animals and plants. Aerobiology thus not simply means the study of microorganisms in the

atmosphere, but it also take into consideration the allergic properties of various bioparticles like pollen and spores. The results of the present study will be valuable in providing insights to the afore mentioned problems.

## Acknowledgements:

The authors acknowledge with profound gratitude, the facilities provided by Principal, Prof. Ramkrishna More Arts, Commerce and Science College, for the completion of the present study.

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## RESEARCH ARTICLE

## AEROBIOLOGY, EPIDEMIOLOGY AND CHEMICAL CONTROL OF BEAN RUST

Meshram BM\* and Shilpa Deotale

Department of Botany, Pune- 411018.

**ABSTRACT:**

Bean rust caused by *Uromyces phaseoli* Artn. is a very common disease. The pathogen causes infection mainly through urediospores. Climatic factors and amount of inoculum in air are the most important in rust incidence. The spread of pathogen is favoured by cloudy humid weather with heavy dew and temperature of 21 – 25 °C. Urediospores repeat the cycle within 5 days under favourable conditions. Three sprays of 0.2 percent Bayleten 45, 60 and 75 days after sowing gave the best chemical control of rust. Volumetric Tilak air sampler was used for air monitoring.

**Key words:** Aerobiology, epidemiology, chemical control, bean rust.

**INTRODUCTION**

French bean rust caused by *Uromyces phaseoli* Artn. is a very common and destructive disease. The disease is world-wide in distribution and occurs in almost every state of India. In addition to *Phaseolus vulgaris* L., the pathogen also attacks different species and cultivars of *Phaseolus*, *Dolichos* and cowpea and may prove much destructive through defoliation in plants.

With 3.0 percent infection the yield loss of dry bean seeds may be up to 21.3 percent and with 6.8 percent infection up to 36.5 percent loss. French bean is cultivated on large scale in India for its young, immature pods used as vegetable and also for its dry seeds (rajma). Seeds are highly nutritious and reach in phosphorus, iron and vitamin B<sub>1</sub>. The present investigations were carried out with the object to understand aerobiology, epidemiology and chemical control of bean rust in relation to climatic factors and amount of urediospores in air associated with disease incidence in the field condition.

**MATERIALS AND METHODS**

Air monitoring was carried out by volumetric Tilak air sampler continuously in french bean field in outskirts of Pune city during kharif season of 2012. (Fig: 1). Crop of cultivar Pusa Parvati was sown on July 3<sup>rd</sup> and harvested on October 2<sup>nd</sup>. Air monitoring was started one week before sowing of crop and continued up to harvesting covering almost a period of 3 months. Concentration of urediospores was calculated using conversion factor, 14 of the apparatus. From time to time leaf surface washing was done to find out the population of urediospores deposited on phylloplane after their dispersal by air. Frequent visits to the growing crop were made to record the disease incidence. Estimation of disease incidence percentage was calculated by using 1-12 grade scale proposed by Horsfall et al. (1955). Record on climatic factors like temperature, relative humidity and rainfall was maintained by obtaining the data from Meteorological Department in Pune to correlate these factors with disease incidence and amount of inoculum in the air. Experiments on spore germination at various temperatures were performed in laboratory to understand favorable conditions.

The experiments on chemical control using three concentrations of various fungicides (Table 2) were performed by spraying the plants raised in specially designed plots of 3X10 m size in the same field.

**\*Corresponding Author**Email: [bmmeshram@yahoo.com](mailto:bmmeshram@yahoo.com)

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## RESULTS AND DISCUSSION

### a. Aerobiology:

Here, aerobiology deals with the source, release, dispersal and deposition of urediospores on the crop surface. The pathogen is an autoecious long cycled rust causing infection mainly through urediospores. The spores were recorded in air in the range of 190-365 one week before the sowing of the crop indicating their probable survival on continuously grown different species of *Phaseolus* and cowpea in the locality. The amount of urediospores was raised up to 782-925 spores/m<sup>3</sup> of air during second week of July with the first infection on leaves associated with temperature range of 18-20 °C and R.H. 58-65 percent. At spore concentration of 1470-1930 spores/m<sup>3</sup> of air, the number of minute whitish uredia increased during first week of August when temperature range was 20-25 °C, R.H. 69-78 percent and 15 mm rainfall. Subsequently, the leaf pustules of rust enlarged gradually to form reddish brown sori during third week of August (Table 1).

With the increase in urediospores concentration at 3050-4500 spores/m<sup>3</sup> of air, sori became surrounded by a ring of secondary sori when temperature was 17-22 °C, R.H. 80-90 percent and 25 mm rainfall at the end of August. The urediospores built up in masses and spread by wind to cause secondary infections leading to large scale disease spread at 6500-8270 spores/m<sup>3</sup> of air covering late

vegetative growth, flowering and pod initiation stage of crop during first 15-20 days of September. (Fig: 2)

This was most favourable period for exist of spores from pustules, their dispersal and deposition on leaves, stem and petiole of crop plants. During this period urediospores repeated the cycle within 5 days and new crop was produced within next 5-10 days leading to high rise in spore concentration in air.

As the plants approach maturity during last half of September pathogen produced telia fewer in number in the place of uredia. At about 26 °C air temperature teliospores showed less infection. No disease development by teliospores was observed at 28-30 °C temperature of air. No day without any urediospores in air was noticed during the entire period of investigation. However, their day to day concentration in air showed great fluctuations depending up on climatic conditions.

### b. Epidemiology:

Epidemiology here mainly deals with the source of primary inoculum of disease incidence in relation to climatic factors and host nature. Bean rust is not seed-borne. This has been confirmed in laboratory by isolation technique. In cold climatic regions survival of pathogen has been reported through teliospores and urediospores in the crop debris left in the field. According to McMullan et al. (2003) aecidia formed on plant debris infect volunteer bean plants forming pycnia and aecidia. During present investigation these spores bodies were not found on the standing crop.

**Table 1:** Range of weekly concentration of urediospores/m<sup>3</sup> of air over bean crop during kharif season of 2012

Month	Weekly spores concentration in air			
	I	II	III	IV
June	-	-	-	195-365
July	406-513	782-925	813-1130	1300-1460
August	1508-1680	1740-1810	2060-2500	3050-4500
September	4604-4830	6500-8270	1806-1903	1500-1705

**Table 2:** Disease reduction (DR) due to spraying of various fungicides at three concentrations in bean rust

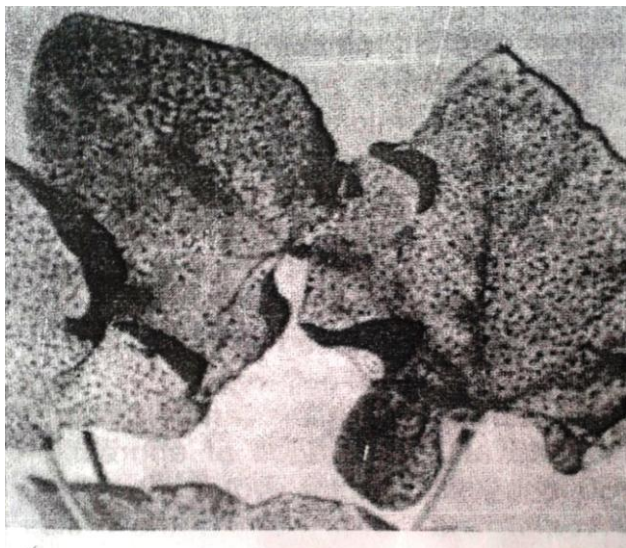
Fungicide	% concentration of fungicide		
	0.1	0.2	0.3
Mancozeb	+	++	+++
Moneb	++	++	+++
Zineb	+	++	+++
Bayleton	+	+++	++
Tridemorph	++	++	+++
INA	+	+++	++

(+less DR, ++ = Moderate DR, +++ = best DR)





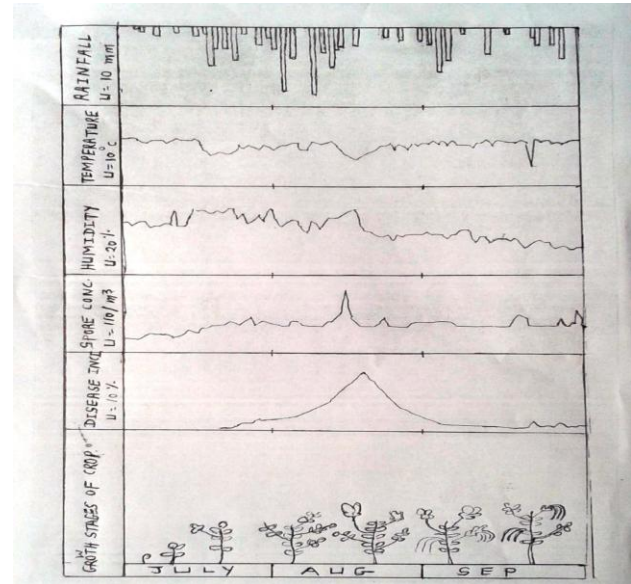
**Fig.1.** Volumetric Tilak Air Sampler operating in bean field for air monitoring of urediospores of uromyces rust during kharif season of 2012 at the outskirts of Pune city.



**Fig. 2:** Heavy pustules of uredia on the leaves of bean plant during first half of September 2012.

In other regions of temperate and subtropical climate including present locality of Pune the pathogen is found to survive through continuously grown beans and other species of *Phaseolus*. During the crop season the disease is found mainly spreading through wind-borne urediospores (Fig: 3). Existences of more than 25 physiological races of this pathogen are reported. Spider mites can also disperse these spores. They are attracted to the uredia (Batra and Stavely, 1994). Intensive cultivation of beans in areas as in present site having high atmospheric humidity is one of the conditions favourable for disease development. If crop is attacked early there may be total loss.

Urediospores germinate best at 15-24 °C of air and it was also confirmed in laboratory germination test. About 93 percent germination was found at 17.5-22.5 °C. Teliospores obtained from crop debris could germinate best at 10-15 °C. These spores produced at temperature above 24 °C showed reduced germination. The spores from pustules on old leaves also showed 30 percent less germination in laboratory testing (fig: 1).



**Fig. 3:** Incidence of Uromyces rust over bean field in relation to climate condition and amount of urediospores in the air at various growth stages of crop during kharif season 2012.

Adhesion of urediospores and germ tubes to the host surface is reported to be governed by surface hydrophobicity (Terhune and Hoch, 1993). Mechanical forces imposed by a combination of turgour pressure and adhesion of appressorium to stomatal surface cause deformities in the lateral and normally erect stomatal guard cell lips become prostrate. According to Rauscher et al. (1995) urediospores produce extracellular proteases and these enzymes break the host cell wall by acting on the fibrous hydroxiprolin-rich proteins in the walls. These proteins are important in plants for cell wall stability and play a role in defense against the pathogen.

Heavy rains wash down the spores from air hence there is less chance of disease spread. Frequency and duration of leaf wetness period are more important than temperature factor in disease spread. Cloudy and humid days permitting night dew to last on the leaves



in morning hours are most favourable in association with 20-25 °C temperature for spore germination and successful infections in the standing crop. If day temperature reaches 34 °C there is no disease development. Long day hours are found favoring the disease spread.

Severity of rust varies among the host species and cultivars. This has been observed by noticing disease incidence on beans and cowpea crops in this locality. Cultivars having moderate or low susceptibility showed only minute pustules. Resistance to rust in *Phaseolus vulgaris* was noted to be related with leaf pubescence. According to Mmbaga et al. (1992) on leaf surface having large number of trichomes the infection is prevented by not allowing the germ tubes of urediospores to contact the leaf surface. Cavello et al. (2002) reported that the cultivars which have resistance to rust through hypersensitive reactions show the accumulation of jasmonic acid. This acid in synergism with ethylene seems to play key role in activating multiple resistance in various host-pathogen combination. More pronounced resistance has been observed in the adult plants than in seedlings.

### c. Chemical control

Chemical control in present studies was performed with some fungicides used for spraying though cultural practices may be helpful in avoiding in rust incidence. Plants raised in specially designed plots were sprayed with Mancozeb, maneb and zineb using 0.1, 0.2 and 0.3 percent concentration 45, 60 and 75 days of sowing gave the best result of chemical control (Table: 2). As the rust occurred earlier during present studies the application of INA (2,6-dichloro-iso-nicotinic acid) in 0.1 percent concentration to the 18-22 days old seedlings provided good protection at least for 5 weeks through induced resistance. Dann and Deverall (1996) also reported INA- induced resistance in beans against various rusts.

### CONCLUSION:

- 1) Studies on aerobiology and epidemiology are very essential to understand various angles of air-borne diseases in crop plants.
- 2) Climatic factors and amount of urediospores in air play key role in bean rust incidence.
- 3) Chemical control becomes necessary when secondary spread of disease takes place on large scale through air-borne inoculum.
- 4) The information data obtained from these studies may be helpful in devising disease forecasting of rusts provided that the study is repeated for more years to reach to appropriate conclusion.

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## RESEARCH ARTICLE

**ASSESSMENT OF METAL CONCENTRATIONS IN THE STREET DUSTS OF DELHI****Verma Srishti, Shridhar Vijay and Panwar Pooja**

School of Environment and Natural Resources, Doon University, Dehradun 248001, Uttarakhand, India.

**ABSTRACT**

Heavy metal concentrations play an important role in affecting the health of people by an overall activation of the haemostatic system upon chronic/acute exposure of these contaminants present in air. Present study was conducted with an objective to estimate metal concentration in the street dust of Delhi. 14 different sites were selected for the study which included industrial, commercial, bus terminals and other areas. Inductively Coupled Plasma-Optical Emission Spectrometer analysis showed that Al had the highest concentration while Cd had the lowest. Pb concentration was recorded to be high in the present study sites, but relatively low as compared to those cited in previous literatures due to an early phase-out of leaded gasoline. Industrial areas reported high concentrations of Cr & Fe, Cu & Zn, and Al & Ca. Besides anthropogenic activities, natural sources do account for heavy pollution of metals in the street dusts of Delhi (Ca and Fe), which are present in its loosely bound soil. Anthropogenic sources are major contributors to the trace metal pollution in Delhi. Residential-cum-commercial area had lowest concentration for all the metals studied. Correlation coefficient analysis indicated that industrial activities are the source of Cd, Ni & Cu, and Pb, Mn, Zn and V. Fe, Mn, Ni, V, Al are a result of dust re-suspension from traffic related sources.

**Keywords :** Street dust, Heavy metals, Delhi, Anthropogenic sources**INTRODUCTION**

Air pollution in less developed countries like India, is one of the major concerns due to increase in urbanization. The growing population, increasing number of vehicles and industries, improper maintenance of vehicles and lack of implementation of stringent emission standards are the chief causes (Srinivas, 1999), which make the problem of air pollution still severe (Mittal & Van-Grieken, 2001). Delhi, being the capital city, the pollution is mostly contributed by the use of vehicles which is 70% (Gokhale & Patil, 2004). Atmospheric air, being the mixture of gases also contains suspended particulate matter emitted from various activities. These particulate matters, on re-suspension, gets settled on the road and become the "road dust". Of the

total air pollution caused in the environment, about half of this is caused due to "road dust". It can be said from the fact that all those metals which are associated with vehicular pollution or industrial activity gets associated with the road dust during the course of time (Khemani et al., 1985; Anju and Banerjee, 2003; Monkkonen et al., 2004; Mouli et al., 2006; Srivastava and Jain, 2006).

In urban areas, sources of heavy metal pollution in road dust includes water transported material from surrounding soils and slopes, road surface wear, road paint degradation, dry and wet atmospheric deposition biological inputs, vehicle wear (tyres, body, brake lining, etc.) and vehicular fluid and particulate emissions (Sutherland and Tolosa, 2000).

The trace metals contamination in road deposited sediments is known to exert a toxic impact on the human system which may be due to biochemical activity of metals (Smith et al., 1997). The trace metals found in outdoor air may also become the source of indoor air pollution (Tong and Lam, 2000;

**\*Corresponding Author**Email: [srishti25verma@gmail.com](mailto:srishti25verma@gmail.com)

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Chattopadhyay et al., 2003; Srivastava and Jain (2007). Direct inhalation, ingestion and dermal contact absorption are three routes for entry of toxic metals in the human body (Ferreira- Baptista and De Miguel, 2005; Ahmed and Ishiga, 2006). Toddlers are more susceptible to ingestion of these air-borne metals (Okorie et al., 2011; Xin-Hu et al., 2011; Guitao et al., 2010) through "Pica" (defined as the mouthing of non-food objects) (Davis et al., 1990; Wijnen et al., 1990; Calabrese et al., 1997). Heavy metals are dangerous because they tend to be bio-accumulated. This focuses the light on the fact that, within a particular biological organism, the heavy metal can be found in higher concentration than that in the environment. Therefore, determination of heavy metals, with regard to its origin, distribution and its level, is possible through the study of road dust.

The present study attempts to assess the trace metal levels of street dust samples collected from the 3 main broad categorized areas- industrial, commercial and residential, of a capital city Delhi and their possible health impacts.

## MATERIAL AND METHODS

**1. Road dust sampling procedure-** Urban street dust was collected from 14 locations of Delhi in the month of April through hand- brushing. The collected dust was transferred to self-sealing polyethene bags (with proper labeling) for transport to laboratory for analysis. Details of sampling sites are given in Table 1.

For quality maintenance, necessary precautions such as one-time usage of gloves were taken so as to avoid contamination and interference of any extraneous matters such as cigarette, etc. The samples were screened through a stainless steel of size 45 micrometer to obtain a desirable fraction of the dust sample which would be preferable for acid digestion and metal analysis in ICP-OES.

**2. Microwave Acid Digestion-** The samples were digested in a MARS microwave system according to the procedure described in household dust levels digestion protocol (Gautam Chattopadhyay et al., 2003). Samples solution was filtered through Whatmann filter paper no.42, and then analyzed using ICP-OES (Prodigy XP from Teledyne leemans Lab U.S.A), for heavy metal analysis.

**3. QA/QC Analysis-** Samples were digested in closed microwave assisted pressurized vessels. Acids of analytical grade (Nitric Acid with 70% purity of Himedia Laboratories Pvt.Ltd, Mumbai, India) were taken for maintaining purity of samples. Millipore ultra pure water was added to dilute the samples.

**4. Statistical Analysis -** Statistical analysis was done using SPSS 17. Mean, Median, Maximum, Minimum, Range and Standard Deviation was calculated for all the metals at different locations. Also, correlation coefficients were obtained between the metals observed at different locations in street dusts of Delhi. It was used to establish the correlation between different metals, further implicating the possible source of their concentration level. One-way ANOVA was used to determine the existence/ non-existence of variations within and between groups for all the sites.

## RESULTS & DISCUSSION:

The concentrations of Cr, Fe, Al and Ca were observed to be high in samples collected from most of the sites (10 sites: Site no. 1, 2,3,4,5,6,7,8, 11 and 14). Site 9 had high concentrations of Cu and Zn along with Cr, Fe, Al and Ca. Site no. 10 and 13 showed little contamination of Zn in the range of 45.6 mg/l and 89.6 mg/l. Higher levels of Cu and Zn were observed in the samples collected from site no.12. These values are quite high in comparison to other reported studies from various countries. Lead concentration was observed to be lower in comparison to previous study from same city.

**Zn** contamination may be a result of its release from vehicle tyre wear and tear process as Zn metal incorporated by rubber manufacturing industry in the vulcanization process. (Smolders and Degryse, 2002).High concentrations of **Cu** in the samples collected from industrial sites, i.e. Site no.9 and 12, were observed. It can be a result of abrasion of vehicle parts. According to Al- Khashman (2004), the Cu and Zn contamination of roadside soils result from the abrasion of vehicle parts such as brake lining, oil leak sumps, and cylinder head baskets. Source of **Cr** contamination can be vehicular pollution (Balachandran et al., 2000; Khillare et al., 2004).



**Table 1:** Delhi samples- location with their description.

SITE	LOCATION/ AREA/DESCRIPTION	DUST COLLECTED (g/cm <sup>2</sup> )
1	Near bus stand Dilshad Garden-Jhilmil area(residential area)	0.016
2	Near Reebok factory price shop, Govindpuri-Kalkaji (residential area)	0.008
3	Opposite Falcov industry Okhla industrial area- Phase II(slum areas)	0.033
4	Stand no.112- Anand Vihar ISBT (bus stand with heavy traffic)	0.010
5	Stand no.18- Anand Vihar ISBT (bus stand with heavy traffic)	0.011
6	Near Gurudwara Govindpuri-Kalkaji (residential-cum- commercial)	0.024
7	Airtel communication, Okhla industrial area- Phase II(industries e.g. paint )	0.128
8	Near petrol pump Way to Anand Vihar from Dilshad Garden	0.118
9	Near classic company Patparganj industrial area ( commercial area)	0.055
10	Front of Radhakrishna mandir Dilshad Garden- (a marketplace)	0.06
11	Delhi Jal Board Okhla industrial area- Phase II (open road side shops)	0.010
12	Backside of Sahwney industry Jhilmil area (heavy traffic load)	0.052
13	Stand no.8- Anand Vihar ISBT (bus stand with heavy traffic)	0.038
14	Opposite Toyota showroom Patparganj (industrial area)	0.22

**Table 2:** Street dusts in different samples of street dusts from different cities/countries- a comparison (values in mg/kg)

City/Country & Reference	Cd	Cr	Pb	Fe	Ni	Cu	Mn	Zn	V	Al	Ca
Delhi (India), Present study	5.711 <sup>a</sup>	4587.74 <sup>a</sup>	179.6429 <sup>a</sup>	18399.84 <sup>a</sup>	83.1028 <sup>a</sup>	1052.32 <sup>a</sup>	413.0 <sup>a</sup>	1504.13 <sup>a</sup>	77.7842 <sup>a</sup>	32425.4 <sup>a</sup>	5786.3 <sup>a</sup>
Calcutta (India) Chatterjee & Banerjee (1999)	3.12	-	536	-	42	44	-	159	-	-	-
Delhi (India), A.D.K.Banerjee(2003)	18.96 <sup>a</sup>	4816.93 <sup>a</sup>	597.63 <sup>a</sup>	-	574.56 <sup>a</sup>	721.50 <sup>a</sup>	-	365.92 <sup>a</sup>	-	-	-
USA, E.Apeageyi et al. (2011)	-	95 <sup>a</sup>	73 <sup>a</sup>	28,091 <sup>a</sup>	-	105 <sup>a</sup>	456 <sup>a</sup>	240 <sup>a</sup>	-	-	9185 <sup>a</sup>
China, T.Yang et al.(2010)	-	75.3 <sup>a</sup>	102.6 <sup>a</sup>	-	27.7 <sup>a</sup>	62.1 <sup>a</sup>	602.9 <sup>a</sup>	224.2 <sup>a</sup>	65.6 <sup>a</sup>	-	-
Paris (France) Pagotto et al. (2001)	1.7	-	1450	-	25	1075	-	840	-	-	-
Kavala (Greece) Achilleas and Nikolaos (2009)	0.2	-	301	-	58	124	-	272	-	-	-
Ottawa (Canada) Rasmussen et al. (2001)	0.37	-	39.1	-	15.2	65.8	-	112.5	-	-	-
Austria, Zechmeister et al. (2005)	0.431 <sup>a</sup>	0.81 <sup>a</sup>	7.3 <sup>a</sup>	259 <sup>a</sup>	16.5 <sup>a</sup>	70 <sup>a</sup>	-	37.4 <sup>a</sup>	1.38 <sup>a</sup>	136.3 <sup>a</sup>	2192 <sup>a</sup>
Girona (Spain) Amato et al.(2011)	2 <sup>a</sup>	-	128 <sup>a</sup>	-	191 <sup>a</sup>	1055	-	1760 <sup>a</sup>	-	-	-

<sup>a</sup> mean value used

All the sites excepting site no.10, 12 & 13 showed high concentrations of **Fe**. The Fe is known to be of crustal origin and is found in Delhi's loosely bound dust (Srivastava and Jain, 2007). **Cd** is released as

combustion in vehicles (Al- Khashman 2007 a, b; Divrikli et al., 2005). Bone damage and kidney dysfunction results due to low levels of Cd (Alfven et al., 2002; Buchet et al., 1990). The street side of Delhi



contains significant amount of **Ca** (crustal re-suspension) (Balachandran et al., 2000; Srivastava and Jain, 2007). **Pb** is ubiquitous in industrialized regions. Among the applications of lead, it can be said that paints, varnishes, pipes, storage batteries and insecticides are the major areas where Pb is used (Venugopal and Luckey, 1978). The major use of **Ni** is in plating the vehicle or its parts. It may be applied on outer part of a vehicle or on the surface of the cylinder and pistons of an engine (Hidayah and Amran, 2008). **Mn** may result as a consequence of industrial activities (V. Shridhar et al., 2010). Site no.8 is a zone near to petrol pump where heavy diesel and petrol vehicles pass through the road. Mn- based additives in gasoline are used to increase gasoline octane, and Mn is emitted by vehicles as brake- dust (A. V. Kumar et al., 2001; A. Tandon et al., 2008).

### CONCLUSION:

Al has the highest mean concentration of all the metals studied, while Mg and Cd had the least. Site no.9, which was an industrial area, had high concentration of Zn, Cu, Fe, Ca, Pb, Ni, Mn, and V. Industrial areas reported high concentrations of Cr, Fe, Al, Ca (11 sites study) along with Cu and Zn (2 sites study). The high concentrations of these metals can be a product of industrial activities and heavy movement of vehicles (accounting for vehicle wear, abrasion parts wear, re-suspension of dust). Besides anthropogenic activities, natural sources of trace metals do account for heavy pollution of metals in the street dusts of Delhi (such as Ca and Fe), which are present in loosely bound soil of Delhi. Thus, an important conclusion which can be drawn is that anthropogenic sources are the major contributors to the trace metal pollution in Delhi, an area which registers high rates of street dust metal pollution as a consequence of traffic emission and industrial establishments.

Correlation coefficient analysis indicated that industrial activities are the source of Cd, Ni & Cu, and Pb, Mn, Zn and V. Fe, Mn, Ni, V, Al are a result of dust re-suspension, while Mn, V, Al and Ca are traffic related sources. One-way ANOVA analysis clearly indicates that there exists a variation in their concentration between the metals studied within groups.

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## PHYTOREMEDIATION PROCESS FOR HYDROPHONIC PLANT OF REFINERY WASTE WATER

Khadye Ravindra A and Patil Vivekkumar V\*

Royal College of Science and Commerce, Dombivli.

### ABSTRACT

Wastewater treatment has been normally carried out by conventional systems. These systems along with advanced technologies being employed at many places are highly dependent upon power availability, skilled manpower and waste load characteristics. In developing countries, some of these could be critical towards efficient waste treatment. The demand of time to develop a sustainable wastewater treatment system overcoming. Phytoremediation Technology based on aquatic plant systems to solve the current runoff and wastewater quality problems. Phytoremediation depends on naturally occurring processes in which plants detoxify inorganic and organic pollutants with the help of degradation, sequestration and/or transformation. Phytoremediation is being used successfully to deal with a wide range of solid, liquid and gaseous substrates.

**Keywords :** Phytoremediation, Hydroponic plant, waste water, HPLC,

### INTRODUCTION

Many of the cities in India have been provided with treatment systems but Pollution Control Boards have not been able to maintain and run the systems properly, leading to non-functionality of most of these waste treatment units. Many pollution Control Boards in the country are in poor shape in terms of finances. The need and priority, therefore, of these Pollution Control Boards are not waste treatment but to give good water supply, health care and solid waste collection. The issue of giving low priority to waste treatment and disposal of wastewater for many Pollution Control Boards is not realized either by residents or people's representatives. Majority of the Pollution Control Boards apply themselves mainly to collect wastewater and channelise them to single or multiple disposal points.

#### *\*Corresponding Author*

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The term phytoremediation (phyto=plant and remediation=correct evil) is relatively new, coined in 1991. Phytoremediation includes variety of research areas including constructed wetlands, oil spills, and agriculture plant accumulation of heavy metals (EPA, 2000). Phytoremediation, the use of plants to remove/reduce the pollutants from the environment studies because of the advantages to its environmental studies because of the advantages of its environmental friendliness, cost effectiveness and the possibility of harvesting the plants for the extraction of absorbed contaminants such as metals that cannot be easily biodegraded for recycling among others (Maine et al, 2001, Maine et al, 2004, skinee et al, 2007; Malik, 2007).

Plants in a natural system provide a substrate (roots, stems and leaves) upon which microorganisms can grow as they break down organic materials and uptake heavy metals (McCutcheon et al 2008). However, as a result of the exponentially increasing demands of human expansion and resource exploitation, it has been recognized that natural



wetland ecosystems cannot always function efficiently for desired objectives and stringent water quality standards (Wetzel, 1993). These and many other factors have led to the rapid development of "constructed wetlands" for waste (especially wastewater) treatment (Wetzel, 1993).

Phytoremediation is the utilization of plants accumulation capabilities to remove contamination from water, soil and air, the capacity of aquatic plants to remove pollutants from water is well documented (Demirezen et al, 2004).

In the last two decades, a special interest in the world is aroused by the potential of using the biological methods in the wastewater treatment, whose application as of natural and not artificial procedures of effluents provides the effluents of required quality in an economically acceptable way in the technically simple structures. Capacity of water hyacinth (*Eichhorniacrassipes* (Martius) Solma-Laubach) as a very promising plant with tremendous application in wastewater treatment is already proved (Jafari and Trivedi, 2005; Trivedi, 2001).

Over the last two decades, phytoremediation has become an increasingly recognized pathway for contaminant removal from water and shallow soils and is an aesthetically pleasing, solar-driven, passive technique useful from remediation of shallow plumes with low to moderate levels of contamination (EPA, 2001).

## MATERIAL AND METHODS

The recent application of phytoremediation technology by duckweed in wastewater treatment and management is quite interesting and revealing. Phytoremediation systems by duckweed are one of the options that have been widely applied for combined handling of wastewater with the nutrients used for poultry and aqua-cultural projects (Gijzen et al, 1997; Naphi et al, 2003).

Lemma minor known as common duckweed is a small, free floating aquatic plant fast growing, adapt easily to various aquatic conditions and play an important role in extraction and accumulation of

pollutants from waters (Kaur et al, 2010). In particular, species of lemma are reported to accumulate toxic metals and therefore are being used as experimental model systems to investigate heavy metal induced responses, Bioavailability and bioaccumulation of various heavy metals in aquatic and wetland ecosystems is gaining tremendous significance globally (Greger, 1999). Aquatic macrophytes take up metals from water producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes found to be potential scavengers of heavy metals from aquatic environment and are being used in wastewater renovation systems (Abbasi et al, 1999, Kadlec et al, 2000). Aquatic plants have shown their efficiency in absorbing nutrients from various sources of polluted water (Cheng et al, 2002; Janjit et al, 2007).

Aquatic macrophytes have great potential for the phytoremediation of water contaminated with heavy metals (Zayed et al., 1998; Zhu et al., 1999; Wang et al., 2002; Miretzky et al., 2004; Peng et al., 2008; Rai, 2009), and several plants species (i.e., *Alyssum bertolonni*, *Brassica juncea*, *Eichhorniacrassipes* and *Iberisintermedia*) have been considered for phytomining of Ni, Co, TI, Ag and Au (Pinto et al., 1987; Robinson et al., 1997 ; Brooks et al., 1998 ; Anderson et al., 1999; Boominathan et al., 2004). However, No previous study has investigated the capacity of aquatic plants from water to accumulate in and few studies have been on Ag accumulation (Pinto et al., 1987; Harris and Bali, 2008; Xu et al., 2010), or the use of plants for combined phytoremediation and phytomining (Robinson et al., 1997).

Macrophytes have been shown to play important roles in marsh biogeochemistry through their active and passive circulation of elements. Through their action as nutrient pumps' (Odum, 1988), active uptake of elements into plant tissue, as seen in wetlands constructed for wastewater treatment (Kadlec and Knight, 1996) and in the use of wetland plants in phytoremediation. Phytoremediation is considered an effective, low cost, preferred clean-up option for moderately contaminated areas. Wetlands are often considered sinks for contaminants, and there are many cases in which wetland plants are utilized for removal of pollutants, including metals. The approach



is generally one of "phytostabilization", where the plants are used to immobilize metals and store them below ground in roots and/or soil, in contrast to "phytoextraction" in which hyperaccumulators may be used to remove metals from the soil and concentrate them in aboveground tissues. These latter plants must be, in turn, harvested and disposed of to prevent recycling of accumulated metals when the plants decompose. However, with few exceptions (e.g. *Ceratophyllum demersum*, a freshwater submerged rooted species, that accumulates arsenic with a 20,000-fold concentration factor- Reay,1972) wetland plants are generally not hyperaccumulators and in any case, the mechanical aspects of harvesting plants would be destructive to wetlands comprised of rooted plants. Therefore, for wetland plants, storing metals below ground is the preferable alternative. While many engineering studies of treatment wetlands use a black box approach analyzing levels in the influent and effluent (for e.g. , Chengetal., 2002), more must be known about patterns and processes of metal uptake, distribution and removal by different species of wetland plants.

The extent of uptake and how metal are distributed within plants can have important effects on the residence time of metals in plants and in wetlands, and the potential release of metals. This information is needed in order to better understand this system and to assure that the wetlands do not themselves eventually become sources of metal contamination to surrounding areas.

Currently, Phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radio nuclides, and landfill leachates. According to a recent report (Best et al., 1997), approximately 80% of the polluted ground waters are within 20 m of the surface. This suggests that a significant number of sites are potentially suitable for low cost phytoremediation applications.

In recent years, a number of articles have addressed the role of plants in remediating contaminated soils and ground waters (Paterson et al., 1990; Shimp et al., 1993; Schnoor et al., 1995; Simonich and Hites, 1995; Watanabe, 1997).

## CONCLUSION:

The study would result in state-of-the-art formulation of selected plant species for the treatment of refinery wastewater. This would also help in development of user- defined or trailer- made systems for treatment of particular pollutant of interest as well as gross wastewater parameters. The study would also reveal an understanding of the mechanisms of action and responsible of the various aquatic plant species for the treatment, which could be then efficiently utilized in various aquatic plant species for the treatment, which could be then efficiently utilized in various permutation and combinations for improved efficiency of wastewater treatment.

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## RESEARCH ARTICLE

**BIOREMEDIATION OF CHLORPYRIFOS USING BACTERIA ISOLATED FROM PESTICIDE CONTAMINATED SOIL****Wahida Rehman and Khan SJ**

The Institute of Science, Mumbai, 15, Madam Cama Road, Mumbai 400032.

**ABSTRACT**

Chlorpyrifos, used as an agricultural pesticide is a cause of great environment pollution and toxicity. Hence exploration of various chlorpyrifos degrading organisms to clean up the pollutant is of immense importance. Present study shows the great efficiency of bacteria in degradation of chlorpyrifos from minimal media where chlorpyrifos is the sole carbon source available for their growth. Four bacterial species isolated from chlorpyrifos contaminated soil showed growth in simulated conditions. These bacterial species were further checked for the extent of degradation. All the four showed degradation above 70%. The most efficient one *Achromobacter spp.* was further studied in detail for degradation along with parallel study of bacteria *Pseudomonas aeruginosa* previously known for degradation capability. Both these species showed degradation above 80%. Selected bacterial species showed promising result in degradation which can be harnessed in clean up technology for chlorpyrifos contaminated environment.

**Keywords :** Chlorpyrifos, toxicity, bacteria, degradation and clean up technology

**INTRODUCTION**

Organophosphate pesticides constitute a group of widely used, very heterogeneous compounds that share a phosphoric acid derivative chemical structure (Abo-Amer, Aly E, 2011) wide use of organophosphorus pesticides has created numerous problems, due to environmental pollution. (Metin Diurak and Ferda Ukazanici, 2001).

Chlorpyrifos (CPF) is a type of organophosphorus pesticide and its chemical name is *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate. It is used worldwide as an agricultural insecticide. The reported half-life of chlorpyrifos in soil varies from 10 to 120 days, with 3, 5, 6-trichloro-2-pyridinol (TCP) as the major degradation product. Attempts to isolate

Chlorpyrifos degrading bacteria from chlorpyrifos treated soils have not been very successful. However, chlorpyrifos has been shown to be degraded co metabolically in liquid media by bacteria (Brajesh K. Singh, Allan Walker et al, 2003; Cho CMH, Mulchandani A & Chen W, Getzin LW, 2002).

It has been suggested that the accumulation of TCP, which has antimicrobial properties, prevents the proliferation of chlorpyrifos-degrading microorganisms in soil (Racke *et al.*, 1990).

In general, microorganisms demonstrate considerable capacity to metabolize many pesticides. They possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds (Bhagobaty, 2007). Bacterial strains, such as *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas diminuta* strain MG, with the capability of hydrolyzing OPs such as diazinon and parathion, were isolated from soils in the Philippines and United States, respectively (McDaniel et al, 1988; Harper et al, 1988). Many microorganisms can specifically hydrolyze the phosphoester bonds of OPs and thus reduce the toxicity of OP pesticides and OP chemical

**\*Corresponding Author**Email: [vaishaliishere@yahoo.com](mailto:vaishaliishere@yahoo.com)

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warfare agents (e.g. sarin). Studies of Munnecke et al (1974) showed that the rate of enzymatic hydrolysis was two to 450 times faster than that of chemical hydrolysis, when parathion was used as a substrate. Considering that chlorpyrifos is one of the most commonly applied insecticides for control of pests and insects, the purpose of this experiment was to isolate and characterize chlorpyrifos degrading-bacteria, to investigate their degradation potential, to assess their adaptation to high concentrations of chlorpyrifos and to determine their usefulness in biodegradation of contaminated soil.

## MATERIAL AND METHODS

**Chemicals:** Samples of chlorpyrifos (97.0 %, pure analytical grade) were obtained from Gharda Chemical Co., Ltd, India. All other Chemicals were also of analytical grade.

**Isolation and culture conditions:** The mineral salt medium (MSM, pH 7.2) was prepared by adding 1.5 g  $K_2HPO_4$ , 0.5  $KH_2PO_4$ , 1.0 g  $(NH_4)_2SO_4$ , 0.5 g NaCl, 0.2 g  $MgSO_4$ , 0.02 g  $FeSO_4$  into 1L of distilled water. The NA medium was prepared with distilled water containing 1 % Peptone, 0.5 % Meat Extract, 0.5 % NaCl, with pH at 7.

One gram of organophosphorus pesticides contaminated soil was added into the flask containing 100 mL MSM with chlorpyrifos at 0.5 mg/ml and cultivated for 3 days under shaking at 150 rpm at 30°C. Then medium from the flask was inoculated into fresh MSM with chlorpyrifos plates. The colonies on the MSM plate with 0.5 mg/ml chlorpyrifos at 30°C were observed after 24 hrs. Then the colonies were selected and purified and their degrading capability was further tested by inoculation in liquid medium. Chlorpyrifos residue was measured by High-performance liquid chromatography according to the method of CIPAC.

**Identification of degrading bacteria:** Isolated bacterial strains were identified from laboratory Metropolis Healthcare Ltd.

**Inoculum preparation for degradation study:** The isolated culture maintained on MSM agar with chlorpyrifos were cultured in the 250 mL flask containing 100mL NB medium supplemented with 0.5

mg/ml chlorpyrifos. Flasks were shaken at 150 rpm and at 30°C for 18 hrs. For all the experiment, 2 ml (OD 0.7nm) of this culture was used.

**Bacterial growth and degradation of chlorpyrifos:** Degradation experiments were conducted at 30°C and at 150 rpm in MSM supplemented with 0.5 mg/ml chlorpyrifos. The fresh MSM medium without the organism was used as control. The degradation efficiency of the strain isolated were determined and estimated by the removal percentage of chlorpyrifos from the liquid culture.

**Extraction of samples for quantification:** Samples were recovered from culture flasks at time interval of 0, 1 and 5 days and centrifuged at 10,000 rpm for 15 min to obtain cell free medium. It was further filtered with 0.3 micron filters.

**Quantification of chlorpyrifos by HPLC:** Chlorpyrifos residue was measured by High-performance liquid chromatography according to the method of CIPAC (CIPAC Handbook 1 C, p. 2028 ) with following conditions: column C18 (150 x 4.6 mm), programmable variable wavelength UV detector, flow rate: 1 ml/min, mobile phase: Acetonitrile + water + glacial acetic acid (82:17.5:0.5). Injection volume 20 microlitre chlorpyrifos was detected at 230 wave length.

**Selection of efficient strain and its degradation study in formulative market sample along with parallel run strain previously known for degradation :** Bacterial strain which showed highest degradation was selected and studied for degradation of chlorpyrifos formulative market sample along with parallel run *Pseudomonas* species which is known to degrade chlorpyrifos. Degradation experiments were conducted at 30°C and at 150 rpm in MSM supplemented with 1 mg/ml formulative chlorpyrifos. The fresh MSM medium without the organism was used as control. The degradation efficiency of the strain isolated were determined and estimated by the removal percentage of chlorpyrifos from the liquid culture.

**Extraction of samples for quantification:** Left over chlorpyrifos in medium after 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> day was extracted by liquid extraction using hexane which was further dried and dissolved in acetonitrile and quantified by HPLC



**RESULTS AND DISCUSSION:****Table 1: Growth response of four isolated bacteria**

NO.	Growth in different concentration(mg/mL) of CPF					
	0.5		0.5		0.5	
B1	+	B1	+	B1	+	B1
B2	+	B2	+	B2	+	B2
B3	+	B3	+	B3	+	B3
B4	+	B4	+	B4	+	B4

(+) Growth (-) No growth

These isolates were identified as gram negative pathogenic organisms (Table 2)

**Table 2: Identification of isolated soil bacteria**

NO	SAMPLE NO.	NAME
1	B1	<i>Achromobacter spp.</i>
2	B2	<i>Enterobacter cloacae</i>
3	B3	<i>Stenotrophomonas maltophilia</i>
4	B4	<i>Pseudomonas aeruginosa</i>

**Table 3: Percentage degradation of Chlorpyrifos by test organisms.**

No.	Bacteria	24hours		120 hrs	
		Amount remaining in medium	% degradation	Amount remaining in medium	% degradation
1	<i>Achromobacter spp.</i>	16.86 %	83.14	0.81 %	99.19
2	<i>Enterobacter cloacae</i>	19.18 %	80.82	19.01 %	80.99
3	<i>Stenotrophomonas maltophilia</i>	7.32 %	92.68	3.8 %	96.2

**Table 4: Percentage degradation of Chlorpyrifos by *Achromobacter spp.* and *Pseudomonas aeruginosa***

No.	Bacteria	% degradation in MS medium		
		24 hours	3 <sup>rd</sup> day	5 <sup>th</sup> day
1	<i>Achromobacter spp.</i>	79.46	80.79	87.4
2	<i>Pseudomonas aeruginosa</i>	10.40	83.92	84.612

**Isolation, adaptation and identification of chlorpyrifos-degrading strain :**

During primary screening four strains were isolated that were capable of utilizing chlorpyrifos (0.5 mg/ml) as the sole source of carbon. The isolates, designed B1, B2, B3, B4, were grown in different

concentrations of chlorpyrifos (0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml & 3 mg/ml). The four isolated bacteria were adapted to grow in presence of chlorpyrifos and utilize it as sole carbon source for their growth. B1 showed to survive the highest concentration of CPF (Table1).



**Biodegradation of chlorpyrifos:** The amount of chlorpyrifos remaining in the medium after degradation of CPF was analyzed by HPLC analysis. It was observed that chlorpyrifos gradually decreased over 5 days. The observation (Table 4) showed efficient degradation by all the four bacteria. *Achromobacter* showed maximum degradation to 99.19 % followed by *Stenotrophomonas\_maltophilia* 96.2 %, *Enterobacter cloacae* 80.99 % and least by *Pseudomonas aeruginosa* 77.14 %. Good amount of CPF was degraded within 24 hours except by *Pseudomonas aeruginosa*

**Selection of efficient strain and its degradation study in formulative market sample along with parallel run strain previously known for degradation.**

*Achromobacter spp.* was selected for further studies as it showed the highest degradation among the four isolated species (Table 3).

*Achromobacter spp.* was run parallel with *Pseudomonas aeruginosa* which is known to degrade chlorpyrifos to study degradation of formulative market sample of chlorpyrifos (Dursban Chlorpyrifos 20EC). Both the species (Table 4) showed degradation in formulative sample also. They showed degradation above 80%.

**CONCLUSION:**

The present study reports the isolation and identification of efficient chlorpyrifos degrading bacteria including *Achromobacter spp.* There were four isolates from soil which degraded (77 - 99 %) CPF within 5 days. Utilization of xenobiotic compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. Results from this study suggest that the isolated strains of bacteria

are able to grow in medium in the presence of added pesticide and may therefore be used for bioremediation of pesticide contaminated soil. This leads us to believe that the soil with previous exposure to chlorpyrifos contains diverse range of bacteria and fungi having novel organophosphorus hydrolase enzyme system for carrying out enhanced biodegradation of this toxic pesticide.

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## RESEARCH ARTICLE

## INTRAMURAL DUST MITES FROM POULTRY AND FLOUR MILL IN PUNE, INDIA

Bansod Vaishali M<sup>1</sup>, Borde Sunita N<sup>2</sup> and Lahot Sapna<sup>2</sup>

<sup>1</sup>Department of Zoology, Prof. R. More ACS College, Akurdi, University of Pune, M.S. India

<sup>2</sup>Department of Zoology, Dr. B. A. Marathwada University, Aurangabad, M.S. India.

### ABSTRACT

The Biodiversity of mites in dust of poultry and flour mill was investigated.. The mites were picked up from intramural dust ecosystem that is poultry farm and flour mill. Analysis of air was also done using Tilak Air Sampler. The dust from poultry farm was collected and scanned. The mite *Dermatophagoides pteronyssinus* were found more in number of the entire mite found in poultry, followed by *Urodia spistecta*, and *Dermanyssus gallinae* which is actual chicken mite. The observation revealed mite fauna exhibiting seasonal fluctuations. The mites were found in highest percentage in rainy season, moderate during winter season and least during summer season. *Acarus siro* mite was observed in the study of flour mill dust.

**Keywords :** Biodiversity, Mites, Prevalence

### INTRODUCTION

Mites are arachnids, cosmopolitan in distribution, and are characterized by the presence of four pairs of legs. They are small, microscopic and are parasitizing majority of animals specially birds and mammals. Mites are free living and are useful in biologic recycling process as scavengers or as saprophagous or parasitic mites (Spieksma F. Th. M- 1997). Mites are the main material found in intramural dust. Dust is fine dry powder and it consists of particles. Dust in home, offices and other home environment contain small amount of plant pollen, human and animal hairs, textile fibers, paper fibers, human skin cells and many others materials which may be found in local environment.

Dust mites found in poultry dusts are allergens causing allergy in sensitive individuals. Some of them

have also been found to cause diseases in poultry birds and poultry workers. They create ecological imbalance in nature. It also results into aero bio pollution problems. Some of them are very tiny and lighter in weight, therefore are suspended in breeze, and forms exclusive part of Aerobiology. Dust mites prefer dark and humid climate. Poultry dust is mixture of bird feed. The rearing bed consists of wood shavings, shreds, straws, gravels, bird droppings, feather and dander (dead skin). The poultry birds affects on the growth of the birds and laying of the eggs. The increasing incidence of a number of diseases in poultry has been associated with ingestion of contaminated feed with biologically active compound. Poultry house provides an environment to dust mites. Exploration about biodiversity of poultry mites is carried out from September 2010 to August 2012.

### MATERIAL AND METHODS

The site selected is the poultry farm and flour mill in Pune. The poultry dust was collected from corner, under the feeder, sides of wall and central part of the poultry house. The surface layer was removed, which is the poultry litter. It is coarse and consists of bigger

#### \*Corresponding Author

Email: [vaishaliishere@yahoo.com](mailto:vaishaliishere@yahoo.com)

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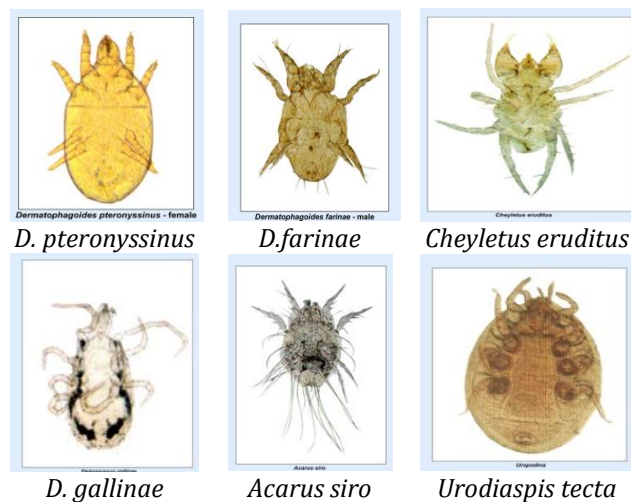
size gravel, saw dust, groundnut hulls, chopped straws and paddy husks. The immediate lower layer below surface is fine which was collected manually. It was then packed in sterilized bag of paper. The time of collection of dust is 4:00 p.m. It was then sieved through 300micronmeter mesh size sieve into Petri dish of 5 cm diameter. It was uniformly spread in thin layer. The Petri plates were then mounted under dissecting microscope with 10X magnification. They were then picked up with the help of needle of 0.2mm, 0.5 mm depending on the size of mite. The tip of needles was moistened with 40% lactic acid, as on touch they stick to moist needle. It was then placed in cavity blocks containing lactic acid for 24 hours. Lactic acid is used as clearing agent and it also paralyzes the mites. It dissolves and clears the sclerotization of the mites, and makes it transparent.

Another site for the collection of flour dust was from flour mill. It is the place where whole grains like wheat, jowar, bajri, and cereals are ground. The flour dust was collected from the side of walls, corners and near the flap of mill. It was collected with the help of brush and collected in paper bags.

Quantification of mites is undertaken so as to find out the number of each species out of total mites present in the dust at given time. The quantification of mites was done every month. It is done by floatation technique. The dust was weighed to 1 gram. It was then centrifuged in 100% kerosene at 2000 rpm for 10 minutes. Supernatant was filtered through filter paper. To the sediment of above, kerosene and carbon tetrachloride in 3:5 ratios is added. It was again centrifuged at 2000 rpm for 10 minutes. It was filtered through the same filter paper. To the above sediment kerosene and CCl<sub>4</sub> was added in 1:3 ratio and filtered through same filter paper. In the last step, in the sediment, pure CCl<sub>4</sub> was added and centrifuged at 2000 rpm for 10 minutes. The filter paper is spread on Petri plate and observed under the microscope and total mites were counted. The mites kept in cavity blocks were then picked up and placed on slide with ventral side up. A drop of freshly melted glycerin jelly was put on the mite and immediately cover slip was mounted over it. It is then pressed slightly. Excess of jelly is removed.

## OBSERVATION:

Mites are placed in phylum Arthropoda in class Arachnida. The mites found belong to order Astigmata, Prostigmata, Mesostigmata. Total 379 specimens were screened to find out the percentage contribution of each species of mite. The mites found in the investigation were *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cheyletus eruditus*, *Dermanyssus gallinae* and *Urodiaspis tecta*. Scanning of dust from the flour mill was also undertaken and only one species of mite *Acarus siro* was found.



Species	% Contribution
<i>Dermatophagoides pteronyssinus</i>	30.34
<i>Dermatophagoides farinae</i>	15.30
<i>Cheyletus eruditus</i>	17.94
<i>Dermanyssus gallinae</i>	7.65
<i>Urodiaspis tecta</i>	28.75

Name of mite	Season	% Prevalence of mites
<i>D.ptronyssinus</i>	rainy	73.04
	winter	24.34
	summer	2.60
<i>D.farinae</i>	rainy	75.86
	winter	22.41
	summer	1.72
<i>C.eruditus</i>	rainy	98.52
	winter	0
	summer	2.94
<i>D.gallinae</i>	rainy	93.10
	winter	6.89
	summer	0
<i>U.tecta</i>	rainy	94.95
	winter	5.50
	summer	0



### Seasonal fluctuation of population of mites in poultry

Systematic study was undertaken to study the seasonal variation in the population of mites of poultry September 2010 to August 2012. The prevalence in percentage contribution of mites found in poultry dust is calculated. Out of all mites, *Dermatophagoides pteronyssinus*, *Cheyletus eruditus* were not found in winter season. *D. gallinae* which is actual chicken mite was found in less number in winter season as compared to *D. pteronyssinus* and *D. farinae*. Highest % contribution of all dust mites is recorded during rainy season that is July, August, September and October. These are congenial environmental conditions, when the temperature is 25°C and humidity between 75% and 85%. There is progressive increase in number of mites per gram of poultry dust from July to September. Our findings revealed that low temperature, low RH, cold condition and rainless days act as adverse condition for the incidence and growth of mites. In such condition the population of mite in the dust of poultry is significantly decreased from November to May that is comprising winter and summer months. The dry period of summer months makes it hard for the survival of mites.

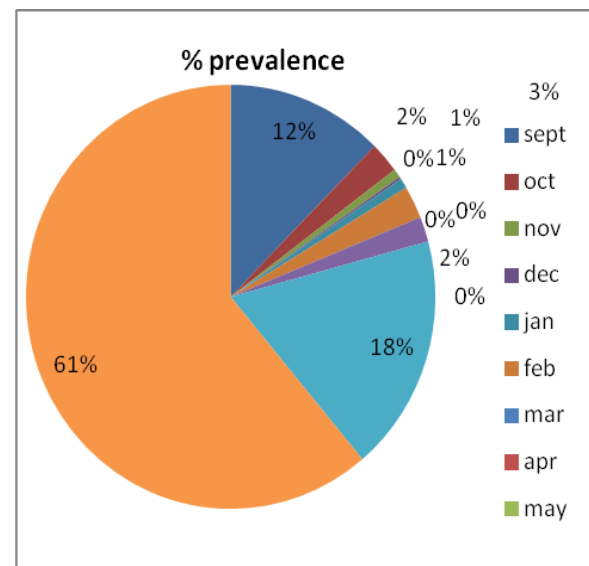
#### Prevalence of Poultry Dust Mites

September 2010 to August 2012 Number of mites/gm dust

Months	2008-09	2009-10	Total	% prevalence
Sept	45	85	130	12.41
Oct	13	12	25	2.38
Nov	3	4	7	0.66
Dec	1	1	2	0.19
Jan	4	4	8	0.76
Feb	14	12	26	2.48
Mar	0	0	0	0
Apr	0	0	0	0
May	0	0	0	0
Jun	10	10	20	1.91
Jul	100	89	189	18.05
Aug	241	399	640	61.12
	128	132	1047	

### RESULT AND DISCUSSION

The study demonstrates that poultry dust mites occur in humid environmental condition. It is known that prevalence is greater in humid geographic areas than the dry areas. Seasonal variation in population of mites is generally consistent with those reported by studies in Bangalore, India. There was gradual increase in the population of mite during the months of rainy season, when the RH and temperature was optimum for the survival of mite fauna.



Very little work has been done on poultry mites and house dust mites in Pune region. In this we performed identification and presence of different types of mites in the poultry farm. During these studies *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cheyletus eruditus*, *Dermanyssus gallinae* and *Urodiaspis tecta* mite species were reported in different concentration, of which *Dermatophagoides pteronyssinus* were reported highest in all season. Moderate temperature, high relative humidity provided most congenial environment for maximum percentage contribution of mites in poultry dust. RH affects the population of mites. The observation is also reported by Spieksma (1997). Whereas low temperature and high temperature were found to be unfavorable for the survival of mites as is revealed in cold dry winter months and hot summers of April and May. It is in agreement with those of Tilak and Jogdand (2009).



In the present study *Dermanyssus* was also found, which is the actual chicken mite. Hughes (1976) has found its distribution cosmopolitan. Work on *Cheyletus eruditus* has also been done by Choudhary and Mukherjee (1971) and have recorded it as a common predatory mite. Further investigations would include exploring more biodiversity of mites in intramural ecosystem, allergen load of mites in dust samples and clinical investigations.

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## RESEARCH ARTICLE

## BIODIVERSITY OF MYCOFLORA IN MANGROVES HABITAT OF MUMBRI CREEK OF SOUTH KONKAN, MAHARASHTRA.

Dekate HM<sup>1</sup>, Yeragi SG<sup>2</sup> and Yeragi SS<sup>2\*</sup>

<sup>1</sup>ICLES Motilal Jhunjhunwala College, Vashi, Navi Mumbai.

<sup>2</sup>K. J. Somaiya College of Science, Vidyavihar, Mumbai-400077.

### ABSTRACT

The present investigation is carried out in Mumbri Creek of Sindhudurg district (Lat. 16° 21' N. Long. 73°25' E). The main aim of this work is to find out the productivity of the Mumbri creek. This abstract consists of major mycoflora such as Phycomycetes, Ascomycetes, *Zygomycetes* and Deuteromycetes. The mycoflora help in making the wetlands highly nutritious which enhances commercially important resource organisms. Pneumatophores of *Avicenniamarina* provide excellent media to grow the fungi on large scale. The Deuteromycetes were dominating group.

**Keywords :** Mycoflora, Mangrove, Mumbri Creek.

### INTRODUCTION

The diversity and density of mycoflora associates with root surfaces of one major mangrove species in Mumbri creek, South Konkan, Maharashtra was studied during Jan.-Sept. 2013. The species recorded in the present investigation belonged to *Phycomycetes*, *Ascomycetes*, *Zygomycetes* and *Deuteromycetes*. The fungi were observed on one dominant mangrove species namely *Avicennia marina*, which harbours 12 genera and 17 species and some unidentified colonies. Among the species, *Aspergillus spp.* was most dominant species on the mangroves.

From the mycofloral point of view, tannin is an important secretion of mangrove that protects the protoplast against desiccational decay which plays an important role in establishment of mycoflora. The tannin along with, entangled fine silt or sand particles, from a film around the roots in which spores and fungi germinate to establish mycoflora in this interesting microhabitat. The film seen as a nutrient broth or medium in which the fungi are trapped and grow luxurious developing colonies of different classes.

#### \*Corresponding Author

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### MATERIAL AND METHODS

Regular fortnight samples of the pneumatophores of *Avicennia marina* were collected for a period during Jan.-Sept.2013. The fungi were obtained by serial washing of roots following Harley and Waid (1955) method.

### RESULTS

In all genera, *Aspergillus spp.* was the dominating genus with highest average percentage to be followed by *Curvularia*. The percentage distribution was *Deuteromycetes* (42.22), *Zygomycetes* (12.88), *Phycomycetes* (16.00), *Ascomycetes* (25.77) unidentified colonies (3.11). *Chaetomium olivaceum* (*Ascomycetes*) was the percentage wise lowest species. The class percentage of fungi was lowest in monsoon compared to pre monsoon and post monsoon periods. The maximum number of species was recorded in the months of October to March, while minimum in July to August. The observed variation in density can be attributed to extreme hydrological condition like heavy rainfall, high velocity of water currents, flooding and mechanical stress due to wind and water currents that prevent formation and stability of slime film formation around roots. In the case of *Avicennia marina*, 12 genera and 17 species



and some unidentified colonies were recorded of mycofloral diversity. Among the fungal class, *Deuteromycetes* was dominating over the other three and *Aspergillus* was the most abundant species.

*Aspergillus niger* or *A. niger* is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called fruit scab on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It was seen that altogether five diseases caused by *Aspergillusniger*, *Aspergillus flavus*, *Alternaria* Sp., *Botrytis cinerea* and *Rhizopus stolonifer* were recorded. However, fruit scab by *Penicillium expansum* was recorded at maximum places and on more varieties. Baviskarand Suryawanshi (2013).

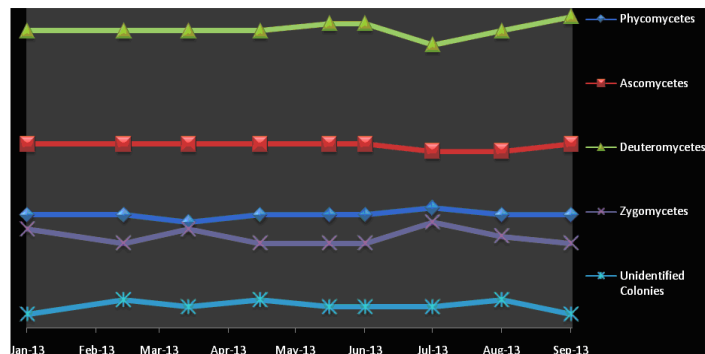
*Aspergillus fumigatus* is a fungus of the genus *Aspergillus*, a saprotroph wide spread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling.

**List of fungal species**

- 1) Phycmycetes
  - a) *Absidia ramose*
  - b) *Rhizopusnigricans*
  - c) *Syncephalastrumolivacum*
- 2) Ascomycetes
  - a) *Emericellanidulans*
  - b) *Cirrenaliatropicalis*
- 3) Deutromycetes
  - a) *Aspergillusfumigatus*
  - b) *A. nidulans*
  - c) *A. niger*
  - d) *A. terreus*
  - e) *A. flavus*
  - f) *Cladosporiumoxysporum*
  - g) *Curvulariaoryzae*
  - h) *C. tuberculata*
  - i) *Fusariumoxysporium*
  - j) *Penicillumnigricans*
  - k) *Trichothesiumroseum*
- 4) Zygomycetes
  - a) *Mucorracemosus*
- 5) Unidentified colonies

**Check list of fungi species isolated from the mangroves.**

Sr. No.	<i>Avicennia marina</i>
01	<i>Absidia ramose</i>
02	<i>Mucorracemosus</i>
03	<i>Rhizopusnigricans</i>
04	<i>Syncephalastrumolivacum</i>
05	<i>Aspergillusfumigatus</i>
06	<i>A. nidulans</i>
07	<i>A. niger</i>
08	<i>A. terreus</i>
09	<i>A. flavus</i>
10	<i>Cladosporiumoxysporum</i>
11	<i>Curvulariaoryzae</i>
12	<i>C. tuberculata</i>
13	<i>Emericellanidulans</i>
14	<i>Fusariumoxysporium</i>
15	<i>Penicillumnigricans</i>
16	<i>Trichothesiumroseum</i>
17	<i>Cirrenaliatropicalis</i>
18	Unidentified colonies



**Fig. 1:** Percentage composition of fungal classes isolated from *Avicennia marina*.



**DISCUSSION:**

Patole (2009) has recorded that the stilt roots are better for the abundant growth of mycoflora than for the pneumatophores of *Avicennia* species. The stilt or prop roots also differentiate that the inner roots show more fungi than those in the outer peripheral region because of comparatively more humidity on the inner side. The humidity is directly proportional to both qualitative as well as quantitative growth of fungi. Babu (1999) noticed that the mycofloral growth is affected in monsoon than in any other season. In monsoon, due to the breaking of the waves on the root surfaces, the freshly developed film gets washed out thus inhibiting the multiplication of mycoflora. Parkinson (1967) described the root surface fungi of rhizoplane fungi. Untawale, A. G., Dwivedi, S. N. and Singbal, S. Y. S. (1973) have investigated rhizoplane fungi of certain plants and also those colonizing intertidal region of the mangrove swamps.

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## REVIEW ARTICLE

## SENSOR TECHNOLOGY FOR ENVIRONMENTAL MONITORING: AN OVERVIEW

Jadav Jagruti N<sup>1</sup>, Maind Sandip D<sup>2a</sup>, Gajbhiye Suraj<sup>2b</sup> and Bhalerao Satish A<sup>1\*</sup>

<sup>1</sup>Environmental Sciences Research Laboratory, Department of Botany, Wilson College, University of Mumbai, Chowpatty, Mumbai-400007, (M.S.), India.

<sup>2a</sup>Department of Chemistry; <sup>2b</sup>Department of Botany, Bharatiya Vidya Bhavan's Hazarimal Somani college of Arts and Science, University of Mumbai, Kulapati K.M. Munshi Marg, Mumbai-400007, (M.S.) India.

**ABSTRACT**

Pollution of the environment is a problem of the modern age. The technology developed and used over the past decades has left serious consequences on human health and environment and implied the need for resolving them. Pollution which contaminates water, air & soil which affects ecosystems and creates new diseases which hazards human being. Preserving quality of water, air and soil are quite common issues in many countries. Sensor technology has significantly improved over the past few years. Sensors are smaller, lighter, more reliable and portable. They are capable of monitoring and measuring certain features of observed phenomena and can be placed anywhere. Sensors should allow one to move the measurement of numerous inorganic and organic pollutants from laboratory to the field and to perform them rapidly, inexpensively and reliably. Thus sensor technology plays a very important role to control and monitor the environment. In the present paper various types of sensors like electrochemical biosensors, chemically modified sensors & stripping based metals sensors and their applications in environmental monitoring and environmental protection is overviewed.

**Keywords :** Sensors, environmental protection, environmental monitoring.

**INTRODUCTION**

Environmental concern is present nowadays all over the world. The diversity and quantity of chemicals released into the environment has risen dramatically in recent years. These emissions and their impacts are varied and usually complex. This causes serious concerns about their adverse effects on the ecosystem and on human health. The legacy of land and groundwater contaminated by human activities affects quality of life. Increasing regulatory and economic requirements to monitor and treat pollution in the environment have created a pressing need for reliable, cost effective monitoring of contaminating compounds in water, soil and sediments. New low-cost effective tools are needed for

monitoring pollution and detecting trends over time. Modern way of living has left some serious consequences on human environment. In recent years, one of the emerging technologies that have had big impact on the field of research is sensor technology and its diversity of features and applications.

Environmental monitoring and protection is an area where these sensor technologies are of huge importance. Data collected by sensors are sent through the network to the control centres where are than being processed and analysed. The environmental monitoring is one of the main areas of application of this technology due to its characteristics that allow the measurement of parameters in different environmental settings such as crop management, protection of forest fires, agriculture, earthquakes, and active volcano. It is also possible to use macro-instruments for measuring parameters of large-scale such as landslides, atmospheric meteorology, and finally pollution studies or even for planetary exploration.

**\*Corresponding Author**

Email: [drsatis16@rediffmail.com](mailto:drsatis16@rediffmail.com)

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At present, global climate changes on the earth made a rational land use, environmental monitoring, forecasting of natural and technological disaster, and the tasks of great importance. The basis for the solution of these crucial applied problems consists in the integrated use of data of different nature: modelling data, in situ measurements and observations, and indirect observation such as airborne and space borne remote sensing data.

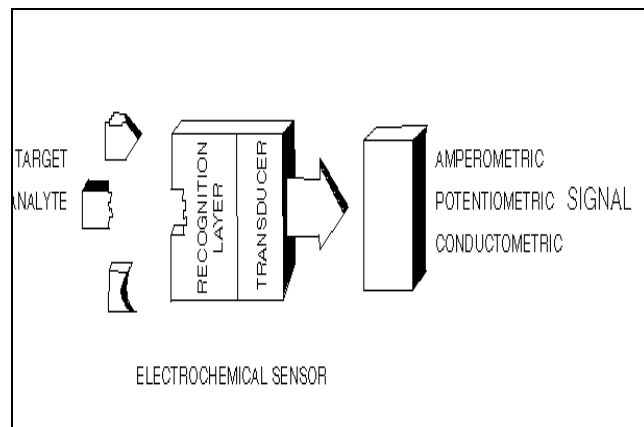
Electro-analytical chemistry can play a very important role in the production of in particular; electrochemical sensor and detectors are very attractive for on-site monitoring of priority pollutants, as well as for addressing other environmental needs and helping for protection of our environment. Such devices satisfy many of the requirements for on-site environmental analysis. Chemical sensors also include a special branch referred to as biosensors for the recognition of biochemical and bio-reaction.

#### ELECTROCHEMICAL BIOSENSORS:

The remarkable specificity of biological recognition processes has led to the development of highly selective biosensing devices. Electrochemical biosensors hold a leading position among the bioprobes currently available and hold great promise for the task of environmental monitoring. Such devices consist of two components: a biological entity that recognizes the target analyte and the electrode transducer that translates the biorecognition event into a useful electrical signal. A great variety of schemes for implementing the electrochemical biosensing approach, based on different combinations of biocomponents and electrode transducers have been suggested. These rely on the immobilization of enzymes, antibodies, receptors or whole cells onto amperometric or potentiometric electrodes. A general schematic diagram for the operation of electrochemical biosensors is shown in Figure 1. Fundamental aspects of these dives have been reviewed in the literature (Turner et al. 1987; Frew 1987 and Kobos 1994).

Electrochemical biosensors have been the subject of basic as well as applied research for nearly fifty years. Leland C. Clark introduced the principle of the first enzyme electrode with immobilized glucose oxidase at the New York Academy of Sciences

Symposium in 1962 (Clark and Lyons was the YSI 23A Blood Glucose Analyzer; Yellow Miroslav Pohanka, Centre of Advanced Studies, Faculty of Military Health Sciences, University of Defense, Hradec Králové, Czech Republic).



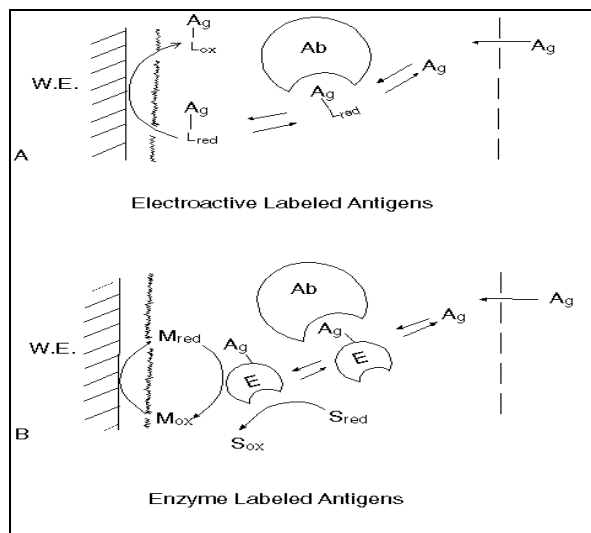
**Figure.1. Electrochemical biosensors: biorecognition and signal transduction**

The first commercially produced biosensor Springs Instruments (Yellow Springs, OH, USA) placed on the market in 1975. This device was applied to the fast glucose assay in blood samples from diabetics. At present, there are many proposed and already commercialized devices based on the biosensor principle including those for pathogens and toxins, some even based on a multi-channel configuration. The most typical part of electrochemical biosensors is the presence of a suitable enzyme in the biorecognition layer providing electroactive substances for detection by the physico-chemical transducer providing the measurable signal. A native enzyme can be used as the biorecognition component. In this case the analyte is equal to the enzyme substrate, alternatively it may function as its inhibitor and in addition, enzymes can be used. Electrochemical biosensors whose principles and applications labels bound to antibodies, antigens and oligonucleotides with a specific sequence, thus providing affinity-based sensors. A rather limited number of enzymes processed in biotechnology were chosen for the monitoring of clinical metabolites and, especially from the group of oxidoreductases: glucose oxidase and glucose dehydrogenase for glucose assays, alcohol oxidase for ethanol, NADH dependent lactate dehydrogenase and lactate: cytochromec



oxidoreductase for lactate, urease for urea and cholesterol oxidase co-immobilized with cholesterol esterase for the cholesterol assay. Peroxidase and alkaline phosphatase are the most common enzyme labels for electrochemical affinity biosensors. Based on their operating principle, the electrochemical biosensors can employ potentiometric, amperometric and impedimetric transducers converting the chemical information into a measurable amperometric signal.

The design of enzyme electrodes is such that the current or potential measured is proportional to the rate limiting step in the overall reaction. For reactions limited by the Michaelis-Menten kinetics, a leveling off of calibration curves is expected at high substrate concentrations. Mass-transport limiting membranes can be used to greatly extend the linear range. This will also lead to a slower response. The signal may be dependent also upon the Ph of the water sample or its heavy metal content that affect the enzymatic activity. Attention should be given also to the long-term stability of these devices, due to the limited thermostability of the biocatalytic layer.



**Figure. 2.** Amperometric immunosensor based electroactive-(A) and enzyme (B) tagged antigen.

Several enzyme electrodes have already proven useful for the task of environmental monitoring. For example, several groups reported on highly sensitive amperometric biosensors for phenolic compounds. Such devices rely on the immobilization of tyrosinase onto carbon- or platinum transducers, and the low potential detection of the liberated quinone product (Figure 2.). Assays of industrial wastes or natural

water have been documented, including possible remote phenol sensing (Wang and Chen) and single-use on-site sensing (Kotte et al. 1995 and Wang and Chen 1995). Similarly, low potential biosensing of organic peroxides or hydrogen peroxides or hydrogen peroxide can be accomplished at peroxidase-modified electrodes (Wang et al. 1991 and Csoregi et al. 1994). "class-selective" enzyme electrodes, based on tyrosinase or peroxidase, can be used for semiquantitative field screening. They can also be used as detectors for liquid chromatography, hence providing quantitation of the individual substrates (Ortega et al. 1994).

### CHEMICALLY MODIFIED SENSORS:

The world seems to have a natural division between chemical and physical sensors. However, there are those that do not classify easily, like relative humidity sensors, a chemical sensor traditionally lumped with physical sensors. Also, sensors are often discussed along with the topic of actuators. Chemical sensors have a chemical or molecular target to be measured. Biosensors are defined as sensors that use biomolecules and/or structures to measure something with biological significance or bioactivity. More appropriately, bio- sensors target a biomolecule of interest for measurement. The biosensor can usually be considered a subset of chemical sensors because the transduction methods, sometimes referred to as the sensor platforms, are the same as those for chemical sensors. A useful definition for a chemical sensor is "a small device that as the result of a chemical interaction or process between the analyte gas and the sensor device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal. The lab-on-a-chip or m-TAS ~micro-Total Analytical System! is considered a sensor in only the broadest of definitions and is really a complete analytical system. The signal from a sensor is typically electronic in nature, being a current, voltage, or impedance/conductance change caused by changing analyte composition or quality. While chemical sensors contain a physical transducer and a chemically sensitive layer or recognition layer, the microinstrument or spectrometer sends out an energy signal, be it thermal, electrical, or optical, and reads



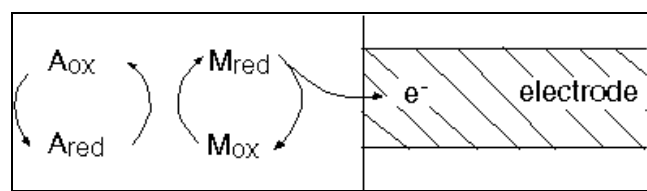
the change in this same property caused by the intervening chemical and this is akin to molecular spectroscopy in the above example. In m-TAS, the system can include sampling system, separation or fluidic instrumentation system, as well as a detector. The users of sensors, of course, do not care about this division, but this paradigm is helpful in explaining the types of systems that exist and understanding how they work, why they have certain properties and analytical performance, and how new developments are made. ECS has had conferences that have included all of these types of sensors. A few types of electrochemical sensors are included in the following discussions. While the topic of sensors of interest to the Society is too broad to cover here, we can discuss a few electrochemical sensors by conventional definition, assigned to three categories: potentiometric, amperometric, and impedance or admittance based devices. Biosensors, while directed toward analysis for a specific or significant biological material or bio-endpoint will utilize one or more of these principles. Optical and acoustic or similar approaches are also included in electrochemical sensors if a broad definition of these terms is used. Electrochemical sensors can be applied for solid, liquid, or gaseous analytes with the latter two most common. High temperatures can be accommodated using solid electrolytes and high temperature materials for sensor device construction. In the following brief discussion, we outline some common electrochemical sensors and, by illustration, the continued ECS interest in sensors.

Chemical layers can also be used for imparting a high degree of selectivity to electrochemical transducers. While conventional amperometric electrodes serve mainly for carrying the electrical current, powerful sensing devices can be designed by a deliberate modification of their surfaces. Basically, the modification of an electrode involves immobilization of reagents that change the electrochemical characteristics of the bare surface. Inclusion of reagents within the electrode matrix (e.g. carbon paste) is another attractive approach for modifying electrodes. Such manipulation of the molecular composition of the electrode thus allows one to tailor the response to meet specific sensing needs. The new "mercury-free" surface address also growing concerns associated with field applications of the classical

mercury drop electrode. Theoretical details on modified electrodes can be found in several reviews (Murray et al. 1987; Wang 1991 and Arrigan 1994).

While sensor based on modified electrodes are still in the early stage of their lifetime, such preparation of structured interfaces hold great promise for the task of environmental monitoring. There are different directions by which the resulting modified electrode can benefit environmental analysis, including acceleration of electron-transfer reaction, preferential accumulation or permselective transport.

Electrocatalysis involves an electron transfer mediation between the target analyte and the surface by an immobilized catalyst (Figure 3.) Such catalytic action results in faster electrodes reactions at lower operating potentials. Various catalytic surfaces have thus been successfully employed for facilitating the detection of environmentally relevant analytes. These include the electrocatalytic determination of hydrazines (Waang and Lu 1989) or nitrosamines (Gorski and Cox 1994) at electrodes coated with mixedvalent ruthenium films, monitoring of aliphatic aldehydes at palladium-modified carbon paste (Cai and Kalcher 1994), Sensing nitrite-based redox polymer (Doherty et al. 1991) of nitrate at a copper modified screen printed carbon electrode (Fogg et al. 1991), monitoring of organic peroxides at cobalt-phthalocyanine containing carbon paste (Wang et al. 1991), and of hydrogen peroxide at a copper heptacyanonitrosylferrate-coated electrode (Gao et al. 1992).



**Figure.3.** Electrocatalysis at modified electrodes; electron transfer mediated reaction between the target analyte and surface-bound catalyst.

#### STRIPPING-BASED METAL SENSORS:

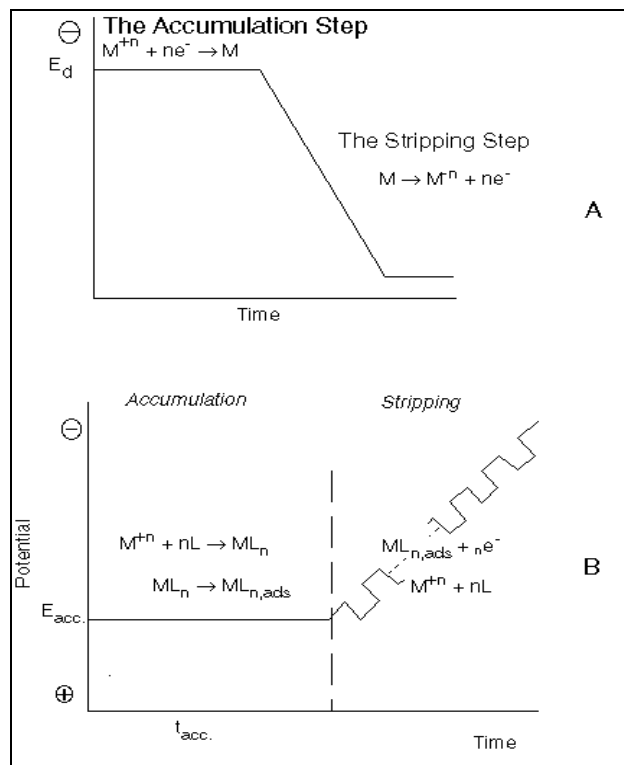
Mercury-modified electrodes coupled with stripping techniques have been recognised as the most sensitive methods for determination of heavy metals. However, the potential dangers associated with mercury have led to developing mercury-free sensors.



Unmodified electrodes like bare carbon, gold or iridium2–4 graphite–epoxy composites5–7 recordable CDs8 or silver-plated rotograved carbon electrodes9 have been used as an alternative to mercury based electrodes. Efforts have been done also to use electrodes modified with various metal affinity compounds such as tetraphenylporphyrin, 10 Nafion11,12 N-p-chlorophenyl cinnamon- hydroxamic acid, 13 dithizone,14 etc. One of the excited alternatives to mercury based electrodes is that based on bismuth. Bismuth film electrodes (BiFE) display an attractive stripping voltammetric performance which compares favourably with that of common mercury-film electrodes (HgFE). The low toxicity of bismuth makes it an alternative material to mercury in terms of trace metal determination. The remarkable stripping performance of BiFE can be due to the binary and multicomponent ‘fusing’ alloys formation of bismuth with metals like lead and cadmium. Bismuth film, with an attractive stripping voltammetric behaviour, prepared by electrodeposition onto the micro disc,17 gold,18 carbon paste,19 glassy carbon, 20–23 rotating glassy carbon disc24 electrodes have been reported. In situ or ex-situ preparation 25 of the BiFE including the effect of bismuth precursor salt used to prepare the film and a variety of substrate surfaces (platinum, gold, glassy carbon, carbon paste, carbon fibre) 26 for bismuth plating were carefully examined for their effects in the preconcentration and stripping steps, including the constant-current potentiometric stripping.

Conducting composites represent another effort in designing mercury free sensors for stripping analysis. The capability of integrating various materials is one of their main advantages. Composite sensors offer many potential advantages including higher signal-to-noise (S/N) ratio 28–32 compared to more traditional electrodes consisting of single conducting phase. Composite electrodes can often be fabricated with great flexibility in size and shape of the material, permitting easy adaptation to a variety of electrode configurations. Their surfaces can be smoothed or polished to provide fresh active material ready to be used in a new assay. Each new surface yields reproducible results because all individual compounds are homogeneously dispersed or compressed in the bulk of the composite. Represents the schematic of the rigid graphite–epoxy composite electrode (GECE)

configurations (I, II) that have been used by our group as well as the new one (III con- figuration) that is presented in this work. The first configura- tion was based on GECE sensors without modifications. These sensors have been studied firstly for PSA determination of heavy metals by using stripping with constant current mode or chemical oxidation by dissolved oxygen. Later on the same GECE without any modification have been characterised in their use in DPASV.6,7 The second configuration, Bi-GECE33 was based on GECE without modification but bismuth film formation due to the presence of bismuth in the measuring solution. In the present work we present a novel configuration (Bi(NO3) GECE, that represents GECE modified internally with bismuth nitrate salt which serves as a built-in bismuth precursor for bismuth film formation. This represents an integrated configuration of bismuth based GECEs for stripping analysis.



**Figure 4.** Steps in anodic (A) and adsorptive (B) stripping voltammetry of trace metals, based on electrolytic and adsorptive accumulation, respectively, of target metal analyte





The most sensitive electroanalytical technique, stripping analysis, is highly suitable for the task of field monitoring of toxic metals. The remarkable sensitivity of stripping analysis is attributed to its preconcentration step, in which trace metals are accumulated onto the working electrode. This step is followed by the stripping (measurement) step, in which the metals are "stripped" away from the electrode during an appropriate potential scan. About 30 metals can thus be determined by using electrolytic deposition or adsorptive accumulation of a suitable complex onto the electrode surface (Figure 4.). Stripping electrodes thus represent a unique type of chemical sensors, where the recognition and transduction processes are temporally resolved.

Various advances in stripping analysis should accelerate the realization of on-site environmental testing of toxic metals. New sensor technology has thus replaced the traditional laboratory-based mercury electrodes and associated cumbersome operation (oxygen removal, solution stirring, cell cleaning, etc.). Of particular significance are new stripping-based tools, such as automated flow systems for continuous on-line monitoring (Zirino et al. 1978; Wang et al. 1992 and Clark et al. 1988), disposable screen-printed stripping electrodes for single-use field applications (Wang and Tian 1992), or remote/submersible devices for down-hole well monitoring or unattended operation (Terciet et al 1990 and Wang et al. 1995).

In addition to trace metal pollutants, it is possible to employ new adsorptive stripping procedures for measuring low levels of organic contaminants that display surface-active properties (e.g. detergents, oil components). However, due to competitive adsorption such schemes usually require a prior separation step. Another version of stripping analysis, cathodic stripping voltammetry can be used for measuring environmentally-relevant anions (e.g.  $S^{2-}$ ,  $I^-$ ,  $Br^-$ ) or sulfur or chlorine containing pollutants (e.g. pesticides) following their oxidative deposition onto the working electrode. Additional background information on stripping analysis and its environmental opportunities can be found in various reviews (Wang 1985; Wang 1982; Tercier and Buffle 1993 and Wang 1994).

## CONCLUSION:

Sensor technology is very much useful for environmental monitoring, though having limited scope and cannot solve all environmental monitoring needs. Yet a vast array of sensor technology has been applied in recent years for monitoring a wide range of inorganic and organic pollutants.

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## REVIEW ARTICLE

## INFLUENCE OF ATMOSPHERIC AEROSOLS ON HEALTH AND ENVIRONMENT-CLIMATE CHANGE

**Sharma Amit S<sup>1</sup>, Maind Sandip D<sup>2</sup>, Kelkar Tushar<sup>1</sup>, Knox Jai<sup>1</sup> and Bhalerao Satish A<sup>1\*</sup>**

<sup>1</sup> Environmental Sciences Research Laboratory, Department of Botany, Wilson College, University of Mumbai, Chowpatty, Mumbai-400007, (M.S.), India,

<sup>2</sup>Department of Chemistry, Bharatiya Vidya Bhavan's Hazarimal Somani college of Arts and Science, Kulapati K.M. Munshi Marg, University of Mumbai, Chowpatty, Mumbai-400007, (M.S.), India.

### ABSTRACT

Atmospheric Aerosols are consisting of very small, microscopic particles in the atmosphere which comes from different sources. Assessment of aerosol- climate interactions requires an understanding of the factors that determine the abundances and properties of aerosols in the atmosphere. From the standpoint of direct radiative forcing, critical aerosol properties are the surface size distribution, particle shape and complex refractive index. From the standpoint of indirect radiative forcing, critical aerosol properties are the number size distribution, hygroscopicity, phase and chemical composition. Present review paper deals with various examples of aerosols e.g. nucleation particles, soot particles, ammonium sulphate particles, sea salt particles, pollen and mineral dust particles and their properties are reviewed. Atmospheric particles – aerosols – are some of the key components of the atmosphere. They influence the energy balance of the Earth's surface, visibility, climate, human health and environment as a whole. According to World Health Organization (WHO), ozone, particulate matter, heavy metals and some hydrocarbons present the priority pollutants in the troposphere. Direct and indirect effects of atmospheric aerosol on climate system and of epidemiologic studies and their causative interconnection between particles and health effects. Impact of aerosols on the clouds is also discussed.

**Keywords :** Aerosol; Health; Environmental effects.

### INTRODUCTION

Atmospheric aerosols consist of very small particles, microscopic particles in the atmosphere which come from two different sources. One of which is of human origin from things like burning fossil fuels and other is natural aerosols which come from things like windblown dust or sea salt from breaking waves.

There are various types of suspended particulate matter in air (aerosols) such as soot (black carbon), organic matters, sea dust, sea salt, and sulfate from sulfur oxides and nitrate from nitrogen oxides. Aerosols have been called SPM (suspended particle matter) on the environmental administration, which

can be classified as PM<sub>10</sub> (defined as collection efficiency of 50% at 10 $\mu$ m aerodynamic diameter) or PM 2.5. As can be seen from that the main cause of Yokkaichi asthma was sulfate aerosol, they affect the respiratory system. PM<sub>2.5</sub> can also have been incorporated into blood from the lungs, leading to cardiovascular diseases. Aerosols also cause less visibility as understood from hazy by air pollution.

Atmospheric particles are a complex mixture of organic and inorganic substances, suspended in the atmosphere as both liquids and solids, and they cover a very wide range from a few nm in diameter to 100 or more  $\mu$ m. They vary also in shape, chemical composition, and optical properties. Particle size is one of the key parameters that govern the transport and removal of particles from the air, their deposition within the respiratory system and is associated with the chemical composition and sources. Sizes of some typical atmospheric particles are presented in Table 1. (Tasić et al., 2006)

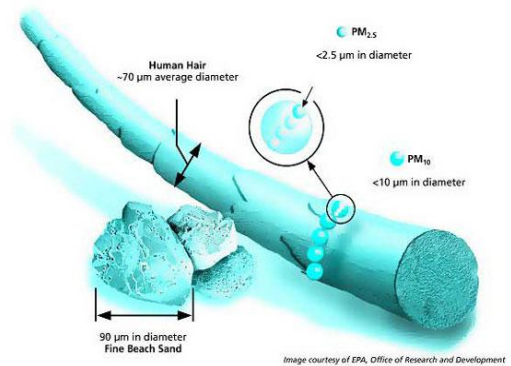
#### *\*Corresponding Author*

Email: [drsatis16@rediffmail.com](mailto:drsatis16@rediffmail.com)

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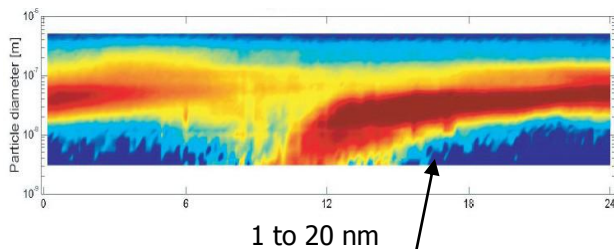
**Figure. 1:** size comparisons for aerosol pollution

**Table .** Sizes of some typical Atmospheric particles

Description	Examples
<b>Very small</b> 0.01 to 5 (µm)	Paint pigments, Tobacco Smoke, Dust, Sea-Salt particles.
<b>Larger</b> 5 to 100 µm	Cement dust, wind-blown soil dust, Foundry dust, Pulverized coal, Milled Flower.
<b>Liquid (Mist)</b> 5 to 10,000 µm	Fog, Smog, Mist, Raindrops.
<b>Of Biological Origin</b> 0.001 to 0.01 µm	Viruses, Bacteria, Pollen, Spores.
<b>Of Chemical Formation</b> 0.001 to 100 µm	Atmospheric sulphur dioxide oxidizes producing sulfuric acid; the acid attracts Atmospheric water forming small droplets (haze). Metal oxides form when Fuels that contain Metals are burned.

**EXAMPLES OF AEROSOLS PARTICLES:**

**Nucleation Particles:**



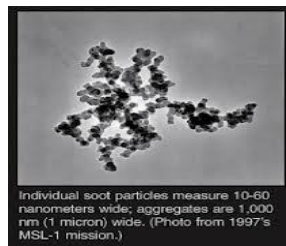
**Fig. 2. Nucleation in the atmosphere**

Size of the nucleation particles are 1 to 20 nm. Sources are various gas-phase precursor compounds, both natural (plants) and anthropogenic. Frequently found near busy roads, but also in pine forests. Formed via photo-oxidation of gas-phase traffic or industrial emissions or organic emissions from pine trees. Appearances are presumably spherical and liquid. Life time is few hours. Mainly limited by

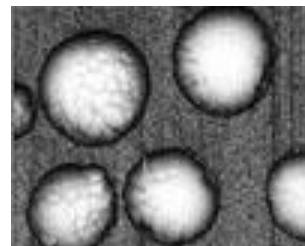
coagulation with larger particles. Other properties are if nucleation particles are observed, then in high number concentrations.

**Soot Particles:**

Soot, a product of incomplete combustion, is major size of soot particles is very variable length in the order of 1000 nm, equivalent diameters in the order of 100 nm. Sources are anthropogenic incomplete combustion of fossil fuel (e.g., diesel engines) also biomass burning. Appearance is fractal-like, complex shaped, black. Lifetime is about one week. The lifetime depends on the aging processes of the soot particles. These processes modify the ability of soot particles to take up water (hygroscopicity) and thereby the efficiency of wet removal. Other properties are cancerogenous, penetrate deep into the lungs, absorb solar radiation (positive direct climate effect), Fresh soot particle are hydrophobic, i.e., they are not water soluble and do not take up water, while aged soot is usually more hygroscopic and can take up water → important for cloud formation.



**Fig.3. Soot particles**



**Fig.4. Ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) particles**

**Ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>):**

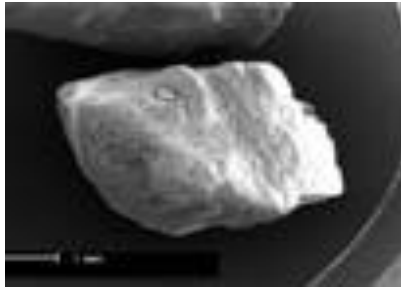
Size are about 100 - 400 nm. Sources are various: formed via chemical processes in the atmosphere (H<sub>2</sub>SO<sub>4</sub>+ NH<sub>3</sub>) → secondary aerosol precursor gases: SO<sub>2</sub> from fossil fuel combustion, NH<sub>3</sub> from industry and agriculture. Appearances are compact, white. Lifetime is about one week. Other properties are highly water soluble, effective for cloud formation, usually in internal mixture with other secondary compounds (NH<sub>4</sub>NO<sub>3</sub>, organics).

**Sea Salt:**

The principal mechanism for emission of sea salt particles is the bursting of air bubbles at the sea surface. Size particles range from 200 nm up to about 10 µm. Sources are evaporation of sea spray produced



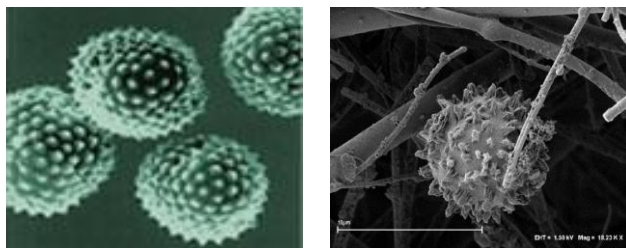
by bursting bubbles or wind-induced wave breaking. Appearance is compact, white. Lifetime hours to a few days. Other properties are composed mainly of NaCl (sodium chloride), also  $\text{NO}_3^-$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ; highly water soluble, effective in cloud formation and no adverse health effect.



**Fig.5. Sea salts particles**

**Pollen:**

Size: around 3 to 100  $\mu\text{m}$ . Sources are plants and vegetal material. Appearance is many different shapes. Lifetime are hours to days. Pollens are effectively removed by precipitation. Other properties are little water soluble, health problem for persons suffering from allergies (hay fever) and Ice nuclei.

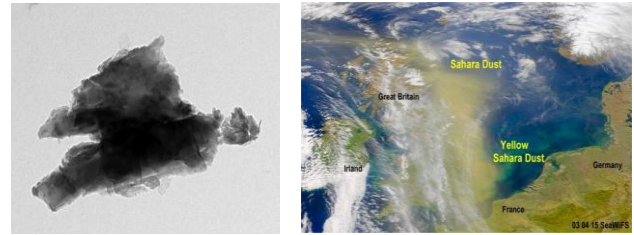


**Fig.6. Pollen**

**Mineral Dust:**

Mineral dust makes a major contribution to the aerosol optical depth. Primary sources are the arid and semi arid regions of the world, which account for about 15% of the global land.

Mineral dust particles are generally between 1 and 20  $\mu\text{m}$  (several up to 100  $\mu\text{m}$ ). Appearance are non-spherical, irregular. Lifetime of mineral dust is hours to days. Sahara dust can be transported to Europe and even to South America. Other properties are not water soluble and good ice nuclei.



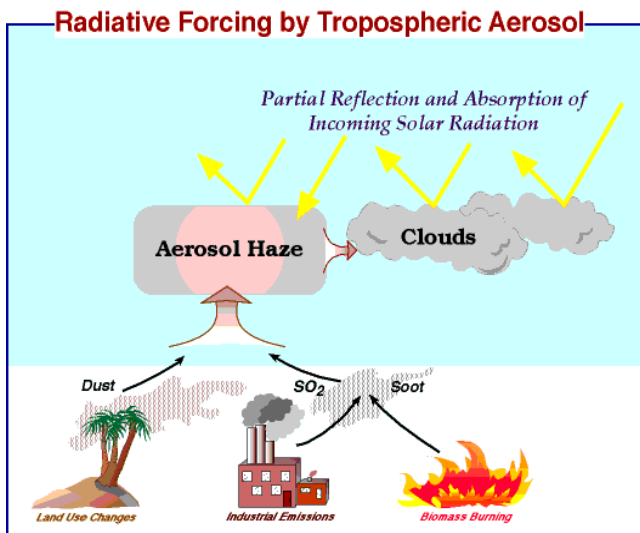
**Fig.7. Mineral dust particles**

**AEROSOLS EFFECT ON CLIMATE SYSTEM:**

Aerosol effects on climate system can be roughly classified into three. The first is the “*direct effect*” in which aerosols scatter and absorb the thermal radiation that is emitted from the Earth’s surface and atmosphere and the solar radiation, leading to a change in radiation balance. (McCormick and Ludwig, 1967; Charlson and Pilat, 1969; Atwater, 1970; Mitchell Jr., 1971; Coakley et al., 1983). The direct effect is much dependent on the physical and chemical properties of aerosols, such as particle size, hygroscopicity, and complex refractive index. For example, sulfate aerosol cools the atmosphere because it mainly scatters the solar radiation back to space. On the other hand, black carbon heats the atmosphere because of efficient absorption of the solar and thermal radiation. The second is the “*indirect effect*”. Aerosols have roles of cloud condensation nuclei and ice nuclei. An increase in the aerosol number concentration without changing the mass of the cloud water results in smaller cloud droplets and ice crystals, leading to stronger scattering and absorption of the solar and thermal radiation (referred to as “cloud albedo effect” or “first indirect effect”). (Gunn and Phillips, 1957; Twomey, 1977; Liou and Ou 1989; Albrecht, 1989). Consequently it causes a change in time to grow to precipitation and snowfall and then in cloud fraction (referred to as “cloud lifetime effect” or “second indirect effect”). In addition, an increase in aerosols which can be ice nuclei, such as black carbon and soil dust, promotes freezing of super cooled cloud droplets. Ice crystals grow to precipitation and snowfall faster than cloud droplets, so that a change in the number concentration of ice nuclei can result in a change in the cloud fraction. The third is the “*semi direct effect*” by aerosols which absorb the solar and thermal radiation, such as black carbon and soil dust. They heat the ambient air and then affect generation



of clouds due to changes in atmospheric stability and in saturated water vapor pressure.



**Fig. 8. Direct and indirect aerosol effects**

**HEALTH EFFECTS OF PARTICLES:**

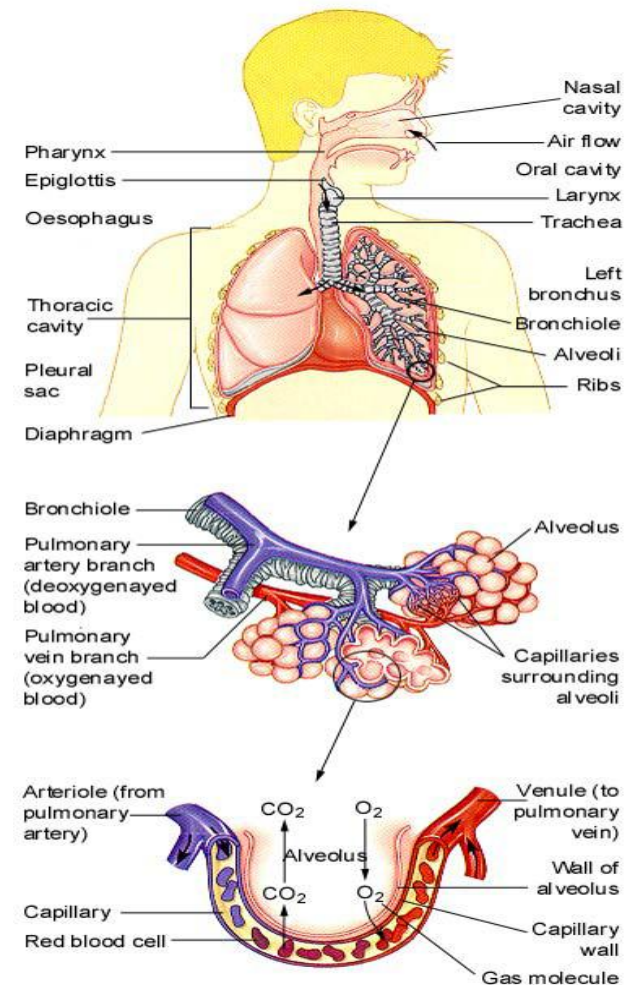
Effects of airborne particles health effects (epidemiology as the main trigger); effects on atmospheric properties; visibility reduction; fog formation and precipitation; solar radiation reduction; temperature and wind distribution alteration (e.g. climate change); effects on materials; effects on vegetation. Health effects of particles – seldom with direct proofs about the mechanism. In general, inhalation of airborne particles contributes to excess mortality and morbidity (not all adverse effects result in death). Specific health “end points” include: Declines in lung function; increased respiratory symptoms such as cough, shortness of breath, wheezing and asthma attacks; chronic obstructive pulmonary disease; cardiovascular diseases (diffusion across the epithelium of alveoli, changes coagulation of blood); Lung cancer.

a) Syndromes, illnesses and sensitivities exhibited or acquired as a result of indoor environment exposures - The indoor exposures causing these responses are believed to be a function of the synergistic effects of two or more pollutants (or even among particles): Sick Building Syndrome (goes away once building is avoided); Building Related Illness (acquisition due to exposure to that building); Multiple Chemical Sensitivity (synergistic effects to a number of chemicals).

b) Factors influencing particle deposition in the respiratory tract: The physio-chemistry of aerosol (particle size/size distribution; density; shape; hygroscopic/hydrophobic character; chemical reactions); The anatomy of the respiratory tract (diameter; length; breathing angles of airway segments); The physiology of the respiratory tract (airflow pattern; breathing pattern).

c) Particle deposition in respiratory tract; can be either total or fractional deposition (extra thoracic in nose or mouth; bronchial; bronchiolar; or alveolar).

d) Determination of particle deposition in respiratory tract can only be determined via experimental studies involving human or animal experiments; lung cast experiments in post mortem. Alternatively, computational modelling: NB: Experimental studies show significantly higher deposition rates than predicted by modelling.



**Fig. 9. Respiratory Tract; schematic diagram of the human respiratory tract and its compartments**

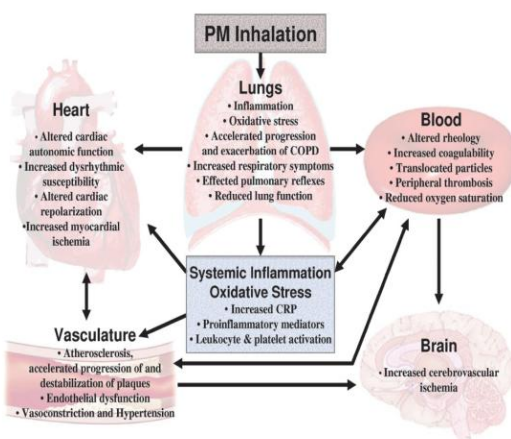


### IMPORTANCE OF AEROSOLS FOR HUMAN HEALTH:

A series of epidemiologic studies has clearly shown the causative interconnection between particles and health effects; frequency rates of chronic obstructive respiratory diseases seem to be increasing. The World Health Organization (WHO) as well as different authorities in Europe and the US, recognized the potential risks of atmospheric particulate matter, PM to public health, and atmospheric pollution by particles has become an important policy theme. Many studies have generally accepted that the ability for particles to cause health effects is dependent on their size [6]. In spite of the fact that particles up to 100  $\mu\text{m}$  enter the body through breathing, only very small particles, below 5  $\mu\text{m}$  aerodynamic diameter can reach deep into the lung and these very small particles have the main potential for causing health effects. The current focus of health-related sampling of particulate matter is on particles with aerodynamic diameter less than 10  $\mu\text{m}$  (PM10) but recent research pointed out the great health effect of fine particles PM2.5, and even PM0.1. The importance of chemical composition of fine particles is also outstanding. Four main fractions are defined: inhalable fraction (E) defined as the mass fraction of total airborne particles which is inhaled through the nose or mouth and for ambient atmosphere is given by:

$$E = 0.5 (1 + \exp[-0.06D]) + 10 - 5U \cdot 2.75 \exp(0.05D)$$

Where D is the aerodynamic diameter of the particle and U is the wind speed (up to 10 m s<sup>-1</sup>).



**Fig.10.** Potential general pathophysiological pathways linking PM exposure with cardiopulmonary morbidity and mortality

*Thoratic fraction*, defined as the mass fraction of inhaled particles penetrating the respiratory system beyond the larynx (median aerodynamic diameter of 10  $\mu\text{m}$ ); *respirable fraction*, defined as the mass fraction of inhaled particles which penetrate to the unciliated airways of the lung with a median aerodynamic diameter of 4  $\mu\text{m}$ , and high risk" respirable fraction for the sick, and infirm or children with a median aerodynamic diameter of 2.5  $\mu\text{m}$

### CONCLUSION:

Air quality investigation in Belgrade urban area has shown that the annual PM mass concentrations, in comparison to majority of European cities are significantly higher. The main sources of suspended particle are traffic, power stations, local heating and dust re-suspension. To predict future trends in the atmospheric loading of aerosols as a result of global change, we must first be able to quantify the sensitivity of the aerosol atmospheric loading.

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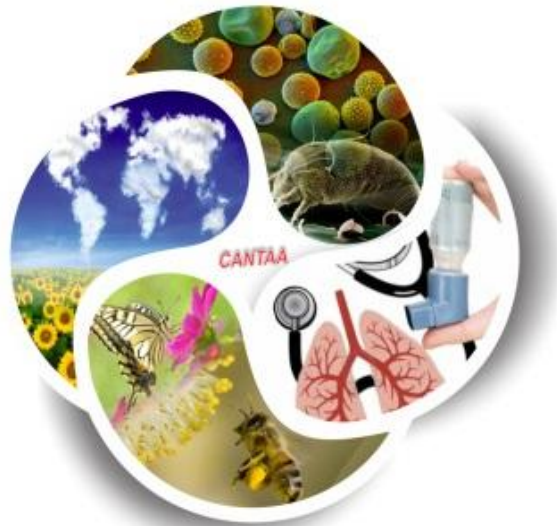
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# Medical / Biological Sciences

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**RESEARCH ARTICLE****EFFECT OF AIR POLLUTION ON HUMAN SKIN IN AMRAVATI CITY,  
MAHARASHTRA****Mankar RN and Ingole SP\***

Department of Environmental Science, Shri Shivaji Science College, Amravati, India -444603

**ABSTRACT**

On the air depends all the life forms of life. The body of humans are cooled by contact of air but there air contain many air pollutant from dust to many toxic gaseous which result ageing of skin and many skin diseases many skin problem comes from the pollution problems, western countries have conducted several studies in this area but there are only few studies in developing countries like India. In this backdrop, a study of air pollution effect on skin in Amravati city of Maharashtra has been undertaken. The study and select was residential area and commercial area. Data shows an increasing in the skin problems due to increasing air pollution decreasing the air quality. The hospital records also support the results obtained from the field study. The children young generations are main victims of the skin disease.

**Keywords :** Air Pollution, Human Health, Skin, Amravati, India.

**INTRODUCTION**

The key to man's health and calm lies largely in his environment. In fact many health problems and skin problems are due to the various pollution such as water pollution air pollution, Noise pollution, soil pollution often man is responsible for the pollution of his environment through urbanization and research. In 1972 the UN conference on the human environment focused world will attention on the environmental hazards that threaten human beings. To facilitate work in this area WHO has compiled a wide ranging survey of environmental hazards with human health.

Man has already control the number of environmental factors as food water, sanitation. This includes the standard of living. And as the new research the old problems are being solved but the new problems are arising. Air pollution problem is of growing concern in many urban areas.

The Main purpose of environmental health is to supply and maintain the environmental and that

give a good health and prevent the hazardous disease and also skin disease the national air quality monitoring programmed sponsored by the central pollution board (PCB) since 1999, has generated data base over last 14 years in 10 major Indian city named Ahmedabad, Mumbai, Kolkata, Delhi, Hyderabad, Jaipur, Kanpur, Kochi, Chennai, Nagpur. The programmed facilitates evolution of long term air quality trends for health related criteria pollutants such as invaluable dust, sulphur dioxide nitrogen, dioxide etc. and has several effect on skin.

The present study is an effort to assess the How skin damage and skin disease by the air pollutant in Amravati city.

**MATERIALS AND METHODS:**

Amravati is the one of the main district in Vidharbha region of Maharashtra. The name is derived from Umbaravati where the umbar plants are found so the name umbaravati is done but it change to Amravati.

Data collection has been done by the questionnaire method covering various households. All aspects of environment of each household like socio-economic status, indoor air pollution and the disease related skin, infectious disease, eye disease, which belongs to the area under study.

**\*Corresponding Author**Email: [sangita\\_pi@gmail.com](mailto:sangita_pi@gmail.com)

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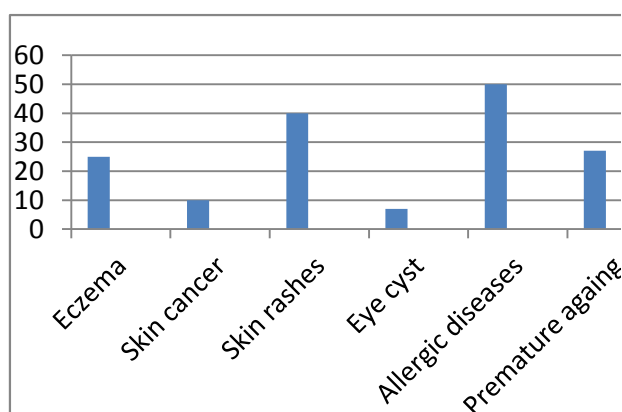


**RESULT AND DISCUSSION:**

**Commercial area:**

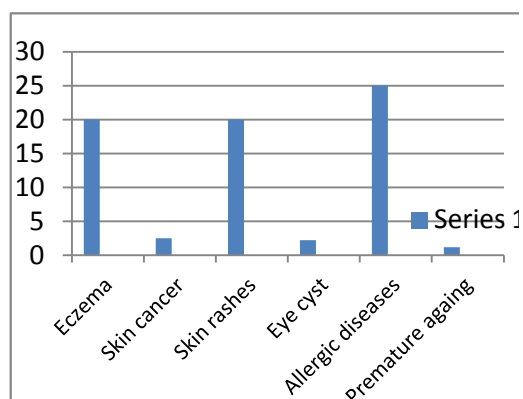
Urbanization has been one of the most striking developments of the 20th century. Sources of urban air quality depends mainly on Vehicular density Worldwide, more than 5000 million urban residents are exposed to health threatening levels of air pollution (UNEP, 2000) in this study, the commercial area includes the market place. (Jaystamb area) The main air pollution source is vehicular, the vehicular exhaust contains. Nox Co, PAHs, Sox and carbon soot particles, which have some direct effect on human health skin and eyes.

PAHs like benzo (a) pyrene has certain carcinogenic property related to the skin concern.



**Fig. 1:** Skin Disease Prevalence in Commercial Area

**Residential Area:** In the residential area most of the people are concern about the environmental problems they are trying to keep their environment clean and this helps to keep away air born disease. Hence indoor air pollution from bio-fuels is not a series problem as most people have switch on the LPG system. The LPG is effect on skin due to the LPG effect the blackening of skin is done but the process is very slow. The people also report some kinds of phto allergic skin problems. Here dust problems is main problem.



**Fig. 1:** Skin Disease Prevalence in residential area

**CONCLUSION:**

The present study has shown in each area has its own environmental problems, as related to the skin disease. In the commercial area eczema and acne, skin rashes, allergic disease prematuring are found in more number as compared to skin cancer, eye cyst and in the residential area skin disease like the allergic disease, eczema & acne, skin rashes are mainly found and skin cancer, premature of ageing and eye cyst are found but in less percentage. An exclusive study on the soureces and sinks of pollutants, its reaction mechanism, skin problem human, short and long terms effects etc. are very essential to manage the risks associated with the degradation of environmental quality, particularly in urban areas.

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## RESEARCH ARTICLE

**AN EXPLORATORY STUDY TO ASCERTAIN THE AEROBIC COCCI PRESENT ON  
DANDRUFF AFFLICTED HUMAN SCALPS****Agrawal Vasudharani<sup>1</sup>, Bhagwat AM<sup>2</sup>, Vishalakshi V<sup>3</sup>, Sawant CS<sup>2</sup>**<sup>1</sup>School Of Science NMIMS Deemed To Be University, Mumbai, Maharashtra; India.<sup>2</sup>Shri C. B. Patel Research Centre for Chemistry and Biological Sciences, Mumbai, Maharashtra, India.<sup>3</sup>Department of Dermatology, CSM Hospital & Rajiv Gandhi Medical College, Kalwa, Thane, Maharashtra, India.**ABSTRACT**

Seborrheic Dermatitis of scalp (dandruff) is a common scalp condition, but the correlation of dandruff formation with aerobic cocci remains unexplored. The purpose of this study was to obtain the prevalence of aerobic cocci on dandruff and healthy human scalps, in order to establish a basis for future research on the possible microbial etiology to dandruff. The study population included 30 subjects (15 clinically healthy and 15 dandruff). Samples were collected using sterile moist swab and the cocci were isolated and identified using culture-based methodologies. A total of 189 cocci, including 14 different species, were isolated. The major organisms found on the healthy scalp were *Staphylococcus varians*, *Cellobiococcus* and *Staphylococcus intermedius*. Amongst the dandruff scalps, the major organisms found were *Micrococcus*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*. The dandruff scalp shows a completely varied microflora in terms of aerobic cocci as compared to the healthy scalp. *Staphylococcus hemolyticus* was the only common organism across the study population. These results elucidate the distribution of aerobic gram positive cocci associated with dandruff and provide new avenues for the potential prevention and treatment of dandruff.

**Keywords:** Seborrheic Dermatitis, Dandruff, aerobic cocci, *Staphylococcus*, *Cellobiococcus*, *Micrococcus*.

**INTRODUCTION**

Seborrheic dermatitis of scalp (Dandruff) is a chronic and relapsing condition, with a worldwide distribution. Its etiology is poorly understood, but earlier literature shows that the disease is associated with the skin commensal yeast belonging to the genus *Malassezia* (Gemmer et al., 2002; Gemmer et al., 2005; Gupta et al., 2001; Klingman et al., 1989; Park et al., 2012). Researchers have reported the presence of *Malassezia* species, aerobic cocci and corynebacterium acne as the normal microflora of the scalp. (Gao et al., 2010; Grice et al., 2008; Grice et al, 2011; Zaidi et al, 2002). The high density of sweat glands, rapid epidermal turnover and presence of large sebaceous glands on the scalp, provide appropriate conditions for the growth and survival of these microorganisms.

Trillions of bacteria, fungi, viruses, archaea and small arthropods colonize the skin surface, collectively forming the skin microbiome (Heidi and Julia, 2012). These microorganism have been classified as transient versus resident or beneficial versus pathogenic or collaborators versus adversaries by various researchers (Heidi and Julia, 2012). Several methods have been used for the identification of microbial inhabitants of the human skin (Gao et al. 2007). Although the presence of aerobic cocci on the human skin has been reported in many studies, the isolation and identification of specific aerobic cocci especially from the human scalp in healthy and diseased condition has not been reported before. The aim of the present study was to isolate and characterize the aerobic cocci present on the human healthy and dandruff afflicted scalp, so as to determine the role of these aerobic cocci in the etiology of dandruff. The results obtained in the study may help in developing a target specific treatment strategy for dandruff.

**\*Corresponding Author**Email: [world.vra@gmail.com](mailto:world.vra@gmail.com)

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## MATERIALS AND METHODS:

**Subjects:** Specimens of scalp skin were obtained from 30 individuals including 15 healthy adult subjects (7 males and 8 females) and 15 dandruff afflicted subjects (7 males and 8 females). The mean age of subjects was 27 years (range, 18-50 years of age). Ethical clearance was obtained from institutional ethics committee at CSM Hospital & Rajiv Gandhi Medical College, Kalwa, Thane. All the volunteers provided written informed consent.

**Sample collection:** Scalp surface samples were collected using a sterile swab moistened in sterile saline. Sterile conditions were maintained during sample collection. Two swabs were collected per sample.

**Sample processing:** Of the two swab samples, one was first transferred to a sterile nutrient agar (NA) plate for isolation and then to trypticase soy broth for enumeration. Similarly the other swab sample was first transferred to a sterile blood agar (BA) plate for isolation and then to trypticase soy broth for enumeration. All the plates and tubes were incubated at 37 degree Celsius for 24 hours. After 24 hours, the colonies obtained on NA and BA plates were gram stained, their colony characteristics noted and only the gram positive cocci obtained were sub cultured for further identification. The broth cultures were streaked on NA and BA plates which were incubated at 37 degree Celsius for 24 hours. Colonies obtained on NA and BA plates after

incubation were processed as earlier. The major test performed for identification of gram positive aerobic cocci included catalase test, coagulase test (slide and tube), haemolysis, sugar fermentation and Voges-Proskauer. All the identification was performed as per Bergey's Manual for Systemic Bacteriology using PIBWin software (Bryant TN, 2004).

**Statistical analysis:** The data obtained was analysed by calculating the percentage prevalence of each cocci isolated. The total percentage prevalence of each cocci across the study population and within each category (healthy scalp and dandruff scalp) was calculated. Also the percentage prevalence of each organism associated with male and female population in each study category was calculated.

## RESULTS AND DISCUSSION:

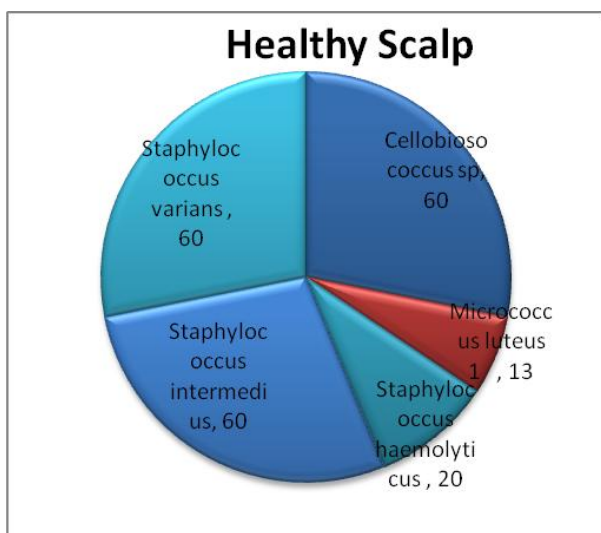
In contrast to the data obtained in previous studies, a large number of gram positive cocci showing significant variation in the dandruff and non - dandruff scalp samples were obtained in the present study. A total of 189 gram positive aerobic cocci were isolated from 30 volunteers. These 189 cocci included 4 different genus and 14 different species. (Table 1)

The major organisms isolated from the healthy scalp included *Staphylococcus varians* (60%), *Cellobiosococcus species* (60%) and *Staphylococcus intermedius* (60%). The other cocci found on the healthy scalp included *Micrococcus luteus* (13%) and *Staphylococcus hemolyticus* (20%). (Fig. 1).

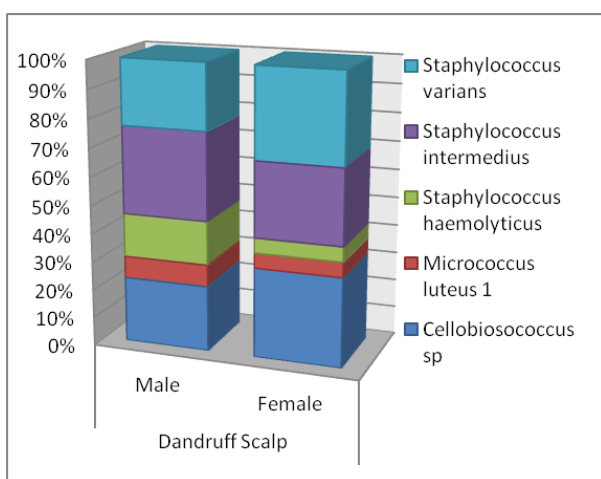
**Table 1: Percentage prevalence of gram positive aerobic cocci isolated from healthy and dandruff afflicted human scalps**

Sr. No.	Organism	Healthy Scalp prevalence %	Dandruff Scalp prevalence %
1.	<i>Cellobiosococcus sp</i>	60	0
2.	<i>Micrococcus luteus</i>	13	0
3.	<i>Micrococcus sp</i>	0	93
4.	<i>Staphylococcus aureus</i>	0	87
5.	<i>Staphylococcus hemolyticus</i>	20	53
6.	<i>Staphylococcus hyicus</i>	0	53
7.	<i>Staphylococcus intermedius</i>	60	0
8.	<i>Staphylococcus saprophyticus</i>	0	93
9.	<i>Staphylococcus sp BP III</i>	0	7
10.	<i>Staphylococcus varians</i>	60	0
11.	<i>Staphylococcus xylosus</i>	0	7
12.	<i>Streptococcus agalactiae</i>	0	47
13.	<i>Streptococcus grp O</i>	0	7
14.	<i>Streptococcus pyogenes</i>	0	53

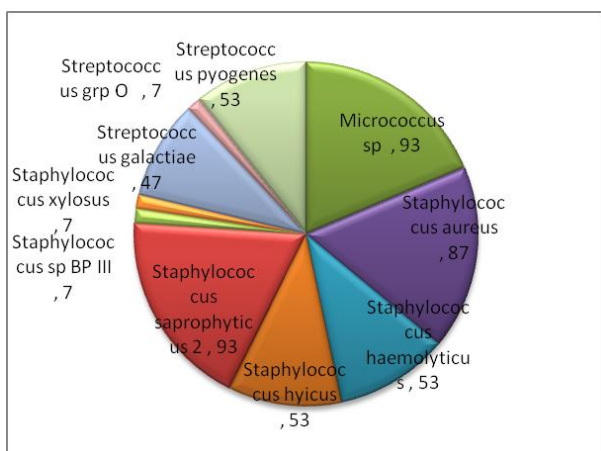




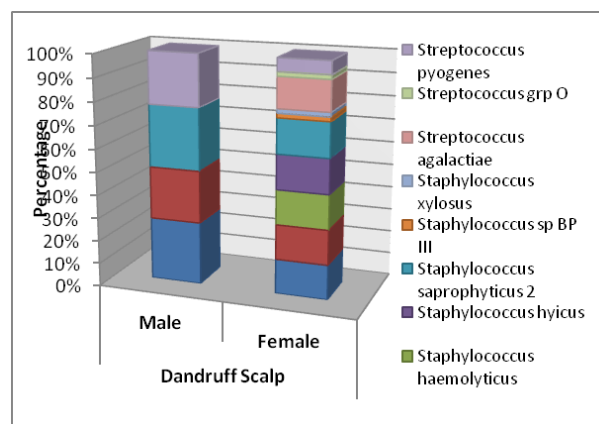
**Fig. 1: Percentage prevalence of Gram positive cocci on Healthy scalp**



**Fig.2: Percentage distribution of Gram positive cocci among male and female population of healthy human scalp.**



**Fig. 3: Percentage prevalence of Gram positive cocci on Dandruff afflicted scalp**



**Fig. 4: Percentage distribution of Gram positive cocci among male and female population of dandruff afflicted human scalp.**

There was no variation in the scalp microflora amongst the male and female in healthy individuals. All the 5 different cocci were isolated from both male and female healthy scalps. (Fig. 2)

On the dandruff scalp the major organism isolated included *Micrococcus species* (93%), *Staphylococcus aureus* (87%), *Staphylococcus hemolyticus* (53%), *Staphylococcus hyicus* (53%), *Staphylococcus saprophyticus* (93%), *Streptococcus agalactiae* (47%), and *Streptococcus pyogenes* (53%). Other organism isolated from the dandruff scalp included *Staphylococcus xylosum* (7%), *Streptococcus grp O* (7%) and *Streptococcus sp BP III* (7%). (Fig. 3)

Significant variation in the scalp microflora among the male and female scalp was observed in case of dandruff afflicted individuals. A larger population of aerobic cocci was isolated from the female scalp as compared to the male scalps in dandruff samples. Of the 10 different organisms found on the dandruff scalps, only 4 gram positive cocci (*Streptococcus pyogenes*, *Micrococcus species*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*) were found on the male dandruff scalps, while all the 10 different cocci were found on the female dandruff scalp. (Fig. 4)

The only common organism found across the study population was *Staphylococcus hemolyticus*, suggesting that this organism may be an opportunist, which is present as a resident microbe turning into a pathogen under specific conditions.

Studies on dandruff have always revealed *Malassezia* as the sole microorganism involved in this condition (Gemmer et al., 2005; Klingman et al., 1976; Park et al, 2012). But, the data obtained in this



study shows that the change in the complete aerobic cocci microflora of the scalp in healthy and diseased condition, may be an indication of the association of these cocci with dandruff along with *Malassezia*.

### CONCLUSION:

In order to understand the function and activities of the human skin, the skin microflora needs to be studied. Researchers have isolated a great variety of micro-organisms from the human skin and arranged them in three groups: transients, microbes which are intermittently found on the skin, arising from the environment and persist on the skin surface for a short duration; residents, which are permanently found on the skin; and the pathogens, which cause disease conditions (Holland et al, 2002). On the basis of the data obtained from this study, the aerobic cocci isolated from the human scalp may be classified into 3 categories depending upon their prevalence.

**Resident Cocci** - *Cellobiosococcus species*,  
*Micrococcus luteus*, *Staphylococcus intermedius*,  
*Staphylococcus varians*.

**Transient Cocci** - *Staphylococcus xylosum*

**Pathogenic Cocci** - *Micrococcus species*,  
*Staphylococcus aureus*, *Staphylococcus hemolyticus*,  
*Staphylococcus hyicus*, *Staphylococcus saprophyticus*,  
*Streptococcus agalactiae*, *Streptococcus pyogenes*.

Diversity of the species identified on the scalp was higher in dandruff scalps than in non-dandruff scalps. It can be said that dandruff may be associated with the differences in the balance between the fungal and bacterial populations on the scalp, and not just to *Malassezia* (Clavaud et al., 2013). Hence, treatment strategies targeting bacteria as well as fungi need attention.

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## RESEARCH ARTICLE

**PREVALENCE OF ALLERGIC RESPIRATORY DISEASES DUE TO CLIMATE CHANGE AND AIR POLLUTION IN SINDHUDURG DISTRICT****Giri Smita S<sup>1</sup>, Rathod SV<sup>2</sup>, Kulkarni Prashant D, Rokade Asha and Jadhav Sangita A**<sup>1</sup> Br. Balasaheb Khardekar , College; Vengurla.<sup>2</sup>Chemistry Research Lab. Bhavans H. Somani College, Chowpatty, Mumbai-7**ABSTRACT**

Sindhudurga district is well known about its Coastal beauty and Variety of Flora and Fauna. Due to coastal effect its air contains moisture in more percentage. In India we have three seasons, namely Monsoon, Summer and Winter. In Maharashtra, Due to global warming and drastic climatic fluctuations we can't predict how many seasons we have. During the past few decades not only in industrialized countries temperature varies dramatically, but the prevalence of asthma and allergic diseases has increased. Due to climate change, air pollution patterns are changing in several urbanized areas of the world, with a significant effect on respiratory health.

Genetic factors are important in the development of asthma and allergic diseases, the rising trend can be explained only in changes occurred in the environment. Air quality is an important concern for public health in the cities throughout the world. Some differences in the air pollution profile and decreasing trends of some key air pollutants. Allergens patterns are also changing in response to climate change and air pollution can modify the allergenic potential of pollens especially in presence of specific weather conditions. Some air pollution-related incidents with asthma aggravation do not depend only on the increased production of air pollution, but rather on atmospheric factors that favor the accumulation of air pollutants at ground level.

The observational evidence indicates that recent regional changes in climate, particularly temperature increases, the data of Sindhudurga districts health and air monitoring strictly indicates that increase in the temperature in summer and October months environment is suitable for growing pollen strength in air comparatively, which is major source of increasing allergic patients mostly in Shiroda taluka followed by Malvan than others. The number of patients increases in summer season and October month particularly due to high temperature, which is favourable for growing number of pollens

**Keywords:** Bronchial asthma, Climate change and allergy, Environment and respiratory allergy, Pollen allergy

**INTRODUCTION**

Sindhudurg district changing its phase very fast due to transportation and increasing urbanization. If we concerning health ratio of these people then question arises, are we allergic to modern life? It is known as to why there has been such a steep escalation in allergic diseases over the past few decades. It may be because allergens have become more aggressive as the environments we live in have

changed. People are now also exposed to a multitude of substances, both natural and synthetic, which were not in our environment 20 years ago (Pulimood, 2007). An allergen is the name given to a substance which can cause an allergic reaction. An aggressive allergen is defined as one that is more likely to cause symptoms than others because the body reacts in a more intense way. Not all allergens directly cause a reaction but the reaction can be triggered when the allergen interacts with other causal factors such as stress or air pollution. There are thousands of allergens, ranging from pollen to shellfish. Most allergens contain proteins, which are to blame for allergic reactions, but there are some, such as penicillin and other medicines, which simply combine with proteins when they enter the body.

**\*Corresponding Author**Email: [smitagiri2012@gmail.com](mailto:smitagiri2012@gmail.com)

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Common allergens can be found all around us and can be ingested through the nose, the eyes, the stomach and lungs, touch the skin or enter directly into the body through an insect sting or bite.

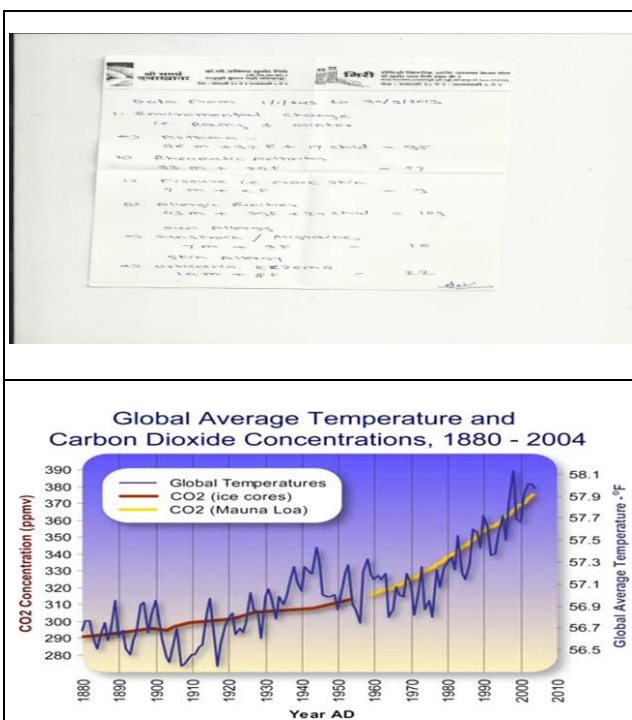
No.	Allergens	Triggers
1	House dust mites	Tobacco smoke
2	Domestic and wild animals/insect venom	Traffic pollution
3	Mould	Air pollution
4	Perfume	Indoor/outdoor temperature
5	Feathers	Humidity
6	Foods and Cleaning products	Stress

particular carbon dioxide (CO<sub>2</sub>) and ozone (O<sub>3</sub>), are greenhouse gases which are involved in the global warming. Changes are also occurring in the amount, intensity, frequency, and type of precipitation as well as the increase of extreme events, like heat waves, droughts, floods, thunderstorms and hurricanes and these are a real and daunting problem. It is now an emerging problem in Sindhudurg. The massive increase in emissions of air pollutants due to mining for economic and industrial growth in the last century made air quality an environmental problem. (Pawankar, 2011).

**Working Group I Report of the Intergovernmental Panel on Climate Change (IPCC) 2007.**

**Global warming** • The global warming average air temperature has increased by 1.0±0.3°F (0.6±0.2°C) since the late 19th century ..• The average surface temperature of the earth is likely to increase by 2 to 11.5°F (1.1-6.4°C) by the end of the 21st century ...• The average rate of warming over each inhabited continent is very likely to be at least thrice as large as that experienced during 20th century

Global warming Climate scenarios for the next century predict that the warming will be associated with more frequent and more intense heat waves in wide areas of our planet with increased risk of wildfires and desertification. In urban areas of Sindhudurg, the effects are higher since climate change influences outdoor air pollution because the generation and dispersion of air pollution is in strictly correlation with local patterns of temperature, wind and precipitation. ]. There is also a link between climate changes and air pollution and an individual’s response to air pollution depends on the source and components of the pollution, as well as on climatic agents . Main determinants of greenhouse gas emissions are energy production, transportation, agriculture and food production and waste management, and attempts at mitigating climate change will need to address each of these. The world will experience more hot days, fewer frost days, and more periods of heavy rain and consequent flooding (Wilkinson et al., 2007). It is important to consider that after CO<sub>2</sub> emissions are reduced and atmospheric concentrations stabilized, surface air temperature continues to rise slowly for a century or more.



**Figure 1:** Global average temperature and carbon dioxide concentrations, 1980–2004.

Indoor and outdoor airborne pollutants are major factors in the allergy epidemic, with a defined link between the increase in air pollution and the prevalence of allergic diseases. Air pollution does not only irritate the airways, it has also been shown to make allergens like pollen more aggressive. The increasing incidence in allergic asthma in children may coincide with modifications to the home, school or day-care centre environment. Changes to bedding and air conditioning units, or increased concentration of humidity and mould, can result in changes to indoor air quality and subsequent exposure to allergy triggers. Several air pollutants, in



### **Air Monitoring Report of Average August 2013 of Sindhudurg District**

**1] MALVAN:** Max Temp.-33.8°C – date-17 th Aug 2013.; Min Temp.-14.2°C – date-27 th Aug 2013.;Max. Rain fall-27.5 – date-27<sup>th</sup> 2013.; Min.Rainfall – 11.8 .

**2] Shiroda:** Max Temp.-32.22°C – date-15 th Aug 2013.; Min Temp.-15.3°C – date-24 th Aug 2013.;Max. Rain fall-7.3 – date-27<sup>th</sup> 2013.; Min.Rainfall – 0 .8.

**3] Vengurla:** Max Temp.-33.6°C – date-15 th Aug 2013.; Min Temp.-19.°C – date-28 th Aug 2013.;Max. Rain fall-22.5 – date-27<sup>th</sup> 2013.; Min.Rainfall – 6.8 .

**4] Sawantwadi:** Max Temp.-34.1°C – date-19 th Aug 2013.; Min Temp.-20.6°C – date-25 th Aug 2013.;Max. Rain fall-62.5 – date-21<sup>th</sup> 2013.; Min.Rainfall – 10.7 .

**5] Kudal:** Max Temp.-36.2°C – date-18 th Aug 2013.; Min Temp.-20.3°C – date-24 th Aug 2013.;Max. Rain fall-27.5 – date-20<sup>th</sup> 2013.; Min.Rainfall – 12.6 .

**6] Kankavali:** Max Temp.-33.8°C – date-12 th Aug 2013.; Min Temp.-18.7°C – date-28 th Aug 2013.;Max. Rain fall-24.9 – date-27<sup>th</sup> 2013.; Min.Rainfall – 11.8 .

**7] Vaibhavwadi:** Max Temp.-33.8°C – date-18 th Aug 2013.; Min Temp.-19.0°C – date-20 th Aug 2013.;Max. Rain fall-27.5 – date-27<sup>th</sup> 2013.; Min.Rainfall – 11.8 .

**8] Deogad:** Max Temp.-33.8°C – date-19 th Aug 2013.; Min Temp.-20.3°C – date-28 th Aug 2013.;Max. Rain fall-37.5 – date-22<sup>th</sup> 2013.; Min.Rainfall – 10.8

### **Report: Sindhudurg District Allergic Diseases Patients Year- 2013.**

#### **2. Weather changes with climate change**

\* More extreme weather patterns, such as increase in thunderstorm..\* High number of thunderstorms in spring and summer at the same

time as high pollen counts..\* Pollen grain rupture with thunderstorm with higher level of respirable allergens; also increased in zone..\* More asthma outbreaks..\*Malvan, Shiroda ,Urban air pollutants. (Losappio, 2011).

The most abundant components of air pollution in urban areas are nitrogen dioxide, ozone and particulate matter. Sulphur dioxide is particularly abundant in industrial areas. It is estimated that more than 50% of the population of the Sindhudurg live in areas where levels of ozone, nitrogen dioxide, sulphur dioxide, and particulates exceed current National Ambient Quality Standards, as monitored by the EPA. A number of experimental and epidemiological studies confirmed the negative effect of urban air pollution on human health and on allergic respiratory diseases and projections of climate variability suggest these effects will increase in the next decades.

**Nitrogen dioxide (NO<sub>2</sub>):** Sindhudurg district has sandy soil, hilly areas, coconut plants, palms, rice as major crop. Thus due to mining and deforestation and district climatic change Air found to be contaminated much in 2013 year in summer and august. NO<sub>2</sub> exposure is associated with increased emergency room visits, wheezing, and medication use among children with asthma Car and trucks exhausts, together with power plants, are the most significant sources of outdoor NO<sub>2</sub>, which is a precursor of photochemical smog found in outdoor air in urban and industrial regions and, in conjunction with sunlight and hydrocarbons, results in the production of ozone. Like ozone, NO<sub>2</sub> is an oxidant pollutant, although it is less chemically reactive. Controlled exposure studies of asthmatics have found that NO<sub>2</sub> can enhance the allergic response to inhaled allergens (D'Amato and Liccardi, 2003

**Tab;e 2: Report: Sindhudurg District Allergic Diseases Patients Year- 2013.**

Diseases	Malvan	Shiroda	Vengurla	Sawantwadi	Kudal	Kankavali	Vaibhavwadi	Deogad
Asthama	159	95	69	57	77	90	84	73
Rheumatic Artries	203	57	56	48	52	43	51	42
Fissure crackskin	85	90	72	59	79	81	79	78
Allergic Rhitnities	173	109	92	87	58	104	106	91
Skin allergy (sun)	111	100	92	76	82	74	97	68
Urticaria Eczema	89	82	78	55	63	79	67	81
Eye irritation	203	108	102	97	88	106	107	93



**Ozone (O<sub>3</sub>):** Ozone is generated at ground level by photochemical reactions involving nitrogen dioxide, hydrocarbons, and UV radiation. Ozone inhalation induces epithelial damage and consequent inflammatory responses in the upper and lower airways, as shown by an increase in levels of inflammatory cells and mediators in nasal and bronchoalveolar lavage. O<sub>3</sub> exposure significantly increases levels of inflammatory cells (in particular neutrophils) and mediators, such as IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and fibronectin, in bronchoalveolar lavage fluid (BALF) of asthmatic subjects. It has long been speculated that O<sub>3</sub> and other pollutants may render allergic subjects more susceptible to the antigen they are sensitized. Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the airways of allergic asthmatics.

**Particulate matter** is a mixture of organic and inorganic solid and liquid particles of different origins, size, and composition. It is a major component of urban air pollution and has the greatest effect on health. Penetration of the tracheobronchial area is related to particle size and the efficiency of airway defence mechanisms. Ultrafine particulate matter (UFP), with diameters of 0.1 µm or less, is a major component of emissions from vehicles. These particles accumulate into larger fine PM with a diameter of ≤2.5 µm (PM<sub>2.5</sub>), within short distances from the point of release). PM<sub>10</sub> (particulate matter with a diameter of 10 µm or less) consists of PM<sub>2.5</sub> and larger particles of mainly crustal or biological origin, including many aeroallergens. PM<sub>2.5</sub> appears to be more potent agent for the development of respiratory and cardiovascular disease compared with PM<sub>10</sub>. A large portion of urban particulate matter originates from diesel engines, the source of diesel exhaust particles (DEPs) which includes other components such as polycyclic aromatic hydrocarbons [PAH]. DEPs account for up to 90% of airborne particulate matter in the world's largest cities and are composed of fine particles (2.5-0.1 µm) and ultrafine (0.1 µm) particles, which can also coalesce to form aggregates of varying sizes (D'Amato et al., 1998)

#### ***The correlation between climate changes, allergenic plants and pollen distribution***

(i) increase and faster plant growth; (ii) increase the amount of pollen produced by each plant;

(iii) increase the amount of allergenic proteins contained in pollen, (iv) increase the start time of plant growth and therefore the start of pollen production, (v) earlier and longer growing pollen seasons.

According to current climate change in Sindhudurg districts scenarios, there will be an increase in intensity and frequency of heavy rainfall episodes, including thunderstorms, over the next few decades, which can be expected to be associated with an increase in the number and severity of asthma attacks both in adults and in children (Table 2).

#### ***Weather changes with climate change***

- More extreme weather patterns, such as increase in thunderstorm..
- High number of thunderstorms in spring and summer at the same..time as high pollen counts..
- Pollen grain rupture with thunderstorm with higher level of respirable..allergens; also increased in zone..
- More asthma outbreaks..-UK, Australia and Italy

#### ***The evidence about thunderstorm-related epidemics of rhinitis and asthma exacerbations***

1) The occurrence of epidemics is closely linked to thunderstorm. 2) The thunderstorm-related epidemics are limited to late spring and summer when there are high levels of airborne pollen grains. 3) There is a close temporal association between the arrival of the thunderstorm, a major rise in the concentration of pollen grains and the onset of epidemics. 4) Subjects with pollen allergy, who stay indoors with window closed during thunderstorm, are not involved. 5) There are not high levels of gaseous and particulate components of air pollution during outbreaks. 6) There is a major risk for subjects who are not under anti-asthma correct treatment (Islam et al., 2007).

**Action on Allergy:** \*Recognize allergic diseases as a public health priority. \*Conduct epidemiological research on allergic disease prevalence and trends. \*Ensure healthcare systems are fully equipped and resourced to provide professional healthcare education, access to reimbursed medication and patient disease information. \*Establish guidelines for the cost-effective management of and therapy for allergy patients. \*Establish programmes to train, educate, empower and rehabilitate allergy patients.



\*Work with the Indian Community to establish an allergy-friendly environment for all.

**In summary** In cases of severe asthma, NO<sub>x</sub> and O<sub>3</sub> may even cause death. A recent study found that NO<sub>x</sub> increased the risk of death in patients with more than one emergency room admission for asthma (Sunyer et al, 2002). In this study, O<sub>3</sub> was also found to increase the risk of death in asthmatic patients during spring and summer. No interactions between air pollutants and pollen or spores were found and there was no significant association between mortality and particles, spores or pollen. ( D'Amato, 2012).

### CONCLUSION:

Citizen and in particular health professionals and societies must raise their voices in the decision process to give strong support for clean policies on both national and international levels (Packe and Ayres, 1985). They made strategies to reduce climate changes and air pollution are political in nature. Collaborative Group survey: High temperature and hospitalizations for cardiovascular and respiratory infections on coastal region of Shiroda and Malvan, Sindhudurg district indicates the evidence about climate change-related epidemics of rhinitis and asthma exacerbations

- 1) There is a major risk for subjects who are not under anti-asthma correct treatment.
- 2) The climate change -related epidemics are limited to late spring and summer when there are high levels of airborne pollen grains.
- 3) Subjects with pollen allergy, who stay indoors with window closed during climate change , are not involved.
- 4) There is a close temporal association between the arrival of the climate change, a major rise in the concentration of pollen grains and the onset of epidemics.
- 5) There are not high levels of gaseous and particulate components of air pollution during outbreaks.
- 6) The occurrence of epidemics is closely linked to climate change.

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## RESEARCH ARTICLE

## PREVALENCE OF ALLERGIC RHINITIS AND CO-MORBIDITIES IN STUDENTS OF COLLEGES IN MUMBAI, MAHARASHTRA

Koshy NE and Ghosh R

Department of Zoology, Wilson College, University of Mumbai, Mumbai 400007, India.

### ABSTRACT

There is a rising trend of allergies especially in developing countries. However, allergies are underdiagnosed due to lack of awareness about them. Therefore, a survey based research study was carried out with the purpose of understanding the prevalence of allergic rhinitis and co-morbidities in students of colleges in Mumbai, Maharashtra. Questionnaires were filled in by 210 college students and were analyzed to determine the incidence of allergic rhinitis and associated co-morbidities such as allergic conjunctivitis, sinusitis, asthma and eczema. The influence of family history and environmental factors on allergies was studied. Triggers that are seen to aggravate the allergy symptoms were identified. Results of analysis showed that having allergies is independent of family history. Only 21.90 % of the total sample were seen to be unaffected by any allergy. Overall it was found that 66.66 % of the sample showed symptoms consistent with allergic rhinitis. Among the associated co-morbidities, 37.61 % had allergic conjunctivitis, 25.71% had sinusitis, 18.57% had asthma and 22.38 % had eczema. Prevalence of seasonal allergy was noted in 65.84 % of the sample and 34.14 % had perennial allergies. The severity of the symptoms in the affected sample was also analyzed and categorized into mild, moderate and severe allergies. Only a small number of the affected students sought medical advice for their allergies suggesting a need for allergy awareness. Timely intervention could enable the affected students to manage their allergies and thus boost their overall productivity, performance and quality of life.

**Keywords:** Allergies, prevalence, college students, triggers, symptoms, awareness, rhinitis, co-morbidities, conjunctivitis, sinusitis, eczema.

### INTRODUCTION

Allergic Rhinitis is a global health problem afflicting 500 million people worldwide and 100 million in India and its neighbouring countries (Bousquet, 2008). Epidemiological surveys have indicated that there has been a notable prevalence of allergic symptoms in young adults (Leynaert, 2000). Allergic rhinitis is defined as "a symptomatic disorder of the nose induced after allergen exposure due to an IgE-mediated inflammation of the membranes lining the nose.

"It comprises more than classical symptoms of sneezing, rhinorrhoea and nasal obstruction (Hansel, 1929). It is associated with co-morbidities like conjunctivitis, sinusitis, asthma and eczema (Jauregui et al, 2009).

The disease has a negative impact on daily life as it induces daytime fatigue, impairment of cognition and memory which affects learning process and performance (Jauregui et al, 2009). There is a lack of documented information about epidemiology of allergic diseases in many parts of the world (Pawankar et al, 2011). Several surveys have been involved with school children on allergic rhinitis and asthma (Saini et al, 2013; Narayanappa et al, 2013). But there is insufficient literature on allergic trends in young adults especially those in crowded metros like Mumbai. Studies have shown that air pollution

#### **\*Corresponding Author**

Email: [ruplekha07@gmail.com](mailto:ruplekha07@gmail.com)

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due to high vehicular emissions, construction activities, urbanization and a westernized lifestyle have led to a marked increase in respiratory allergy prevalence in urban areas (D'Amato and Cecchi, 2008).

The objective of this study was to conduct a questionnaire based survey on allergic rhinitis and associated co-morbidities in students of colleges in Mumbai, Maharashtra. This was done to detect allergy prevalence among students and to identify the triggers escalating these allergies. Along with this, the study highlights the level of awareness among college students about effects of these allergies on the quality of life.

### **MATERIALS AND METHODS:**

The main objectives of this study were two-fold. The primary aim was to detect the prevalence of allergic rhinitis and associated co-morbidities like allergic conjunctivitis, sinusitis, asthma and eczema. Another objective was to gauge the understanding and awareness level that college students have about allergies.

210 college students were selected for this study by random sampling. The participants belonged to the age group of 16 to 23 years.

The tool used for this study was a questionnaire. After reviewing extensive literature and questionnaire forms (such as ISAAC and ARIA) and other allergy patient evaluation questionnaires used by medical practitioners, a modified questionnaire was prepared. The allergy questionnaire was modified to suit the needs of this study. It was adapted keeping in mind the age group of the sample, the vocabulary of students and made relevant to the Indian context.

The questionnaire consisted of six main sections. It was divided into symptoms, duration of symptoms, triggers affecting symptoms, environment survey, medical history and family history. The former three sections comprised the core questionnaire and data from these sections were predominantly analysed for this research paper.

The first section in the questionnaire dealt with common symptoms experienced by the participant even when they were not suffering from a cold and fever. The classification of severity of the symptoms ranged from mild, moderate to severe. Working epidemiological definitions were used to recognize

allergic rhinitis, allergic conjunctivitis, sinusitis, asthma and eczema in the sample under study. The symptoms considered consistent with allergic rhinitis were sneezing, rhinorrhoea, nasal itching and blocked nose. The working definition used for allergic conjunctivitis was redness, itching and watery eyes. For sinusitis, the symptoms considered were headaches, post-nasal drip and catarrh. Presence of chest tightness, wheezing, breathlessness and dry cough was the working definition used for asthma. Eczema was supposedly present if a respondent suffered from rashes, itching, scaling and hives.

The second section of the questionnaire was related to the duration of the symptoms and it helped identify whether the participant faced seasonal or perennial allergies. The third section focussed on the triggers that aggravated the allergy symptoms. These triggers were broadly classified as indoor and outdoor allergens. The latter three sections had 21 close ended questions regarding the respondent's environment, medical and family history. This questionnaire was administered to the respondents after they gave their informed consent for participation in this study.

Statistical analysis of the data was performed using Minitab and Microsoft Office Excel 2010 statistical software. Overall prevalence of allergic rhinitis and co-morbidities and age related differences were studied. Triggers causing the allergic symptoms were analyzed using Pareto chart. Gender analysis of allergy symptoms and association of family history with prevalence of allergies were analyzed in this study by the Pearson's chi-square test of independence.

### **RESULT AND DISCUSSION:**

The main demographic groups of interest for this questionnaire based analysis were students of colleges in Mumbai, Maharashtra. The response rate was high (95.46%). Out of the 220 questionnaire forms distributed, 210 were returned fully filled in. The male-female ratio of the cohort was 0.69:1

Prevalence of allergic rhinitis in different age groups was as follows: 16-17 years (78.08%), 18-20 years (60.90%) and 21-23 year (59.25%) (Fig.1). Research studies have also confirmed that allergic rhinitis is present in all age groups but with a higher incidence in children and young adults (Sainiet al, 2013; Greiner et al, 2011). Allergic rhinitis can be



classified as mild, moderate and severe on the basis of severity of symptoms (Bousquet et al, 2008). Overall prevalence of allergic rhinitis was 66.66% in the examined sample (Fig. 2a). Out of this, 45% had mild symptoms, 26.42% had moderate symptoms and 28.57% had severe symptoms of allergic rhinitis (Table 1 and Fig. 2b).

According to Saini et al (2003) a large proportion of those affected with allergic rhinitis have one or more associated co-morbidity. Allergic rhinitis also has a link with conjunctivitis with ocular complaints such as redness, watery eyes and itching (Schwartz, 2007). An involvement of allergic conjunctivitis with allergic rhinitis was evident in our sample which was consistent with previous literature (Pelikan, 2009). Among the associated co-morbidities, 37.61% in our sample had allergic conjunctivitis. Of which, 69.62% showed mild, 17.72% showed moderate and 12.65% showed severe allergic conjunctivitis (Table 1).

Shah and Pawankar (2009) explained that sinusitis symptoms compound the effects of allergic rhinitis. The examined sample showed 25.71% had sinusitis. Among those affected with sinusitis, 87.03% had mild symptoms, 11.11% had moderate symptoms and 1.85 % had severe symptoms (Table 1).

Studies have shown a close association of allergic rhinitis with asthma (Bousquet et al, 2001; Ciprandi et al, 2004). An editorial by Shah (2000) aptly reinforced this point "Rarely does one hear a wheeze without a sneeze". Bousquet et al (2005) explained that usually less than 2% of asthmatics do not show allergic rhinitis. In the sample, 18.57% were asthmatic, of which 51.28% had mild symptoms, 17.94% had moderate symptoms and 30.76% had severe symptoms (Table 1). This was consistent with findings from other studies that showed 10 to 40% patients with allergic rhinitis also had asthma. (Linneberg et al, 2002 ; Leynaert et al, 2004; Downie et al, 2004).

Musharrafieh et al (2009) also found that eczema was a common co-morbidity along with allergic rhinitis. Presence of any allergy condition in an individual can increase the chances of allergic rhinitis, asthma or eczema by more than double (Musharrafieh et al, 2009). In the sample 22.38% had eczema, of which 76.59% had mild symptoms, 10.63% had moderate symptoms and 12.76% had severe symptoms (Table 1). Overall prevalence of allergic rhinitis and co-morbidities is depicted in Table 2 and Fig.3.

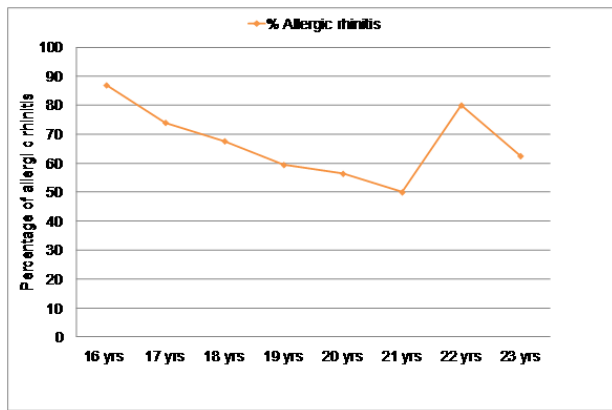
**Table 1: Percentage of severity of different allergies in the affected sample.**

Allergies	% Severity of symptoms in the affected sample		
	Mild	Moderate	Severe
Allergic rhinitis	45.00	26.42	28.57
Conjunctivitis	69.62	17.72	12.65
Sinusitis	87.03	11.11	1.85
Asthma	51.28	17.94	30.76
Eczema	76.59	10.63	12.76

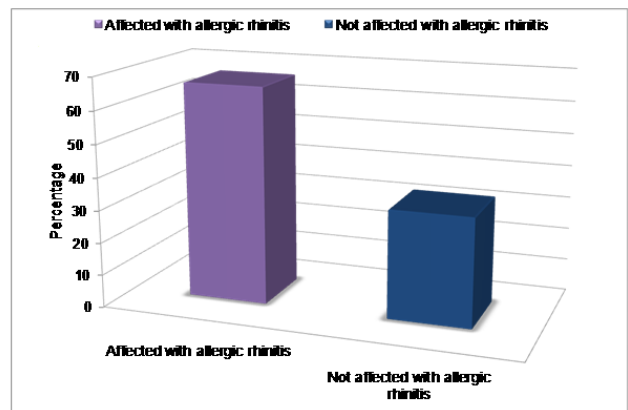
**Table 2: Prevalence of allergic rhinitis and co-morbidities in the sample.**

Allergies	Gender		Percentage in Total Sample
	Percentage in Males	Percentage in Females	
Allergic rhinitis	74.41	61.29	66.66
Conjunctivitis	41.86	34.67	37.61
Sinusitis	20.93	29.03	25.71
Asthma	26.74	12.90	18.57
Eczema	19.76	24.19	22.38
None	17.44	25.00	21.90

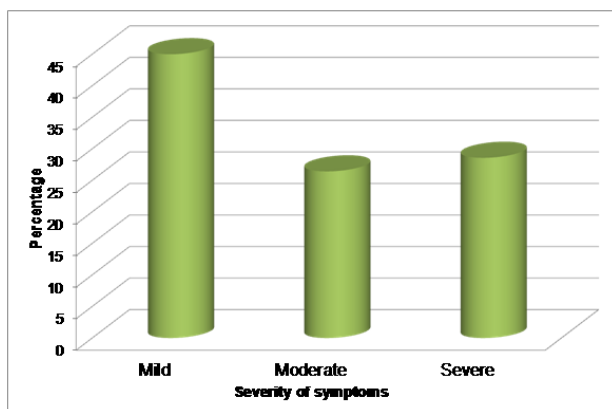




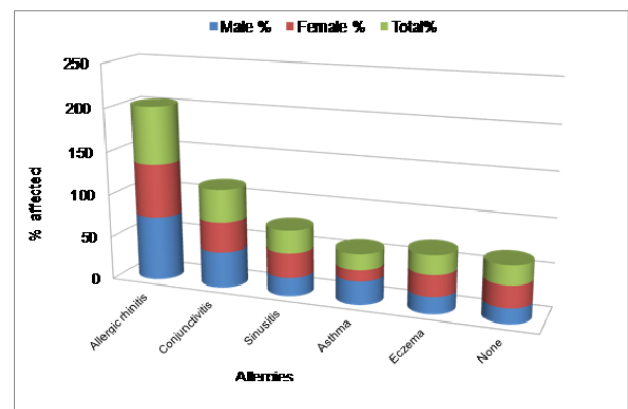
**Fig. 1** Age wise distribution of allergic rhinitis in the sample population



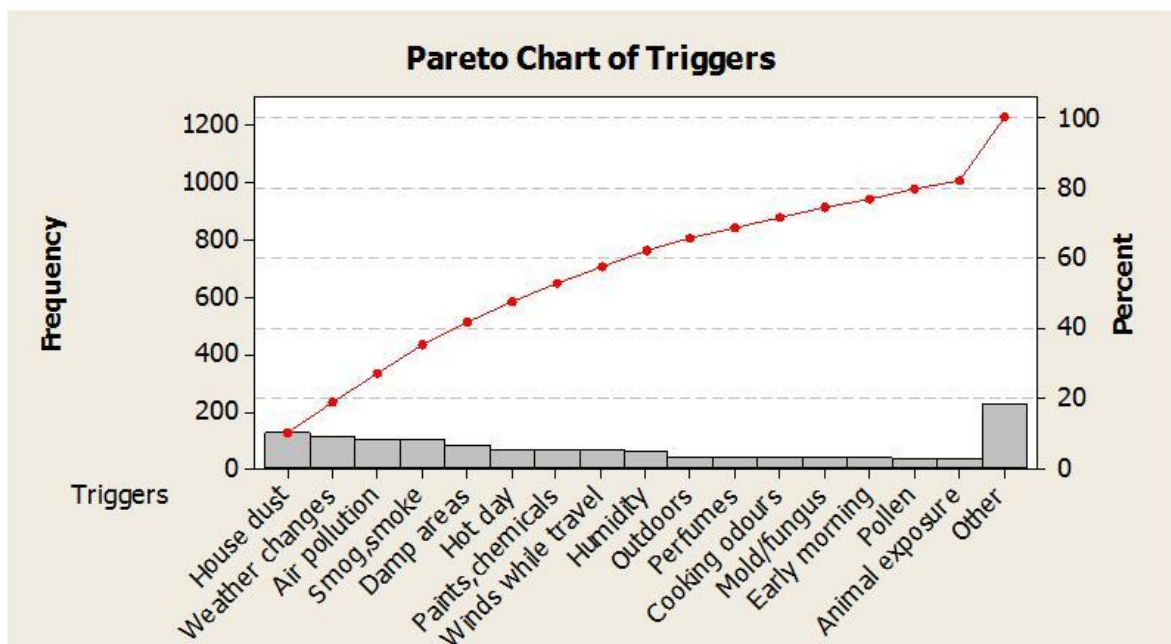
**Figure 2a:** Prevalence of Allergic rhinitis in the sample population



**Fig. 2b:** % Severity of allergic rhinitis in affected samples.



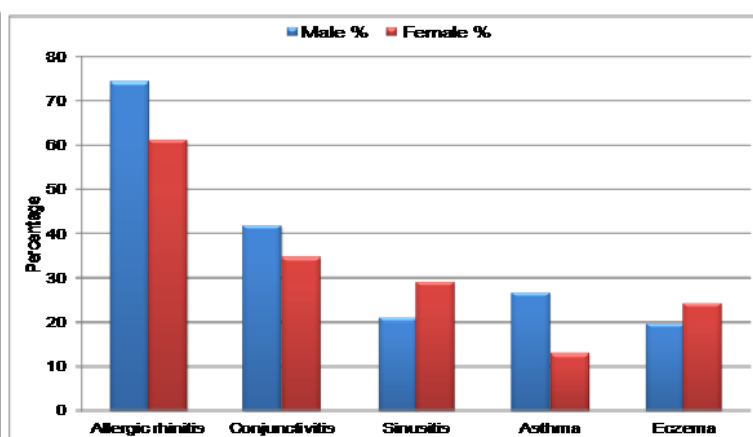
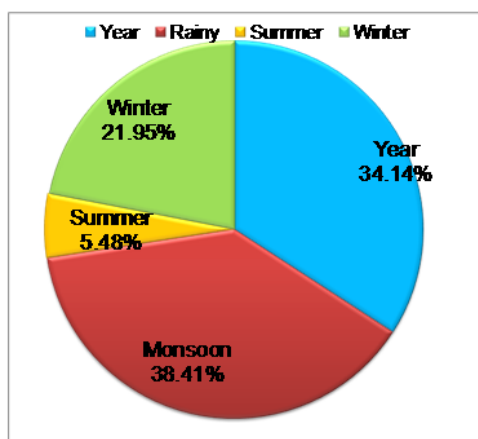
**Fig 3.** Prevalence of allergic rhinitis and co-morbidities in males, females and total sample



**Figure. 4:** Pareto chart showing triggers aggravating allergies in the overall sample







**Figure 5:** Percentage distribution of perennial and seasonal allergies in the affected samples **Figure.6:** Gender wise Prevalence of allergic rhinitis and co-morbidities in different genders

Majority of the triggers causing allergies are aeroallergens (Boulet et al, 1997; Platts-Mills et al, 1998; Marogna et al, 2006). Indoor allergens like house dust, pets/animal dander and dust mites are commonly implicated in allergies. Outdoor allergens responsible for triggering allergies are pollen, fungal spores and insects (Burge and Rogers, 2000). Triggers that were seen to aggravate allergic symptoms in the respondents were evident through a Pareto chart. Overall the triggers affecting 80% of the sample are seen to be house dust, weather changes, winds while travelling, outdoor humidity, pollen, molds/fungus, perfumes, cooking odours, early morning time and animal exposure (Fig. 4). 80% of males were seen to suffer due to triggers such as house dust, weather changes, air pollution, damp areas, hot day, smog or smoke, paints and chemicals, winds while travelling, outdoors, humidity, pollen, molds/fungus and dry weather. Whereas 80% of females showed susceptibility to all the above and in addition were affected by triggers such as cooking odours, early morning time, perfumes and animal exposure.

Incidence of seasonal allergies was noted in 65.84% of the sample, whereas 34.14% had perennial allergies. Seasonal variation was observed with a peak in monsoon followed by winter (Fig. 5). Outdoor allergens are most likely to trigger seasonal allergies (Braun-Fahrlander et al, 1997). Respondents suffering from perennial rhinitis are more at a risk from common indoor allergens (Gergen and Turkeltaub, 1992).

A study on gender analysis of allergy symptoms was also done on the sample population. A chi-

square test of independence showed that allergic rhinitis and asthma were gender dependent (since  $p < 0.05$ ). Allergic rhinitis and asthma prevalence was higher in males than females in our examined population. This is consistent with a study on asthma prevalence in different Indian cities (Pal et al, 2009). Whereas, by the chi-square test of independence, allergic conjunctivitis, sinusitis and eczema were seen to be independent of gender (Fig. 6).

Association between prevalence of allergies and family history was also analyzed using the Pearson's chi-square test of independence. The analysis showed that there was no statistically significant association between family history and incidence of allergies in students (since  $p > 0.05$ ). This indicates that allergies are most likely triggered due to environmental factors (Castro et al, 2013).

It is distressing to note that only a small percentage (13.41%) of the affected college students have sought advice from a medical practitioner for their allergic symptoms. This suggests a greater need for allergy awareness programs.

## CONCLUSION:

The prevalence of allergic rhinitis and associated co-morbidities was seen to be quite high in college students in Mumbai due to a constant exposure to a plethora of indoor and outdoor allergens. This increasing trend is probably because of life in an overcrowded metro where there are high levels of suspended particulate matter due to air pollution and weather changes (Kumar et al, 2007). Several studies have indicated a possible



synergistic effect between allergens and air pollutants (Bugiani et al, 2005). This exercise prompted the suggestion that a similar survey on a larger scale spanning this large island city could help in getting an update of allergic rhinitis and associated co-morbidities in Mumbai. Since the study found allergies to be independent of family history, the rising trend in allergies could be attributed to mainly environmental factors. The recognition and the avoidance of environmental triggers seemed to be an important step towards a better management of allergies. Therefore, it is recommended that students be aware of their allergies, especially those with severe allergic symptoms. They should consult medical practitioners to effectively manage and alleviate their allergies through timely testing and treatment. This could go a long way towards improving their quality of life and performance. There is no real consensus on the actual national prevalence of allergies and therefore it is imperative for more efforts to be focused on allergy awareness.

The limitation of a study based only on screening questionnaire is that it is not substantiated clinically with skin tests and IgE level detections. Therefore, formulation of a standardized allergy questionnaire in India is relevant and opens up avenues for subsequent epidemiological studies.

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## RESEARCH ARTICLE

**BIODIVERSITY OF MITES IN POULTRY DUST AT RAJNANDGOAN DISTRICT IN CHHATTISGARH STATE, INDIA**

Damle Kiran Lata

Dept. of Zoology, Govt. Digvijay College, Rajnandgaon. (C.G.)

**ABSTRACT**

Mites are the allergens in sensitive individual human beings. Sometimes they are parasites causing diseases in human beings, animals or plants. They are vectors carrying diseases from place to place. So to study the biodiversity of dust mites, a general study was carried out for three years. A regular record of temperature and relative humidity was maintained for one poultry farm at village Ghorda, at Rajnandgaon District in Chhattisgarh State in India. Simple pick up method for qualitative, and centrifugation method for quantitative detection of mites was performed. After screening, three mite species *Dermanyssus gallinae* (35%), *Dermatophagoides pteronyssinus* (17%) and *Ornithonyssus bursa* (10%) were found in poultry dust. The temperature and relative humidity directly affected the mite incidence. Poultry dust mites found peak in autumn season during the study year, when temperature was around 25° and relative humidity ranged between 75° to 85°. Protonymphs and duetonymphs larvae of chicken mite's incidence were also found

**Keywords:** Biodiversity, allergy, species, poultry mites, environmental parameters, poultry workers, Ghorda.

**INTRODUCTION**

Mites are Arthropods, belonging to the order Acarina of subclass Acari and class Arachnids without neck, microscopic, around 300-500 microns in length and 100 microns in diameter, oval, opaque or transparent or variously colored, preys, predators or parasites, terrestrial or aquatic, contaminants, or free-living bio control agents, decomposers decomposing various organics matters or dead parts of plants and animals helping the recycling of materials in an ecosystem. They are also allergens in sensitive individual human beings. Sometimes they are parasites causing diseases in human beings, animals or plants. They are vectors carrying diseases from place to place.

In the world 36 species of house dust mites have been reported, out of which 29 have been reported from India. 17 species from Kerala and 20 from Maharashtra. Approximately 30,000 known species

of mites are soil dwellers. Further investigations of house dust mites have revealed different groups of mites like animal mite, cat mite, rat mite, pig mite, cattle mite, poultry mite, etc.

Some house dust mites found in the poultry dusts are allergens causing allergy in sensitive human beings and animals. Some of them have also been found to cause diseases in poultry birds and poultry workers, and create ecological imbalance in nature. It may result into aerobot pollution problems. The incidence, growth and development, increase in the load, distribution of house dust mites and allergy causation due to mites in the individuals is greatly influenced by various factors like types of dwellings, temperature and relative humidity. In India three seasons are visualized, i.e. autumn (June to October), winter (November to February) and summer (March to June). These three seasons' exhibit three different environmental parameters which effect the percentage contribution of dust mites greatly.

Hence the study of biodiversity of mites in poultry dust was carried out at Rajnandgaon. So far no work has been done in poultry mites as well as house dust mites in Rajnandgaon district rather Chhattisgarh State region around.

**\*Corresponding Author**Email: [majorkld12@gmail.com](mailto:majorkld12@gmail.com)

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**MATERIALS AND METHODS:**

To study the biodiversity of poultry mites, a general study was carried out from June 2010 to June 2013. A regular record of temperature and relative humidity was maintained for one poultry farm at village Ghorda, at Rajnandgaon District in Chhattisgarh State in India. About 300 samples were collected twice a month from the different corners and the central part of the farm, sieved through a sieve sized 300 meshes (Kashiram Maurya and Zafar Jamil 1980). Isolation of mites was done by simple pickup method. The sieved dust was placed in Petri dish of 5.5 cm diameter. Permanent slides were prepared in melted glycerin jelly by direct picking up the mites by a needle dipped in lactic acid and observed under Stereoscopic Binocular Research Microscope for identification. After screening, three mite species *Dermanyssus gallinae* (35%), *Dermatophagoides pteronyssinus* (17%) and *Ornithonyssus bursa* (10%) were found in poultry dust. For quantification of mites centrifugation method was adopted which was used at Bangalore:

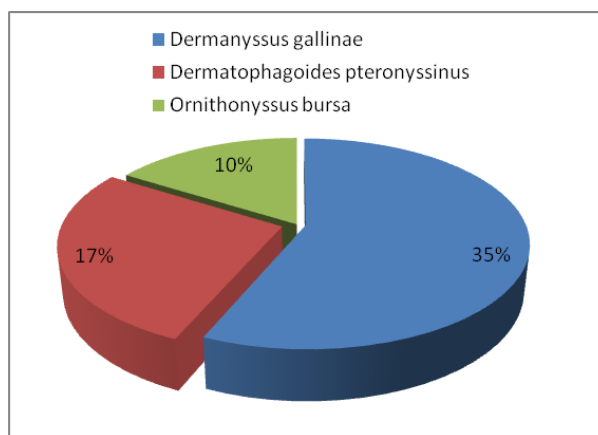
The dust samples were sieved through 500 mesh sieve, weighed one gram and mixed thoroughly with kerosene oil. The mixture was centrifuged at 2000 rpm for 10 minutes. Supernatant was filtered through filter-paper marked in the squares of one square inch each and mites were counted under a stereo-binocular microscope. Sediment was put into a mixture of kerosene oil and carbon tetrachloride (3:5), and the process was repeated. Sediment was mixed with a mixture of kerosene and carbon tetrachloride (1:3), and the process was repeated. Sediment was put in pure carbon tetrachloride and the process was repeated and observed under Stereoscopic Binocular Research Microscope.

**Identification:** The mites were identified according to the key given by different authors from time to time and available updated literature like Hughes (1961), Fain (1965, 1966, 1997, 1979), Bruce and Johns (1969), Spiexsma (1990, 1992) etc.

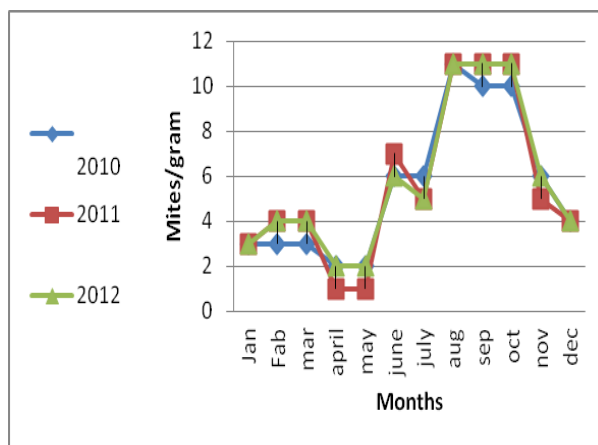
**RESULTS AND DISCUSSION:**

About 300 samples were screened and the population of mites was observed. Three species of mites *Dermanyssus gallinae* (35%), *Dermatophagoides pteronyssinus* (17%) and *Ornithonyssus bursa* (10%) were found in poultry dust during these studies. Refer Figure. 1.

Mites showed variation round the year. They were found significantly high from August to October. Moderate in July and November, comparatively less from December to March, and very few or practically nil from April to June. This monthly variation in mite incidence indicates that environmental conditions of mites vary seasonally. Thus our findings revealed that low relative humidity, high temperature, extremely cold condition and rainless days act as adverse condition for the incidence and growth of mites. Under these conditions, the mite population was significantly decreased between November to May comprising winter and summer season.



**Fig. 1. Percentage of Poultry mites**



**Fig.2. Variation of Poultry mites during June 2010 to June 2013**

The average results for monthly concentration of poultry dust mites revealed peak in autumn season during the year, when temperature was around 25° and relative humidity ranged between 75 % to 85 %. Thus showing seasonal variation, Temperature and relative humidity directly affected mite incidence showing indirect role affecting temperature and relative humidity. Figure.2.



**CONCLUSION:**

No work has been done on poultry mites and house dust mites in Rajnandgaon District, Chhattisgarh state region concerning the types of mites found in the poultry dust. In this study we performed identification and presence of different types of mites in the poultry dust for three years. During this study three mite species were reported of which *Dermanyssus gallinae* (35%), *Dermatophagoides pteronyssinus* (17%) and *Ornithonyssus bursa* (10%) It was found that moderate temperature and high relative humidity provided most congenial environment for maximum percentage contribution of mites in poultry dust. Relative humidity effects the population of mites, (supported by Spieksma, 1997.), whereas low temperature and high temperature were found unfavorable for the survival of mites as is revealed in cold dry winter months and hot summers of April and May, (Jogdand, 1997.). Thus the population of mites shows variations in different seasons.

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## RESEARCH ARTICLE

**POLLEN GRAINS OF *Parthenium hysterophorus* AS POTENT SOURCE OF ALLERGIES****Solanke AK and Kondulkar SR**Mahatma Fule Arts, Commerce and Sitaramji Choudhari Science Mahavidyalaya, Warud,  
Amravati (Maharashtra) India.**ABSTRACT**

*Parthenium hysterophorus* commonly known as congress grass or *gajar gavat* is a notorious weed in India. It grows abundantly during the month of July and August. It causes allergies, itching, coughing and shortness of breath. This weed also causes respiratory problems, cardiovascular diseases and asthmatic allergies. Patients with grass pollen allergy, commonly called pollinosis, often present reactivity to pollen allergens from a number of grasses due to cross-reactivity of IgE antibodies to pollen proteins present in pollen grasses. In India, congress grass has been considered a major sensitizing agent in patients with pollinosis.

**Keywords:** Weed, Allergy, Pollinosis, Antibodies.

**INTRODUCTION**

Pollen allergy is the most common form of seasonal respiratory allergic disease all over the world. Pollens are carried from one part to another by wind, insects. The pollens of grasses are light weight and produced in large amount. We may have noticed that allergy symptoms seem to change with weather. That is due to the production, dispersal and quantity of pollen grains in the air are strongly related to weather patterns.

The distance pollens travel from the plant depends on the nature of the carrying agents, environmental conditions and pollen grain size. Allergens are rapidly released when pollen comes into contact with the mouth, nose or eyes.

**RESULTS AND DISCUSSION:**

In the present study, a survey has been carried in Warud town in the area where *Parthenium hysterophorus* is abundantly grown in the month of August. About 100 persons were monitored in the month of August. It was noticed that about 10-15% of people suffered with pollinosis including symptoms like allergies, itching, shortness of breath and asthma.

**\*Corresponding Author**

Email: ashok.solanke@rediffmail.com

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Congress grass grows in an uncontrolled manner in open land throughout India during the month of July–Aug. It produces a large number of anemophilous pollen grains. It is estimated that about 100 square meters produce about 100 kg of pollen grains. These are dispersed by wind and water to a vast area.

Many studies have shown an association between grass pollen allergens and atopic diseases. *Lolium perenne* pollens have been associated with seasonal allergic rhinoconjunctivitis in temperate climatic countries.(1) Pollens of *Lolium multiflorum* commonly called rye grass, known to cause pollinosis in Brazil(2). Pollen grains when hydrated may release a variety of enzymes including proteases that may damage epithelial cells.(3). Experimental studies have suggested that both environmental and genetic factors influence the induction of an IgE response to inhaled aeroallergen. In susceptible individuals, if the integrity of the airway epithelial barrier is breached at the time of initial exposure, for example by infection or exposure to environmental pollutants, an IgE response may be stimulated.(4). Enzymes including proteases in the pollens are released in high concentration upon deposition on the upper respiratory mucosal surfaces, which are able to disrupt epithelial integrity and probably facilitate access of allergic protein components to subepithelial antigen-presenting dendritic cells(5). Pollutants such as diesel particles and other airborne matter which adsorb pollen particles and



starch granules from ruptured pollen act similarly rendering city dwellers more susceptible to pollinosis(6, &7).



**Fig. 1: *Parthenium hysterophorus***

An important feature of pollinosis is annual periodicity with symptoms' usually occurring at the same time of the year during pollination(8).

The use of mixed extract containing pollen from different grass species has been recommended as there may be cross reactivity between grasses for repetitions of classical symptoms(9).

Prophylaxis is extremely difficult in pollinosis. It is difficult to reduce or avoid environmental exposure as people work and play in that same environment. When the quantity and propagation of pollen in the atmosphere is significant ,such as dry, warm and windy days, patients are advised to remain in closed environments, if possible with filtered air conditioning,and use glasses when riding bicycle or motorbike and avoid grass cutting or gardening.

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## REVIEW ARTICLE

## HEALTH EFFECTS OF PARTICULATE MATTER: A REVIEW

Poojari Anukthi C<sup>1</sup>, Maind Sandip D<sup>2</sup> and Bhalerao Satish A<sup>1\*</sup><sup>1</sup> Environmental Sciences Research Laboratory, Department of Botany, Wilson College, University of Mumbai, Chowpatty, Mumbai-400007, (M.S.), India.<sup>2</sup>Department of Chemistry, BharatiyaVidyaBhavan's Hazarimal Somani college of Arts and Science, University of Mumbai, Kulapati K.M. MunshiMarg, Chowpatty, Mumbai-400007, (M.S.), India.

## ABSTRACT

PM is a widespread air pollutant, consisting of a mixture of solid and liquid particles suspended in the air. PM is a mixture with physical and chemical characteristics varying by location. Common chemical constituents of PM include sulfates, nitrates, ammonium, other inorganic ions such as ions of sodium, potassium, calcium, magnesium and chloride, organic and elemental carbon, crustal material, particle-bound water, metals (including cadmium, copper, nickel, vanadium and zinc) and polycyclic aromatic hydrocarbons (PAH). In addition, biological components such as allergens and microbial compounds are found in PM. Particles can either be directly emitted into the air (primary PM) or be formed in the atmosphere from gaseous precursors such as sulfur dioxide, oxides of nitrogen, ammonia and non-methane volatile organic compounds (secondary particles). Particles come in a wide variety of sizes and have been historically assessed based on size, typically measured by the diameter of the particle in micrometers. PM<sub>10</sub> refers to particles that are 10 micrometers in diameter or less. PM<sub>2.5</sub>, or fine PM, refers to particles that are 2.5 micrometers in diameter or less. Pollution in urban areas has changed. PM posed health problems, but at lower concentrations their effects were confounded by other factors. Many scientists disagreed with the main conclusions and through the careful analysis of data supported that PM has adverse health effects even at relatively low concentrations (28, 29). The reason that PM became very important air pollutants in recent decades and their adverse health effects became more hazardous is that air. Fine and ultrafine particles from vehicular exhausts are affecting heart rate variability, blood viscosity and blood coagulability, cardiac arrhythmia, deep vein thrombosis, atherogenesis, and destabilization or rupture of atheromatous plaques. Cancer risks in relation to airborne PM after long-term exposure in urban areas have been studied with different epidemiological methodologies, mainly ecologic, cohort, and case-control studies. Several studies in the 1990s collected adequate epidemiological data on air pollution and lung cancer, and their evidence was reviewed. Particulate matter, especially traffic-related airborne particles, contains a large number of genotoxic/mutagenic chemical substances, which can cause DNA damage and promote malignant neoplasms.

**Keywords** Particulate matter (PM), air pollution- adverse effects, Environment and public health, Environmental pollutant, Particulate matter- analysis

## INTRODUCTION

PM is a widespread air pollutant, consisting of a mixture of solid and liquid particles suspended in the air. Commonly used indicators describing PM that are relevant to health refer to the mass concentration of particles with a diameter of less than 10 µm (PM<sub>10</sub>) and of particles with a diameter of less than 2.5 µm (PM<sub>2.5</sub>). PM<sub>2.5</sub>, often called fine PM, also comprises ultrafine particles having a dia-

meter of less than 0.1 µm. In most locations in Europe, PM<sub>2.5</sub> constitutes 50–70% of PM<sub>10</sub>. PM between 0.1 µm and 1 µm in diameter can remain in the atmosphere for days or weeks and thus be subject to long-range trans-boundary transport in the air. PM is a mixture with physical and chemical characteristics varying by location. Common chemical constituents of PM include sulfates, nitrates, ammonium, other inorganic ions such as ions of sodium, potassium, calcium, magnesium and chloride, organic and elemental carbon, crustal material, particle-bound water, metals (including cadmium, copper, nickel, vanadium and zinc) and polycyclic aromatic hydrocarbons (PAH). In addition, biological components such as allergens and microbial compounds are found in PM.

**\*Corresponding Author**Email: [drsatis16@rediffmail.com](mailto:drsatis16@rediffmail.com)

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### Sources of PM:

Particles can either be directly emitted into the air (primary PM) or be formed in the atmosphere from gaseous precursors such as sulfur dioxide, oxides of nitrogen, ammonia and non-methane volatile organic compounds (secondary particles). Primary PM and the precursor gases can have both man-made (anthropogenic) and natural (non-anthropogenic) sources. Anthropogenic sources include combustion engines (both diesel and petrol), solid-fuel (coal, lignite, heavy oil and biomass) combustion for energy production in households and industry, other industrial activities (building, mining, manufacture of cement, ceramic and bricks, and smelting), and erosion of the pavement by road traffic and abrasion of brake sand tyres. Agriculture is the main source of ammonium. Secondary particles are formed in the air through chemical reactions of gaseous pollutants. They are products of atmospheric transformation of nitrogen oxides (mainly emitted by traffic and some industrial processes) and sulfur dioxide resulting from the combustion of sulfur-containing fuels. Secondary particles are mostly found in fine PM.

### Difference between PM10 and PM2.5:

Particles come in a wide variety of sizes and have been historically assessed based on size, typically measured by the diameter of the particle in micrometers. PM10 refers to particles that are 10 micrometers in diameter or less. PM2.5, or fine PM, refers to particles that are 2.5 micrometers in diameter or less. (Note: a human hair is about 70 micrometers in diameter and a grain of sand is about 90 micrometers in diameter). Areas of the country are designated nonattainment or attainment separately for the PM10 and PM2.5 standards. Both PM10 and PM2.5 have two standards related to the average concentration over different time periods:

#### PM10 Annual 50 µg/m<sup>3</sup> \*\*

To attain this standard, the expected annual arithmetic mean PM10 concentration at each monitor within an area must not exceed 50 µg/m<sup>3</sup>.

#### PM10 24-hour 150 µg/m<sup>3</sup>

Not to be exceeded more than once per year.

#### PM2.5 Annual 15.0 µg/m<sup>3</sup>

To attain this standard, the 3-year average of the annual arithmetic mean PM2.5 concentrations from

single or multiple community-oriented monitors **must** not exceed 15.0 µg/m<sup>3</sup>.

#### PM2.5 24-hour 65 µg/m<sup>3</sup>

To attain this standard, the 3-year average of the 98th percentile of 24-hour concentrations at each population-oriented monitor within an area must not exceed 65 µg/m<sup>3</sup>.

\*\* µg/m<sup>3</sup> is micrograms per a cubic meter

### Health Effects of Airborne Particulate Matter:

The introduction of new clean air regulations and the successful abatement of traditional air pollutants in the 1960s and 1970s in many industrialized countries led to the reduction of pollution episodes in urban areas, elimination of winter smog (London-type pollution), and significantly improved levels of photochemical pollutants (California-type pollution) because of on vehicle exhausts and industrial fumes. But the problem of adverse effects and excess deaths by airborne particulate matter (PM) in urban areas (Holland et al) was argued that high levels of PM posed health problems, but at lower concentrations their effects were confounded by other factors. Many scientists disagreed with the main conclusions and through the careful analysis of data supported that PM has adverse health effects even at relatively low concentrations (Shy CM et al). The reason that PM became very important air pollutants in recent decades and their adverse health effects became more hazardous is that air pollution in urban areas has changed. Air pollution from combustion of traditional fossil fuels (biomass, coal, wood, crude oil, diesel with high content in sulfur) is now in much lower concentrations than 30 to 40 years ago because of cleaner technology, but other pollutants have gained prominence, such as fine and ultrafine PM, because of a dramatic increase in motor vehicle worldwide with the consequent rise in exhaust emissions in urban areas. Airborne PM is found not only in big cities but also in small and large towns, and their size distribution and composition (heavy metals, PAHs, etc.) has changed, resulting in higher oxidative cellular damage and toxicological effects.

In the 1990s, epidemiologists focused on day-to-day variations in air pollution over long periods as determinants of day-to-day variations in mortality, hospital admissions, and lung function changes. These time-series studies have several advantages, with large differences in exposure overtime and lots of collected data, leading to greater statistical power



to detect small increases in adverse health effects of air pollution. Health effects from airborne fine particles were revealed by two very important cohort studies in the United States. Both studies were based on observations the late 1970s through the late 1980s. Then, another study significant health effects (increased mortality from non-malignant respiratory diseases and lung cancer in male non-smokers) for high concentrations of PM with aerodynamic diameter less than  $10 \mu\text{m}$  (PM<sub>10</sub>) (Abbey et al.). Additional epidemiological studies have provided more quantification of subtle health effects associated with fine PM, which is common in the contemporary urban areas and big cities in the developed countries, by better definitions and measures of air pollution exposures and health endpoints. In, advanced biostatistical and econometric techniques for longitudinal or cross-sectional analysis have greatly expanded the evaluation of health effects.

#### **EPIDEMIOLOGICAL EVIDENCE:**

##### ***Short-Term Health Effects:***

Numerous studies from the 1980s onwards evaluated the short-term effect of air pollution on health, with emphasis on mortality and hospital admissions. The most interesting studies were those that observed changes in daily death counts associated with short-term changes in PM air pollution. Although precise comparison between studies is difficult, due to differences, most of the results suggest that a  $10 \mu\text{g}/\text{m}^3$  increase in PM<sub>10</sub> was associated with a 0.5% to 1.5% increase in daily mortality.

##### ***Long-Term Health Effects:***

Short-term studies are based on air pollution episodes or on days with severe or very high concentrations of air pollutants. But, what happens with long-term cumulative exposures at low or very low concentrations of particulates? Are standards and health guidelines sufficient to protect human health? Epidemiological studies were designed to study the associations between cumulative exposures (months, years, decades) to suspended particulate air pollution and long-term effects on morbidity endpoints. Studies that measured deaths in short-term PM exposure capture only the very frail people who would have died in a few days anyway, whereas studies on long-term exposures to

PM measure the overall health effects for varied concentrations and length of exposure. Most of these studies have been cross-sectional, assuming that current air pollution exposures are representative of long-term previous exposures.

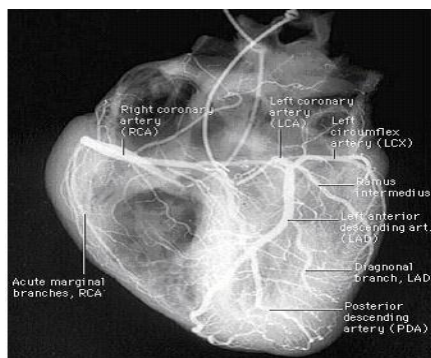
Epidemiological research has found consistent and coherent associations between long-term exposure and various health outcomes, such as reduced lung function, respiratory symptoms, chronic bronchitis, relative increase of lung cancer risk, and cardiopulmonary mortality. Scientists used the respiratory health effects in adults and in children as an example for long-term effects of particulate air pollution. There are now several studies on potential mechanisms by which long term exposure to particulate air pollution affects the cardiovascular system. Fine and ultrafine particles from vehicular exhausts are affecting heart rate variability, blood viscosity and blood coagulability, cardiac arrhythmia, deep vein thrombosis, atherogenesis, and destabilization or rupture of atheromatous plaques.

Lung cancer risk and particulate air pollution was the subject of many recent studies. Large epidemiological cohort studies in the United States and in Europe assessed the relationship between long-term exposure to fine particulate air pollution (PM<sub>10</sub> and PM<sub>2.5</sub>) and increased mortality from lung cancer, especially in combination with other known risk factors, such as smoking, passive smoking, and occupational exposures. One of the most important studies on long-term effects of fine particulate air pollution (PM<sub>2.5</sub>) and its relationship with all-cause, lung cancer, and cardiopulmonary mortality was conducted in the United States by Pope et al. (1995). The study enrolled 1.2 million adults in 1982 (data from the American Cancer Society, Cancer Prevention II study). Participants completed a questionnaire that detailed individual risk factor data (age, weight, smoking, diet, occupational exposure, etc.). The risk factor data for 500,000 adults were linked with air pollution data from metropolitan areas throughout United States and were combined with data of causes of death up to 1998. Fine particulate and SO<sub>2</sub> air pollution (the integrated average of PM<sub>2.5</sub> concentrations was estimated, first by site and then by metropolitan area) were associated with increased mortality. Each  $10 \mu\text{g}/\text{m}^3$  elevation in fine particulate matter was associated with approximately a 4%, 6%, and 8%



increased risk of all-cause, cardiopulmonary, and lung cancer mortality, respectively. It is interesting that data of coarse particles and TSP were not consistently associated with mortality. A recent review of epidemiological evidence for long-term exposures suggests the existence of a dynamic exposure-response relationship between PM and mortality risk. Data showed that adverse health effects are dependent on both exposure concentrations and length of exposure, and that long-term exposures in PM have larger, more persistent, and cumulative effects than short-term exposures.

Research data collected during the past 20 years in many industrialized countries on air pollution, especially particulate matter (PM), contributed to various estimates of public-health impact on mortality and morbidity. One of the first studies estimated the contribution of air pollution and traffic-related air pollution in Austria, France, and Switzerland. A  $10 \mu\text{g}/\text{m}^3$  increase in PM was used to quantify the effects of air pollution. The findings of the study showed that air pollution caused 6% of total mortality (or more than 40,000 attributable cases per year) and about half of all mortality was attributed to motorized traffic. In addition, air pollution caused more than 25,000 new cases of chronic bronchitis (adults); more than 290,000 episodes of bronchitis (children); more than 500,000 cases of asthma attacks; and more than 16 million person-days of restricted activities. Similar results of increases in mortality (all-causes and cardiovascular/cardiopulmonary) from long-term exposures in particulate air pollution were estimated in the United States. Therefore, the next appropriate step in the scientific research was to investigate the role of the size and composition of PM and especially the traffic related fine PM.



**Fig.1.** Heart disease by short term exposure to fine particles of air pollution.

### Lung Cancer Risk:

Cancer risks in relation to airborne PM after long-term exposure in urban areas have been studied with different epidemiological methodologies, mainly ecologic, cohort, and case-control studies. Several studies in the 1990s collected adequate epidemiological data on air pollution and lung cancer, and their evidence was reviewed. The problem with most of the cohort investigations was that they did not contain information on smoking for all study subjects, and findings for non-smokers were too small for a meaningful interpretation of urban to rural differences. In most of the case-control studies, the increased lung cancer risks were seen primarily in smokers. Ecologic studies generally were not suitable for assessment of causal relationships. A number of studies have been performed on populations living near industries with heavy emissions of air pollutants. Most of the studies on lung cancer and air pollution gave somewhat inconsistent results and positive interactions with smoking (additive or multiplicative).

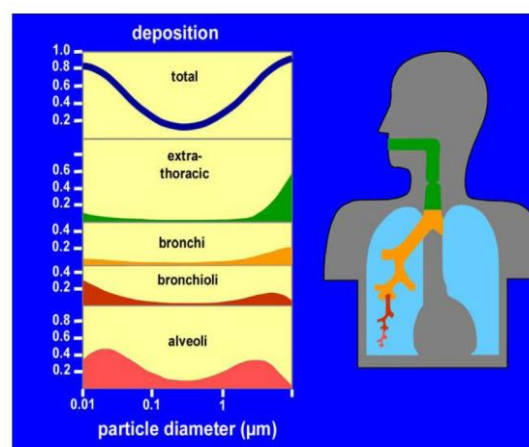
The relationship among long-term exposure to ambient PM and lung cancer was established for the first time by three cohort studies in the United States. The first study was the AHSMOG study based on 6,338 non-smoker adults (Seventh Day Adventists) followed for 15 years. The investigators reported relative risk (RR) increases of lung cancer mortality among men in relation to long-term exposure to PM<sub>10</sub> (measured initially from total suspended particulates, TSP, and as PM<sub>10</sub> in the past 5 years). The RR was 3.36 [(95% confidence interval, (CI): 1.57–7.19] associated with an interquartile range of PM 24  $\mu\text{g}/\text{m}^3$ ). Although the mean ozone concentration was not associated with lung cancer incidence, there was an association in males when exposure was formulated as the number of hours per year with elevated ozone concentrations (RR = 4.19, 95% CI: 1.81–9.69) (.The second study was based on 8,111 residents of six US cities followed in the period of 1974–1989. Differences in the long-term average PM concentrations; between the most and least polluted cities was 20  $\mu\text{g}/\text{m}^3$ . Relative risks after adjustment (age, smoking habits, education, body mass index, etc.) were to 1.37, which is estimated as a 19% increase per 10  $\mu\text{g}/\text{m}^3$ . The third and largest US investigation was published in 2002 by Pope et al.



This study was based on the mortality of approximately 500,000 adult men and women followed for 17 years. Personal information and other confounders or risk factors were collected with questionnaires (age, race, marital status, smoking, body mass, occupational exposures, diet, etc.). For each metropolitan area, PM<sub>2.5</sub> concentrations were compiled from several data sources. The investigation found a significant increase in mortality from lung cancer. Relative risk was 1.14 (95% CI: 1.04–1.23), or 14%, for a difference of 10  $\mu\text{g}/\text{m}^3$  of PM<sub>2.5</sub>. The importance of these three studies is that they avoided various limitations of previous geographic comparisons (case-control studies) and controlled the various confounders or risk factors, especially smoking habits. The European studies on the relationship of long-term exposure to PM and lung cancer risk are considered more valuable because the ambient levels of PM are more variable in European cities and usually higher than in the United States, and the European populations have a wide range of different exposures and living habits (especially smoking and diet), which might modify the final risk. The first study (case-control) did not control the various confounders. It was conducted among 755 men in Trieste (Italy) who died from lung cancer and 755 controls who died from other causes in the period of 1979–1981 and 1985–1986. The RRs were 1.1 (95% CI: 0.8–1.5) for PM exposure less than 30  $\mu\text{g}/\text{m}^3$  and 1.4 for more than 30  $\mu\text{g}/\text{m}^3$ . The second study in the Netherlands was a cohort study (on Diet and Cancer, NLCS) that examined the long-term exposure of 4,492 participants, and their deaths from lung cancer were recorded for the period of 1986–94. Risk of lung cancer was, after adjustment for several confounding factors, 1.06 (95% CI: 0.43–2.63) for an increase of 10  $\mu\text{g}/\text{m}^3$  (black smoke), but the number of lung cancer cases were too small ( $n = 60$ ).

The third study was a French cohort study and was based on 14,284 adults (in 24 areas of France), with 178 deaths recorded from lung cancer between 1974–2000. Relative risk for lung cancer associated with an increase in exposure to 10  $\mu\text{g}/\text{m}^3$  of TSP was 0.97 (95% CI: 0.94–1.01) when all 24 areas were considered, and 1.0 when the analysis was restricted to 18 areas. A nested case-controlled European study was conducted in seven countries with over half a million volunteers (35–74 years of age) between 1993 and 1998. The study investigated the association between lung cancer and long term

exposure to PM<sub>10</sub>. Cancer cases were recorded from cancer registries. Overall, 271 lung cancer cases and 737 controls were included. Data for PM<sub>10</sub> exposure were available for 113 cases and 312 controls only. Relative risks were 0.91 (95% CI: 0.70–1.18) for an increase in PM<sub>10</sub> of 10  $\mu\text{g}/\text{m}^3$  and 0.98 (95% CI: 0.66–1.45) for exposures over 27  $\mu\text{g}/\text{m}^3$ . Various other studies were conducted in Norway, Greece, and Sweden for the investigation of the association of lung cancer risk and long-term PM exposure, but their results were not consistent, or the age-adjusted RR was small and not statistically significant, or no association was observed. A recent review describes in detail all European studies and investigated the association of lung cancer risk and long-term exposure to ambient particulate matter.



**Fig.2.** Regional deposition of particles in the human respiratory tract.

### Genotoxicity:

Particulate matter, especially traffic-related airborne particles, contains a large number of genotoxic/mutagenic chemical substances, which can cause DNA damage and promote malignant neoplasms. In recent decades, a number of experimental studies that use different short-term assays have provided evidence for the mutagenic potential of airborne PM. Most studies focused their observations on the genotoxicity of extractable organic compounds and mixtures but also on the water-soluble substances (such as metals) and volatile organic compounds. The genotoxicity of PM was extensively studied with the *Salmonella typhimurium* assay (Ames test) by various research groups and reviewed by Claxton et al. Studies showed that the mutagenicity of airborne PM is due to at least 500 identified organic compounds from varying chemical classes. Mutagenicity was



associated with moderately polar/highly polar classes of substances that tend to contain nitroaromatics (nitro-PAHs), aromatic amines, and aromatic ketones. These compounds are produced in the atmosphere when organic compounds (even non-mutagenic) are exposed to NO<sub>x</sub> and sunlight. Combustion emissions were associated with much of the mutagenicity and carcinogenicity of urban PM. Human-derived cell lines have been used to investigate DNA damage induced by extractable organic material (especially PAH-containing mixtures bound onto PM<sub>10</sub> particles using a variety of end-points). Target cells used were human leukocytes, human alveolar carcinoma, human myeloid leukemia, human tran-cheo-bronchial epithelia, human fibroblasts, and so on. Air borne PM used in these studies were TSP, PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>2.5-10</sub>, residual oil flyash (ROFA), and other types of PM. All studies showed positive DNA damage, single-strand breaks, micronuclei sister chromatid exchange, and oxidative DNA damage.

Measurements were analyzed by alkaline single-cell gelelectrophoresis and comet assay. These studies suggest that the mechanisms of genotoxicity of PM is the result of adduct-forming compounds (through cell particulate interactions) and oxidizing DNA damage. Apart from organic-soluble fractions of PM, other studies focused on the water-soluble fractions (mainly transition metals) and compared their DNA damage potential. Results of these studies showed that the constituents of the water-soluble PM extracts are more likely to induce oxidative DNA damage than the organic compounds. A series of studies in the past decade suggest that after inhalation and deposition of PM in the lung, alveoli are able to stimulate the formation of reactive oxygen species (ROS), especially hydroxyl (HO•) and superoxide anion radicals (O<sup>•-</sup><sub>2</sub>). These ROS, which can be generated by transition metals and/or quinoid redox cycling, initiate a cascade of reactions and can play an important role in oxidative damage to cellular membrane lipids, proteins-enzymes, and DNA. In addition, ROS can initiate pulmonary inflammation and, through complex mechanisms, might contribute to the impairment of excision repair mechanisms of DNA and activation of oncogenes. A recent review summarizes the toxicological assessment of various biological mechanisms behind the associations of ambient particulate matter and health risks to humans.

## CONCLUSION:

There is emerging evidence from various scientific investigations for the relationship between fine airborne PM and health risk to human beings. Airborne PM is reportedly known to cause wide ranging health effects. PM disperses in air is normally classified as (ultrafine, size ranges less than 0.1 μm), (fine, 0.7-1.0 μm) and (course, 1-200 μm). Many epidemiological studies published strongly indicates that the long term exposure to even a low level of PM are linked with deleterious health problems including Asthma, bronchitis, Chronic pulmonary diseases, pneumonia and upper respiratory track and lower respiratory track disorder. PM is also risk factor for premature mortality cardiopulmonary and lung cancer mortality. Alarming levels of PM reported in all metropolitan cities and other urban areas. Immediate measures are essential to stream lines recording the morbidity in more detailed fashions. Data should be made easily available to research community by publishing on the web or making available statistical documents in public domain.

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## REVIEW ARTICLE

## IMMUNOPHARMACOLOGICAL STUDIES OF AEROMYCOFLORA OF MUMBAI

Mishra VK<sup>1</sup> and Tripathi DM<sup>2</sup><sup>1</sup>K. M. Agrawal College, Kalyan (W) 421301<sup>2</sup>Hon. Allergologist, Bombay Hospital, Mumbai.

## ABSTRACT

Aeromycological and immunopharmacological studies were conducted, to find out the allergenicity of airborne fungal spores. Aeromycological survey of two Industries of Mumbai was undertaken for two years using gravity Petridish method and a conventional Rotorod Sampler. Altogether 47 genera were isolated and identified. Out of them five fungi were selected for Immunopharmacological studies in guinea pigs. Animals responded positively to three fungal extracts viz. *Paecilomyces fusisporus*, *Phoma hibernica* and *Trichoderma viride* whereas negative response was noted with *Cladosporium cladosporioides* and *Penicillium nigricans*. Present study thus supports the allergenicity of fungi.

**Keywords :** Aeromycoflora, occupational environs, Hypersensitivity..

## INTRODUCTION

Air-borne fungal spores have been considered responsible for causing various allergic ailments like rhinitis, nasobronchial asthma, conjunctivitis, dermatitis, urticaria etc. in sensitive human population. Probably Floyer (1726) was the first to report the case of fungal sensitivity. Next was Blackley (1873) who demonstrated his own allergy to the spores of *Chaetomium* and *Penicillium glaucum*. Van Leeuwen (1924) suggested that in Holland, asthma was caused by 'Miasmata' or 'Climate allergens. The climate allergens were *Aspergillus*, *Mucor* and *Penicillium*. Cadham (1924) reported three cases of allergy to *Puccinia graminis* and was first to use the fungal extracts for desensitization treatment of allergic disorder. Hansen (1928) reported 15% positive skin reactions to one or more fungal extracts. Hopkins *et. al.* (1930) recorded the case of asthma due to *Alternaria*. Hyde and Co-workers (1956) reported that 8.3% of the 627 patients gave positive reaction to *Cladosporium*. Menezes *et. al.* (2004) found relationship between air-borne fungi and respiratory allergy in Brazil.

(Shivpuri and Singh, 1965 & Agarwal and Shivpuri, 1974). Kothari *et. al.* (1993) found correlation between *Alternaria* spores and bronchial asthma. Singh and Kumar (2002) found various fungi as aero-allergens.

Allergy is the hypersensitivity in the form of immune response in which antibodies (Proteins) are released in provocation by a different foreign protein termed antigen. In some individuals, due to some blemish in the immune system, the body starts producing certain harmful Reagins (IgE antibodies) on exposure to harmless substances. Such individuals suffer from allergic disorders whenever they are re-exposed to the same substance (the specific allergen). This happens due to the allergen-antibody interaction.

Due to this interaction, certain mediators of allergy such as histamine, 5-hydroxytryptamine, SRS-A (slow reacting substance of anaphylaxis), some kinins etc are released. These mediators act on the tissues and cause precipitation of symptoms of allergic disorders such as breathlessness in the patients of asthma, sneezing and running nose in the patients suffering from rhinitis (allergic colds) and itching and rashes in patients of urticaria etc.

In USA 10-20% of the total population suffers from allergic disorders (Urbach and Gottlieb, 1946) and in Great Britain allergic ailments are found in 11.8% population. The incidence of allergic disorders in India is reported to be quite high. With

**\*Corresponding Author**

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regard to the prevalence of all major allergic diseases in India the total incidence estimated in the neighbourhood of 10% of population (Vishwanathan 1964)

Mumbai, being one of the largest and thickly populated cities of India, it is necessary to take qualitative as well as quantitative account of prevalence of aeromycoflora of the atmosphere of the city and to find out their allergenicity.

The present work was undertaken to investigate the air-borne fungal flora of indoor as well as outdoor air of two industries of Mumbai and to study their allergenic behaviour.

### MATERIALS AND METHODS:

Aeromycological study of indoor as well as outdoor atmosphere of two industries of Mumbai, was undertaken for two years, using gravity Petridish method and a conventional Rotorod sampler.

In the present study, 73 fungal species belonging to 47 genera were trapped, isolated and indentified. Some of the dominant genera are *Aspergillus*, *Cladosporium*, *Curvularia*, *Penicillium*, *Trichoderma*, *Paecilomyces* *Alternaria*, *Fusarium*, *Rhizoctonia* *Cephalosporium* and *Rhizopus*. These eleven genera together contributed to 90.10% whereas the remaining 36 genera accounted to only 7.06%.

In order to study the allergenicity of some of these fungi, Immunopharmacological studies were performed, in guineapigs with allergen extract of five types of fungal spores by giving them microanaphylactic shock. These fungi are 1) *Cladosporium cladosporioides* 2) *Paecilomyces fusisporus* 3) *Penicillium nigricans* 4) *Phoma hibernica* & 5) *Trichoderma viride*. Extracts of these fungi were prepared by following mass culturing, harvesting & drying, grinding, defatting, extraction and sterilization steps.

For the present study, guineapigs of either sex weighing 300-400g were sensitized using water in oil suspension of each of the above mentioned allergen extracts and complete Freund's adjuvant (Lakdawala et.al. 1982).

Each extract was suspended in 0.5% carboxyl methyl cellulose at the concentration of 20 mg/kg and then mixed with equal amount of complete Freund's adjuvant. The uniform suspension was achieved by mixing them thoroughly and finally

testing it for water in oil suspension. Each guineapig, received 0.125 ml of above suspension, subcutaneously on the nape of the neck. Twenty days later, each animal was challenged by exposing it to the aerosol of 0.5% solution of the sensitizing antigen effected through EEL atomizer at a constant air pressure of 180 mm Hg. The onset time of asthmatic attack was noted (Lakdawala et.al. 1984).

The contact sensitivity was assessed by challenging the sensitized guineapig with intradermal injection of 0.1 ml. of antigen. The reaction was seen as a wheal or Oedema at the site of challenge. Control animal received the vehicle.

### RESULT AND DISCUSSION:

In the present study animals responded positively to three out of five allergen extracts, *Paecilomyces fusisporus*, *Phoma hibernica* and *Trichoderma viride* whereas negative response was noted among the animals tested with *Cladosporium cladosporioides* and *Penicillium nigricans* as no sensitization could be achieved with these two allergen extracts, thus showing them inactive as shown in the table 1:

**Table 1:** Type of response observed in Guineapigs to different allergen extracts.

Sr.No.	Fungal Extracts	Response
1	<i>Cladosporium cladosporioides</i>	Negative
2	<i>Paecilomyces fusisporus</i>	Positive
3	<i>Penicillium nigricans</i>	Negative
4	<i>Phoma hibernica</i>	Positive
5	<i>Trichoderma viride</i>	Positive

To the best of our knowledge, no such report is available, except that of Thakur (1986) who observed positive response in guineapigs for *Prosopis juliflora* pollen extract, hence comparison could not be made.

### CONCLUSION:

Above study in guinea pigs support the earlier studies and proves that the fungal spores possess the property of allergenicity Aeromycological study indicates the abundance of fungal spores in the air of Mumbai which may be due to the effect of high humidity and moderate temperature throughout the year and also due to unhygienic conditions in and around industries. Considering the results of the present study, authors would like to suggest to adopt measures, to reduce the air-borne fungal flora,



by maintaining clean / unpolluted environment in the localities.

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**RESEARCH ARTICLE****LIMNO-ECOLOGICAL ASSESSMENT OF LENTIC ECO-HABITAT, GAONDEVI TALAO FROM MALWANI, MUMBAI (INDIA)****Shingadia Hitesh U and Vaidya Meenakshi**SVKM's Mithibai College of Arts, Chauhan Institute of Science and A. J. College of Commerce and Economics,  
Vile Parle - West Mumbai 400 056**ABSTRACT**

Limno-ecological study plays a very crucial role in decision making processes with regards to dam construction, pollution control, aquaculture practices, declaration of recreation zone and ecotourism. Considering immense importance & utility of the freshwater body a few hydrobiological parameters were analyzed in relation to diversity. Analysis was conducted from July 2006 to June 2007 with respect to important physico-chemical parameters like temperature, turbidity, conductivity, TS, TDS, TSS, pH, DO, free CO<sub>2</sub>, acidity, alkalinity, total hardness, Ca, Mg, nutrients like phosphates, silicates, nitrates, BOD & COD. Results revealed a significant seasonal variation in a few physico-chemical parameters while few others were in the near to normal range that indicated better quality of aquatic habitat. The phytoplankton diversity showed dominance of organisms belonging to Cyanophyceae, Bacillariophyceae & Chlorophyceae respectively. The lake was observed to be moderately nutrient enriched that supported phytoplankton production & luxuriant growth of hydrophytes predominantly aquatic weeds.

**Keywords:** talao, Limno-ecological Status, Seasonal variation.**INTRODUCTION**

Lakes & reservoirs form an integral part of our eco-heritage. It is also a legacy of traditional wisdom of people from different agro-climatic zones that provides freshwater, one of the five elements of the so-called "Panchmahabhuta" for the very existence of life. Lake maintains ecological balance, flora and fauna inter-relationship, there by regulating surrounding climate and recharges ground water. The quality of water resources is usually described according to its physical, chemical and biological characteristics. For confirming the good quality of water resources large number of physico-chemical parameters, magnitude and source of any pollutional load must be known for which monitoring of physico-chemical parameter and pollutant is essential (Reddi et al., 1994).

**\*Corresponding Author**E-mail: [hiteshshingadia26@gmail.com](mailto:hiteshshingadia26@gmail.com)

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Assessment of water resource quality from any region is an important aspect for the developmental activities of the region, because rivers, lake and man made reservoir are used for water supply to domestic, industrial, agricultural, fish culture and also for enhancing aesthetic value of the environment (Jain and Seethapathi, 1996). Vast literature is available on limnological studies of lakes in India (Pendse et al. 2000; Sivakumar et al. 2000 and Borse et al. 2003; Khapekar and Deshpande 2007). The biodiversity study shows that the distribution of planktonic life in a water body is influenced by its physico-chemical nature. Algal flora constitutes a vital link in the food chain and its productivity depends on water quality at given time (Meshram and Dhande, 2000). The phytoplankton is primary producers of the aquatic ecosystem. Algae are the natural inhabitants of water and form an important group of plant kingdom. In natural ecosystem growth of algae is usually limited by small quantities of inorganic nutrients dissolved in surface waters. In Maharashtra, hydrobiological study of algae was done by More and Nandan (2000); Kumavat and Jawale (2003); Jindal and Gusain (2007).



Lakes and reservoir are the terms that encompasses a wide variety of natural and manmade fresh water surface resources like perennial ponds, tanks, natural lakes and small and medium man-made reservoirs with definite non-riverine catchments.



**Fig. 1:** Satellite image of Gaondevi Talao, Malvani - Mumbai (India). (Source:www.google.com)

The Gaondevi Talao is almost circular fresh water lake situated at latitude  $72^{\circ} 48' 47''$  E and longitude  $19^{\circ} 11' 44''$  N in Malvani a western suburb in the city of Mumbai state of Maharashtra, India (Fig. 1). It is a perennial lake with a depth 20 mts. and total area of 23,500 sq. mts. approximately. Gaondevi talao is also called Kamal talao rightly said so because the lake supports luxuriant growth of colourful lotus that adds to the aesthetics beauty of the lake. The Gaondevi talao also supports the religious sentiments of the people of the villages in the vicinity of the lakes viz. Malvani and Kharodi. Although through survey of all the wetlands from India is lacking, several studies on the subject done in past give us the degree of deterioration of these aquatic resources (Tekale and Kodarkar 1999; Diwan and Kodarkar 2000 & Sakhre and Joshi 2003).

## MATERIALS AND METHODS:

The study area was surveyed once a month from July 2006 to June 2007 for the period of one year. Surface water samples were collected using clean plastic containers for the analysis of various physico-chemical parameter viz. Temperature, Turbidity, Conductivity, Total Solids, TDS, TSS, pH, Dissolved Oxygen, Free Carbon dioxide, Acidity, Alkalinity, Hardness, Calcium, Magnesium, Phosphate, Silicate, Nitrates, BOD & COD. The

physico-chemical parameters were analyzed as per standard methods prescribed in APHA (2005) and Trivedy & Goel (1986). For the phytoplankton density one liter sample water was collected separately once in a month. The samples were allowed to settle by adding Lugol's iodine, centrifuged and the concentrate was made up to 20 ml with 4% formalin. Density of phytoplankton was studied by using Lackey's drop method (1938). The sample period was divided into three main season's viz. summer, monsoon and winter seasons.

## RESULTS AND DISCUSSION:

The results of physico-chemical analysis and phytoplankton diversity of Gaondevi Talao for summer, monsoon and winter are summarized in Table 1, Fig. 2.

### Temperature

Temperature is key factor for seasonal productivity of phytoplankton as observed in the present study. Similar observation was also reported by Guatam et al. (2007). Temperature varies with respect to depth, season and environment. Low temperature in winter favour diffusion of DO (Khatavekar et al 2002 & khare et al., 2002). Jindal and Gusain (2007) reported negative relationship between temperature and DO in Bichali pond. They attributed this condition to the fact that at increased temperatures, the dissolved oxygen content of the water decreases as observed as observed in the present study. Temperature of the lake ranged between  $27-29^{\circ}\text{C}$ , this variation could be with respect to depth, season and environment. The depth of the lake water varies. The central area approximately has a depth of 20 mts, which decreases to merely 5 mts at the peripheral boundaries of the lake. Temperature is one important physical parameter that directly influences some chemical reactions in aquatic ecosystem. The low temperature recorded during the monsoon months could be due to rain, cloudy sky and cold weather (Gopinathan 1985).

### Turbidity, Conductivity, TDS and TSS

Turbidity ranged between 9.2 to 12.5 NTU. The maximum turbidity during monsoon could be due to rains that results in to turbulence of water and influx of particulate matter due to water run-off causing



turbidity. Determination of turbidity is interfered by presence of debris and other rapidly settleable matter. It was observed that due to an increase in turbidity the rate of photosynthesis decreases leading to decline in growth of phytoplankton. The electric conductivity of the surface water ranged from 0.7 to 1.1  $\mu\text{sm}^{-1}$ . The maximum conductivity was reported in monsoon (1.15 mS) followed by winter (0.83 mS) and summer (0.76 mS). Higher values of TS, TDS and TSS during monsoon indicate high concentration of organic waste. This factor could be responsible for increasing the turbidity caused by dense phytoplankton population in winter. Increase in TDS could be due to increase in level of salts containing carbonates and bicarbonates which is reflected in the values of conductivity in monsoon.

### pH

pH is an important indicator of freshwater body. Factor like exposure to air, temperature and disposal of sewage bring about changes in pH. Slightly alkaline pH as reported in present study could be due to rise in temperature with increase in rate of photosynthesis and resultant higher consumption of  $\text{CO}_2$ . The  $\text{CO}_2$  in turn is obtained from conversion of carbonates to bicarbonates with

rise in pH (Wetzel, 1983). Similar observations were also reported by Khare (2002).

### Dissolved Oxygen

Oxygen content is one of the important parameter in water quality assessment as it affects the solubility and availability of many nutrients. Decrease in concentration of DO during summer might be due to its utilization in decomposition of organic matter. Direct correlation between DO and phytoplankton growth as observed in present study was also reported by Borse et al. (2003), high pH, DO and COD promotes the growth of Phytoplankton.

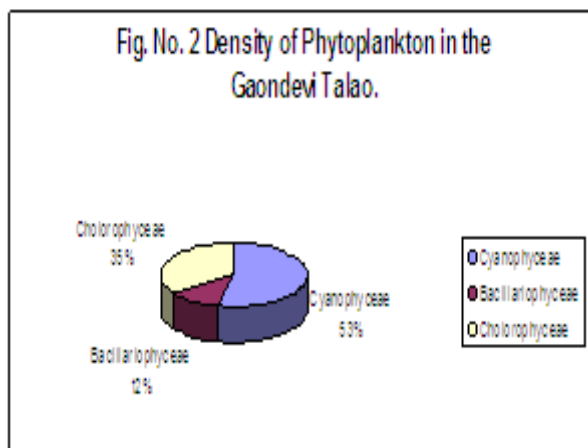
### Free Carbon dioxide:

Carbon dioxide is added to the aquatic ecosystem by directly being mixed from atmosphere. In addition to this, the other sources are rain water, inflowing ground water & the respiration of aquatic flora & fauna. Moderate amount of free  $\text{CO}_2$  in the present study could be a result of decomposition of organic matter from the aquatic ecosystem. However the photosynthetic activity of the aquatic flora could be a factor responsible for the lower values of free  $\text{CO}_2$  during winter season.

**Table 1: Seasonal variation in physico-chemical characteristics of Gaondevi Talao**

Sr. No.	Parameters	Seasons		
		Summer	Monsoon	Winter
1	Temperature ( $^{\circ}\text{C}$ )	29.5 $\pm$ 0.05	27.6 $\pm$ 0.08	26.2 $\pm$ 0.03
2	Turbidity (NTU)	9.2 $\pm$ 0.21	12.5 $\pm$ 0.19	9.95 $\pm$ 0.11
3	Conductivity (mg/L)	0.76 $\pm$ 0.65	1.15 $\pm$ 0.52	0.83 $\pm$ 0.83
4	Total Solids (mg/L)	540 $\pm$ 0.16	710 $\pm$ 0.13	608 $\pm$ 0.07
5	T.D.S (mg/L)	385 $\pm$ 0.03	524 $\pm$ 0.07	441 $\pm$ 0.08
6	T.S.S. (mg/L)	155 $\pm$ 0.25	186 $\pm$ 0.19	167 $\pm$ 0.16
7	pH	7.2 $\pm$ 0.67	7.9 $\pm$ 0.54	7.5 $\pm$ 0.19
8	Dissolved Oxygen (mg/L)	5.32 $\pm$ 0.81	6.8 $\pm$ 0.14	7.55 $\pm$ 0.67
9	Free $\text{CO}_2$ (mg/L)	6.51 $\pm$ 0.09	6.35 $\pm$ 0.16	5.1 $\pm$ 0.71
10	Acidity (mg/L)	184 $\pm$ 0.45	162 $\pm$ 0.56	189 $\pm$ 0.68
11	Alkalinity (mg/L)	264 $\pm$ 0.07	205 $\pm$ 0.09	265 $\pm$ 0.04
12	Hardness(mg $\text{CaCO}_3$ /L)	58 $\pm$ 0.73	62 $\pm$ 0.36	66 $\pm$ 0.24
13	Calcium(mg/L)	11.5 $\pm$ 0.17	9.6 $\pm$ 0.22	12.8 $\pm$ 0.25
14	Magnesium(mg/L)	12.6 $\pm$ 0.51	17.42 $\pm$ 0.57	11.96 $\pm$ 0.66
15	$\text{PO}_4\text{-P}$ (mg/L)	2.01 $\pm$ 0.08	3.1 $\pm$ 0.05	2.69 $\pm$ 0.08
16	$\text{SiO}_3\text{-Si}$ (mg/L)	43.9 $\pm$ 0.93	48.5 $\pm$ 0.81	38.5 $\pm$ 0.76
17	$\text{NO}_3\text{-N}$ (mg/L)	11.89 $\pm$ 0.05	13.5 $\pm$ 0.07	8.9 $\pm$ 0.09
18	BOD (mg/L)	3.75 $\pm$ 0.19	4.5 $\pm$ 0.11	4.6 $\pm$ 0.17
19	COD (mg/L)	28.0 $\pm$ 0.07	24.5 $\pm$ 0.05	26.5 $\pm$ 0.03
20	Cyanophyceae per Litre	31,875 $\pm$ 1.05	29,157 $\pm$ 0.923	54,765 $\pm$ 2.012
21	Bacillariophyceae per Litre	32,944 $\pm$ 0.911	15,991 $\pm$ 0.654	26,650 $\pm$ 1.067
22	Chlorophyceae per Litre	1,825 $\pm$ 0.096	2,509 $\pm$ 0.621	3,139 $\pm$ 0.087





### Acidity & Alkalinity:

The minimum value of acidity was observed during monsoon (162 mg/L) & maximum during winter season (189 mg/L). Alkalinity in the water is primarily a function of carbonate, bicarbonate & hydroxide ions. During premonsoon & post monsoon alkalinity was recorded higher than during monsoon. Chaudhary et al. (2005) also reported high alkalinity in ground water in Faridabad during winter & summer seasons. In the present investigation, the total alkalinity values were high that indicates the productive nature of the lake

### Calcium and Magnesium

The calcium and magnesium forms the most abundant ions on freshwater. Calcium is linked to  $\text{CO}_2$  and is important constituent of the skeletal structure of organism. Magnesium is essential for chlorophyll bearing organism, since it goes into the composition of the pigments. The presence of calcium and magnesium along with carbonate and sulphate make the water hard. Decrease in value of magnesium especially during winter may be due to uptake by the phytoplankton as reported by Rath et al. (2000). Nutrient vary with the seasons and also affected by overturning of the water especially during monsoon as observed in the present study, which brings decomposed bottom material to the photosynthetic zone. During the present investigation maximum Calcium was reported in winter (12.8 mg  $\text{CaCO}_3/\text{L}$ ) while maximum Magnesium reported during monsoon (17.42 mg/L). The maximum hardness was reported in winter (66 mg  $\text{CaCO}_3/\text{L}$ ).

### Nutrients(Phosphate, Silicate & Nitrate)

Among the nutrients Phosphate is considered as the most critical single element responsible for the biological productivity of the ecosystem. Increase in the value of phosphate in monsoon (3.1 mg/L) could be due to decaying organic matter, runoff and washing activities around the lake causing eutrophication. Phosphorus along with nitrogen causes explosive growth of algal species. Rainfall plays a significant role in increasing the concentration of nitrates (13.5 mg/L) as reported in the present study. In natural waters, amount of silicate range between 2-25 ppm. Higher silicate concentration during monsoon (48.5 mg/L) could be attributed to the sediment particles and land runoff. Diatoms require silica for the formation of skeletal structure that represented the abundant growth of Bacillariophyceae during summer months (43.9 mg/L) thus decreasing amount of silicates from the lake. Decomposition of organic matter, nitrifying bacteria and run off might cause increase in nitrate concentration in the aquatic environment. Lower level of nutrients like phosphate (2.69 mg/L), silicate (38.5 mg/L) and nitrate (8.9 mg/L) during winter season directly correlates with the blooming phytoplankton production, being consumed as major nutrients for the growth.

### BOD and COD

The BOD and COD are appropriate indices for assessing the status water bodies. Throughout the study period both these parameter were observed to be moderately high. This could be due to the degradation of the organic waste & accumulation of wastes due to anthropogenic activities. Maximum BOD values reported in the monsoon (4.5 mg/L) & winter (4.6 mg/L) while minimum value of BOD was reported during summer (3.75 mg/L). The BOD values are more than one in all the seasons of the year hence the water cannot be used directly for the drinking purpose without conventional treatment. High COD indicate the presence of chemically oxidizable carbonaceous matter as well as inorganic matter present in the aquatic environment. The maximum COD recorded during summer (28 mg/L) could be due to high temperature & increased rate of evaporation of water & consumption of oxygen for degradation of organic matter & load of chemical waste discharged into the lake by the inhabitants in





the vicinity. The lowest COD value was reported during monsoon (24.5 mg/L).

### Phytoplankton Density

According to Reid (1961) the plankton population on which the whole aquatic life depends directly or indirectly is governed by the interaction of a number of physical and chemical and biological conditions and the tolerance of the organisms to variations in one or more of them. The productivity of an aquatic ecosystem is directly correlated with the density of phytoplankton that in turn depends on the nutrient like phosphate, nitrate & silicate present in the environment. Thus over enrichment resulting into eutrophication causing phytoplankton bloom.

The physico-chemical characteristics of the lake are favourable for the growth of phytoplankton. Gaondevi talao showed a distinct seasonal variation in the distribution of phytoplankton (Fig. 2). Cyanophyceae (53%) was dominant over other groups almost throughout the study period in monsoon & winter. However diatoms (Bacillariophyceae) marginally dominated during the summer season. Next to Cyanophyceae was Bacillariophyceae (35%). Chlorophyceae was represented by only 12% of the total phytoplankton. The total algal population was represented by the diversity of 15 different genera. Since the lake was moderately nutrient enriched it showed an increasing trend towards eutrophication.

### Recommendations:

- Prevention of human intervention like washing, cleaning, bathing of cattle etc.
- Social abstinence from disposing waste of any kind to restore the lake
- Reclamation & desilting for restoration of habitat.
- Use of lake for aquaculture and recreation purposes.
- Thus enhancing the aesthetic value of the aquatic habitat.

### Acknowledgement:

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## RESEARCH ARTICLE

## AN INSIGHT IN URBAN INDIAN POPULATION WITH THE AWARENESS OF PLASTIC AS A POLLUTANT

Vora Jyoti D<sup>1</sup>\*, Shah Vidhi A<sup>2</sup> and Pednekar Sneha R<sup>3</sup>

<sup>1</sup>Department of Biochemistry & Food Science and Quality Control, Ramnarain Ruia College, Matunga, Mumbai – 400019.

<sup>2</sup>Presenting authors, Department of Biochemistry & Food Science and Quality Control,

<sup>3</sup>Ramnarain Ruia College, Matunga, Mumbai – 400019

### ABSTRACT

Plastic is one of the novel chemical materials which cause serious environment pollution and is certainly the worst 'cancer in nature'. Plastic pollution can adversely affect lands, waterways and oceans. Disposing plastic into water or land causes contamination and even increases the mortality rate of animals. A significant contamination of atmosphere results from the burning of plastic due to the release of poisonous, obnoxious chemicals. The damage inflicted by the usage of plastic has been unprecedented. The most sustainable management of plastic pollution would be to first educate the user on the detrimental nature of plastic and then substantially reduce the demand of plastic. Therefore, the present study was carried out to create awareness about the usage of plastic and its various ill-effects on health and environment. A carefully structured questionnaire was administered to the urban Indian subjects in the age group of 18 – 25 years with the view of obtaining a holistic picture of their perspective towards plastic and its impact on the environment. A total of 60 subjects were studied. The findings were statistically analyzed. It was found that 73% of the subjects under study were aware of the ill-effects caused by plastic. Around 93% of the subjects under study agreed that stringent laws should be implemented on usage of plastic. In addition 59% of the subjects under study were of the opinion that manufacturing of plastic materials should be banned. The results are indicative of important benchmarks of psychosocial aspects of environmental study. The research endeavor mirrors the 'correct' picture of prevalence of anomalies as far as man-made molecules are concerned among young urban Indian population. Hence, awareness and education are the first steps towards environmental management.

**Keywords:** Plastic, pollution, obnoxious chemicals, sustainable management of pollution, ill-effects of plastic, non-ecofriendly.

### INTRODUCTION

Plastics are organic polymers of high molecular mass. They are usually synthetic, most commonly derived from petrochemicals, but many are partially natural. There are two types of plastics: thermoplastics and thermosetting polymers. Thermo plastics are the plastics that do not undergo chemical change in their composition when heated and can be molded again and again. These include polyethylene, polypropylene, polystyrene and polyvinyl chloride. Thermosets are the plastics that can melt and take shape once; after they have solidified, they stay solid.

Due to their relatively low cost, ease of manufacture, versatility, and imperviousness to water, plastics are used in an enormous and expanding range of products, from paper clips to spaceships. They have already displaced many traditional materials, such as wood, stone, horn and bone, leather, paper, metal, glass, and ceramic, in most of their former uses.

In developed countries, about a third of plastic is used in packaging and another third in buildings such as piping used in plumbing or vinyl siding. Other uses include automobiles (up to 20% plastic), furniture, and toys. In the developing world, the ratios may be different - for example, reportedly 42% of India's consumption is used in packaging.

Plastic is one of the novel chemical materials which cause serious environment pollution and is certainly the worst 'cancer in nature'.

#### \*Corresponding Author

Email: [drjyotivora@yahoo.co.uk](mailto:drjyotivora@yahoo.co.uk)

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Plastics are durable and degrade very slowly; the chemical bonds that make plastic so durable make it equally resistant to natural processes of degradation. Plastic pollution can adversely affect lands, waterways and oceans. Serious environmental threats from plastic include the increasing presence of microplastics in the marine food chain along with the many other highly toxic chemical pollutants that plastic attracts and concentrates, and larger fragmented pieces of plastic called nurdles. A significant contamination of atmosphere results from the burning of plastic due to the release of poisonous, obnoxious chemicals. If the plastic is incinerated, it increases carbon emissions; if it is placed in a landfill, it becomes a carbon sink.

Disposing plastic into water or land causes contamination and even increases the mortality rate of animals. The damage inflicted by the usage of plastic has been unprecedented. The most sustainable management of plastic pollution would be to first educate the user on the detrimental nature of plastic and then substantially reduce the demand of plastic. Therefore, the present study was carried out to create awareness about the usage of plastic and its various ill-effects on health and environment.

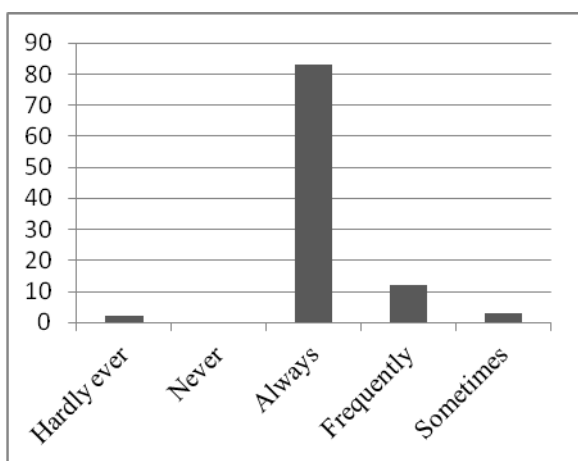
**MATERIALS AND METHODS:**

A carefully structured questionnaire was administered to the urban Indian subjects in the age group of 18 – 25 years with the view of obtaining a holistic picture of their perspective towards plastic

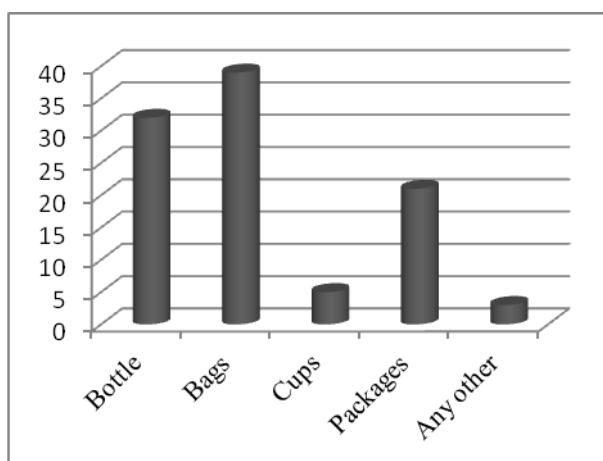
and its impact on the environment. A total of 60 subjects were studied. The findings were statistically analyzed. The conclusions on these perspectives were drawn on the basis of the results obtained.

**RESULTS AND DISCUSSION:**

The usage of plastic by the subjects under study was phenomenally high. Around 26% of the subjects under study preferred plastic items over items made from other materials. It was found that about 73% of the subjects under study were aware of the ill-effects caused by plastic. Most of the subjects reused the plastic items. However, only 58% of the subjects agreed that plastics are not totally inert. In addition, about 39% of the subjects were aware that plastics could be carcinogenic. All the subjects agreed that plastics are responsible for global warming and the effluents given out during the manufacturing of plastic can be hazardous. Around 93% of the subjects under study agreed that stringent laws should be implemented on usage of plastic and 59% of the subjects under study were of the opinion that manufacturing of plastic materials should be banned. In addition 87% of the subjects were of the view that using modern technology and with the aid of biotechnology, renewable biomass should be used for the manufacturing of plastics and also wherever possible substitute for plastics should be used such as to minimize the damage cause to the environment. The results are indicative of important benchmarks of psychosocial aspects of environmental study.

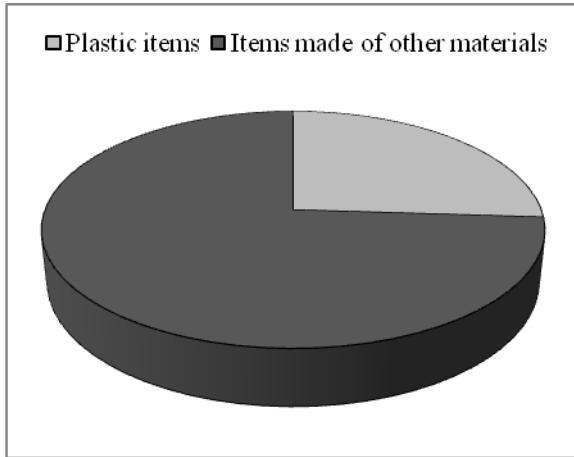


**Fig. 1:** Use of plastic by the subjects under study

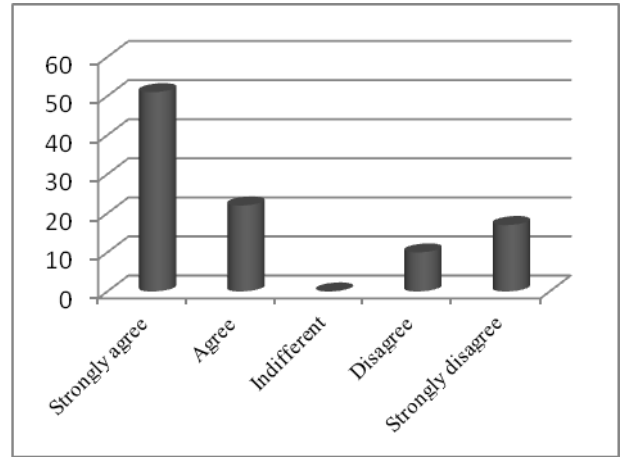


**Fig. 2:** Different forms in which plastic is used

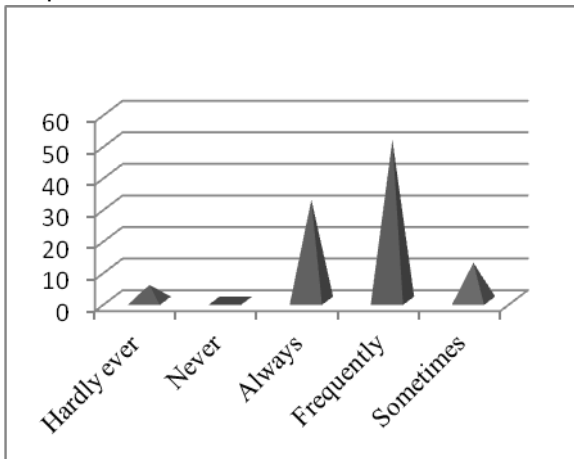




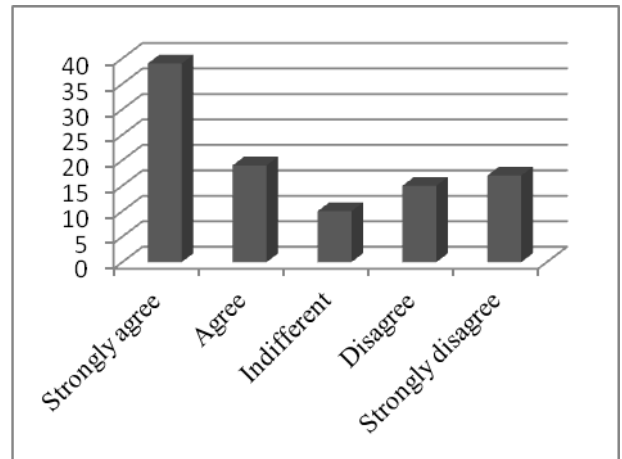
**Fig. 3:** Preference of items made of other materials over plastic items



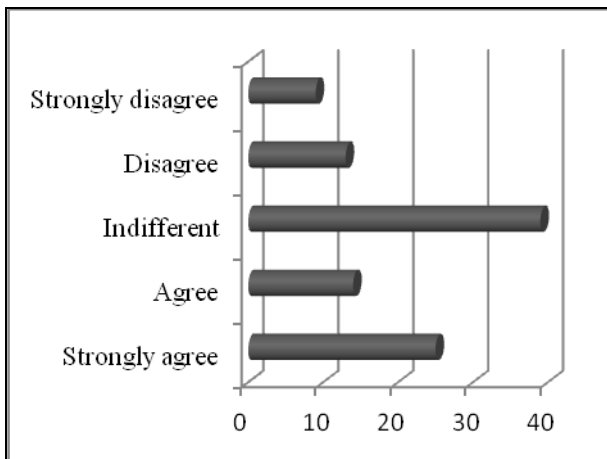
**Fig. 4:** Plastics have ill-effects on living organisms



**Fig. 5:** Reuse of Plastic



**Fig. 6:** Plastics are not totally inert



**Fig. 7:** Plastics are carcinogenic

**CONCLUSION:**

The research endeavor mirrors the ‘correct’ picture of prevalence of anomalies as far as man-made molecules are concerned among young urban Indian population. Hence, awareness and education are the first steps towards environmental management.

Motivated by the finiteness of petrochemical reserves and threat of global warming, bioplastics are being developed. Bioplastics are made substantially from renewable plant materials such as cellulose and starch. Biodegradable plastics degrade upon exposure to sunlight (e.g., ultra-violet radiation), water or dampness, bacteria, enzymes, wind abrasion, and in some instances, rodent, pest, or insect attack are also included as forms of biodegradation or environmental degradation. Some modes of degradation require that the plastic be exposed at the surface, whereas other modes will



only be effective if certain conditions exist in landfill or composting systems. Starch powder has been mixed with plastic as a filler to allow it to degrade more easily, but it still does not lead to complete breakdown of the plastic. Some researchers have actually genetically engineered bacteria that synthesize a completely biodegradable plastic, but this material, such as Biopol, is expensive at present. Companies have made biodegradable additives to enhance the biodegradation of plastics.

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## RESEARCH ARTICLE

**ANTIOXIDANT AND ANTIFUNGAL ACTIVITY OF “THE PERFECT AQUATIC WEED”****Mestry Asmita and Gupta Abhisheek**Department of Botany Bharatiya Vidya Bhavan's Hazarimal Somani college of Arts and Science,  
Mumbai-400007, (M.S.) India.**ABSTRACT**

The presence of natural antioxidant in plants is well known. This study was undertaken to investigate the antioxidant activities of aqueous and ethanolic extracts of *Hydrilla verticillata* (Linn.f) stem and leaves. The analysis carried out was total phenolic content and ferric reducing power test. From the analysis it was observed that aqueous and ethanolic extracts increases with increase in concentration. The extracts were compared to standard gallic acid and ascorbic acid. All extracts showed increase in the total phenolic content and ferric reducing antioxidant power. The extracts of leaves showed high phenolic content and reducing power compared to stem. However the efficacy of the extracts of leaves were tested for antifungal activity using *Aspergillus niger* and *Candida albicans*. The present study showed that the perfect aquatic weed with antioxidant and antifungal potentials and may be useful for developing alternative compounds to treat infectious diseases caused by fungal pathogens.

**Keywords:** *Hydrilla verticillata* L., Aquatic Plant, Antioxidants, Antifungal

**INTRODUCTION**

In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Arnous *et al.*, 2001). Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Lu and Foo, 1995). ROS, which include free radicals such as superoxide anion radicals (O<sub>2</sub><sup>-</sup>), hydroxyl radicals (OH<sup>•</sup>) and non free-radical species such as H<sub>2</sub>O<sub>2</sub> and singlet oxygen (<sup>1</sup>O<sub>2</sub>), are various forms of activated oxygen. These free radicals may either be produced by physiological or biochemical processes or by pollution and other endogenous sources. All these free radicals are capable of reacting with membrane lipids nucleic acids proteins and enzymes and other small molecules resulting in cellular damages (Shivaprasad *et al.*, 2005).

*Hydrilla verticillata* (Linn.f) is a slender submerged perennial aquatic herb. The plant is a rich source of variable nutrients and chemical constituents like saponins, vitamins, minerals, antioxidants, aminoacids, detoxifying agents etc.

The plant may be used in digestion and gastrointestinal functions, improves blood circulation, helps in detoxification and increases cardiovascular functions helps in blood sugar control and strengthens immune system. This present study therefore aimed at the evaluation of antioxidant and antifungal activities of these extracts.

**MATERIALS AND METHODS:**

The plant material *Hydrilla verticillata* (Linn f.) was collected in the month of July – August, 2013 from the Bhavans college, Andheri(W). The completely dried material was coarsely powdered and stored for further use.

**1. Determination of Total phenolic content:**

Various concentrations of the plant extracts in water and ethanol were mixed with 2.5 ml of Folin ciocalteu phenolic reagent (10 %) and 2 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was incubated for 30 min at room temperature in dark condition and the absorbance was measured at 760 nm. Control was prepared in similar manner excluding samples. Gallic acid at various concentrations was used at standard. Increased absorbance of the reaction mixture indicates increase in total phenolic contents. The total phenol content for 1ml is calculated with reference to standard gallic acid.

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**2. Protocol for reducing power:**

Various concentrations of the plant extracts in the corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50° C in water bath for 20 minutes. After cooling 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used at standard. Increased absorbance of the reaction mixture indicates increase in reducing power. The reducing power is calculated as:  $\text{Reducing Power \%} = \frac{A_{\text{Test}}}{A_{\text{Cont}}} \times 100$ .

**3. Paper disc diffusion method:**

To determine the antifungal activity of the ethanolic extract, 0.1µl individual culture were poured with a potato dextrose agar medium 30ml in petriplates. Sterilized filter paper disc Whatman No.1.6mm in diameter soaked in different beakers containing the dissolved extracts of different concentrations were taken out with sterilized forceps and air dried and placed on plates on plates with the different organisms. The plates were incubated at 37°C after incubation the zones of inhibition in millimeter diameter using transparent ruler.

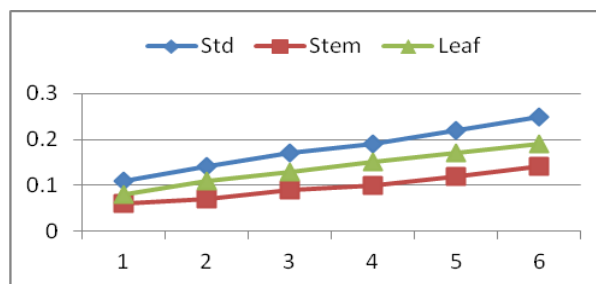
**RESULTS AND DISCUSSION:**

The perfect aquatic weed *Hydrilla verticillata* (Linn.f) has antioxidant property. Stem and leaves of the plant were selected for antioxidant activity. Aqueous and ethanolic extracts were concentrated and the residue was used for making different concentration. These concentrations were used for analysis of total phenolic content and reducing power. It was observed that leaves showed maximum phenolic content and reducing power compared to stem. (Table-I) Fig-I. Show effect of total phenol content in aqueous extracts. The total phenol content in 1mg of stem extract is 0.38mg/ml and in 1mg of leaf extract is 0.44mg/ml. whereas (Table II), Fig-II shows total phenol content in ethanolic extracts. The total phenol content in 1mg of stem extract is 0.52mg/ml and 1mg of leaf extract

is 0.66mg/ml. This shows significant antioxidant property in ethanolic extracts when compared with aqueous extracts. Some previous results give evidence of our results where Surendraraj et al., (2013) reported that ethanolic extract of *Eichhornia crassipes* contained high amounts of phenolic acids, whereas aqueous extracts contain less amounts of a varied number of phenolic acids.

**Table 1:** Effect of Aqueous extract of *Hydrilla verticillata* L. in Total Phenolic Contents

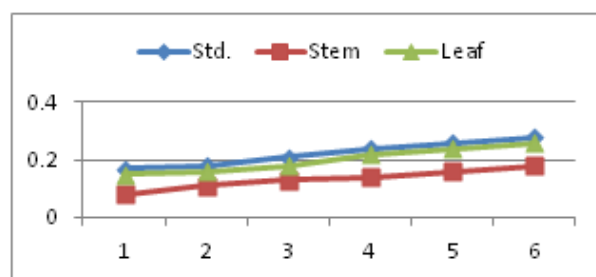
Sr. No	Conc. mg/ml	Gallic acid	Steam	Leaf
1	0.2	0.11	0.06	0.08
2	0.4	0.14	0.07	0.11
3	0.6	0.16	0.09	0.13
4	0.8	0.19	0.11	0.15
5	1.0	0.22	0.12	0.16
6	1.5	0.25	0.14	0.19



**Fig. 1:** Comparative effect of Aqueous extract of *Hydrilla Verticillata* L. In Total Phenolic Content.

Sr. No	Conc. mg/ml	Gallic acid	Steam	Leaf
1	0.2	0.17	0.08	0.15
2	0.4	0.18	0.11	0.16
3	0.6	0.21	0.13	0.18
4	0.8	0.24	0.14	0.22
5	1.0	0.26	0.16	0.24
6	1.5	0.28	0.18	0.26

**Table 2:** Effect of Ethanolic extract of *Hydrilla verticillata* L. in Total Phenolic Contents.



**Fig.2 :** Comparative effect of Ethanolic extract of *Hydrilla Verticillata* L. In Total Phenolic.

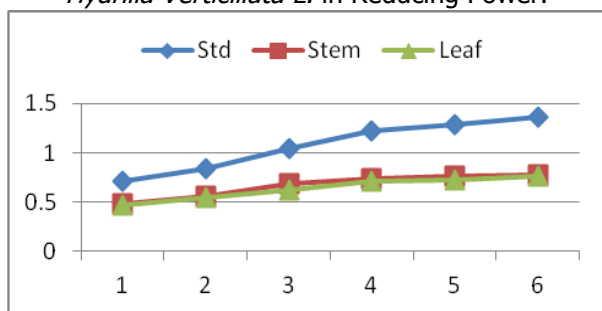




**Table I.** Effect of Aqueous extract of *Hydrilla verticillata L.* in Reducing Power

Sr.no	Conc.mg/ml	Ascorbic Acid	Steam	Leaf
1	0.2	0.71	0.48	0.47
2	0.4	0.84	0.56	0.54
3	0.6	1.04	0.68	0.62
4	0.8	1.22	0.74	0.71
5	1.0	1.29	0.76	0.73
6	1.5	1.36	0.78	0.76

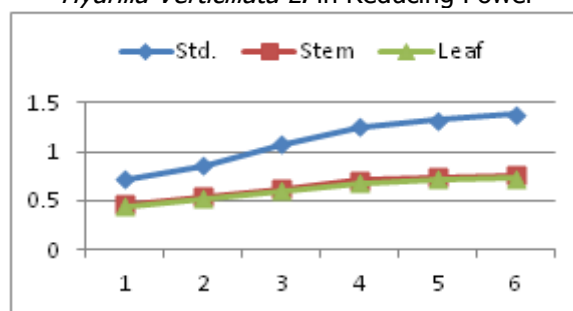
**Fig. 1I.**Comparative effect of Aqueous extract of *Hydrilla Verticillata L.* in Reducing Power.



**Table II:** Effect of Ethanolic extract of *Hydrilla verticillata L.* in Reducing Power

Sr.no	Conc.mg/ml	Ascorbic Acid	Steam	Leaf
1	0.2	0.72	0.46	0.44
2	0.4	0.86	0.54	0.52
3	0.6	1.08	0.62	0.60
4	0.8	1.26	0.71	0.68
5	1.0	1.32	0.75	0.72
6	1.5	1.38	0.76	0.78

**Fig. 1I.**Comparative effect of Ethanolic extract of *Hydrilla Verticillata L.* in Reducing Power



**Table III:** Zone of inhibition (mm) of fungal organism at various concentrations of extracts.

Sr. No.	Conc.mg/ml	Ketaconazole		<i>A.niger</i>		<i>C.albicans</i>	
		A.niger	C.albicans	Aqueous	Ethanol	Aqueous	Ethanol
1	2	-	-	-	-	-	-
2	4	-	-	-	-	-	-
3	6	0.2	-	-	-	-	-
4	8	0.3	-	-	0.1	-	-
5	1	0.4	-	-	0.3	-	0.2
6	1.5	0.5	0.2	0.2	0.5	0.1	0.3

(-)ND

The reducing power of all extracts increased with increase in concentration. (Table-II) Fig II shows increase in absorbance as the concentration increases. The reducing power for 1ml of aqueous extract of stem is 41.0% and in leaf it is 43.4%.Where as in ethanol extract of stem the reducing power is 43.9% and in leaf it is 45.5%. Previous study explained that the reducing power of ethanolic extract of plant *Eichhornia. crassipes* increased with the increasing amount of the sample and all ethanolic extract showed significant activities when compared to standard Ascorbic acid (Lalitha and Jayanthi 2012b). The higher antioxidant activity of *Hydrilla verticillata(Linn.f)* may be due synergistic effect of its components and phenolic compounds.

In addition to antioxidant potency the plant extract was further evaluated against *Aspergillus niger* and *Candida albicans* using leaf extract of *Hydrilla verticillata (Linn.f)* the result were compared with the standard ketaconazole .The aqueous extracts was less potent than ethanolic extracts. Natrajan *et al.*, (2005) reported the antifungal properties of three medicinal plant extracts against *Cercospora arachidicola*. They reported that fungal growth was gradually suppressed with increasing extract concentration (Table III). Similar findings have been reported by Lucia *et al.*, (2002) on the antifungal properties. They stated that ethanolic extracts of the plants showed higher antifungal activity. The findings from this study are similar to the report of these authors.



**CONCLUSION:**

The Aqueous and ethanolic leaf extract of *Hydrilla verticillata*(Linn.f) exhibited antioxidant and antifungal activities this may be due to many biologically active compounds such as phenols and saponins. The present study showed that the perfect aquatic weed possess antioxidant activity which is believe to be one of the most important component for many pharmacological activity. In addition, the extracts possess antifungal potential which could provide an affordable platform for newer drugs.

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## RESEARCH ARTICLE

## BIOSYNTHESIS OF SILVER NANOPARTICLES USING *ACHYRANTHES ASPERA* LEAVES EXTRACT AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

Basarkar UG\* and Nikumbh PS

P. G. Department of Botany, HPT Arts and RYK Science College, Nashik – 422 005.

### ABSTRACT

The ecofriendly synthesis of silver nanoparticles through various biological means helps to explore various plants for their ability to synthesize silver nanoparticles. In the present study, biosynthesis of silver nanoparticles carried out using leaf extract of *Achyranthes aspera* at room temperature. Silver nanoparticles were characterized for UV-Vis Spectrophotometer, SEM, FTIR, XRD & EDX. The antimicrobial activity of silver nanoparticles was evaluated on gram positive (*S. aureus*) gram negative (*E. coli*, *S. paratyphi*, *K. pneumonia*) & fungi (*C. albicans*). *S. aureus* & *C. albicans* were found to be more susceptible to silver nanoparticles. MIC study revealed that 1% concentration of *C. albicans* & 3% concentration of *S. aureus* found to be effective inhibitory concentration.

**Keywords:** Characterization, biosynthesis, leaf extract, silver nanoparticles, antimicrobial activity.

### INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process. Nanomaterials have a long list of applicability in improving human life and its environment. The synthesis and assembly of nanoparticles would define from the development of clean, nontoxic and environmentally acceptable “green chemistry” approaches for nano particles. Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications (Mani Aparna *et al*, 2012). Nearly 80% of the world’s population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. Nanoparticle can be used in combination therapy for decreasing antibiotic

resistance. Antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune- suppression and allergic reactions. There is a need to develop new antimicrobial drugs for treatment of infectious diseases. Because of their high reactivity due to large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media.

Green synthesis of silver nanoparticles has been reported using extracts of various plants such as *Allium cepa* (Benjamin *et al*, 2011), *Andrographis paniculata* Nees (Paneerselvam *et al*, 2011), *Datura metel* (Ojha *et al*, 2013), *Indigofera aspalathoids* (Krishnasamy *et al*, 2012), *Achyranthes aspera L* is known as uttareni belongs to family Amaranthaceae is an important medicinal herb found as a weed throughout India. It is an erect or procumbent, annual or perennial herb, 1 to 2 meters in height, often with a broody base commonly found as a weed of waysides, on roadsides. (Fig.1 a). This plant was reported to contain saponins A and B. Saponin A was identified as D-glucuronic acid and Saponin B was identified as β-D-galactopyranosyl ester. It is widely used in traditional system of medicine as alterative & antiperiodic, antiphlegmatic, purgative, laxative,

#### \*Corresponding Author

Email: [majorkld12@gmail.com](mailto:majorkld12@gmail.com)

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liver complaints, rheumatism, scabies and other skin diseases. The plant is indigenously used as diuretic, spermicidal, anti-allergic, cardiovascular, nephroprotectives, antiparasitic, hypoglycemic, analgesic and antipyretic. In view of this following study was undertaken to synthesize the silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous leaf extracts of *Achyranthes aspera*.

## MATERIALS AND METHODS:

For the synthesis of silver nanoparticles, plants were collected from the college campus and from nearby area of Nasik city. The extract was used for reducing and capping agent. Silver nitrate (Qualigens make), was purchased from Fisher Scientific India Pvt. LTD, Business Park, Powai, Mumbai, India. Culture of micro organism was procured from the Department of Microbiology and BAC Test Lab, Nasik. The nutrient media used here were supplied by Hi media.

**Preparation of plant extracts:** The leaves of the plant *A. aspera* was collected from college campus and nearby areas of Nasik city. The leaves were allowed to dry at room temperature and powdered. The plant leaf broth solution was prepared by taking 20 gm of finely powdered leaves in 500 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 10 min. It was then filtered to obtain the plant extract and stored at 4°C.

### 1) Synthesis & characterization of silver nanoparticle:

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 5 ml of plant extract was mixed with 25 ml of 3 mM silver nitrate and kept in dark for synthesis of silver nanoparticles. Then solution is stored at room temperature for 24 hrs for complete settlement of nanoparticles.

#### 1) pH analysis

3mM silver nitrate ( $\text{AgNO}_3$ ) solution shows pH, there is concerned change in pH was determined of silver nano particle synthesis using extract of plant which was determined by using digital pH meter Systronic. (Ojha *et al*, 2013). Change in color of the solution was noted after 24 hours.

#### 2) UV-Visible spectra analysis

UV- visible spectroscopy analysis was carried out on a Systronic UV- Visible absorption spectrophotometer 2450 with a resolution of  $\pm$  nm between 200-1000nm processing a speed of 200 nm/min. The progress of the reaction between metal ions and leaf extract were monitored by UV-Visible spectra of silver nanoparticles in aqueous solution after diluting a small aliquot of 100  $\mu\text{l}$  of the sample with 1 ml of deionized water in different wavelengths i.e. 200, 400, 600, 800, 1000 nm in 1<sup>st</sup> and 6<sup>th</sup> hour interval of time at 380 nm.

#### 3) FT-IR measurement

FT-IR measurement of sample was performed using Nicolet Avatar Model FT-IR Spectrophotometer in a diffuse reflectance mode at a resolution of 4cm<sup>-1</sup> in KBr pellets. The synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 minutes. The pellet was washed thrice with 5 ml of deionized water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. This was analyzed by FTIR ((Priya *et al*, 2011)

#### 4) Scanning electron microscope study

SEM analysis was done using Hitachi S-3500SEM machine The pellet was subjected for SEM analysis. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis (Priya *et al*, 2011).

#### 5) Energy dispersive X-ray spectrometers

EDX take advantage of the photon nature of light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra low noise preamplifier connected to the low noise are a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses with in a so called Multi Channel Analyzer, a complete image of the X-ray spectrum is building up almost simultaneously. This digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the x-rays together with processing electronics to analyses the spectrum (Saraniya *et al*, 2012)



### 6) X-ray diffraction study

A thin film of the silver nanoparticles was made by dipping a glass plate in a solution and carried out for XRD X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks, using Debye- Scherer formula,

$$D = 0.94\lambda / \beta \cos \theta$$

Where D is the average crystalline domain size perpendicular to the reflecting planes.  $\lambda$  is the X ray wavelength,  $\beta$  is the full width at half maximum and  $\theta$  is the diffraction angle. (Priya *et al*, 2011).

### II) In vitro antimicrobial assay

The antibacterial assays were done on bacterial organisms like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella paratyphi* & one fungal strain *Candida albicans* by well diffusion method (Dhanalakshmi *et al*, 2012). Luria bertani (LB) broth/agar medium was used to cultivate bacteria & PDA (Potato Dextrose agar) for fungal growth. Fresh overnight culture of inoculums (100  $\mu$ l) of each culture was spread on to Muller Hinton Agar (MHA) plates. Five wells 6 mm diameter made and were labeled properly and 200 microlitre of the working suspension/ solution of different silver nanoparticles plants extracts and same volume of distilled water (100 $\mu$ g/ml) as control or solvent for control was filled in the well with the help of micropipette. The plates were incubated at 37°C for overnight. Next day the inhibition zones around the discs were measured in mm.

### III) Minimum inhibitory concentration study

The determination of MIC of extracts was conducted according to standard procedures (Eloff, 1968). The MIC method was applied on extracts that proved their high efficacy against microorganisms by the well diffusion method. The highest dilution of the plant extract that still retains an inhibitory effect

against the growth of a microorganism is known as MIC.

Selected silver nano particle plants extracts were subjected to a serial dilution using sterile nutrient broth medium as diluents. In a series of test tubes a loopful of an individual microorganism was loaded and incubated at 37 °C for 24 hrs. After incubation the MIC was determined by transferring a loopful of culture on agar surface; incubating the inoculated plates at 37°C for 24 hrs. The highest dilution of the silver nano particle plants extracts retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as MIC value of the extract.

## RESULTS AND DISCUSSION:

### I) Synthesis & characterization of silver nanoparticles

Fig 1 represents change into reddish color due to reduction of silver ions (Table-1) and reducing pH of the solution which may be an indication of formation of silver nanoparticles. In this analysis, it was observed that pH changed from high acidic to low acidic (Table 2).

Fig. 2 represents the UV-Vis spectra of aqueous component as a function of time variation of leaf broth with 3 mM aqueous AgNO<sub>3</sub> solution. Absorption spectra of Ag nanoparticles formed in reaction mixture at different time intervals at nm showed the particle has increasingly sharp between 1<sup>st</sup> to 6<sup>th</sup> hour i.e. particles are polydispersed to 380 nm throughout the reaction period indicates that the particles are dispersed in the aqueous solution. It was observed that the nanoparticles solution was stable for more than six months with little signs of aggregation FTIR measurements (Fig.3) were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by leaf broth.

**Table – 1 Change in color of solution during synthesis of silver nano particles**

Sr. No.	Name of the plant samples	Color change		Color intensity *	Time (Hrs)
		Before reduction of Ag	After reduction of Ag		
1	<i>A. aspera</i>	Red	Very dark Reddish brown	+++	1
2	AgNO <sub>3</sub> Solution	Colorless	Colorless	-	2

**Table 2: pH Analysis**

Sr. No.	Plant name	Plant part used	pH change in plant extract samples during synthesis of Ag nano particles		UV range (nm)	Result
			Before	After		
1	<i>A. aspera</i>	Leaves	05	04	380	+ve



**Table-3: In vitro antimicrobial activity of silver nanoparticles of *A .aspera* plant extract**

Name of plant the extract	Zone of inhibition (mm)				
	Bacterial strains				Fungal strain
	E. coli	S. aureus	K. pneumonia	S. paratyphi	C. albicans
<i>A.aspera</i>	08	09	-	-	13
Distilled water	09	12	-	-	-

**Table 4: Minimum inhibitory concentration of silver nanoparticles of *A .aspera* plant extracts**

Name of the plant sample	Concentration (%)	Zone of inhibition (mm)	
		S. aureus	C. albicans
<i>A. aspera</i>	1	10	09
	2	08	-
	3	11	06
	4	05	05
	5	06	06
	control	18	-



a) *Achyranthes aspera*

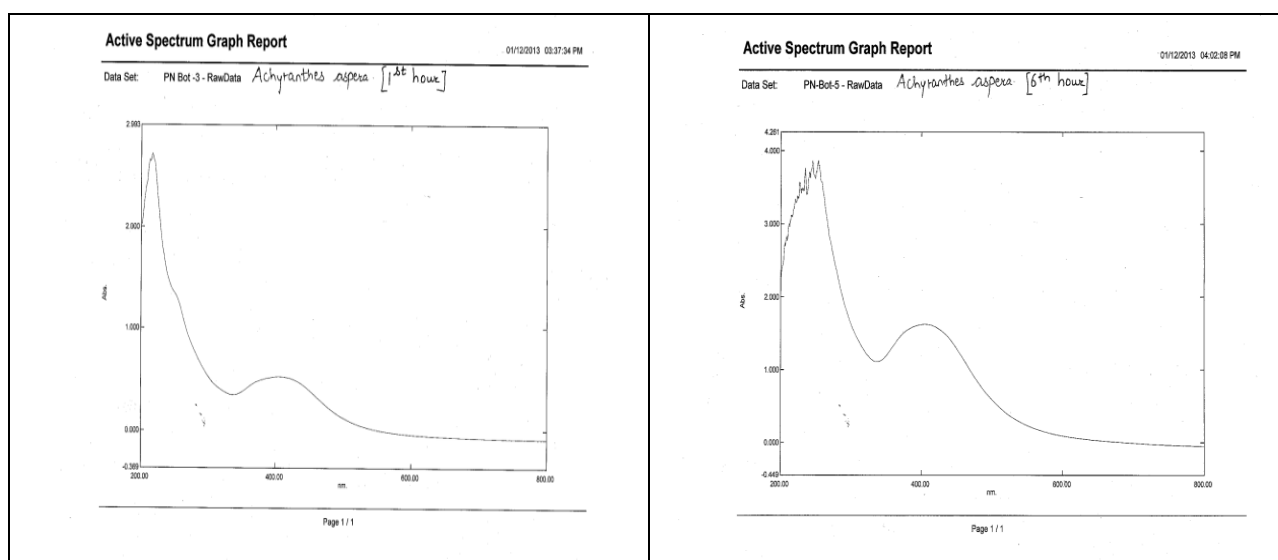


b) Before



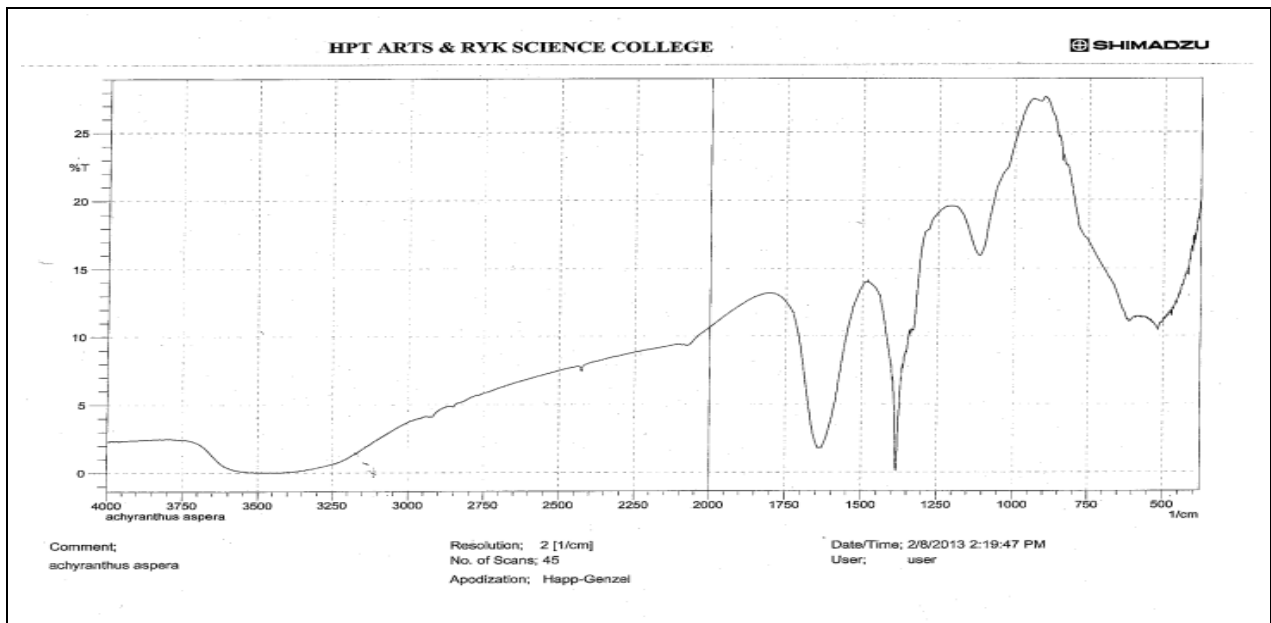
c) After

**Fig. 1. (a)** *A. aspera* plant aqueous solution of 3mM AgNO<sub>3</sub>, *A. aspera* leaf extracts (b) before adding the leaf extract (c) after addition of leaf broth.

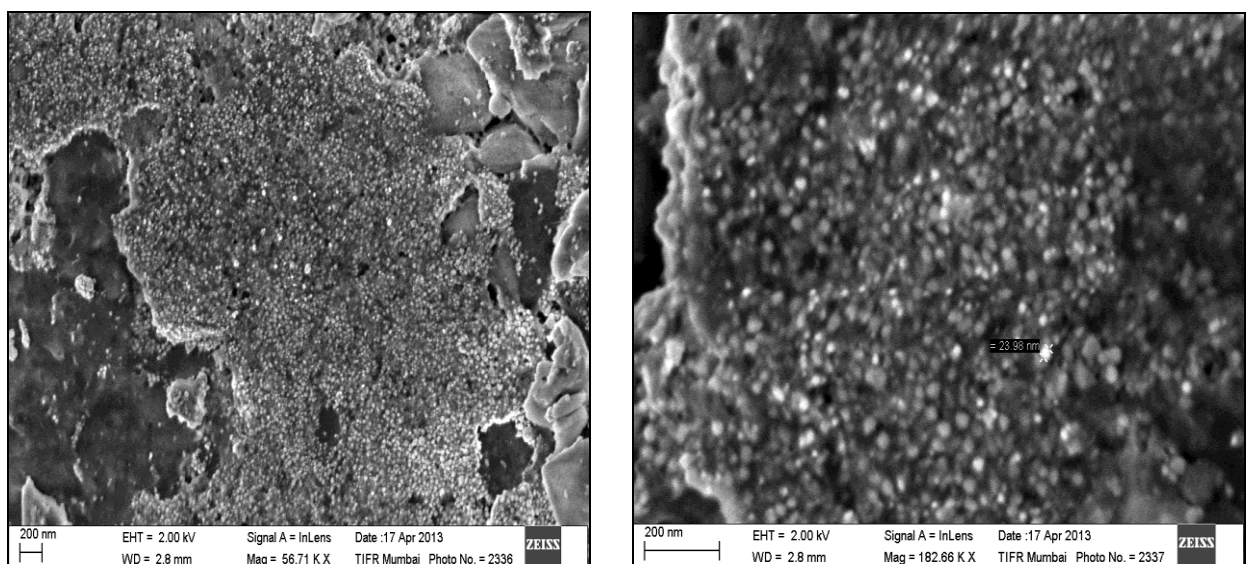


**Fig.2-UV-Vis spectra of *A. aspera* with interval of 1<sup>st</sup> to 6<sup>th</sup> hour range 200-800 nm.**

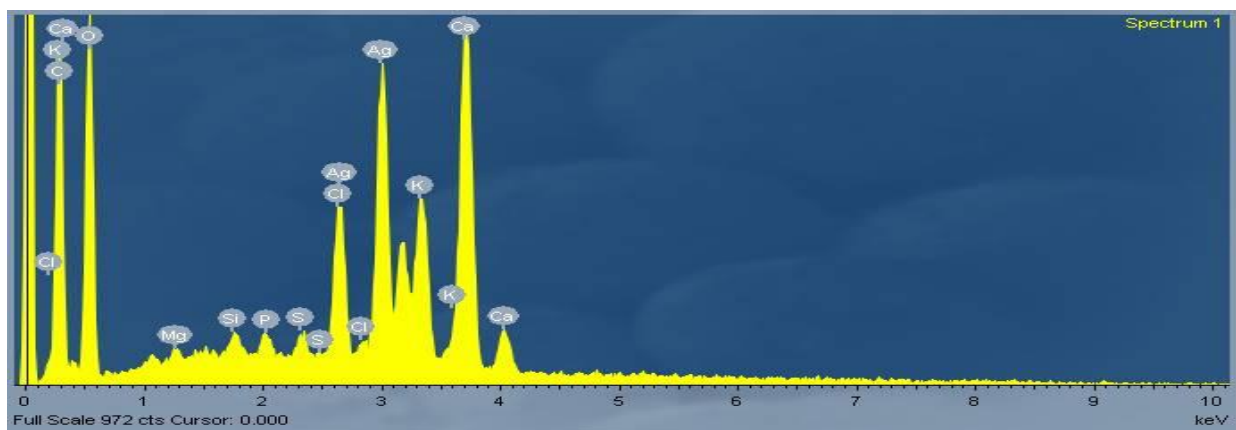




**Fig.3-FTIR of silver nanoparticles**

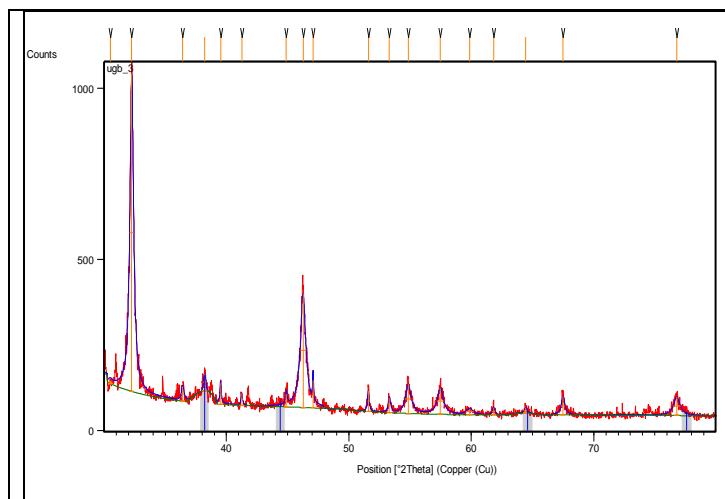


**Fig. 4-SEM image of silver nanoparticles**

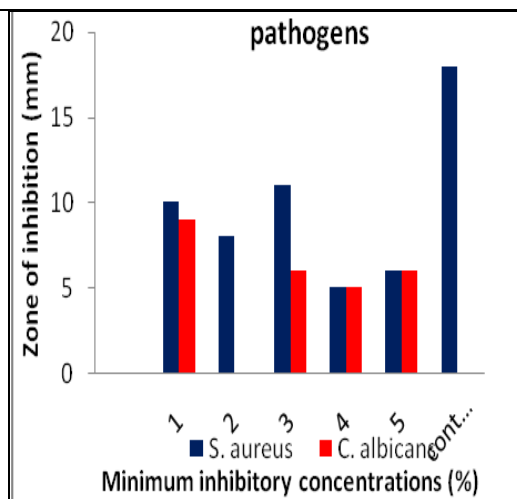


**Fig. 5(a) EDX image of silver nanoparticles**





**Fig. 5(b)**-XRD pattern from drop-coated films of synthesized silver nanoparticles



**Fig. 6:** Minimum inhibitory concentration of *A. aspera* for pathogens

In *A. aspera*, the peaks near 3600 cm<sup>-1</sup>, 2900 cm<sup>-1</sup>, 2800 cm<sup>-1</sup>, assigned to OH stretching and aldehydic C-H stretching respectively. The weaker band at 1639 cm<sup>-1</sup> corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1100 cm<sup>-1</sup> corresponds to C-N stretching vibration of the amine. The peak near 1741 cm<sup>-1</sup> corresponds to C=C stretching (non conjugated), the peak near 600cm<sup>-1</sup> and 620 Cm<sup>-1</sup> assigned to CH out of plane bending vibrations are substituted ethylene systems -CH=CH (cis). IR spectroscopic study confirmed that the carbonyl group from amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e. capping of silver nanoparticles) to prevent agglomeration and thereby stabilized the medium. This suggests that the biological molecules could possibly perform dual function of reduction and stabilization of silver nanoparticles in the aqueous medium.

The SEM image shown high density Ag nanoparticles synthesized by *A. aspera* plant extract further confirmed the presence of Ag nanoparticles (Fig.4). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. Under careful observation, it is evident that the silver nanoparticles surrounded by a faint thin layer of other materials, which we suppose are capping organic material from *A. aspera* leaf broth. The obtained nanoparticles are in the range of sizes 9–34 nm and few particles are agglomerate.

EDX micro analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen which shows the EDS spectrum recorded in the spot-profile mode (Fig.5a). The optical absorption peak is observed at 3 KeV, which is typical for the absorption of metallic Ag nanoparticles. Strong signals from the silver atoms are observed, while weaker signals from Cl, C, K, Ca, O, Mg, Si, P and S atoms are also recorded. Those weaker signals are likely to be due to X-ray emission from the plant leaves extract. From the EDX spectrum's it is cleared that Ag nanoparticles reduced by plant *A. aspera* have the weight percentage of silver which supports the XRD results.

XRD analysis of Ag nanoparticles using *C. igneous*, *E. hirta* and *A. aspera* plant extracts further confirmed the presence of Ag nanoparticles (Fig.5b).The XRD pattern showed intense peaks in the whole spectrum of 2θ values ranging for 34 nm for *A. aspera*. The typical XRD pattern revealed that the sample contains a mixed structure of silver nanoparticles. The average estimated particle sizes of the samples were calculated using the Debye-Scherrer formula. A number of Bragg reflections corresponding to the sets of lattice planes are observed which may be indexed based on the face centered cubic structures of silver, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver. Peaks marked with yellow background are from silver and average crystallite size is 34 nm.





## II) In vitro antimicrobial assay

The biosynthesis of silver nanoparticles, *A. aspera* were studied for antimicrobial activity against pathogenic microorganism by using standard zone of inhibition microbiology assay. Ag nanoparticles of the plant extracts were found highly effective in their antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* than distilled water. Bacterial membrane, proteins and DNA make perennial sites for silver nanoparticles interactions as they possess sulphur and phosphorous compounds and silver has higher affinity to react with these compounds. Highest zone of inhibition was shown by extract of *A. aspera* (13 mm) against *C. albicans*. No any response observed with *K. pneumonia* and *S. paratyphi* against any plant extract. Remarkable result seen in control as distilled water for *S. aureus* (12 mm) and *E. coli* as (9 mm), (Table-3).

## III) MIC study

MIC study revealed that no any aqueous concentration proved to be strongly susceptible likely (injury) for any of the pathogen. This might have resulted from minimum concentration used 1 % from *A. aspera* found susceptible to *C. albicans*. The results shown that the plant extracts silver nanoparticles were found effective against bacterial and fungal strain. The *S. aureus* and *C. albicans* mostly related to skin infection, food poisoning, (Table-4, Fig. 6).

## CONCLUSION:

The bio-reduction of aqueous Ag<sup>+</sup> ions by the leaf extract of the plants, *A. aspera* has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. The use of medicinally important plants *A. aspera* has added advantage that these highly medicinally important plants can be used by nanotechnology processing industries for pharmaceutical formulations.. Toxicity

studies of silver nanoparticles on human pathogens open a door for a new range of antibacterial agents. Thus present study showed a simple, rapid and economical route to synthesize silver nanoparticles. Additionally it can minimize the dose of pharmaceutical formulations.

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## RESEARCH ARTICLE

## PREPARATION OF HAY WITH GREEN FOLIAGE OF SORGHUM AND LUCERNE

Bendre KB<sup>1</sup> and Rathod MM<sup>2</sup>,

<sup>1</sup>Nanasaheb Y.N. Chavan Arts Sci. and Com. College, Chalisgaon -424101 (MS)

<sup>2</sup>SSMM Arts, Science and Commerce College, Pachora -424201 (MS)

### ABSTRACT

The ideal, suitable and simple method of conservation of fodder grown in rainy season under irrigation in dry months is drive off the surplus moisture in the fresh grass or forage crops (Narayanan and dabadghao, 1972, Mukherjee etal, 1981 and Patil and Mungikar 1991). Sorghum and Lucerne varieties resulted into good hay with 90.0% dry matter 2.4 % relative water content (RWC) and 83.3% dry matter and 3.5% relative water content respectively, when dried in sun for six days.

**Keywords:** Hay, Sorghum, Lucerne, Dry Matter, RWC.

### INTRODUCTION

In view of the demand of fodder in bulk, great importance is being laid on the involvement of high yielding, short duration and nutritive varieties of fodder crops. As a result there is a glut of fodder during the peak periods of growth and scarcity during other periods, particularly in summer. The most practical solution of such fodder scarcity during summer lies in the conserving supplies of green fodder available during flush season either as hay or silage, so as to use of during scarcity period.

Hay is the main source of feed for cattle during lean months (Sohane and Chaudhary, 2001). The forage crop is cut before it is fully ripe and dried for storage as hay. It is leafy, green and free from moulds, weeds and dust and has pleasant characteristic smell and aroma. The present communication deals with the hay process for green foliage of Sorghum and Lucerne.

### MATERIALS AND METHODS:

Green fodder of Sorghum ( Sorghum bicolor (L) Moench cultivar - Sweet Sorghum) and Lucerne (Medicago sativa L, cultivar - T9 ) was used for hay

### \*Corresponding Author

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making. The crop was harvested early in the morning at prevlowering stage and batches of 1.5 kg green foliage were dried in different conditions i.e. in an oven under sun or in shade, with or without cover, with or without turnings. The batches of green foliage were kept for drying under a gives conditions in sun. The weight of the individual batch was recorded at the same time on subsequent day till constant weight. The dry matter (DM) content in the foliage was calculated with the figures obtained for oven dried samples. In order to assess water loss from the samples relative water content (RWC) was calculated as described by Harris and Thaine (1975) using following equation.

$$RWC (\%) = \frac{Wt - Wd}{Ws - Wd} \times 100$$

Where,

*Wt* is the sample weight at time *t*;

*Ws* - is the saturation or initial weight and

*Wd*- is the dry weight.

### RESULTS AND DISCUSSION:

The crops selected for this investigation resulted in good hay after drying. On the basis of color, it was observed that when the sample was dried in oven, it gave off its green colour resulting into pale green or gray hay. The sample, which was covered with polythene paper, resulted into bad product with yellow colour and undesirable odour. This was



**Table 1:** Drying Rates of Sorghum (sweet sorghum) And Lucerne (T9) Under Various Conditions Duration from 18th Feb. 2002 to 24th Feb. 2002.

. Crop & Cultivar	Drying Period (days)	METHODS OF DRYING									
		Oven (60°C)		Sundrying				Shade drying			
		Weight (g)	R.W.C. (%)	Weight (g)	R.W.C. (%)	Weight (g)	R.W.C. (%)	Weight (g)	R.W.C. (%)	Weight (g)	R.W.C. (%)
Sorghum (sweet sorghum)	0	1500	100	1500	100	1500	100	1500	100	1500	100
	1	810	43.9	1035	62.2	1050	63.4	1150	71.5	1255	80.1
	2	515	19.9	745	41.8	800	43.1	885	50.0	930	53.6
	3	270	0.0	650	30.9	680	33.3	780	41.4	855	47.5
	4			510	19.5	530	21.1	600	26.8	645	30.8
	5			395	10.1	420	12.2	450	14.6	470	16.2
	6			300	2.4	330	4.8	350	6.5	370	8.1
	% DM of day		100		90		81.8		77.1		72.9
	Mean		774		590		615		930		846
	S.D.		230.4		155.4		152.3		147.9		134.5
CV.(%)		29.7		26.3		24.7		15.9		15.8	
Lucerne (T9)	0	1500	100	1500	100	1500	100	1500	100	1500	100
	1	645	32.9	855	49.4	885	51.7	875	50.9	930	55.3
	2	310	6.7	445	17.2	555	25.8	615	30.5	750	41.2
	3	225	0.0	390	12.9	435	16.4	460	18.4	625	31.4
	4			320	7.4	330	8.2	370	11.3	465	18.8
	5			300	5.8	315	7.0	325	7.8	360	10.5
	6			270	3.5	285	4.7	280	4.3	300	5.8
	% OM of day		100		83.3		78.9		80.3		75.0
	Mean		670		583		615		632		704
	S.D.		228.9		157.6		154.7		154.6		145.1
C.V. (%)		34.1		27.1		25.2		24.5		20.6	

due to the fermentation process, which took place under anaerobic condition created by the polythene. The pH of polythene sample was 4.2 indicating acid fermentation. The paper cover was better than the polythene cover as the sample could retain its colour, however, the rate of drying was poor. The sample, which was dried in shade, resulted into green hay, particularly when frequent, turnover was giving. The fodder dried in sun made hay in less time. But the resulting hay was not as green as that resulted from shade drying.

Table 1 gives an account of drying rates of fodder species selected for hay making under different conditions the table gives information on decrease in weight and relative water content of the fodder under oven, sun and shade drying. The table also provides information on percent dry matter of resulting hay, green fodder of sweet sorghum variety of Sorghum and T9 variety of Lucerne resulted into good hay with 90% dry matter and 2.4% relative water content and 83.3% dry matter,

and 3.5% relative matter content respectively when dried in sun for six days.

Statistical studies shows that there was maximum average decrease in weight in Sorghum (930 gm) than in Lucerne (704 gm) However, the variation in decrease in weight was more in Lucerne (27.1%) than Sorghum (26.3%) as indicated by the value of coefficient of variation (c.v.)

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All correspondence concerning submission should be addressed to the

**EDITOR-IN-CHIEF:**

**DR. Arvind Chavhan**

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G-3, Dattamandir, Vinayak Nagar, Amravati- 444603

Email: [editorirjse@gmail.com](mailto:editorirjse@gmail.com)

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## BHAVAN'S FAITH

Let me see for the Bhavan's faith for the benefit of the new students and members, for it is necessary that they should understand it clearly and imbibe its spirit.

The Bhavan stands for re-integration of India's culture.

In a world falling to pieces under the impact of an amoral technological avalanche, it tries to hold fast to the fundamental values for which our culture stands: RITA, SATYA, YAGNA and TAPAS.

FAITH in God who informs the Cosmic Order;

TRUTH which is an accord between mind, word and deed.

DEDICATION which offers all movements of life as an offering to God;

SUBLIMATION which purifies the body and mind and transmutes instincts, passions and emotions into things of beauty.

This, regardless of forms and doctrines, is Dharma, the three-fold aspects of which are SATYAM, SHIVAM, SUNDERAM – Truth, Love and Beauty.

For these values our forefathers lived and died. So did Shri Ramkrishnan Paramhansa, Swami Dayananda Saraswati, Swami Vivekananda, Gandhiji and ShriAurobindo, among the moderns.

They are embedded in our National outlook.

We can look forward to the future with confidence only because they have the vitality which gives power to vindicate their validity even in this fear-and-avarice-ridden age of ours.

We the Bhavan's family, whether it is the smaller one or the larger one, must make every effort in restoring an awareness of these values in personal and collective life.

## IDEALS OF BHARATIYA SHIKSHA

1. Bharatiya Shiksha must ensure that no promising young Indian of character having faith in Bharat and her Culture, Bharatiya Vidya should be left without modern equipment by reason merely for want of funds.
2. Bharatiya Shiksha must be more formative than informative and cannot have as its end mere acquisition of knowledge. Its legitimate sphere is not to develop natural talents, but to shape them so as to enable them to absorb and express the permanent values of.
3. Bharatiya Shiksha must take into account not only the full growth of a student's personality, but the totality of his relations and lead him to the highest self-fulfilment of which he is capable.
4. Bharatiya Shiksha must involve at some stage or other an intensive study of Sanskrit or Sanskritic languages and their literature ancient and modern.
5. The integration of Bharatiya Vidya, which is primary object of Bharatiya Shiksha, can only be attained through a study of forces, movements, motives, ideas, form and art of creative life energy through which it has expressed itself in different areas as a single continuous process.
6. Bharatiya Shiksha must stimulate the student's power of expression both written and oral at every stage, in accordance with highest possible ideals.
7. The technique of Bharatiya Shiksha must involve:
  - (a) The adoption by the teacher of the *Guru* attitude which consists in taking a personal interest in the student, inspiring & encouraging him to achieve distinction in his studies, entering to his life with a view to form ideals and remove psychological obstacles, and creating in him a spirit of consecration and
  - (b) The adoption by the student of the *Shishya* attitude by development of
    - i) respect of the teacher
    - ii) a spirit of enquiry
    - iii) a spirit of service towards the teacher, the institution, Bharat and Bharatiya Vidya.
8. The ultimate aim of Bharatiya Shiksha is to teach the younger generation to appreciate and live up to permanent values of Bharatiya Vidya, Which flowing from the supreme art of creative life-energy as represented by Shri Ramchandra, Shri Krishna Vyasa, Buddha and Mahavira have expressed themselves, in modern time the lives of Shri Ramkrishna Paramahansa, Swami Dayanand Saraswati, Swami Vivekanand, ShriAurobindo and Mahatma Gandhi.
9. Bharatiya Shiksha, while equipping the student with every kind of scientific and technical training, must teach the student not to sacrifice an ancient form or attitude to an unreasoning passion for change not to retain a form or attitude which, in the light of modern times, can be replaced by another.



**Founder: Kulapati Dr. K. M. Munshi**



**Bharatiya Vidya Bhavan, Mumbai**