Original Research Paper

Volume - 15 | Issue - 01 | January - 2025 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Botany

ASSESSMENT OF ENVIRONMENTAL FUNGAL BIO-POLLUTION WITH VOLUMETRIC AIR SAMPLER

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ABSTRACT Environmental bio pollutant includes mostly the emission of pollen grains produced by flowering plants and spores produced by various types of fungi. These bioparticles are microscopic. These pollen and spores in the atmosphere are referred to as aeroallergens and produce allergic manifestations in sensitive human beings. Significant qualitative and quantitative variation occurred in aeroallergens in the indoor and outdoor atmosphere. Spore trap samplers are capable of capturing all spores and particulate matter in the air. While many mould spores have a unique morphology and are identifiable by direct microscopic examination. To study the qualitative and quantitative prevalence of bio-pollutants in an outdoor environment, the volumetric Rotorod air sampler (Model-40) was installed on the roof of S.K.Porwal College, Kamptee for consecutive one year (May 2015 to April 2016). A total of 67 pollen types and 24 species of fungal spores have been identified. From the allergy point of view, pollen types such as Parthenium hysterophorus, Casuarina equisetifolia, Poaceae pollen grains, Ricinus communis, Cassia species, Eucalyptus species and fungal species like Cladosporium, Nigrospora, Smut spores, Alternaria, Helminthosporium, Penicillium and dominant Aspergillus spp. were found to be predominant types in the atmosphere of Kamptee.

KEYWORDS : Aeroallergen, Aspergillus sp., Bioparticles, Environmental bio pollution, Volumetric air sampler.

INTRODUCTION

Bioaerosol in indoor environments includes a substantial portion of fungi which are considered as 'Silent killers'. Air is the mixture of gases, dust particles, water vapour, bioparticles (bacteria, fungi, protozoa) and non-living materials. Fungi are ubiquitous. The hazardous effect of fungi on the health of humans, animals and plants can be minimized by monitoring the quality of air for knowing the diversity, abundance and variations according to seasonal changes.

Fungal spores contribute a major fraction of airborne particles. The main aim and objective of this study were to daily monitoring of airborne pollen grains and fungal spores and to make an approximation of airborne content along a year's study. To investigate the relationship between air quality and related health issues, the knowledge of bioaerosol relationships with meteorological parameters, particulate matter chemical components, and sources identifying the factors responsible for the bioaerosol community structure and its seasonal variation [1]. Fungal spores are present in the atmosphere in concentrations considered greater than those of pollen grains. Many surveys have been carried out in different geographical locations with various kinds of volumetric samplers [2-7] to determine the atmosphere & the sources of allergenic species of fungi and to record their seasonal variation.

For the proper diagnosis and treatment of health problems, this type of aerobiological survey on monthly and seasonal fluctuation patterns of airborne fungi is very essential to the aim of the study.

MATERIALAND METHODS

The aerobiological sampling was carried out by the Volumetric Rotorod Air Sampler (Model 40) which was placed for consecutive one year from May 2015 to April 2016 in the extramural environment on the Terris of Seth Kesarimal Porwal College, at the height of 45 feet from the ground level.

A Rotorod sampler is a volumetric, rotation impaction device capable of quantitatively and qualitatively sampling airborne particles in the size range of 1 to 100 µm at sampling rates up to 120 liters of air per minute (L/min.). The sampler consists of a constant speed motor of 2400 RPM and aerodynamically designed collector of two Lucite rods (1.3 mm in width) which are rotated by the sampler motor at 2400 rpm. The retracting head holds two rods within the protective housing when the sampler is idle and when the sampler is activated, the rods get extended to a position perpendicular to the head. Rods are inserted in the pivot blocks and fastened with small thumbscrews.

The particles get impacted on one face of the rod, which has been smeared with adhesive (glycerin jelly) every minute after every nine minutes of rest time when it remains folded and static. The exposed rods are mounted on a grooved stage adaptor, which consists of four parallel grooves of approximately the same width as the rod. By placing a cover slip carefully the rods are microscopically examined thoroughly under 40X objective and 10X eyepiece. After correct identification of the trapped airborne fungal spores, their percentage frequency is expressed as a number per m^3 of air sampled.

The result of daily scanning of a single rod is tabulated as follows;
Volume= Rod height x rod width x path diameter x π x RPM x Time
Rod height = 2.2 cm
Rod width = 0.159 cm
Path diameter = 8.6 cm
π = 3.14
RPM = 2400
Time = 143 minutes (10% duty cycle)
Volume of air sampled (V) = 2.2 x 0.159 x 8.6 x 3.14 x 2400 x 143
= 3241866.9 cm^3
= 3.24 m^3 for one rod

The atmospheric concentration (C) of the particle (p) trapped on the collector rods is a function of the volume of air (V) sampled by the rods.
So,
C= p/V
C= 1/3.24
= 0.3086
Conversion factor = 0.3086 x A (where A is the total number of fungal spore count on one rod).

The slides were prepared and observed under the microscope in low and high magnification and identified with reference slides and standard literature [8-15].

Observations

Knowing the prevalence of fungal spores, in the atmosphere, is of paramount importance. From the time of the earliest investigation on fungal spore contents in air, it has been known that the atmospheric concentration of spores is not the same all year round. During certain periods of the year, concentrations build up to a much higher level than those that exist through the remainder of the year. This period of greater abundance of spores in the air has been generally termed the 'fungal spore season'.

However, not all fungal spores contribute to the seasonal increase in atmospheric concentrations. Some spores appear to occur in more or less the same numbers throughout the year, while other fungal spores show a seasonal rhythm in atmospheric concentrations. The season varies from one group of fungal spores to another [16-17].

The present aerobiological study in Kamptee has undertaken an account of the relatively high incidence of allergic disorders among its population, with airborne fungal spores being recognized as one of the significant offenders. High fungal counts, in the air, account for increased allergic symptoms as reported by Flannigan et al. 1990.

According to the present investigation, fungal spores comprised a

large portion of the aerospora. In the present studies, the ratio of occurrence of pollen to fungal spores in the air was 1:40. During the present study, over 24 types of airborne fungal spores have been recorded (Table 1).

Table 1. Fungal species recorded for a sampling period (May 2015 to April 2016))

Sr. No.	Fungal Types observed during study period	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
1	Alternaria	210	140	154	168	182	168	140	168	140	140	168	252	2030
2	Artrinium	28	42	84	112	112	140	84	98	70	56	28	28	882
3	Ascospores	140	112	140	224	210	252	182	112	140	112	112	84	1820
4	Beltrania	28	70	98	154	168	168	140	140	112	112	70	42	1302
5	Bispora	70	56	84	112	98	84	56	56	56	42	70	56	840
6	Cercospora	0	14	14	14	0	0	0	0	0	0	0	0	42
7	Chaetomium	0	0	28	42	56	42	42	28	28	14	0	0	280
8	Cladosporium	0	182	210	252	308	350	336	280	308	252	252	140	2870
9	Curvularia	168	196	238	224	280	280	308	210	196	210	140	182	2632
10	Didymosporium	28	56	56	56	70	42	14	28	42	14	0	0	406
11	Epicoccum	0	0	0	0	28	42	28	28	28	0	0	0	154
12	Fusariella	0	0	28	14	14	56	28	42	70	42	56	56	406
13	Helminthosporium	70	112	70	84	42	70	56	28	28	28	56	84	728
14	Hirudinaria	0	0	14	14	14	28	28	14	14	0	0	0	126
15	Leptospheria	14	42	14	0	0	28	28	28	0	0	0	0	154
16	Mold spores	392	476	560	602	504	560	476	504	476	420	378	350	5698
17	Nigrospora	0	0	14	84	84	84	56	56	42	28	0	0	448
18	Pithomyces	0	0	56	84	42	56	70	70	28	42	28	0	476
19	Rust spores	28	84	42	14	56	42	70	56	42	56	56	28	574
20	Smut spores	280	140	140	84	112	140	168	168	140	112	98	70	1652
21	Spegazzinia	0	14	0	14	14	28	56	56	42	56	28	0	308
22	Tetraploa	0	14	14	14	28	14	42	28	28	14	0	0	196
23	Torula	0	0	0	0	0	42	56	42	42	28	0	0	210
24	Others	140	168	224	196	210	280	322	336	252	210	168	140	2646
	Total	1596	1918	2282	2562	2632	2996	2786	2576	2324	1988	1708	1512	26880

The total contribution, a maximum 26880 spores/m^3 was calculated for 24 different fungal types. Mold spores contributed the highest with 5698 spores/m^3 followed by Cladosporium with 2870 spore/m^3, while the least contribution was seen by Cercospora with 42 spores/m^3 only followed by Hirudinaria and Leptospheria with 126 and 154 spores/m^3 respectively.

A difference was seen in each season, weather conditions and according to sampling heights. A good diversity and count were seen in winter. Moderate rainfall blooms spores in the air while heavy rainfall washout fungal spores. Temperature between 25 to 30°C and relative humidity between 50 – 80 % favors the release of spores in the atmosphere. The most common species of Aspergillus (Figure1h and 1i), Penicillium (Figure1j), Cladosporium and Rhizopus/Mucor (Figure 1a) were termed as non-seasonal perhaps their presence throughout the year while species such as Chaetomium, Geotrichum, Helminthsporium, and Fusarium (Figure 1g), are truly seasonal.

RESULTS

The one year of continuous aerobiological monitoring showed that the atmosphere was never free from fungal spores. Their number and types vary with time of the year, weather, and season of location. Airborne fungal spores exhibited monthly variations through no distinct clear out seasons that could be demarcated.

However, there was a distinct increase in the total count of airborne fungal spores in the months of March –April and September –December. The total monthly abundance was higher in the months of May, November and December. The highest total monthly count of fungal spores was recorded in the month of May 516.12/m^3, November 537.25/m^3 and December 577.34/m^3. The lowest fungal spores count was in March 271.64/m^3.

Most of the airborne fungal spores were representatives of the three major fungal groups i.e. Deuteromycotina, Basidiomycotina and Ascomycotina. The Deuteromycotina constituted the largest fraction accounting for 60-80% of the total catch every month. A total of 24 genera were identified. The total spore counts of Deuteromycotina were higher in the months of May 134.85/m^3 and December 126.85m^3. The lowest count was recorded in the month of August 39.01/m^3. Generally, a sudden rise in counts was observed in the months of April and November. Alternaria sp. (Figure 1b), Cladosporium sp. (Figure 1e), Curvularia sp. (Figure 1d), Drechslera sp. (Figure1f), Helminthosporium, Nigrospora and Periconia sp. were the regularly occurring spores of Deuteromycotina. The other common types were Botryodiplodia sp., Cercospora, Epicoccum, Fusariella, Pithomyces, Torula sp.

The number of Basidiomycotina was at their high from October - January and low in number (11.25/m^3 and 10.26m^3) from April to May. The Basidiomycotina formed the second most dominant group comprising basidiospores from 12 genera. The representatives of Basidiomycotina most common were Ganoderma, Urediospores and Smut spores. The Ascomycotina were highest in number during January, August and October. The lowest fungal spores count, during present studies, was in the month that had a total monthly count of 5.10/m^3.

Predominant Types Of Fungal Spores

The common spores were consistent in their abundance across months, though their abundance rank varied. Only a few spore species were common, all the rest were uncommon. However, the differences in the number of types recorded between consecutive months were much less compared to the large differences in spore concentration between consecutive months. This indicates that the increased concentration was due to the abundance of a few types only.

The most predominant types were Alternaria, Cladosporium, Curvularia, Dreshslera (Figure 1f), Helminthosporium, Nigrospora and Periconia sp. The maximum number of spores of Cladosporium spp. was recorded in April, May and July. The Cladosporium sp. spores were recorded as having the highest representation in the monthly average total of 15.21% for May.

The total monthly average numbers of Alternaria spp. spores were higher in April (10.94/m^3). The highest number of spores for Curvularia sp. (7.05/m^3) and Helminthosporium sp. (6.69/m^3) spores were recorded in November. The spores of Nigrospora sp (14.57/m^3) and Periconia sp. (24.56/m^3) were recorded at their maximum in December.

Correlation of density and diversity of fungal spores with weather parameters

Table 2. Meteorological data of sampling period (May 2015 to April 2016)

Sr. No.	Sampling months	Max. Temp. (in °C))	Min. Temp. (in °C)	Average Temp. (in °C)	Average Relative Humidity (in %)	Average Rain fall (in mm)
1	May 2015	42.6	27.9	35.25	51	16.3
2	June 2015	37.8	26.3	32.05	77	172.2

3	July 2015	32	25	28.5	67	271
4	August 2015	30.4	23.6	27	80	291.6
5	September 2015	35	29	32	53	176.9
6	October 2015	35	30	32.5	68.37	58.3
7	November 2015	33	28	30.5	67.71	19.6
8	December 2015	29	12	20.5	59	9
9	January 2016	28.6	12.4	20.5	59	10.2
10	February 2016	32.1	15	23.55	58	12.3
11	March 2016	36.3	19	27.65	55	17.8
12	April 2016	40.2	23.9	32.5	48	13.5

***Source:** Regional Meteorological Department, Nagpur.

Table 3. Descriptive Statistics Of Meteorological Parameters

	N	Mean	SD	Sum	Min	Max
Max.Temp.	12	34.25	4.47325	411	28	42.6
Min.Temp.	12	22.675	6.48188	272.1	12	30
Ave.Temp.	12	28.4625	4.9242	341.55	20	35.25
R Humidity	12	61.67333	10.24207	740.08	48	80
Rainfall	12	89.78333	107.7896	1077.4	9	291.6

The average maximum temperature of 26˚C has a positive effect on the diversity of aerospora, increasing their taxa by almost 60 types. A gradual rise in average maximum temperature to 29-30˚C hurts total spore taxa. An average maximum temperature between 26-28˚C has a positive effect on the total spore counts.

But when the temperature rose to 28 -30˚C the spore counts decreased. However, a further increase in the maximum temperature range to 32-35˚C made the total spore counts shoot up to 500/m^3. This illustrates that temperature variations affect the number of taxa as well as the total fungal spore counts (Table 2 and 3).

The average minimum temperature shows that only in one case, which is when the temperature is in between 15-16˚C the number of taxa peaks to 65 types and the total spore counts reaches 580/m^3. Any further increase in the average minimum temperature has a negative effect on the number of taxa. However, a further increase in temperature, that is upwards of 21˚C makes the total spore count regain to around 500/m^3.

The average relative humidity has a favorable region, anything below or beyond which the taxa and the spore counts get affected adversely. As it is shown when the average RH is lower high, there is a decrease in the total number of taxa as well as total spore counts. As the humidity increases, the types of fungal taxa and the spore count increase gradually. They reach a maximum between the average RH of 50-65% taking the total spore count to more than 500/m^3 and the taxa to the peak of more than 60 types.

Rainfall has a positive effect on spore taxa and total spore count. Increased rainfall makes the spore taxa as well as the total spore count fall. A range between 0-25mm is ideal for spore density and diversity, with over 30 taxa and a peak of 577/m^3.

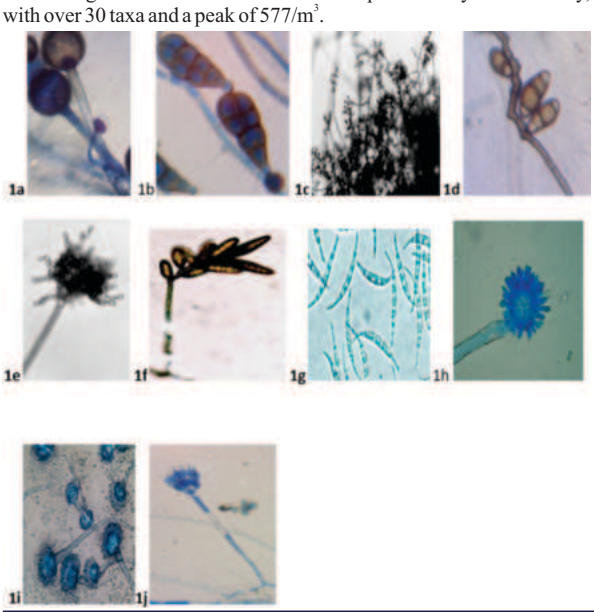

Figure 1. Some fungal species recorded during study period
1a- Mucor sp. **1b-** Alternaria sp. **1c-** Trichoderma sp. **1d-** Curvularia sp. **1e-** Cladosporium sp. **1f-** Dreschelera sp. **1g-** Fusarium sp. **1h-** Aspergillus sp. **1i-** Aspergillus sp. **1j-** Penicillium sp.

The average wind velocity also affects the diversity and density of spores. Average wind speeds between 4.5 to 7 km/h are ideal for a high record of total spore types and counts, with more than 60 types of taxa and 577/m^3 of spores counted. Wind speeds lower or higher than this hurt the total spore counts and taxa (Table 2 and 3).

The average cloud cover also exhibits an ideal range for high spore density and diversity. When the average cloud cover decreased or increased beyond a range between 4.6 -6 octas, the total types of spores and their counts decreased. They reach a density ranging around 500/m^3 and a high of more than 60 types of taxa at this ideal cloud cover range.

CONCLUSION

As the occurrence of fungal spores cannot be controlled, avoiding fungal spores and taking medication in peak season are the main strategies for allergic individuals. However, this requires proper warning of aeroallergen load level, so this type of investigation will be very useful/ helpful for allergists and clinicians for understanding fungal spore load in the air and for making people and students aware of preventing consequent health problems.

The impact of airborne fungal spores including their release, dissemination, deposition and effect is of great significance to identify the health hazards and physiological disorders in living beings. The study of this aspect is highly interdisciplinary and has tremendous scope to find significant applications in human health. Exposure to outdoor and indoor airborne inhalant mould allergies develops airway diseases and allergies. Meteorological parameter has the greatest influence on the concentration of spores and that this could rise with climate change, it is advisable to continue monitoring the air to verify the findings of this analysis over the long term. Due to extreme adaptability, the fungal spores are encountered more or less throughout the year and thus in a real sense there is no 'Aero allergenic fungal spore-free period in the environment.'

Acknowledgement

The Author is thankful to the University Grants Commission, New Delhi for financial support in the form of a Major research project and also thankful to the Principal and Staff of Seth Kesarimal Porwal College, Kamptee for their kind support.

Conflict Of Interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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