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Aerobiological survey over the Mangrove forests of Sewree Creek

Satnam Singh Sohal*

Department of Botany
Maharshi Dayanand College of Arts, Science & Commerce,
Parel, Mumbai-400012 (India)
satsohal777@gmail.com

Abstract

Aerobiology deals with the study of the movement and dispersal of living as well as non-living material through the atmosphere. Pollen grains & Fungal spores are amongst the most common airborne particles present in air. Pollens & Fungal spores can result in a variety of adverse health effects, which includes infectious diseases like skin irritation, reddening of eyes, Hay fever, respiratory allergies etc. Fungal spores & pollen count can directly influence the manifestation of allergic symptoms in sensitive individuals. Creek areas as of Sewree with rich diversity of Mangrove vegetation contributes significantly to release allergenic pollens in the atmosphere. The study was conducted to analyze contribution of Mangrove pollens and fungal spores along with miscellaneous bioparticles as bio pollutants. Total of 58422 pollens & fungal spores were recorded with 10878 (18.62%) of pollen grains& 47544 (81.38%) of fungal spores. *Aspergillus* species dominated the fungal spore type (25.42%) and Grasses dominated among pollen types (2.493%).

Aerobiology deals with the study of the movement and dispersal of living as well as non-living material through the atmosphere. Pollen grains &Fungal spores are amongst the most common airborne particles present in air. Mangroves are also subjected to the growth of the fungi along with Marine fungi on driftwoods 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. Creek areas as of Sewree with rich diversity of Mangrove vegetation contributes significantly to release allergenic pollens in the atmosphere. The study was conducted to analyze contribution of Mangrove Pollens and fungal spores along with miscellaneous bioparticles as bio pollutants. For the preparation of the manuscript relevant[1-21] literature has been consulted.

Selection of Site :

Initially a detailed survey of Mangrove

*Associate Professor

vegetation of Sewree and surrounding area was undertaken to select the site, keeping in mind the objectives of the study. This resulted in the selection of site as Sewree mangroves forest and Surrounding area.

Floristic surveys :

Regular periodic visits were made to the Mangrove vegetation, 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.

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 exposure was done at a height of 2 meters, daily for a duration of 7 consecutive days a month inside the library and surrounding area forone year between 2018-19.TheGlycerine jelly had the following constituents:

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

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 $1^{1/2}$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. safranin 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 hours. 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 quadrate of exposed area of 3.24 cm^2 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[12] 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[2,19,20].

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^2 from the constant exposed area of 3.24 cm^2 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.

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 Mangrove vegetation at sewree creek. 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	**-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.

The fungal colonies were also sub cultured on PDA slants and were sent for identification to Agharkar Research Institute, Pune.

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.

Volumetric sampling using Tilak Air sampler:

The standard Tilak Air sampler was employed for continuous volumetric sampling of air for 8 days a month for one year, *i.e.,* from 1st November 2018 to 31st October 2019, at the Mangrove vegetation of Sewree area.

Tilak Air sampler runs on electric power supply (AC-220V) and provides a continuous sampling of air for 8 days. An electric clock is fitted in the instrument and is synchronized with a drum.Air is sucked in through the orifice of the projecting tube at the rate of 5 liters per minute and it impacts on the transparent cello tape which is 1.5 cm in breadth and stuck on the slowly rotating drum. The drum completes one rotation in 8 days, thus giving the trace of catches for 8 days. The cello tape is coated with glycerine jelly. The mounting of cello tape is done after dividing the tape into 8 sections which are mounted on 8 separate slides for microscopical examination.

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 m3 of air.
For example, 10 spores X 14=140 spores in m3 of air.
Thus, the data provided in the tables are after using the conversion factor=14

Volumetric sampling using Rotarod Air Sampler :

The rotarod air sampler was also employed for this study along with Tilak Air sampler. The data provided is on the basis of Tilak Air sampler analysis. The rotarod sampler was originally devised by Perkins[14]. The device relies upon the high efficiency with which small airborne particles are deposited on narrow cylinders oriented at right angles to high velocity winds[9].

The sampler consists of a small constant speed, battery operated motor, which makes the sticky coated brass rod to whorl around its axis.The collecting arms are made up of brass rod having 0.15 cm cross sectional area, which were bent in the form of 'U'. The two vertical arms measuring 7cm long and

3.5cm apart from the axis.Each D.C. motor with 6-volt battery gives rotation speed of 2500 r.p.m. The section facing the wind is stuck with sticky cello tape along with full length. The surface of the tape is uniformly coated with glycerine jelly as a mountant.

Air sampling was carried out at the desired site at regular intervals.

Calculation and conversion factor :

$$C = \frac{\text{No of spores counted (N) X Total area of strips(A)}}{\text{Area of strips counted (a) X V X time of exposure (min) T}}$$

C = 4 spores/m^3 (rounded)

In the present study of large number of Pollen grains along with Mangroves & associate species, fungal spores, dust mites and miscellaneous air borne material like Plant cells fragments,Algal filaments, insect parts & trichomes were recorded.

- Total **10878 pollen grains** type belonging to **27 genera** and **12 fungal spore genera, total 47544 belonging** to **17 species** were recorded in a period of **one year from November 2018 to October 2019** in the mangrove forest & surrounding area.
- Out of 58422 total no. of **10878 (18.62%) pollen and 47544 (81.38%) fungal spore type** were recorded in the Mangrove vegetation area of Sewree.
- Total of **1890 (3.297%)** pollen and fungal spores remained unidentified.798(1.39%) pollen grains and **1092 (1.907%)** fungal spores.
- Throughout the period of study, *Aspergillus* sp. spores were recorded with highest percentage of 25.42% with total 14546 spores throughout the year The spores were recorded throughout the year with a peak season from July to October 2011, showing maximum during September 2019
- Total 5 species of Aspergillus were recorded *i.e, Aspergillus flavus, A. fumigatus, A. nidulans, A. niger and A. oryzae.*
- Among the fungal spores, *Cladosporium* spores were recorded the second largest in percentage (24.558%) with total 14154 spores throughout the year.
- *Cladosporium* was followed by *Alternaria alternata* (11.058%), *Rhizopus* sp. (5.357%), *Curvularia* sp. (3.228%), *Trichoderma* sp. (2.323%), Dreschlera sp. (1.957%), *Fusarium* sp. (1.5645%), *Absidia* sp. (1.051%), Basidiospores (0.997%), *Cunninghamella* sp. (0.66%) and *Penicillium* sp. (0.341%) recorded the lowest among fungal spores.
- The **highest no. of fungal spores**was recorded in between **June 2019 to October 2019** with a **peak at September 2019**. This can be correlated with low temperature and high humidity during rainy season. **Lowest no**. of fungal spores was recorded during the month of **May 2019.**
- Among the pollen grains,Grasses recorded the highest percentage of 2.493% with a total of 1428 pollen grains throughout the year. The peak season for Grass pollens was from Dec 2018 to June 2019 with maximum in October 2019.
- *Clerodendron* sp. with a total of 1022 pollens (1.74%) was the second largest followed by *Amaranthus*/*Chenopodium* type (1.712%), *Acacia auriculiformis* (1.249%), *Cyperus rotundus* (1.1255), *Cocos nucifera* (0.929), *Cassia siamea* (0.807%), *Carica papaya* (0.734), *Bougainvillea spectabilis* (0.708%), *Delonix regia* (0.587%), *Hibiscus rosasi-*

nensis (0.513%), *Lantana camara* (0.489%), *Samanea saman*(0.463%), *Tridax procumbens* (0.463%), *Peltophorum pterocarpum* (0.415%), *Moringa oleifera* (0.367%), *Ricinus communis* (0.342%), *Lagerstroemia speciosa* (0.293%), *Nerium indicum* (0.244%), and *Syzygium cumini* (0.097%) with lowest recorded among the pollen grains.

Pollen grains :

The pollen grains types of Mangroves along with other pollens recordedan average percentage contribution of 18.62 % to the total air spora in the Mangrove vegetation of Sewree & surrounding area. Some of the common pollen types recorded are as follows (Table-1)

Table-1. The plants whose pollen grains were observed in the Sewree and Surrounding area with the percentage of occurrence

Sr. No	Pollen type	Family	Total No	Percen-tage
1	*Acacia auriculiformis* A. Cum ex Benth	Mimoseae	714	1.247
2	**Acanthus ilicifolius* L.	Acanthaceae	112	0.197
3	**Aegiceras corniculatum* (L.) Blanco	Myrsinaceae	168	0.287
4	*Amaranthus/Chenopodium type*	Amaranthaceae	980	1.712
5	**Avicennia marina* (Forssk.) Vierh	Verbenaceae	308	0.527
6	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	406	0.708
7	**Bruguiera cylindrica* (L.) Blume	Rhizophoraceae	84	0.143
8	*Carica papaya* L.	Caricaceae	420	0.734
9	*Cassia siamea* (Lam) Irwinet Barneby	Caesalpineae	462	0.807
10	**Ceriops tagal* (Pers) C.B. Rob.	Rhizophoraceae	196	0.335
11	**Clerodendron inerme* (L.) Gaertn	Verbenaceae	1022	1.74
12	*Cocos nucifera* L.	Palmae	532	0.929
13	*Cyperus rotundus*. L.	Cyperaceae	644	1.125
14	*Delonix regia* (Boj.ex Hook.) Raf.	Caesalpineae	336	0.587
15	*Grasses*	Poaceae	1428	2.493
16	*Hibiscus rosa-sinenis* L.	Malvaceae	294	0.513
17	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	168	0.293
18	*Lantana camara* L.	Verbenaceae	280	0.489
19	*Moringa oleifera* Lam.	Moringaceae	210	0.367
20	*Nerium indicum* Mill.	Apocynaceae	140	0.244
21	*Peltophorum pterocarpum* (DC.) K. Heyne	Caesalpineae	238	0.415
22	*Ricinus communis* L.	Euphorbiaceae	196	0.342
23	*Samanea saman* (Jacq.) Merr.	Mimoseae	266	0.463
24	**Suaeda fruticosa* Forssk. ex J.F. Gmel.	Chenopodiaceae	70	0.119
25	*Syzygium cumini* (L.) Skeels	Myrtaceae	56	0.097
26	*Tridax procumbens* L.	Asteraceae	266	0.463
27	**Typha angustata* Bory & Chaub	Typhaceae	84	0.143
28	*Unidentified pollen*		798	1.39
	**Mangroves & Associates*			
			10878	18.62%

Acanthuus ilicifolius Linn. Pollen grains are 3-zono colpate, Prolate, Grain size range (7.40-8.25 X 3.34-3.38 µm) Exine surface reticulate in polar axis and regulate in equatorial side. Colpus margin thick. Operculum present.

Aegiceras corniculata (L.) Blanco Pollen grains are 3-zono colporate, Prolate-Spheroidal. Grain size range (3.24-3.39 X 2.73-2.84 µm) Exine surface punctate in equatorial axis and reticulate in polar axis. Ridges found inside the colpus.

Avicennia marina (Forssk.) Vierh. Pollen grains are 3-zono colporate. Oblate-Spheroidal. Grain size range (4.00-4.42 X 3.58-4.00 µm). Exine surface ornamentation reticulate. Colpus margins smooth. Colpus end tapering and acute.

Bruguiera cylindrica (L.) Blume Pollen grains are 3-zono colporate, Oblate-Spheroidal. Grain size range (2.53-2.96 X 2.75-2.95 µm). Exine surfaceornamentation reticulate in equatorial view andpunctate (depressions minute) in polarside; low depressions of various size and shape. Colpus long and colpus membrane granular.[15]

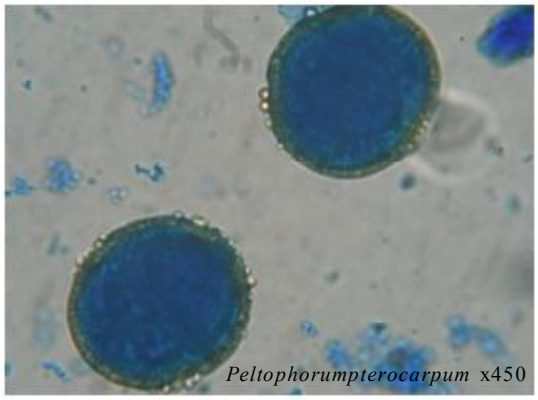
Fungal spores :

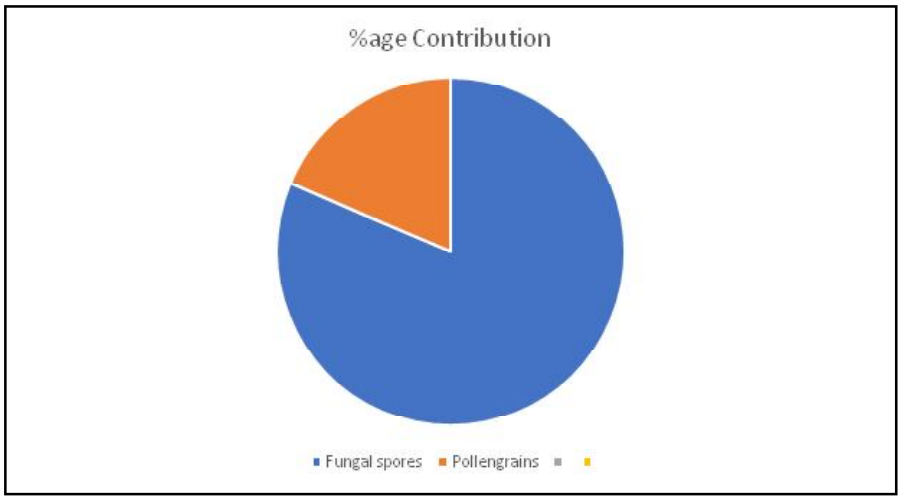
Overall, in the present study fungal spores **81.38%** were recorded in the Mangrove environment. Also increase in fungal spore was recorded with increase in % age humidity in the atmosphere**.** The common fungal spores trapped during the present study from the intramural & extramural environment of library includes the following (Table-2)

Table-2. The fungal spores encountered and their percentage during the present study from the intra and extramural environment of library in the study area

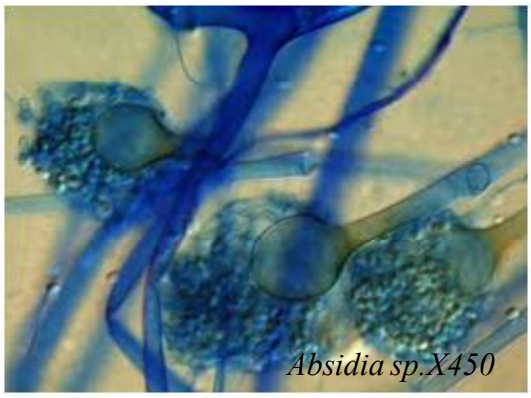
Sr.No	Fungal spore	Total	Percentage
1	*Absidia* sp.	602	1.051
2	*Alternaria alternata*	6328	11.058
3	*Aspergillus flavus*	0	0
4	*Aspergillus fumigatus*	0	0
5	*Aspergillus nidulans*	14546	25.42
6	*Aspergillus niger*	0	0
7	*Aspergillus oryzae*	0	0
8	*Basidiospores*	602	0.997
9	*Chaetomium globosum*	1386	2.421
10	*Cladosporium* sp.	14154	24.558
11	*Cunninghamella* sp.	378	0.66
12	*Curvularia* sp.	1848	3.228
13	*Dreschlera* sp.	1120	1.957
14	*Fusarium* sp.	896	1.564
15	*Penicillium* spp.	196	0.341
16	*Rhizopus* spp.	3066	5.357
17	*Trichoderma* sp.	1330	2.323
18	Unidentified sp.	1092	1.907
		47544	81.38%


Samanea saman x450


Peltophorumpterocarpum x450


%age Contribution
Fungal spores
Pollengrains


Alternaria alternataX450


Absidia sp.X450

Miscellaneous type

Plant fragments : -

It consists of different kinds of epidermal hairs of Malvaceae, Grasses, and multicellular trichomes of dicotyledonous species.Also, epidermal peelings of leaves of Grasses and some other dicotyledonous plants were trapped from the sampling site.

Hyphal filaments : -

Hyphal filaments of fungi of various types, long, short, simple, branched septate, colored, hyaline *etc*. were encountered under this category. Most of these were in broken form. Their presence was recorded throughout the year at all sites.

Insects/Insect parts: -

Whole small insects were encountered in the trappings during the present study. Insect parts such as scales, wings, legs, antennae were very common in the trappings.Dust mites were also recorded which are presented as a separate chapter.

Algal filaments/spores: -

Fragments of dried algal filaments were often present in the trapping.

Aerobiological study over the Mangrove vegetation of Sewree area was significant and provided data which can be utilized to understand concentration &distribution of different pollen types & fungal spores during various seasons.

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