CHAPTER III

MATERIALS AND METHODS
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Monitoring of the airspora is preceded by field botanical studies.

a. FIELD BOTANICAL STUDIES.

Field botanical studies are prerequisite to aerobiological studies, as a thorough knowledge of the ground flora is necessary for the identification of airborne pollen and spores. Two sites, the Visveswaraya Industrial Museum of Bangalore, a representative of the urban area and Attibelle a village near Hosur representing the rural area were selected for the study. These two sites are situated at a distance of 40 km away from each other. Field trips were made every week to collect the samples and to study the flora and flowering periods of plants growing around the sites from August 1985 to July 1987. During field trips detailed information was collected on the grasses, herbs, shrubs and trees found growing in and around the sampling sites. Observations were recorded on the initiation, cessation, duration, intensity and peak of flowering periods.
Identification of Plants

During weekly field trips, fresh specimens of plants with twigs bearing flowers were collected for the preparation of herbarium. The plants collected were identified with the help of Flora of Presidency of Madras (Gamble, 1967), Flora of Bangalore District (Ramaswamy and Razi, 1973), Flora of Hassan District (Saldahna and Nicolson, 1976), Manual of cultivated Plants (Bailey, 1949) and many other books. In addition, collection of fungal specimens of saprophytic forms or parasitic forms on diseased plants was made.

Pollen and Spore Herbarium

An extensive collection of pollen and spores of Pteridophytes, gymnosperms, monocotyledons and dicotyledons growing in and around the areas under investigation was made and reference slides were prepared from them. During field trips fresh flowers were fixed in 70% alcohol. For the preparation of slides, fresh matured, dehiscent pollen materials were mounted in glycerine jelly on glass slides and sealed with paraffin wax. Permanent slides were also prepared by using Wodehouse technique (1935) and Erdtman's acetolysis technique (1960). These slides helped in detailed microscopic study of pollen characters such as
shape, size, colouration, nature of aperture, exine characters etc. A pollination calendar was prepared for both the urban and rural sites giving the details of distribution of various plants, their habits, modes of pollination and flowering period.

**Preparation of Glycerine jelly (mounting medium)**

Glycerine jelly was used for the preparation of reference slides and mounting the exposed tapes. It was prepared as follows:

**Chemicals with their quantities required:**

- Gelatin (Bact) -- 100 gms.
- Glycerine -- 300 ml.
- Distilled water -- 350 ml.
- Phenol crystals -- 10 gms.

First gelatin is soaked in the distilled water and is kept for about 2 - 3 hours. Gelatin absorbs water. Then the supernatent water is thrown away. This mixture is then boiled for 2 - 3 hours in the hot water both. Towards the end phenol crystals are added. It is then filtered through glass wool when hot and stored in the petri dishes.

**Acetolysis technique**

A couple of anthers were crushed gently with a glass
rod in a drop or two of 70% alcohol taken in a centrifuge tube. More of alcohol was added and the mixture was washed down through a sieve (mesh size ca. 0.2 mm) into another centrifuge tube and centrifuged. The pollen residue got was treated with glacial acetic acid for a few minutes. After centrifuging, the pollen residue was treated with a fresh mixture of 9 parts of acetic anhydride and one part of concentrated Sulphuric acid. The mixture was then heated in a boiling water bath till the pollen on testing under a microscope was found to have a clear and light brown exine. The mixture was centrifuged and the residue was first washed with glacial acetic acid and finally with water. Slides were then prepared by mounting the pollen material in glycerine jelly.

b) **AIRSPORA SAMPLING TECHNIQUE.**

Air sampling was done by using an automatic 7-day volumetric Burkard's spore trap. It is based on the principle of suction and impaction and has a suction pump working on A.C. Mains. The pump draws 10 litres of air per minute through 2 mm wide orifice. The air drawn in impacts on a tape coated with vaseline adhesive. The tape is mounted on a clock work mechanism which moves at 2 mm/hr. 345 mm of the tape wound on the clock driven drum which completes one rotation after 7 days. After exposure, the tape was cut into seven segments and mounted
MAP-1 BANGALORE CITY SHOWING CENTRES OF AERIOBIOLOGICAL SURVEY

1 VIM
2 AB
with glycerine jelly. The pollen and spores trapped were counted as number per cubic meter of air. Pollen grains present on the trace were scanned using a microscope fitted with a mechanical stage and a 10 X objective and 10 X eye piece. A scanning width of 1800 \( \mu \text{m} \) was selected. The exposures were scanned by a short traverse method to obtain 2 hourly data. The pollen and spores were counted under the scanning width of 1800 \( \mu \text{m} \).

The pollen and spore counts were converted to a number per cubic meter of air samples as follows:

**Fraction scanned on each traverse:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of movement of tape</td>
<td>2 ( \text{mm/h} )</td>
</tr>
<tr>
<td>Length of trace in a day</td>
<td>24 \times 2 = 48 ( \text{mm} )</td>
</tr>
<tr>
<td>Width of scanning traverse</td>
<td>1800 ( \mu \text{m} ) or 1.8 ( \text{mm} )</td>
</tr>
<tr>
<td>Fraction of total deposit scanned on each traverse</td>
<td>( \frac{1.8}{48} = 0.0375 \text{ mm} )</td>
</tr>
</tbody>
</table>

**Volume of air sampled:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction rate</td>
<td>10 ( \text{lit/min} )</td>
</tr>
<tr>
<td>Volume of air sampled in a day on 48 mm long trace</td>
<td>( 10 \times 60 \times 24 ) ( \text{m}^3 )</td>
</tr>
<tr>
<td></td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>( = 14.4 \text{ m}^3/\text{day} )</td>
</tr>
<tr>
<td>Volume of air scanned in each traverse</td>
<td>( 0.0375 \times 14.4 )</td>
</tr>
</tbody>
</table>
Conversion factor for estimating the number of spores per cubic meter of air

\[
\frac{1}{0.54} = 1.85
\]

rounded to 1.9

No correction for the efficiency of trap has been made.

Aerobiological studies were conducted at two different urban and rural sites situated 40 kms apart to study the quantitative and qualitative variation in the airspora. Urban site selected was in the heart of Bangalore city, while the rural site selected was situated in a village. Both the sites had their own characteristic vegetation.

The trap in the city was installed on the terrace of a 4 storied building at an approximate height of 50 feet above the ground level in Visveswaraya Industrial Museum which is surrounded by parks, commercial establishments, residential areas and wide roads with avenue trees. A similar trap was installed at Rolofon, Attibelle on Hosur road representing the rural site at an approximate height of 20 feet from the ground level. The trap in the rural area was surrounded by crop fields, groves of *Casuarina*, *Eucalyptus*, *Cocos* as well as natural vegetation consisting of herbs, shrubs, and trees. Both the traps were operated for two years from Aug. 1985 to July 1987.
PLATE I.  Figs. 1 & 2

1. The Volumetric Burkard spore trap used for the aerobiological studies conducted at urban and rural sites.

2. View of the adjacent road of the urban site.
PLATE II. Figs. 1 & 2

1. View of the building on which the sampler was installed at the urban site.

2. View of the building on which the sampler was installed at the rural site.
CHAPTER IV

RESULTS
**Photomicrographs**

The photomicrographs have been taken with Zeiss phase contrast and fluorescence microscope. It has a built-in camera and self-timer. Depending on the colour of pollen different filters were used. The aperture and diaphragm were adjusted for sub-stage light intensity which was kept in between minimum and maximum voltage. NP 55 ORWO black and white film was used. Most of the photographs were taken in oil immersion with X 100 objective and X 10 eye piece.

**Meteorological Data**

Meteorological information regarding temperature, relative humidity, wind velocity, rainfall, cloud conditions in Bangalore for the period from August 1985 to July 1987 was obtained from Central Observatory, Bangalore.