Chapter 3

Material and Methods
The present study was carried out for two years from June 2005 to May 2007. Two sites were selected in Panaji city, one at Miramar, at sea level, the second at Altinho, 2.07 km. from Miramar, at a height of 58m. The first site, Miramar, is at sea level and has sand-dune vegetation and various types of trees, shrubs and herbs along the road and unoccupied areas. It also has garden vegetation. The second site Altinho has roadside seasonal as well as perennial vegetation, besides garden plants. The third study site, ICAR (Indian Council of Agricultural Research) Complex, Old Goa, lies at a distance of 11.4 km from Miramar. It has plantation of horticultural importance as well as wild plants. Besides the above main sites, five more sites were also selected to study the seasonal variations of airspora, just for the purpose of comparative study. They were (1) Mollem Sanctuary, a protected wildlife reserve covering 240 sq. km. of tropical evergreen forest, semi-evergreen and moist deciduous forest, situated about 46 kilometres from Panaji, (2) Cotigao Sanctuary, the southern-most wildlife reserve in Goa comprising mostly of moist-deciduous and evergreen forests (3) Mandrem, a coastal village located 21 kms north of Panaji thickly populated with plantations of local commercial crops such as betelnut and cashews along the coastline (4) Farmagudi, a plateau located 21 kms from Panaji, comprising of mountainous terrain and (5) Margao, the commercial capital of Goa located 34 kms from Panaji to the south, mostly comprising of residential and commercial buildings.

The geographical location of the sites where the study was carried out, are shown in the map of Goa in Fig. 1.
The numbers 1 to 8 in the Fig. 1 above, indicate the following sites:

1. Miramar (N 15°28' E 73°48')
2. Altinho (N 15°29' E 73°49')
3. ICAR, Old Goa (N 15°29' E 73°55')
4. Mollem Wild Life Sanctuary (N 15°22' E 74°13')
5. Cotigao Wild Life Sanctuary (N 14°58' E 74°08')
6. Mandrem (N 15°39' E 73°44')
7. Farmagudi (N 15°24' E 73°59')
8. Margao (N 15°16' E 73°59')
Air sampling was done by exposing glycerine-jelly coated slides, for 24 hours. Identification of pollen grains was done with the help of literature and reference slides prepared by collecting the flowers from the surrounding locality. Fungal spores were identified with the help of works of S.N. Agashe, P.K.K. Nair, K.R. Shivanna, T.S. Nair and others.

**Composition of glycerine jelly is as follows:**

1. Gelatin 50 g.
2. Glycerine 150 ml.
3. Distilled water 175 ml.
4. Phenol crystals 7 g.

**Preparation of glycerine-jelly for the gravity-slide technique:**

Gelatin and distilled water were mixed together in a beaker and boiled in a water bath till the gelatin dissolved. After half an hour, glycerine was added to the mixture and again heated for one and half hour. Phenol crystals were added and mixed thoroughly. After cooling glycerine jelly was preserved at room temperature.

**Preparation of Slides:**

The microslide (75x25mm) was washed thoroughly with water and soap and then cleaned with distilled water.

A major portion of the slide was coated with a thin film of glycerine jelly and a part was left uncoated for fixing a label. For coating the slide, a small lump of glycerine jelly was kept on the slide, heated on a spirit lamp flame as shown in Fig. 2.
Then a thin smear was made with the help of another slide as shown in Fig. 3. The slides were kept for exposure at around 9:30 a.m. in the morning and removed at 9:30 a.m. on the following day. At Miramar and Altinho the slides were exposed at a height of 12 meters, at ICAR complex it was done at a height of 8 meters and the other five sites the slides were exposed at a height of 2 meters. After exposing the coated slide for 24 hours, it was carried to the laboratory in a slide box. A drop of fresh molten glycerine was added again and a coverslip was placed. The coverslip was sealed with molten paraffin wax. The slides were later observed under the microscope as shown in Fig. 4 for identifying the pollen grains, fungal spores and other material. A total of eight sites were selected for the study. The construction of the spore trap and slide fixture are as shown in Fig. 5 and Fig. 6 respectively.
Fig. 2 - A small lump of glycerine jelly was kept on the slide, heated on a spirit lamp flame.

Fig. 3 - The slide was coated with a thin film of glycerine jelly.
Fig. 4 - Observation of exposed slides under the microscope for identifying pollen and spores.

Fig. 5 - Spore-trap showing the mounting of slide
Goa has three distinct seasons: pre-monsoon (February, March, April and May), monsoon (June, July, August and September) and post-monsoon (October, November, December and January). This classification based on rains is a generalized one and the length of the season may vary marginally, depending on the onset, intensity and duration of monsoon.
Table 1 - Location of study sites and sampling frequencies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name and number of Site</th>
<th>Location</th>
<th>Sampling Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Miramar-I</td>
<td>N 15° 28' E 73° 48' 38.30&quot;</td>
<td>Everyday for two years</td>
</tr>
<tr>
<td>2</td>
<td>Altinho-II</td>
<td>N15° 29' 32.37&quot; E 73° 49' 39.96&quot;</td>
<td>Everyday for two years</td>
</tr>
<tr>
<td>3</td>
<td>Indian Council of Agricultural Research (ICAR)-III</td>
<td>N 15° 29' 56.50&quot; E 73° 55' 0.900&quot;</td>
<td>Once in a month for two years</td>
</tr>
<tr>
<td>4</td>
<td>Mollem-IV</td>
<td>N 15° 22' 36.09&quot; E 74° 13' 41.65&quot;</td>
<td>Everyday for a week in each season for two years</td>
</tr>
<tr>
<td>5</td>
<td>Mandrem-V</td>
<td>N 15° 39' 51.25 E 73° 44' 20.00&quot;</td>
<td>Everyday for a week in each season for two years</td>
</tr>
<tr>
<td>6</td>
<td>Margao-VI</td>
<td>N 15° 16' 44.88&quot; E 73° 59' 06.54&quot;</td>
<td>Everyday for a week in each season for two years</td>
</tr>
<tr>
<td>7</td>
<td>Farmagudi-VII</td>
<td>N 15° 24' 44.93&quot;E 73° 59' 20.77&quot;</td>
<td>Everyday for a week in each season for two years</td>
</tr>
<tr>
<td>8</td>
<td>Cotigao-VIII</td>
<td>N 14° 58' 33.054&quot; E 74° 08' 29.41&quot;</td>
<td>Everyday for a week in each season for two years</td>
</tr>
</tbody>
</table>

Data from triplicate sampling is averaged and presented. Weather parameters were obtained from India Meteorology Department Altinho, Panaji. Data of rainfall represents the total rainfall for the entire month whereas for parameters such as temperature, humidity and wind velocity is the average for the month. These parameters are applicable to first two sites only viz. Altinho and Miramar.
Statistical Analysis:

Based on the aerobiological studies carried out for the two-year period between 2005 and 2007, the recorded data was subjected to statistical analysis of pollen grains and fungal spores. The data was analyzed for diversity index, evenness and species richness. This is an attempt to quantify the diversity of the airspora. All statistical calculations were based on the following formulae:

**Species diversity \((H')\)**

Species diversity or diversity index was calculated using the Shannon-Weiner Index (Pielou, 1975).

\[
H' = \sum_{i=1}^{S} p_i \log_e p_i
\]

where \(p_i\) is the proportion of individuals of the \(i^{th}\) species and \(S\) is the number of species.

**Evenness \((J')\)**

Evenness was computed as:

\[
J' = \frac{H'}{\log_e S}
\]

where \(H'\) is the Shannon-Weiner index and \(S\) is the number of species.

**Species Richness (SR)**

Species Richness was calculated as follows:
S. R. = \( \frac{S - 1}{\log_e(N)} \)

where \( S \) is the number of species and \( N \) is the number of individuals in the collection.

**Correlation**

The correlation coefficient provides the magnitude of variation between two variables. It is symbolized by ‘\( r \)’. The correlation between various environmental parameters such as rainfall, humidity, temperature, and wind velocity and airspora was calculated by using standard Pearson’s coefficient of correlation formula for twelve months data of Miramar and Altinho only. Students – ‘\( t \)’ test was performed for each value of correlation to determine their statistical significance. The formula used for determining students-‘\( t \)’ test was as follows:

\[
t = r \sqrt{\frac{(n - 2)}{(1 - r^2)}}
\]

where \( r \) = Pearson’s coefficient

\( n = 12 \) for 12-month data based on which \( r \) has been calculated

The parameters which are significantly correlated to the pollen and spore counts are determined from the standard table available for the value of ‘\( t \)’ (for \( n = 12 \)). For those parameters where the value of ‘\( t \)’ is less than the threshold (1.782 for \( P < 0.05 \)), the correlation is assumed to be insignificant.