Methods of Study
METHODS OF STUDY

AIR SAMPLING TECHNIQUES

During the course of the present investigation, two volumetric air samplers, viz. Burkard Seven-day Recording Volumetric Spore Trap (referred to as Burkard Spore Trap in the text), and an Andersen 6-stage Viable Particle Sampler (referred to as Andersen Sampler in the text) were used to monitor the airborne pollen, fungus spore types and culturable moulds. While the former was used to collect continuous data on the airborne pollen and fungus spore types, the latter was used to collect data on culturable moulds of the atmosphere.

Location of the Sampler and Air sampling schedule

The Burkard Spore Trap was installed on the terrace of the Life Science Building in the Guindy Campus of the Madras University, with its orifice kept at 18 meters above ground level (Plate I). Air sampling was carried out over a period of 2-years, i.e. from 1st April 1996 to 31st March 1998.

The Andersen Sampler was operated in the close proximity of Burkard Spore Trap on the terrace of Life Science Building with its orifice facing upwards. The sampling was carried out for a period of 18 months starting from April 1996 to September 1997 at weekly intervals. Air was sampled between 10.30 - 10.45 AM and the duration of sampling was 15 minutes as this was found to yield optimum number of colonies.
PLATE I

Burkard Spore Trap on the roof of the Life Sciences building, University of Madras.
DESCRIPTION OF THE AIR SAMPLERS

i) Burkard Spore Trap.

The Burkard Spore Trap (Burkard Manufacturing Company Ltd. Rickmansworth, Hertfordshire, England) is a compact unit with built-in vacuum pump designed to sample airborne particles such as fungus spores and pollen grains continuously for periods up to 7 days without attention. The collection characteristics of Burkard Spore Trap are similar to that of Hirst Spore Trap (Hirst, 1952) except for some minor differences. It differs from the Hirst trap in that instead of a sticky microscope slide moving at 2 mm per hour behind the impactor slit, particles are impacted on adhesive coated transparent plastic tape supported on a clock work driven drum rotating once in 7 days so that the trap will run for seven days without attention.

Preparation of trapping surface

The trapping surface of the drum was cleaned with xylene every time before mounting the tape. A clean strip of 'melinex' tape (supplied by Burkard Co. England along with the Trap) was wound round the drum tightly and the cut ends were stuck with a piece of double sided sticky cello tape in the marked area on the drum. A thin and uniform coating of vaseline dissolved in xylene was applied to the outer surface of the tape with a fine brush. The drum was fixed in the correct starting position over the clock and fixed to the trap. Air was sampled continuously at the rate of 10 l/m and the inflow rate was checked at frequent intervals with the help of a flow meter supplied by Burkard Co. The airborne particles got impacted on to the sticky tape on the
rotating drum. The rotation of the drum (2 mm/hour) was regulated by the clock work mechanism lying beneath the air tight cover. A trace of 336 mm length and 14 mm breadth was obtained in one week’s sampling. After a 7-day exposure the drum was removed and a freshly prepared drum was refixed. The drum was replaced around 10 a.m every Monday.

**Preparation of slides**

The exposed melinex tape was carefully detached from the drum, ensuring that no adhesive tape is attached, and placed on the acrylic cutting bar provided by the manufacturer so that the start of the trace lines up with a time marker. The cutting bar is divided into eight large segments, each 48 mm long, by lines across the full width and the first segment is again divided by short lines into 24 segments, each of 2 mm. The starting point of the trace was adjusted to the corresponding time of start of sampling and the tape was cut into 8 strips. Except the first and the last strip the other six strips represent full day’s trace (48 mm lengths from midnight to midnight each day). The segments of the tape were mounted on clean labelled slides using 'glycerine jelly' with Calberla’s stain as mountant and covered with a cover slip of 60 x 24 mm. The slides were kept flat in a dust proof slide cabinet and allowed to dry and were scanned after sufficient drying of the mountant.

**Scanning of slides**

The slides were scanned by 'short traverse method' (Hirst, 1953) at 2-hourly intervals. A scanning width of 330 μm was selected for counting the
pollen and fungus spores from the trace under 10 x 40 X magnification with the help of a marked graticule in the eyepiece.

**Estimation of pollen and spores per m³ of air**

The pollen and spore counts were converted to number per cubic meter of air by multiplying with a conversion factor of 10. The conversion factor is calculated as follows.

Rate of movement of drum = 2mm/hour
Length of the trace in a day = 24x2 = 48mm
Width of the scanning traverse = 330 µm (0.33mm)
Fraction of total deposit scanned in each traverse = 0.33

Rate of suction of air = 10 liters/min
Volume of air sampled in a day = 10 x 60 x 24 = 14.4 m³

Volume of air sampled in each traverse = 14.4 x 0.006875

Conversion factor for estimating the number per m³ of air = 1

--- = 0.099
48

--- = 10.10
0.099
rounded to 10.

**ii) Andersen sampler**

The Andersen sampler [Andersen, (1958)- supplied by Anderson Inc. Georgia, USA] is highly specialized instrument devised to collect and
enumerate all air borne microorganisms into six aerodynamic size fractions. The sampler is made entirely of aluminium alloy with stainless steel fittings. It is 8" high 4 1/4" in diameter excluding fasteners and outlets and weighs 3 1/3 lb without petriplates. It consists of six aluminium stages that are held together by three spring clamps and sealed with O-ring gaskets. Each stage has an integral air inlet section that contains 400 orifices (Plate II). The stages are numbered 1 to 6 from top to bottom. The diameter of all the stages is approximately 3.125". The size of the orifices are progressively smaller from top to bottom stages ranging from 0.0465" diameter in stage 1 to 0.0100" diameter. Consequently, the jet velocity is uniform in each stage, but increase in succeeding stage. Air is drawn through the device at the rate of 28.3 liters/min. and the instrument is run on 12 V car battery. Each succeeding stage will remove a top fraction (largest particles). Each stage holds a glass petriplate containing 27ml of culture medium which served as a collection surface.

**Preparation of the sampler**

The six separable metal fractioning units were sterilized in a hot air oven at 140°C for 1 hour before using. Petriplates of 9 cm diameter with solidified 2% Malt extract were used for exposure. The composition of the medium is as follows:

- Malt Extract = 20 gms
- Agar = 20 gms
- Water = 1000 ml
PLATE II

a) Andersen Sampler

b) Fraction Plates
To suppress bacterial contamination 1% Strepto-penicillin was added to the medium at the rate of 8 ml/l.

**Examination of exposed Petri plates**

The exposed petriplates were incubated at room temperature in a sterilised glass chamber for a period of 4 days, the growing colonies were counted and identified up to species level. Colonies of *Aspergillus* were subcultured on Czapek - Dox agar (HIMEDIA) supplemented with antibiotic for species identification. The composition of the medium is as follows:

- Sodium nitrate (NaNO₃) = 2.0 gms
- Potassium dihydrogen phosphate (KH₂PO₄) = 1.0 gms
- Magnesium sulphate (MgSO₄·7H₂O) = 0.5 gms
- Potassium chloride (KCl) = 0.5 gms
- Ferrous sulphate (FeSO₄·7H₂O) = 0.01 gms
- Sucrose = 30 gms
- Agar = 20 gms
- Distilled water = 1000 ml.

**Slide preparation**

Lactophenol and Lactophenol with cotton - blue (for hyaline colonies) were used as mountants for examining the cultures and the slides were sealed with DPX for future use.
The colonies were converted to number/m$^3$ of air by multiplying with a multiplication factor and expressed as Colony Forming Units (CFU). The multiplication factor is calculated as follows.

\[
\text{Amount of air sampled} = 28.3 \text{ liters/min.}
\]

\[
\text{Duration of each sampling} = 15 \text{ min}
\]

\[
\text{Amount of air sampled in 15 min} = 28.3 \times 15 = 424.5 \text{ lt.}
\]

\[
\text{Let the number of colonies recorded} = X
\]

\[
\text{The number of colonies } /m^3 \text{ of air} = \frac{1000}{424.5} \times X
\]

\[
\text{Multiplication factor} = 2.35
\]

Field Survey

Regular field trips were conducted at 15 day intervals to record the flowering periods of plant species and their prevalence in the locality. This also helped in the identification of plant taxa contributing to the airborne pollen. The distribution of plants present around the sampling site was graded as A - abundant, C - common, S - sparse and R - rare. The flowering period of plants present around the trapping site was also recorded. The intensity of flowering of some common plants was recorded based on visual observation and classified into 5 stages: beginning of flowering, flowering, peak flowering, decrease in peak flowering and end of flowering.
Identification of Flowering Plants

During the field survey the fresh plant materials were collected and brought to the laboratory and identified with the help of published literature (Gamble 1967; Matthew 1991).

Identification of Pollen

The pollen grains trapped on the trapping surface were identified in most cases by comparing them with pollen reference slides prepared from live plant material. Fully developed anthers of fresh flowers were collected in the morning hours and from these the pollen were dusted on clean and fresh microscope slides containing a drop of alcohol. These slides were kept for drying for 1 to 2 h and mounted in glycerine jelly pre-stained with Pollen stain - Calberla’s Red. Adopting the standard procedure (Wodehouse, 1935), the permanent slides of acetylolyed pollen were also prepared as they are useful in the study of pollen morphology.

The pollen were identified on the basis of their size, shape, wall ornamentation and other characters. The identifications were confirmed by referring to earlier publications (Tilak, 1989; Nair et al., 1986) and comparing with the reference slides.

Identification of fungal spore types and culturable moulds

The fungus spores trapped were identified to generic level wherever possible based on the visual characters such as spore shape, colour, size and
septation and by consulting standard publications and monographs (Tilak, 1989).

The fungal colonies were identified on the basis of colony characters, colour, conidial morphology and conidium ontogeny and with the help of text-books and monographs (Ellis, 1971,1976; Subramanian, 1971; von Arx, 1974; Onions et al., 1981).

Presentation of data

The results obtained with Burkard Spore Trap are described under two separate heads: "Airborne pollen types" and "Airborne fungal spore types". The data obtained with Andersen Sampler is described under the title "Airborne culturable moulds". The pollen and fungal spore counts are expressed as number/m$^3$ while the colony counts are expressed as CFU/m$^3$ of air. The colony counts are not transformed for multiple impaction.

While presenting the data, three terms, viz. 'daily mean concentration', 'frequency of occurrence' and 'percent contribution' have been used in the text of the thesis.

The 'daily mean concentration' represents mean of total number of sampling days - 730 days during a 2-year period and 365 days during a one year period.

The term 'Frequency of Occurrence' has been used to describe the number of days on which a particular pollen or fungal spore type has been
recorded as against the total number of days of sampling (730). Similarly, in the case of culturable moulds, the term denotes the number of sampling days in which a particular fungus was recorded as against the total number of sampling days (72) spread over 18-month period. On the basis of percent frequency the occurrence of individual pollen, fungal spore type and culturable mould is designated as

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most common</td>
<td>81-100%</td>
</tr>
<tr>
<td>Common</td>
<td>61-80%</td>
</tr>
<tr>
<td>Frequent</td>
<td>41-60%</td>
</tr>
<tr>
<td>Occasional</td>
<td>21 to 40%</td>
</tr>
<tr>
<td>Sporadic</td>
<td>upto 20%</td>
</tr>
</tbody>
</table>

The term ‘Percent Contribution’ refers to the contribution of individual pollen, fungal spore types and culturable moulds to the total of respective type. It is calculated as follows:

\[
\text{Concentration of individual pollen type/spore type/culturable mould} \times 100
\]

\[
\frac{\text{Total concentration of all pollen types/spore types/culturable moulds}}{X}
\]

**Statistical analysis**

To find out the correlation, if any, between the airborne pollen and fungal spore types and meteorological parameters such as maximum and minimum temperature, relative humidity, wind speed and rainfall the data has been statistically analysed by computing the correlation coefficient and using multiple regression analysis.