



CHAPTER - II

MATERIALS AND METHOD

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The methods of sampling aims at collecting spores and other microbial populations from the air into the surface for microscopic observations and analysis. This sampling method of air provide both qualitative and quantitative assessment of aeromicrobiota. The qualitative analysis is the determination of the types of microparticles within a particular place and quantitative analysis is the determination of concentration of different types of particles occurring in the sampled air.

During the present investigation the Rotorod Air Sampler devised by Perkins (1957) and later the model devised by Tilak (1982) was used. The air sampler operate on some basic principle. These principles are categorized according to physical process like impaction by wind by which one can collect the air spora from desired place. The rotorod sampler relies upon the high efficiency with which small air borne particles are deposited on narrow cylinders oriented at right angle to high velocity of winds. The principle on which the rotorod air sampler is based is that, instead of increasing the speed of air flow towards the trapping surface to impact particles, the surface is rapidly moved through the air. A small constant speed battery operated motor is used to whirl thin brass rods coated with sticky substances like petroleum jelly about its axis at a constant high speed. It has been developed into a cheap and portable as well as high efficiency model with high

sensitivity by Tilak (1982). As it is battery operated it is well fitted to use in the field and relatively independent of external wind speed.

The model employs a miniature D.C. motor with controlled speed of the type used for record players. With the rods in the proper position the motor gives about 2300 r.p.m. Collecting arms of the model are made up of 0.159 (1/16 inch) square section brass rods slightly bent inwards. The vertical arms are 6 cm. long and 4 cm. from the axis. According to May (1967) for a wind speed of $1-6 \text{ ms}^{-1}$ the efficiency for rotorod was between 80-100% for particles larger than 20μ diameter. According to Gregory (1951) the width should give more than 60 to 70% efficiency of deposition for 20μ diameter spores at wind speed above 4 m.p.h. (2 mm/sec.)

In rotorod sampler, with the spinning of 2500 r.p.m.; the arms move at the rate of 10.5 ms^{-1} . The sampling efficiency is nearly 100% for particles greater than 15μ diameter in still air. With strong wind the efficiency decreases and may be up to 80% to 85%.

Sample Rate :

The sampling rate is the volume swept by the collecting surface per unit time. The dimensions selected make this as -

$$\begin{aligned} & 2 \text{ (arms)} \times 0.159 \text{ cm} \times 6 \text{ cm} \times 8 \times 2300 \text{ r.p.m.} \times 10^{-3} \\ & = 48.0 \times 10^{-3} \times 2300 \text{ lit/min.} \\ & = \text{approximately } 110 \text{ lit/min.} \end{aligned}$$

Sampling Methods :

For present investigation,, the sampling was carried out by operating rotorod air sampler. This sampler is used for a wind variety of air borne particles. The collecting efficiency of this model is 85%. The petroleum jelly is used as a adhesive on cellotape. After the application of jelly to the cellotape, the edges of the cellotape are trimmed back to the width of the rods with sharp razer blade. (The alternative would be apply the transparent cellotapes trim and then coat with adhesive). The cellotape is cut into four equal parts 1.5 cm. length, before adhesive is applied and after applying the adhesive these are exposed for one hour and then mounted beneath a cover glass with suitable mountant like Glycerine jelly.

Scanning :

Under compound microscope with 10 x 45 x eye piece, objective combination, the spores trapped on cellotape were observed and counted regularly. The number of spores per unit volume of the air was computed with the help of conversion factor and efficiency. With the help of conversion factor the trapping efficiency assuming to be 85%, the number of spores counted on the tape of known area was readily converted into an estimated number of spores per cubic meter of air. The sampling was done for one hour daily from 10.00 a.m. to 11.00 a.m. according to Indian Standard,time (IST). The identification of spores was based on the comparision

with parasitic and saprophytic fungi material collected in and around the field, and studied microscopically and comparing with the reference slides. Also for identification the books -

- 1) Aerobiology by S.T.Tilak 1982.
- 2) Air borne pollen and fungal spores by - S.T.Tilak 1989 were used.

The percentage contribution of each spore type to the total aerospora was calculated by random counting of the each type.

Sampling Site :

Kolhapur is situated between $16^{\circ}43'$ north latitude and $74^{\circ}14'$ east longitude. The height from sea level is 557.21 meters. The annual rainfall is about 900-1000 mm. The sampling site Shahu Market Yard is located in the eastern part of the city (Map.No.1). It is mainly the whole sale market for jaggery. In addition there is fruit and vegetable whole sale market. In the ware-houses where the sampling was carried out the cereal grains like Jowar, Wheat and Rice are stored.

Rotorod air sampler was kept inside the seed godown at a place about 4 to 5 feet above ground level.

Period of Investigation :

The air spora of the seed godown of Shri. Shahu Market Yard, Kolhapur was investigated for a period of six months, from 1st January 1992 to 30th June 1992.

Weather :

During the period of investigation daily record of temperature and humidity were obtained from office of seed godown management located at Shri.Shahu Market Yard, Kolhapur (Table No.2,Histogram No.2).

KOLHAPUR CITY

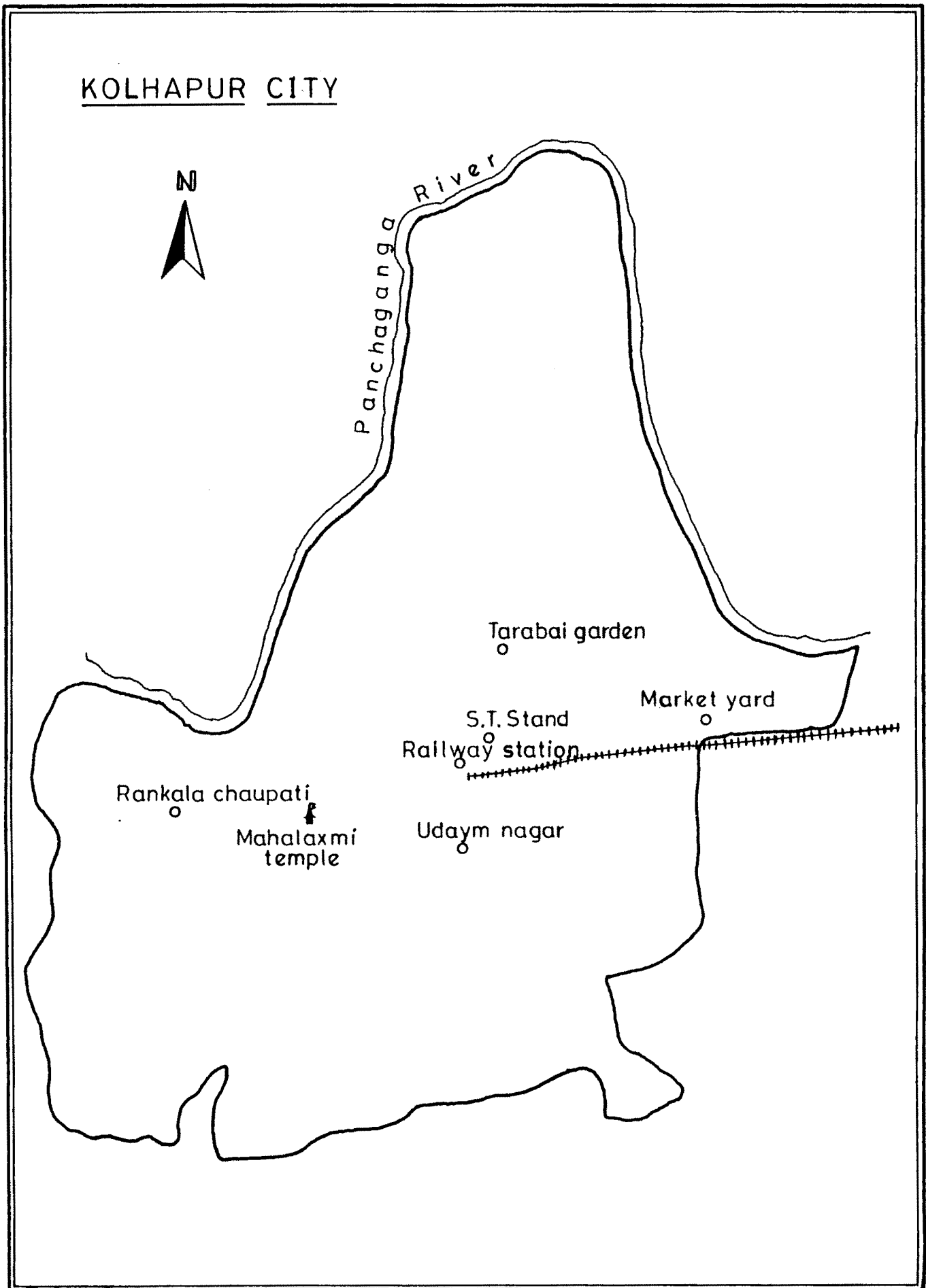
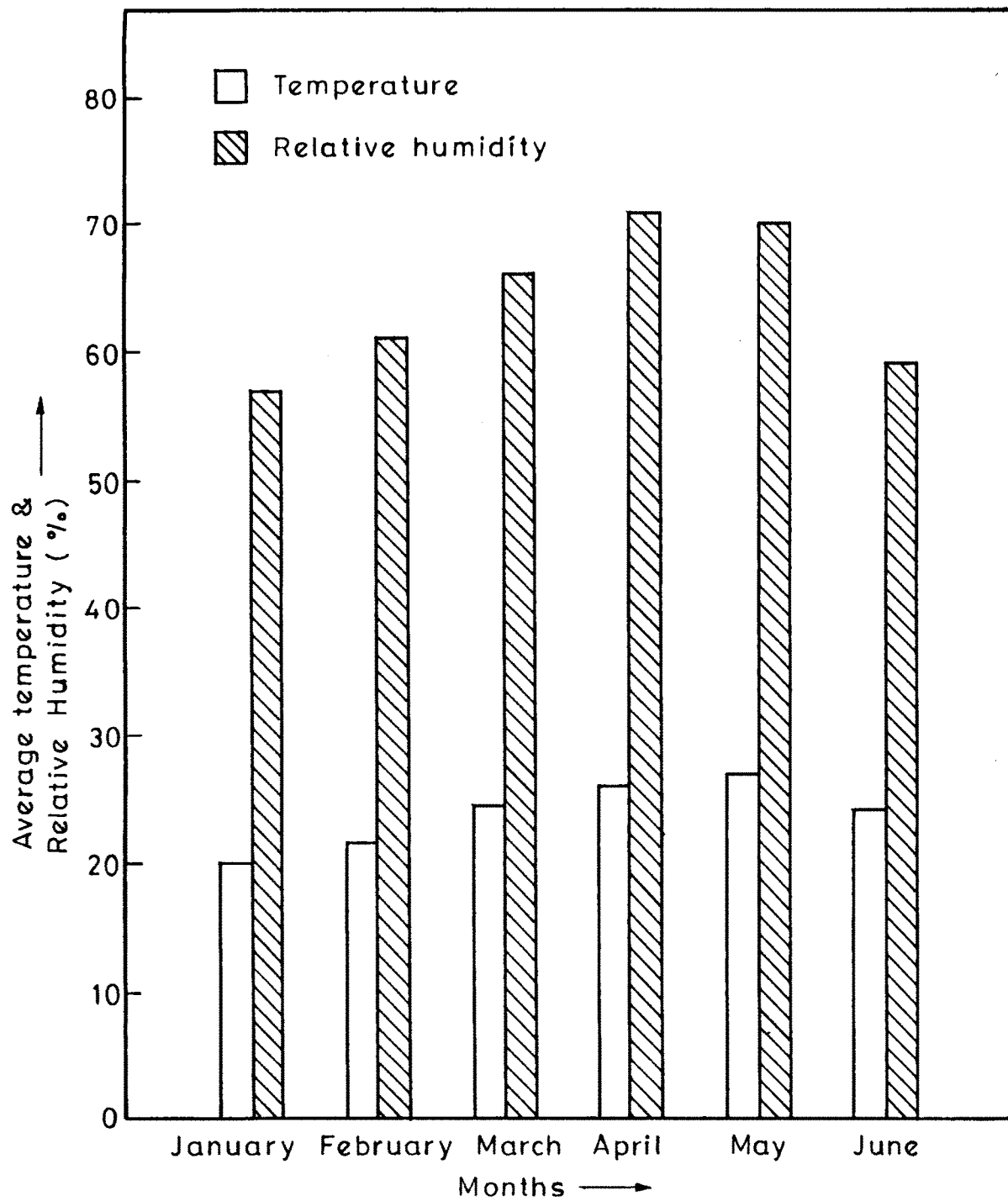


Table No.2
Average monthly temperature and relative humidity for the period
January to June 1992

Months	Average temperature units ^o C	Relative humidity
January	19.95	57%
February	21.45	61%
March	24.45	66%
April	26.1	71%
May	26.75	70%
June	24.3	59%



Histogram No.2 - AVERAGE MONTHLY TEMPERATURE AND
RELATIVE HUMIDITY FOR THE PERIOD
JANUARY TO JUNE 1992.