

CHAPTER 2

Material and Methods

MATERIAL AND METHODS

An ecological study is based on the survey and field observations. This study was performed from September 1991 to December 1992. The present study was undertaken at four different estuaries viz., Ganapatipule, Are, Sakhartar and Shirgaon (along the Ratnagiri coast in Maharashtra). Selection of sites was done on the basis of Avicennia species distribution. Distribution, zonation and Association pattern was recorded from all the sites. For phenological investigations, different phenophases were recorded for each species periodically. Phenophases like floral initiation, budding, blooming, fruiting and seedling development were noted. Continuous observations from September 1991 to September 1992 were made to collect data. In leaf behaviour studies stomatal resistance, transpiration rates, leaf temperature were measured with the help of steady state porometer. (LI Cor. in Model LI-1600, of USA) for three species of Avicennia namely A. officinalis, A. marina and ~~A.~~ ^{Avicennia} dwarf species. Diffusive conductance and diffusive resistance for CO₂ were calculated with the help of following formula

$$\text{Diffusive conductance for CO}_2 = \left(\frac{1}{LR} + \frac{1}{UR} \right) \times 1.605$$

$$\text{Diffusive resistance for CO}_2 = \frac{1}{DC}$$

- (LR = Lower leaf resistance,
UR = Upper leaf resistance
DC = Diffusive conductance)

Sampling :

Soil and plant samples were subjected to analysis of physical and chemical parameters. The samples were collected from four sites.

Soil was sampled from 0 - 15 cm zone of each species. The plants occurring at collection sites were brought to the laboratory in air tight polythene bags. Sampling was done seasonally. The months of August, December and April were chosen for sampling representing monsoon, winter and summer respectively.

Analytical Methods : Methods used for analysis are described in brief below :

Soil : (1:5 aqueous solution for 1 to 4 below)

- | | |
|-----------------------------------|--|
| 1) Electrical conductivity | Field conductivity meter
(Elico Model PE-133)
(mS/cm) |
| 2) Hydrogen ion concentration, pH | Digital pH meter
(Elico Model LI-10T) |
| 3) Chlorides | Titration against silver nitrate
(g/100 g) |
| 4) Salinity | From chlorinity (‰) |

Salinity was calculated from chlorinity according to Knudsen (1901) using the formula

$$\text{Salinity } \text{‰} = (\text{Chlorinity} \times 1.805) + 0.03$$

- | | |
|-------------------|---|
| 5) Some elements | Estimated from 1:5 aqueous solution of an air dry soil. (g/100 g) |
| 6) Organic matter | - ,, - (%) |

Plant (Physical/Morphological analysis)

Morphological variation in leaves, seedling (root-shoot ratio), flowers, pollen and fruits of three different species was assessed.

To study physical properties of leaf, mature leaves from apex, middle and base (each 50) were selected. Each leaf was weighed, the area was marked on the graph paper and the thickness was measured with the help of micrometer screw.

To study morphological characters flowering twigs of three species were collected as per the season. Size of the bract, calyx, corolla, stamen and entire flower were also measured. Structure of pollen grains were studied with the help of light microscope and discussions were recorded using micrometry.

Trichome analysis for each species was done with micrometry.

Pneumatophore characteristics were also recorded from three species. Measurements with respect to height, girth and number of pores per centimeter were taken.

The mature propagulos of three species were collected from different sites and brought to the laboratory. 25 mature propagulos were selected from each species and measurements were taken with respect to area, length and breadth.

Mature leaves of Avicennia species were collected from the same sample sites. Observations of T.S. of leaf, stem and pneumatophore, trichomes and salt glands are based on thin hand cut sections. Sections were studied under (Zeiss microscope) using photomicrographic technique.

Starch distribution pattern was studied to support leaf anatomy in different Avicennia species. Mature leaves of different species were placed in 80% (v/v) boiling ethanol until the chlorophyll was fully extracted, then it was placed in 10% NaOH for clarity. The transparent material was obtained after 10 days. It was then rinsed thoroughly with distilled water, stained with I₂KI solution, observed under microscope and were photographed for distribution of starch in mesophyll and bundle sheath cell. The results are presented by microphotograph.

Plant analysis : Parameters	Method and units
1) Moisture	Drying in oven at 60°C till constant weight (%)
2) Chlorophylls	Arnon (1949) (mg/100 g f.wt.)
3) Polyphenols	Horwitz (1965) (g/100 g f.wt.)
4) Carbohydrates	Nelson (1944) (g/100 g d.wt.)
5) Proteins	Lowry et al (1951) (g/100 g d.wt.)
6) Elements	Flame photometrically (Oven dry material was used for wet digestion)
7) Chlorides	Volhard (1956) (g/100 g)
8) Energy content	Titrimetric method (cal/g) (Karzikhin and Tarkovaskya (1964)

PLATE - 1

Avicennia dwarf species

Plate - 1

