# III MATERIAL AND METHODS

The phenomenon of salt excretion exists in some of the mangroves like <u>Aegiceras</u>, <u>Avicennia</u> and <u>Acanthus</u>. For the present study, two mangroves, <u>Acanthus ilicifolius</u> L., and <u>Avicennia marina ((Forsk) Vierh)</u>, have been selected. The plants were obtained from Ratnagiri which is at  $17^{\circ}0'$  N and  $73^{\circ}3'$  E, the place nearly 136 Km. away from the Kolhapur, the laboratory place. Kolhapur is situated on the eastern foot hills of the western ghats. The city is situated at  $16^{\circ}$  42' N and  $74^{\circ}$  14' E.

Salt glands in the leaves of <u>Acanthus</u> <u>ilicifolius</u> have been microscopically observed for their frequency as well as structure. For this purpose peel from 1st, 2nd, 3rd, 4th and 5th leaf has been used.

#### a. Hoagland culture

Ny.

Seedlings of <u>A</u>. <u>ilicifolius</u> and <u>A</u>. <u>marina</u>, collected from Ratnagiri in October (1981) were washed and kept in two separate bottles containing Hoagland nutrient solution which was 4 times diluted. Hoagland was prepared in tap water. Constituents of Hoagland solution are given in Table - A. After 4 days 81.7% survival of <u>A</u>. <u>ilicifolius</u> and 63.6% survival of

<u>A. marina</u> was observed. Based on this, for further study <u>A. ilicifolius</u> was selected.

Second collection of <u>Acanthus</u> seedlings was done in November, 1981. This time Hoagland was prepared in distilled water. It was used in different concentrations ranging from full strength Therefore, to 4 times. No plant could survive. seedlings were once again collected, one week after the first collection. Hoagland medium was prepared in tap water. This solution was further diluted to 4 times, 10 times, 20 times and 30 times. In each concentration 10 plants were kept to test the It was found that nutrient solution survival. diluted 30 times suited best. So plants were grown in 30 times diluted nutrient solution. But here also only 25% survival was observed.

7

For excretion study, plants were placed in bottles, each containing about 500 ml. nutrient solution and a single plant. Bottles were numbered. T he solutions were aerated every alternate day. The nutrient solution was changed once a week. A simultaneous sample was also run with daily change of nutrient solution.

After three weeks, salt excreted by leaves

was washed out daily with distilled water and collected to study the constituents of excreted salt. From this wash, Na, K, Ca and Cl excreted by each plant after every 24 h. were determined. For Na, K, Ca flamephotometry was used while chloride was determined by titrating the leaf wash against  $AgNO_3$  (0.01 N).

For uptake of  ${}^{36}$  Cl two bottles were selected. To each bottle 3 ml of  ${}^{36}$  Cl (Sp.Act -262  $\mathcal{A}$  ci/gm of Cl.) previously equilibrated, was added. After 48 h. salt observed on leaves was washed out. Leaves, roots, stems were crushed separately in 80% alcohol. Care was taken to wash the roots perfectly to avoid  ${}^{36}$  Cl from the root surface. Samples were filtered and condensed under reduced pressure. Volume of each sample was noted. 200  $\mathcal{A}$  l were loaded on the planchett and B - radiations were noted as counts per minute on PCS counter (Electronics Corporation of India Ltd.).

### Table - A

Salt	Amount	Distilled water	Use in preparation of nutrient solution.
	g	ml.	ml.
NH4H2P04	115.04	1000	1
KNO3	101.10	1000	6
$Ca(NO_3)_2$	164•10	1000	4
MgSO4	120.39	1000	2
<u>Micronutrients</u>			
<sup>H</sup> 3 <sup>BO</sup> 3	2.86	) )	
MnCl <sub>2</sub> , 4H <sub>2</sub> 0	1.81	) ) 1000	
ZnS0 <sub>4</sub> , 7H <sub>2</sub> 0	0.22		1
CuSO <sub>4</sub> , 5H <sub>2</sub> O	0.08		
H <sub>2</sub> Mo0 <sub>4</sub> , H <sub>2</sub> O	0.02	<b>)</b>	
Iron solution			
Iron tartarate	5	1000	1

## Hoagland constituents

### b. <u>Soil culture</u>

<u>A. ilicifolius</u> plants were collected in the month of November, December 1981 and March 1982. At each time plants were washed and grown in pots, containing equal amount of soil. Plants dried slowly from top upto certain length. Then from the green part of the plant, leaves appeared on opposite sides. Four leaves were observed on each side. These four leaves were opposite, deccussate.

In March, again <u>A. illicifolius</u> and <u>A. marina</u> plants were collected. Some of <u>A. illicifolius</u> and <u>A. marina</u> plants were treated with G.A. About 12% survival of <u>A. illicifolius</u> and in case of <u>A. marina</u> 77% survival was observed. For <u>A. illicifolius</u> 18 pots were used. Each pot containing a single plant. For <u>A. marina</u> 20 pots were used, each pot containing 2-4 plants.

Salt treatment was given to plants, cultured in the soil, after their establishment in the soil. For treatment NaCl in the concentration of 0.05 M, 0.1 M, 0.2 M, 0.3 M and 0.5 M was used. For each treatment 3 plants of <u>A. ilicifolius</u> and 6 plants of <u>A. marina</u> were selected. Plants were treated twice a week, on Monday and Thursday. After first week (3 treatments) salt excreted by leaves of each plant was washed out to calculate Na, K, Ca and Cl.

After this wash, plants received one treatment. After a gap of 3 days, leaf washes were taken and Na, K, Ca, Cl were determined.

Productivity of <u>A</u>. <u>ilicifolius</u> was determined in terms of fresh weight as well as dry matter and the effect of salinity on productivity has been recorded after five consecutive salt treatments.

Plant material was dried in an oven and powdered. Na, K, Ca, Cl and P from dry powdered material were calculated. Flamephotometer was used for first three elements, chlorides were determined by Volhard's method while phosphorus was estimated by the method of Sekine (1965).