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Original Paper

SALINITY INDUCED CHANGES IN THE LEAF ANATOMY OF THE MANGROVE *Avicennia marina* ALONG THE ANTHROPOGENICALLY STRESSED TROPICAL CREEK

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ABSTRACT

The mangrove Avicennia marina is a dominant mangrove along the anthropogenically stressed tropical Thane creek, west coast of India. Leaf anatomy of the mangrove along the Thane creek, was assessed in relation to stationwise and seasonwise variations in salinity. It was noticed that under the conditions of higher salinity, Avicennia marina showed increased thickness of hypodermal water storage tissue in the leaf (for conservation of water) and produced taller salt extruding glands at the lower epidermis to eliminate more salt; whereas, the thickness of the photosynthetic mesophyllic tissue significantly reduced. At lower salinity or with reduction in salinity in monsoon, contrary to above occurred. These changes probably explain the stunted growth of Avicennia marina in high salinity environment and its vigorous growth at lower salinity.

Key Words: *Avicennia marina ;* salinity *;* water storage tissue ; stunted growth ; thane creek

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INTRODUCTION

Mangroves are salt tolerant angiosperm plants, which are commonly present along the banks of coastal water bodies such as estuaries and creeks. Arroyo (1977) defined mangroves as “A small group of true mangrove plants and associated species belonging to systematically unrelated families, possessing similar physiological characteristics and structural adaptations with common preference to the intertidal habitat.” The special adaptation of these plants is that they are tolerant to fluctuating water salinity. The prop roots and the stilt roots form a firm anchorage in the sinking substratum, also trap the fine suspended particles in water leading to accumulation of sediment and formation of the mudflats. The mudflats enhance the settlement and growth of different mangrove species and their associate plants there by developing mangrove forest or “mangrove ecosystem”.

The mangrove forests grow by taking nutrients from the tidal waters and in turn provide natural food to the mangrove dwelling fauna (Odum *et al*., 1982). The forest detritus, comprising of fallen leaves and branches from the mangroves, provides nutrients to the coastal environment and support immense variety of sea life in intricate food webs, associated directly through detritus, and indirectly through the planktonic and epiphytic algal food chain. The organic detritus, according to Darnell (1967), is “All types of biogenic materials in various stages of microbial decomposition which represent potential energy sources for consumer species.” Simenstad (1983) expanded this definition to include ‘any free particles of organic matter, which no longer produce carbon through photosynthesis.’ In this, he included all the biogenic particles of both plant and animal origin; as well as free formed (through chemical or geological processes) particles including associated, adsorbed, or dissolved substances and residing microbes. According to Jiunn (1995), the mangrove mudflats receive nutrients not only from the mangroves but also from other sources such as terrestrial run off and

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estuary outflow, depending upon the geomorphology of the basin and tidal amplitude. The mangrove ecosystems serve as sinks for macro and micronutrients and also the heavy metals and other toxicants, whenever there is pollution. The nutrient rich mangrove ecosystems support a complex community assemblage in their vicinity, and also export organic and inorganic nutrients to the open seas through tidal cycles and enrich them to increase their productivity.

The mangroves have adapted themselves best in the shallow sea coast, sheltered estuaries and deltaic zones of the tropics and subtropics with characteristic environmental conditions viz. temperature ranges between 20^{o}C to 30^{o}C, moderate to high monsoon precipitation i.e. 1000 – 3000 mm/ annum, high humidity but with coastal aridity. On the sea-land interface in global mangrove systems, there are gradual, slow or quickly changing ecological or geomorphological conditions to which mangroves have to adapt. The human thrust or pressure, other biotic factors and interactions also cause changes in mangrove ecosystems. Due to this the mangroves are now very dynamic but have become fragile through out the globe.

The salinity tolerant plants in mangrove ecosystem are commonly known as halophytes. They are adapted to survive in a range of saline environments. In any environment the halophytes require water for growth and development. To obtain sufficient water, osmotic adjustments have to be made. For this the halophytes take up ions to increase the osmotic level in their tissues, which permits moisture to move from soil into the tissues. On the other hand excess salt ions can produce toxic effects on plant cells. To counter these potential toxic effects of high concentration of ions different mechanisms are used by the halophytes such as exclusion of salts by the roots, dilution of ions through succulence, synthesizing organic osmotic compounds that can reduce the need for salt ions, and distribution of excess salt ions into tissues, organs, or the vacuoles of the cells. The halophytes need to obtain sufficient ions to maintain growth while avoiding water deficit or an excess of ions. In a world where most of the water is saline halophytic plant are examples of effective adaptations to increased salinity (Rao and Tan, 1984). These adaptations have provided study subjects to many researchers.

The mangrove *Avicennia marina* is the dominant mangrove species along the Thane creek that occurs through out the length of the creek. The mangrove *Avicennia marina* has salt storing glands, salt extruding glands, water storage tissue, ability to synthesize and store tannin etc. The changes in the leaf anatomy of the mangrove along the anthropogenically stressed Thane creek (Quadros *et al*., 2004 & 2009) west coast of India, were assessed in relation to stationwise and seasonwise variations in salinity. Being the dominant species it was studied to assess salinity induced changes on the leaf anatomy.

MATERIALS AND METHODS

Sample Collection

Samples of leaves of *Avicennia marina* (**Fig. 1**) were collected seasonally for four seasons viz. monsoon or rainy season (June to September), early post monsoon or the recovery period, when the salinity in the creek which is lowered in monsoon, recovers (October - November), late post monsoon or winter season (December to February) and premonsoon or summer (March to May).

Two stations were selected along the 26 km stretch of the Thane creek. According to Miller and Ambrose (2000), species are rarely dispersed uniformly in nature; hence the ecological field studies should be designed accordingly.

Of the two stations selected, one was on the riverine end of the creek; the other station was at the seaward end of the creek. Thus the stations represent low and high salinity regions of the creek respectively. The profile of the sampling stations is as follows:

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Fig.1. Mangrove *Avicennia marina* from Thane creek

Station 1 – (Near Kharegaon village)

This station is on the east bank of the Thane creek situated about 1.5 km downstream from the place where the creek meets the Ulhas river estuary by a narrow connection (**Fig.2**). It has relatively more influence of fresh water. At this station the mudflats were about 15 meters wide from the midstream, black in colour, soft and sinking type. The total width of the creek during the peak high tide was barely 100 meters, and the water depth about 3 meters. This station faced a large amount of anthropogenic pressure in terms of human activity, and the sewage and solid waste disposal.

Fig. 2. Station 1 Kharegaon at the riverine end

Station 2 – (Near Trombay)

The second station was on the southern end of the Thane creek, on its west bank near Trombay, close to the Mumbai harbour. (**Fig.3).** The mudflats were soft sinking type, dark brown in colour, having the breadth about 100 meters.

The dominant mangrove species was *Avecinnia marina* with height about 3 to 5 meters; *Avicennia officinalis* and *Sonneratia apetala* were absent. This station had high anthropogenic activity, as it is close to a fish-landing site.

As mentioned earlier, samples were collected during 4 seasons.

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Fig. 3 Station2 Trombay at the seaward end

In every season at least 3 samples were taken at intervals and average value was considered to represent the season

This aspect was taken for study because a close scrutiny of literature revealed lack of information about the changes in the anatomical structures corresponding with the changes in the salinity. The present study was focused mainly on *Avicennia marina* Photoplate 1: including two varieties *Avicennia marina var. marina* and *Avicennia marina var. acutissima* because they occurred at different salinity environments along the creek. *Avicennia officinalis* occurred mainly at the riverine side.

Salinity measurement

The salinity estimations were done by Argentometric titrations (APHA, 1981).
Along with the leaves water sample was also collected from these two stations to study the difference in salinity. Station 1 (one at the riverine end with low average salinity and station 2 at the seaward end with high average salinity. Samples were collected during different seasons viz. monsoon or rainy season (June to September), early post monsoon or the recovery period, when the salinity in the creek which is lowered in monsoon, recovers (October - November), late post monsoon or winter season (December to February) and premonsoon or summer (March to May). (**Table 1**).

Table 1. Stationwise and seasonwise salinity (ppt.)

	stn1	stn2	stn3	stn4	Average
Premonsoon	31.2	17.62	13.67	31.98	23.61
Monsoon	16.09	20.76	18.25	30.8	**21.48**
Early post monsoon	17.89	25.7	29.7	31.26	**26.14**
Late post monsoon	23.63	27.22	10.08	33.42	23.59
Average	22.20	22.83	17.92	31.86	23.70
Minimum	16.09	17.62	**10.08**	30.8	
Maximum	31.2	27.22	29.7	**33.42**	
Std. Dev.	6.80	4.43	8.53	1.14	

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Anatomy Analysis

Leaves, twigs and the roots of *Avicennia marina* were collected in every season namely premonsoon, monsoon, early postmonsoon, late postmonsoon and from the stations at the riverine and the seaward end.

The anatomical peculiarities were studied in suitably stained transverse sections, using compound microscope. Suitable transverse sections were taken, stained with safranin and prepared for microscopic studies. The tissue organization was studied to detect the anatomical variations (if any) with season and station.

Data Analysis

Microphotographs of the sections were taken. Measurements of the tissue parts were taken with the help of occulometer and stage micrometer. Z test was applied to analyse the significance of difference in the mean values. The significance of the difference in the average values was assessed using statistical Z Test of significance.

RESULTS AND DISCUSSION

The leaf of *Avicennia* is dorsiventral. Most of the characters show xeric adaptations for conservation of water (Naskar and Rabinchanath, 1999). A typical transverse section shows the following parts: 1) Upper epidermis, 2) Hypodermis, 3) Mesophyll, 4) Lower epidermis. (**Fig. 4**)

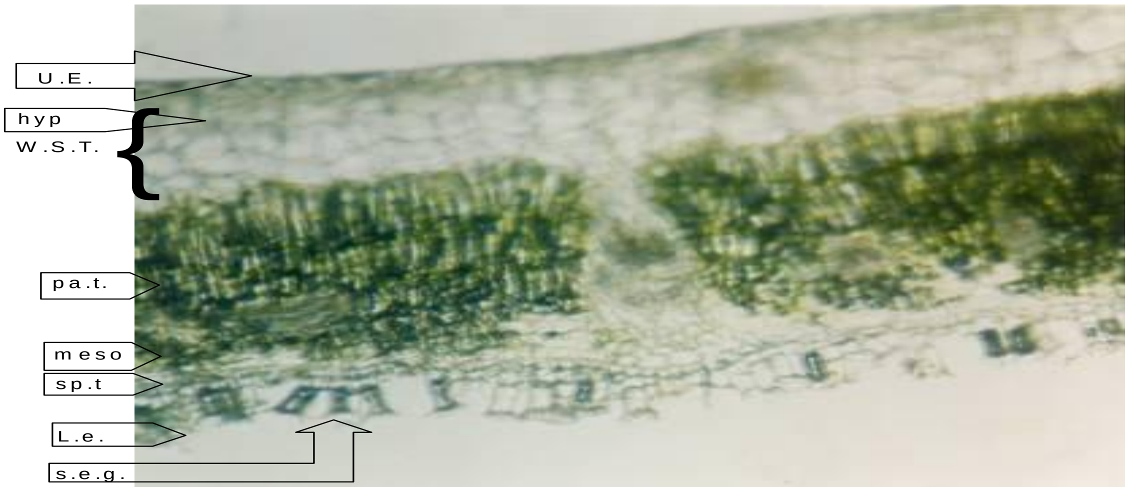

Fig. 4. A typical transverse section of leaf of *Avicennia marina* showing upper epidermis (U.E.), Hypodermis (hyp), Mesophyll (meso), lower epidermis (L.e). Hypodermis- water storage tissue (W.S.T.)
- Mesophyll—palisade (pa.t.), spongy tissue (sp.t.)
- spongy tissue more with reduced salinity
- lower epidermis—salt extruding glands. (s.e.g.)

The upper epidermis is the outermost layer, one cell thick, covered with a thick cuticle. It has salt accumulating glands at intervals (**Fig. 5**). The hypodermis has water storage tissue which is 4-5 layers in thickness. The water storage tissue plays an important role in conservation of water (Nair and Govindakutty, 1972; Walsh, 1974). As the leaf matures the sclerides get stored in the water storage tissue. Mesophyll is differentiated into two parts, the palisade tissue towards upper epidermis and the spongy tissue towards the lower epidermis. The palisade tissue has 2-3 layers of cells and is below the hypodermis. Spongy tissue follows the palisade layer, which are 2-3 cell layers thick. Both these tissues have chloroplast and are photosynthetic. The lower epidermis has sunken stomata and on its outer side there are salt extruding glands, produced by the cells of lower epidermis (**Fig. 4**). The epidermal cell divides and cuts off a

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basal cell on the outer side, which then forms the salt extruding gland. This gland is multicellular, it accumulates and extrudes the salt (mainly NaCl) to outside. All these leaf anatomical characteristics showed significant changes according to stationwise and seasonwise variations in salinity.

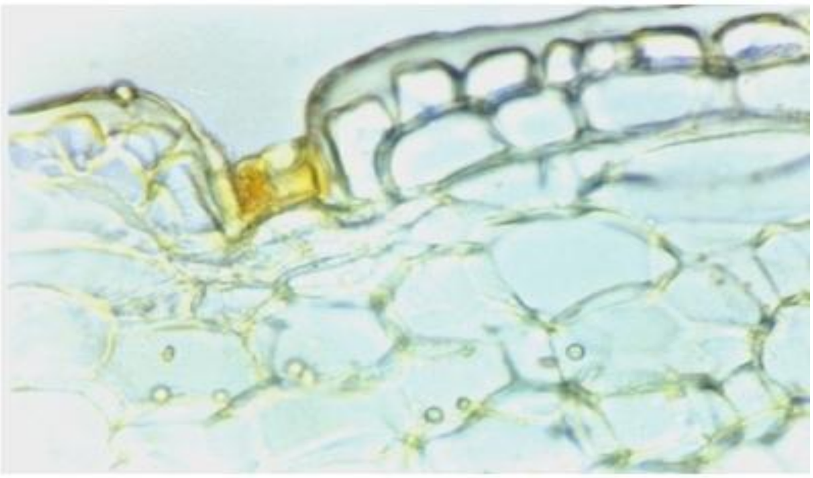

Fig. 5. Upper epidermis covered with a thick cuticle. It has salt accumulating gland at intervals.

Salt extruding glands of lower epidermis: It was observed that the length of the salt glands varied according to the salinity of the environment. At lower salinity the gland cells were shorter (**Fig. 6**) in height where as at the higher salinity the gland cells were taller. (**Fig. 7**).In summer season at the riverine end the average height of the salt gland cell was 0.096mm(96μm), which at the seaward end with relatively high average salinity was 0.125 mm (125μm), the difference being statistically significant (**Table 2**). The change in the tallness of the salt gland cell was also noticed with the season. In the monsoon season there was significant drop in salinity. During this period the salt gland cells were shorter in height as compared to summer season. The gland cell average height in summer (salinity 22.2 ppt; (**Table 1**) at riverine end station was 0.096mm (96μm) which changed to 0.082mm (82 μm) in monsoon (salinity 16.09 ppt). The above differences were statistically significant when Z test was applied.

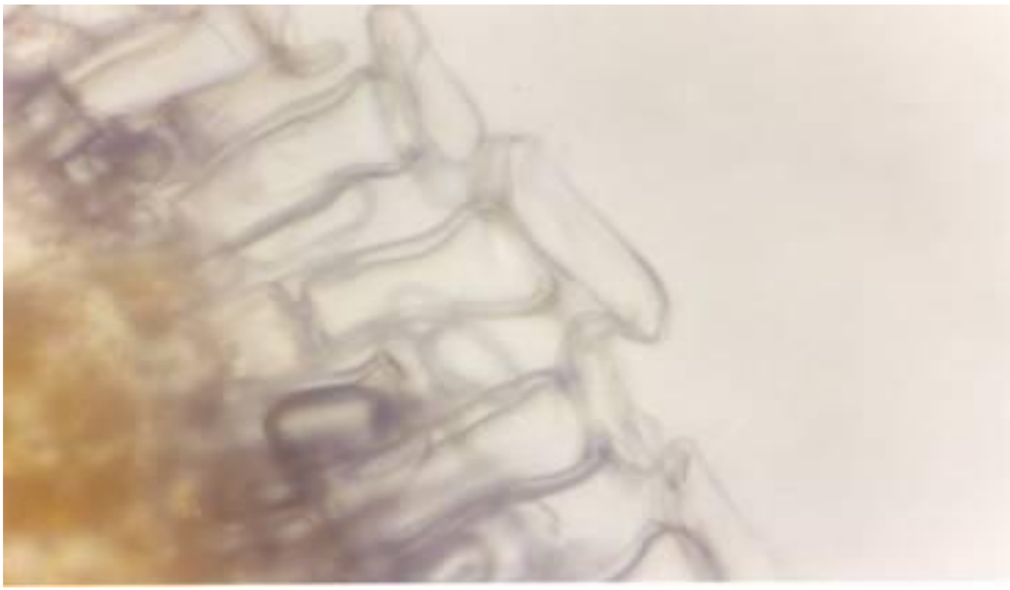

Fig. 6. At lower salinity salt extruding glands are shorter and broader.

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Table 2. Comparison of the length (mm) of salt extruding gland cell of lower epidermis at higher and lower salinities using Z test of significance.

Leaf salt gland Mean thickness mm	x1	x2	s1	s2	n1	n2	S.E	Calculated Z
Seaward end salinity 31.86ppt (High salinity)	0,1254		0.0136		45			
Riverine end salinity 22.2ppt (Low salinity)		0.09683		0.025		40	0.00444	6.439 significant
stn 1 summer avg. Salinity 31.2 ppt	0.09683		0.025		40			
stn 1 monsoon avg. Salinity 16.09 ppt		0.0823		0.039		45	0.00523	7.096 significant
Stn1 winter Salinity 23.63 ppt.		0.0638		0.038		35	0.0075	4.404 significant

Significance | Z | > 1.64 Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (S), Sample size (N), Standard error (S.E.)

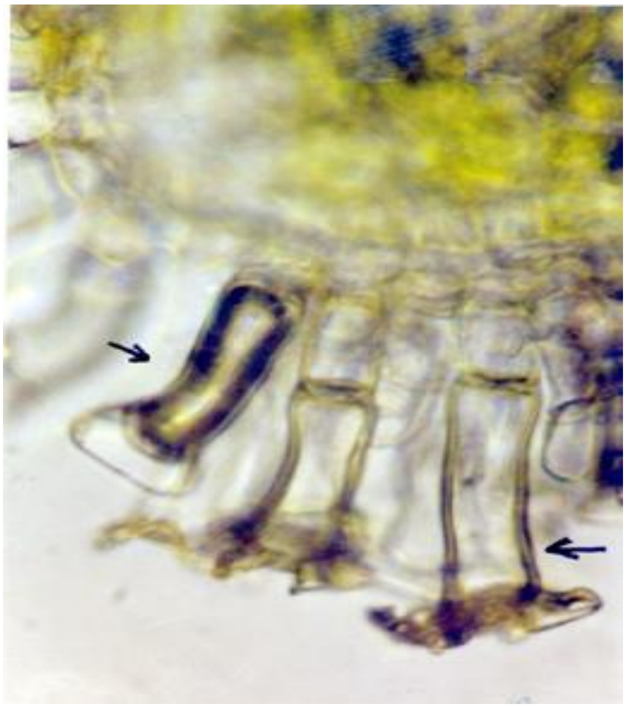

Fig. 7. At higher salinity salt extruding glands are taller and slender

Water storage tissue: (**Table 3**) shows comparison between the thickness (mm) of the water storage tissue at the lower and higher salinities at different stations and seasons. At the riverine end station 1, in summer, when the average salinity was 22.2 ppt, the average thickness of water storage tissue was 0.1519 mm (152µm). Whereas, in monsoon when average salinity was 16.09 ppt it was 0.0823mm (82µm), the difference was statistically significant. At seaward end, in summer when the average salinity was 31.86 ppt (**Table 1**) the water storage tissue had more width (0.1889)mm or 189 µm (**Fig. 8**) as compared to that (152µm) at the riverine end in the same season with average salinity 22.20 ppt. (**Fig. 9**) (**Table 1**).

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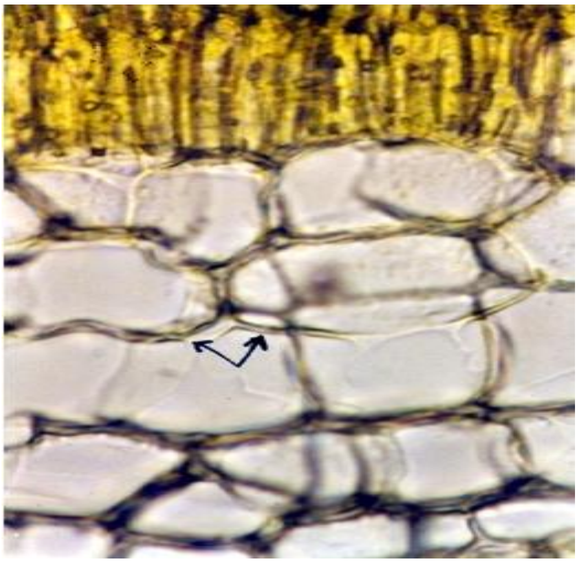

Fig. 8. At seaward end with higher salinity water storage tissue is more in thickness.

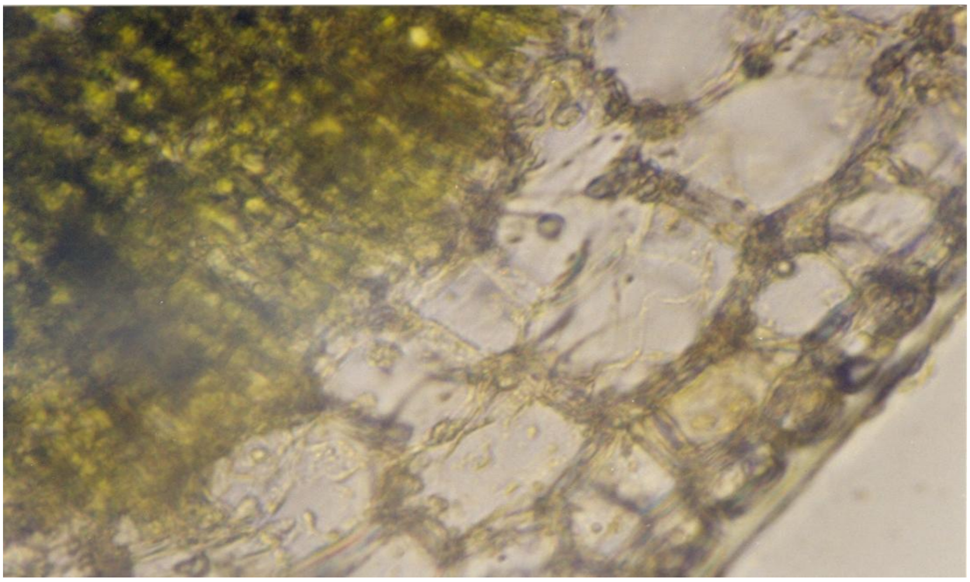

Fig. 9. At riverine end with lower salinity less thickness of water storage tissue.
--- Palisade layer more in thickness.

All these differences were statistically significant. Thus it can be inferred that at lower salinity the thickness of the water storage tissue reduced significantly probably because plants do not require more succulence.

Palisade layer: Comparison of the thickness (in mm) of palisade tissue in different salinities is shown in (**Table 4**). This tissue has chloroplast and hence photosynthetic activity. It was evident that in monsoon season when there was significant lowering of salinity, the palisade tissue showed slight increase in thickness (**Fig. 9**). The change however was not statistically significant. In non-monsoon seasons also the salinity differed from station to station with lower average salinity at the riverine end and higher average salinity at the seaward end. However, this change in salinity did not cause significant change in the thickness of the palisade tissue.

Table 3. Comparison of the thickness (mm) of water storage tissue at lower and higher salinities using Z test of significance.

	Mean thickness (mm)							
	x1	x2	s1	s2	n1	n2	S.E.	Calculated Z
Seaward end salinity 31.86ppt (High salinity)	0.1889		0.0227		45			
Riverine end salinity 22.2ppt (Low salinity)		0.1519		0.038		40	0.0069	5.36 significant
stn 1 summer avg. Salinity 31.2ppt	0.1519		0.038		40			
stn 1 monsoon avg. Salinity 16.09ppt		0.0823		0.039		45	0.0083	8.38 significant
stn 1 winter Salinity 23.63 ppt		0.1326		0.042		30	0.00974	1.9815 significant

Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (s), Sample size (n), Standard Error (S.E.)

Table 4. Comparison of the thickness (mm) of palisade tissue at higher and lower salinities using Z test of significance.

	Palisade Mean thickness mm							
	x1	x2	s1	s2	n1	n2	S.E	Calculated Z
Seaward end salinity 31.86ppt (High salinity)	0.1143		0.02665		45			
Riverine end salinity 22.2ppt (Low salinity)		0.1064		0.0323		40	0.00647	1.22 insignificant
stn1 summer avg. Salinity 31.2 ppt	0.1064		0.0323		40			
stn1 monsoon avg. Salinity 16.09 ppt		0.117		0.031		45	0.00689	1.536 insignifiacnt
stn 1 winter avg. Salinity 23.63 ppt.)		0.09975		0.03		30	0.00749	0.887 insignificant

Significance | Z | > 1.64 Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (S), Sample size (N), Standard error (S.E.)

Spongy tissue is in continuation of palisade tissue and is photosynthetic due to the presence of chloroplast. The thickness of this tissue was more during monsoon or at lower salinity (**Table 5**). In summer season at the riverine end station the average thickness of the spongy tissue was 0.088mm, where as at the seaward end station in the same season it was lower (0.0115 mm). At the riverine end the average thickness of the tissue increased to 0.102 mm in monsoon (**Fig. 1**). All these differences were statistically significant when tested by Z test (**Table 5**). Thus the thickness of photosynthetic spongy tissue increased with reduction of salinity.

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Table 5. Comparison of the thickness (mm) of spongy tissue at higher and lower salinities using Z test of significance.

	Spongy tissue Mean thickness mm							
	x1	x2	s1	s2	n1	n2	S.E	Calculated Z
Seaward end salinity 31.86ppt (High salinity)	0.01151		0.0293		45			
Riverine end salinity 22.2ppt (Low salinity)		0.088		0.038		40	0.0074	10.336 significant
stn1 summer avg. Salinity 31.2 ppt.	0.088		0.038		40			
stn1 monsoon avg. Salinity 16.09 ppt.		0.102		0.03		45	0.00689	1.867 significant
stn 1 winter avg. Salinity 23.63 ppt.		0.094		0.03		30	0.00749	0.73 insignificant

Significance | Z | > 1.64 Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (S), Sample size (N), Standard error (S.E.)

CONCLUSIONS

The mangroves have adaptive structures to cope stress factors, especially salinity. However it was not reported whether they show modifications in the structures according to environmental variations. In the present study it became apparent that the mangrove *Avicennia marina* showed certain anatomical variations with change in environmental salinity. At higher salinity they showed, increased length of the salt extruding glands on the lower epidermis of the leaf, more thickness of the leaf water storage tissue, lesser thickness of photosynthetic spongy tissue. At lower environmental salinity at the riverine end or during monsoon they showed shorter cells of salt extruding glands on lower epidermis of the leaf, as well as less thickness of leaf water storage tissue as succulence were not required due to availability of fresh water, with more thickness of photosynthetic spongy tissue.

The plants in Thane creek were observed to spend more energy on growth of salt extruding glands and water storage tissue and have relatively less photosynthetic tissue in high saline environments which lead to their stunted growth. Opposite changes in these structures, at lower salinity in monsoon season and at riverine end stations, favour growth of the mangroves.

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Table 4: Comparison of the thickness (mm) of palisade tissue at higher and lower salinities using Z test of significance.

	Palisade Mean thickness mm							
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Seaward end salinity 31.86ppt (High salinity)	0.1143		0.02665		45			
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stn 1 winter avg. Salinity 23.63 ppt.)		0.09975		0.03		30	0.00749	0.887 insignificant

Significance | Z | > 1.64 Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (S), Sample size (N), Standard error (S.E.)

Table 5: Comparison of the thickness (mm) of spongy tissue at higher and lower salinities using Z test of significance.

	Spongy tissue Mean thickness mm							
	x1	x2	s1	s2	n1	n2	S.E	Calculated Z
Seaward end salinity 31.86ppt (High salinity)	0.01151		0.0293		45			
Riverine end salinity 22.2ppt (Low salinity)		0.088		0.038		40	0.0074	10.336 significant
stn1 summer avg. Salinity 31.2 ppt.	0.088		0.038		40			
stn1 monsoon avg. Salinity 16.09 ppt.		0.102		0.03		45	0.00689	1.867 significant
stn 1 winter avg. Salinity 23.63 ppt.		0.094		0.03		30	0.00749	0.73 insignificant

Significance | Z | > 1.64 Significance | Z | > 1.64 at 5% level of significance
Mean (x), Standard deviation (S), Sample size (N), Standard error (S.E.)

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Photoplate 1: Mangrove *Avicennia marina* from Thane creek

Photoplate 2 : Station 1 Kharegaon at the riverine end

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Photoplate 3 :Station2 Trombay at the seaward end

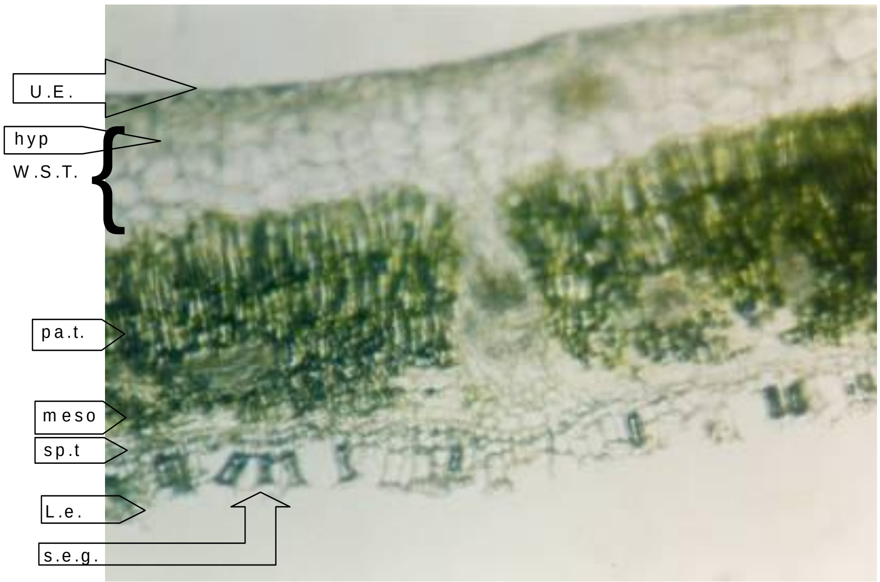

Photo plate 4: A typical transverse section of leaf of *Avicennia marina* showing upper epidermis (U.E.), Hypodermis (hyp), Mesophyll (meso), lower epidermis (L.e). Hypodermis- water storage tissue (W.S.T.)
-Mesophyll—palisade (pa.t.), spongy tissue (sp.t.)
- spongy tissue more with reduced salinity
- lower epidermis—salt extruding glands. (s.e.g.)

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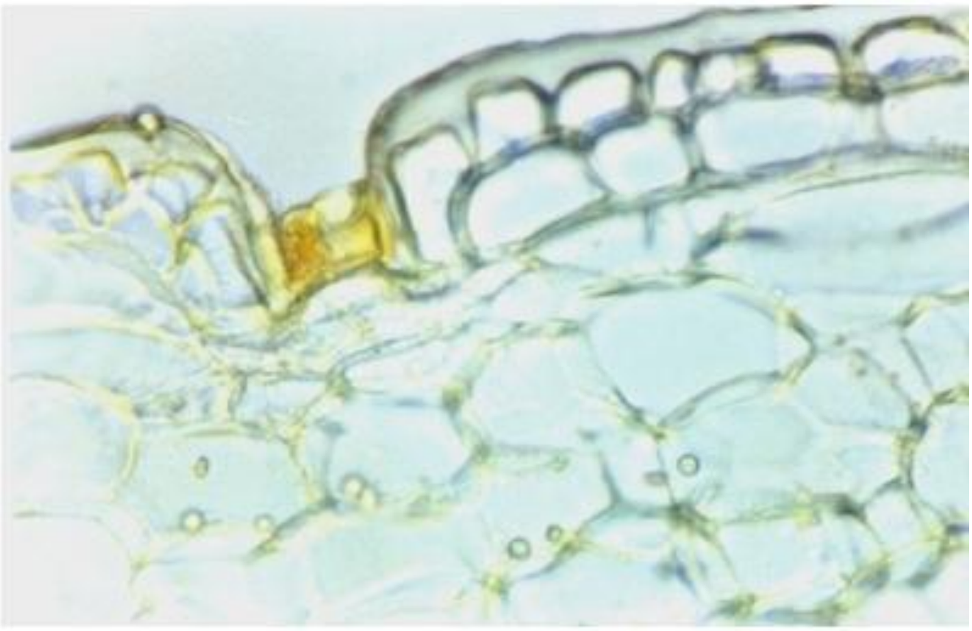
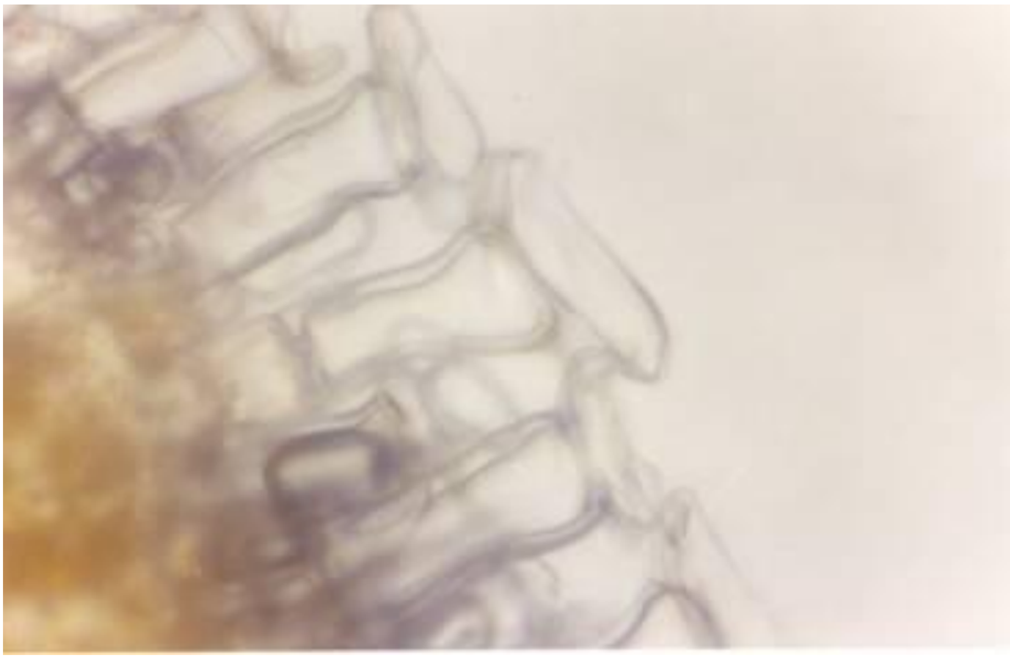


Photo plate 5: Upper epidermis covered with a thick cuticle. It has salt accumulating gland at intervals.



Photoplate 6: At lower salinity salt extruding glands are shorter and broader.

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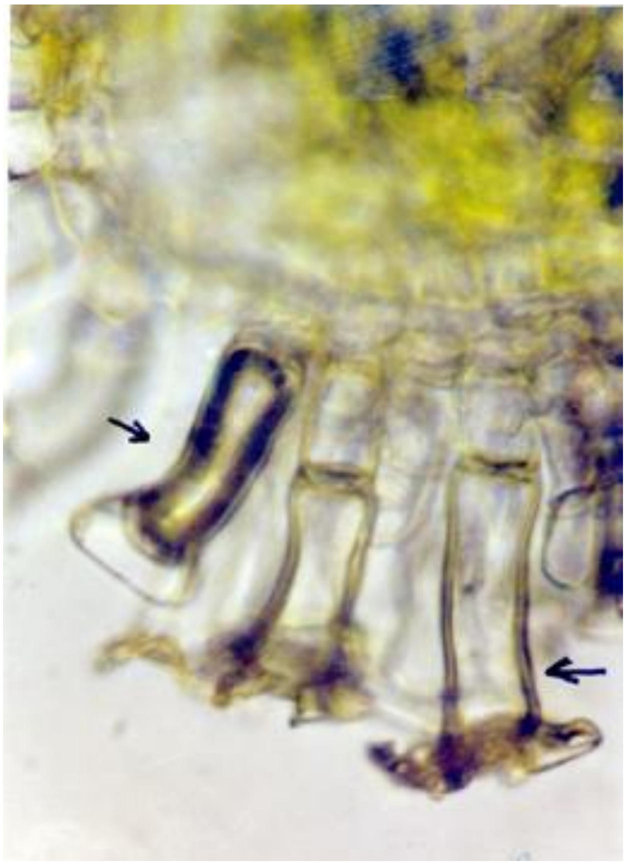

Photo plate 7: At higher salinity salt extruding glands are taller and slender.

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Photo plate 8: At seaward end with higher salinity water storage tissue is more in thickness.

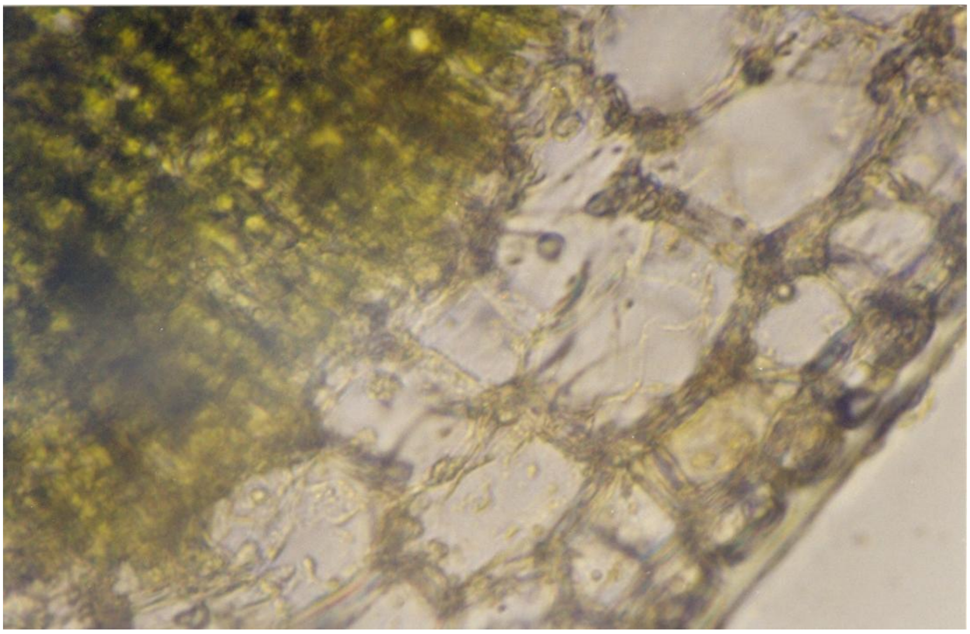

Photo plate 9: At riverine end with lower salinity less thickness of water storage tissue.
--- Palisade layer more in thickness.

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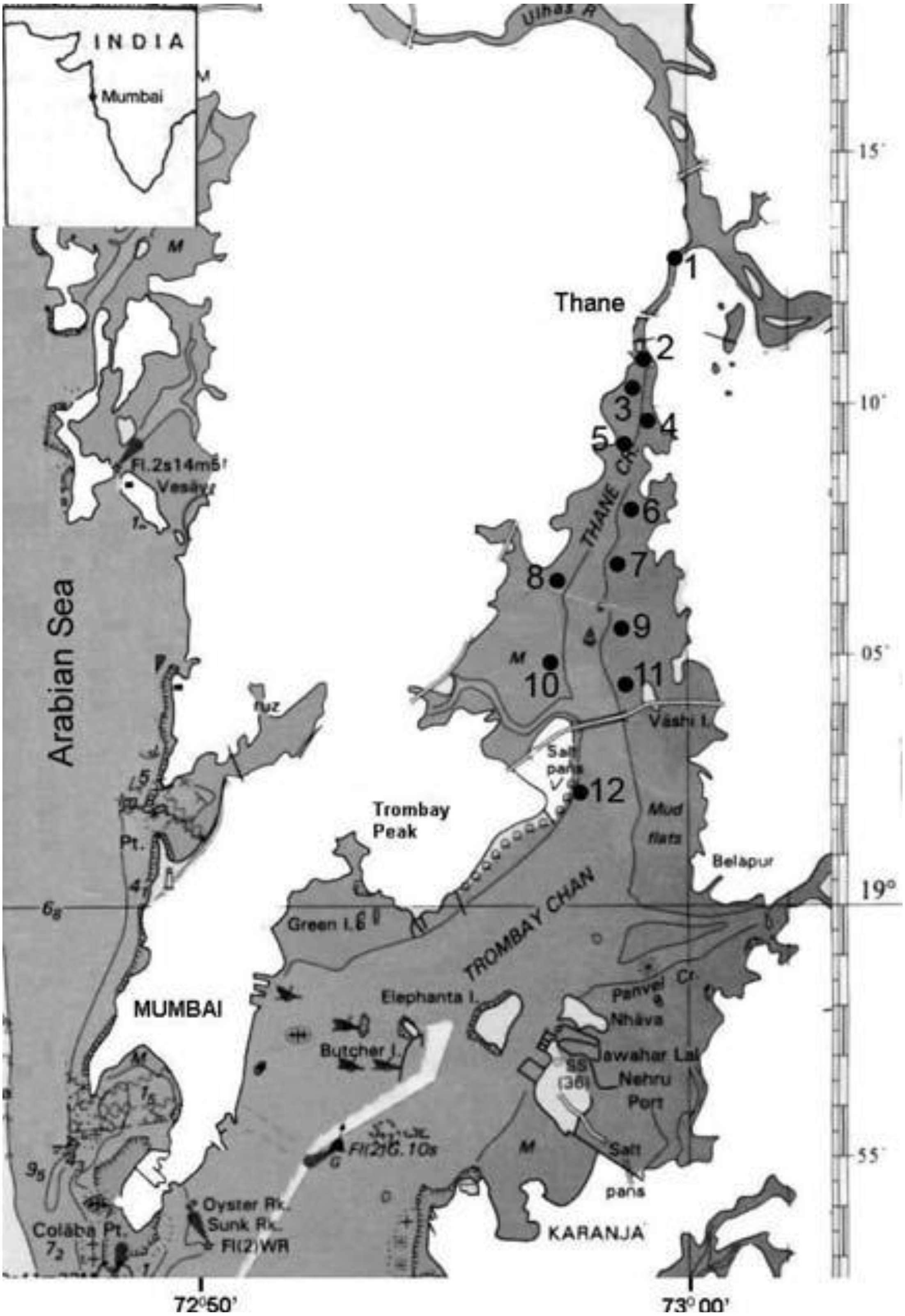

Map 1: Map of Thane creek showing the sampling stations point 1 being the riverine end and point 12 is the sea ward end.