



SPATIAL VARIATION IN PHYSIOLOGY OF *AVICENNIA MARINA* ALONG THE JAMNAGAR COAST, GUJARAT.

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ABSTRACT: The biochemical study of *Avicennia marina* was carried out to understand the variation of the antioxidants and stress parameters along the Jamnagar coast of Gujarat. The present study deals with the chlorophyll levels, antioxidants and Malondialdehyde (MDA) recorded in the leaves of *Avicennia marina* present at different area with differences in the landuse of nearby area *i.e* industrialization and urbanization. The study also reveals that the mangroves are also affected by the landuse of the area and has an impact on the growth and development of mangroves. With the help of biomarkers like MDA and antioxidants like Ascorbic acid, Peroxidase and Total Glutathione the stress levels has been recorded in the leaves of the plant.

Key words: *Avicennia marina*, Total chlorophyll, Antioxidants, MDA

Abbreviations: Tchl-total chlorophyll, POD-peroxidase, AA- Ascorbic acid, GSH-total glutathione, MDA-malondialdehyde, FW-fresh weight, DW-Dry weight

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INTRODUCTION

Mangroves play a vital role in the functioning of the coastal ecosystem. The Mangroves forests in India covers an area of 4,740 sq km and has the world's third largest mangrove cover [1]. Mangrove cover in Gujarat is second after Sundarbans in India accounting to 1,017 sq km. Mangroves are distributed all along the coastline of Gujarat except the Saurashtra coast. There are about 14 species of mangroves recorded in South Gujarat, where as in Gulf of Kachchh only four species is recorded. The most dominant communities found in gulf of Kachchh is *Avicennia marina*, *Ceriops tagal* and *Rhizophora mucronata* [2]. Apart from naturally occurring mangroves, plantation has been done in different areas along the coastline of Gujarat [3].

Literature review of mangroves indicates ample research on the vegetation structure, density and physiology in India [4]. Few studies exist on the relation between the salinity and the chlorophyll under control conditions in the mangroves of coastal region of south India and West Bengal [5, 6 & 7]. Relation between chlorophyll content and secondary metabolites has been found in the seedlings of 14 different mangroves species found in the Bhitarkanika delta region, Orissa [8]. Mangroves are continuously exposed to increase in various kind of pollution resulting from various anthropogenic activities due to rapid industrialization and urbanization in coastal areas [9, 10 & 11]. The mangroves are continuously exposed to various kind of stress like salinity, pollution, heavy metals, etc. Salinity stress has adverse impact on the growth and development of plant by disturbing the ionic and osmotic equilibrium in the cells. Similarly, pollution has an impact on the environment conditions of mangroves and hence leading to the disturbance in the growth and productivity of the plants. Apart from these mangroves are also exposed to various biotic stress such as pest, microbial diseases etc. all these stress factors results into the production of reactive oxygen species (ROS) within the cell. Although the mangroves has been adapted to the naturally occurring biotic stress through the morphological and physiological modifications and has developed an efficient antioxidative defense system composed of complex enzymatic and non-enzymatic system [12].

Plants have a good mechanism to detoxify ROS in the form of antioxidant enzyme system and non-enzymatic system and protect the plants [13, 14 & 12]. The present study aims to monitor any damage to the *Avicennia marina* due to anthropogenic activity with the help of antioxidant enzymes, oxidative stress indicator (MDA), pigments and anatomical variations. Mangroves in Jamnagar district cover an area of around 159 sq km [1] with multiple anthropogenic activities like industrial cluster, salt pans and urban centers. In Gujarat, studies on physiology of mangroves are lacking and the present study is the first attempt to record the variations in the physiology and anatomy of *Avicennia marina* in the Gulf of Kachchh along the Jamnagar coastline.

Study area:

The study area lies in the southern coast of the Gulf of Kutch from Okha to Rozibeyt dotted with industries ranging from fertilizer plant to refinery and cement plant. The description of seven different sites selected is as follows:

Station 1 (S-1): Okha: is situated near the Okha port with high tourism and fishing activity. There is high vehicular movement in the region due to presence of salt pans.

Station 2 (S-2): Pindara: The area lies in bay and surrounded by the rocky beach and calcareous layers of the mollusks. There is bauxite-mining activity in the nearby area.

Station 3 (S-3): Salaya: is a small port town with high fishing activity. The area has high anthropogenic activity, there is dumping of solid waste, and fish waste in the coastal region.

Station 4 (S-4): Near Dhani: it is a creek area with good density mangrove density. There are salts pans in the area that release high brine.

Station 5 (S-5): Narara Beyt: lies within the Marine National Park, with dense mangrove cover and diversity also.

Station 6 (S-6): Sikka: This area is highly urbanized and major industries present are cement industry, Thermal Power plants, petrochemical industries, fertilizer industry etc.

Station 7 (S-7): Rozy beyt: There are two jetties at this station handling coal transportation.

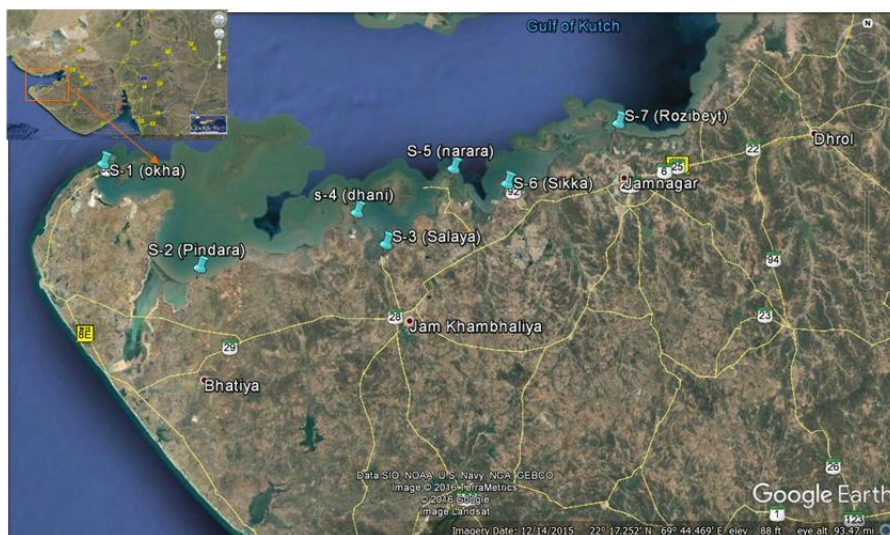


Figure 1: Location map

Method and methodology

Fresh leaves collected in triplicates from *Avicennia marina* from all the locations in three different seasons i.e. pre-monsoon, monsoon and post monsoon. Individual with similar girth at breast height (GBH) selected to elucidate errors related to difference in age of the plants. Further, 3rd leaves from the each branch tip was sampled from all the stations. The leaves washed thoroughly under water before the fresh analysis performed. The leaves collected from the site were analyzed immediately for chlorophyll and antioxidant study.

Standard methods were followed for analysis; chlorophyll was estimated using DMSO method [15], Ascorbic acid was determined using Roe [16] method, total Glutathione content (GSH) as per Patterson & Lazarow method [17] and Peroxidase (POD) following Shanon et al, [18].

The leaves were oven dried in microwave at 48⁰ C for further dry analysis. Lipid peroxidation (MDA) content determined following Heath and Packer method [19].

Seasonal sampling was done at all the sites for all the parameters; however the *t*-test analysis showed low significant value and therefore for the purpose of the present paper the average value of all the three seasons was considered. Statistical analyses performed using PAST and SPSS software.

Table 1: Variations in the different parameters in the study area

Station no.	TCHL (mg/g F.W)	POD units/min/mg FW	GSH mg/g FW	AA mg/g FW	MDA mg/g DW
S-1	1.48±0.001	20.47±0.1	73.45±0.1	42.55±0.1	154.53 ±1
S-2	0.97±0.001	18.84±0.1	102.68±0.2	40.60±0.1	216.55±3
S-3	1.74±0.001	21.77±0.2	70.81±0.1	41.37±0.1	185.05±2
S-4	1.37±0.001	25.80±0.1	89.39±0.1	52.26±0.1	181.68±1
S-5	0.86±0.001	24.23±0.1	77.25±0.1	49.52±0.2	149.52±1
S-6	1.03±0.002	25.91±0.1	60.54±0.1	52.27±0.1	206.58±2
S-7	1.08±0.001	22.38±0.1	97.44±0.1	56.26±0.1	168.27±1

RESULT AND DISCUSSION

The total chlorophyll values in the leaves varied from 0.86 to 1.78 mg/g fresh weight for all the sites. Similar results were observed in the seedlings under controlled conditions in *A.marina* (1.75 mg/g FW) [15]. The total Chlorophyll was high in S-3 and low at S-5. The total chlorophyll in case of 1.5 year seedlings of *A.officinalis* reported was 0.46 mg/g dry wt. [8]. However, in present study the sampled trees represented more than 10- 15 years old and the low chlorophyll value at S -5 indicates presence of stress condition.

POD activity is related to detoxification of oxidative stress in mangroves. Study conducted in Sundarban mangroves where POD activity was recorded indicated tolerance of *A. marina* to high salinity as compared to *Heriteria fomes*. The POD activity in *A.marina* was 8.83 units/mg of protein/min [21] whereas, in the present study it ranged from 18.84 to 25.91 units/min/mg FW, indicating presence of oxidative stress in the plants. Further, the high salinity regimes and the arid conditions prevalent in the study area induced generation of free radicals and high production of radicals scavengers like POD and GSH. The salinity varied from 43.8 ppt to 33.4 ppt observed at Vadinar station during the annual monitoring done in 2011 [22]. Ascorbic acid (AA) content in the present study was reported high at S-7 (40.66) and S-6 (56.26 mg/g FW). Ascorbic acid plays a key role in salt tolerance in many halophytes [23]. Cheesman et, al [24] experimentally showed ascorbate and POD activities in the field grown mangroves. Similar results were found in present study.

Total Glutathione content in the present study ranged from 102 to 60.54 mg/g FW with higher value in S-2 and lowest in S-6. Lower GSH activity was observed in other studies [25]. In plants, Glutathione reductase and POD were considered to be associated with a number of essential metabolic processes, such as cell elongation, lignification, phenolic oxidation, pathogen defense and defense against stress [26, 27]. The glutathione antioxidant system is one of numerous protective mechanisms against hydroperoxides. Glutathione peroxidase (GPx) is an antioxidant enzyme that catalyses the reduction of hydroperoxides employing the glutathione (GSH) pool, thereby protecting the cell against oxidative damage [28]. The presence of higher glutathione content indicates stress condition at site 2 followed by S-7. There is coal handling in the jetties at S-7, which could have accounted for higher oxidative stress in the tissues.

Malondialdehyde (MDA): In the present study high MDA levels were reported from S-2 (216 mg/g DW) to S-6 (206 mg/g DW) indicating presence of oxidative stress condition and damage to internal tissue. Low MDA was reported from S-5(149.52 mg/g DW). As the peroxidation product of membrane lipid, MDA is a reliable indicator of membrane injury caused by ROS [29]. Y. Peng et al, [30] reported increase in MDA in *A.marina* due to chilling stress which indicates the membrane injury. It may result in the breakdown of chlorophyll. In the present study also there is increase in MDA levels in S6 with simultaneous low value of chlorophyll indicative of damage to chloroplast cells due to high oxidative stress. S-6 is located close to industrial cluster and release of air pollutants could have an impact on the site.

Mangroves are vulnerable to oxidative stress and damages due to increase in salts or heavy metal contamination therefore; these plants have higher antioxidant and phytochemicals which prevent plant against oxidative stress [21].

The cluster analysis of all the parameters showed that the response of *A.marina* in different sites is clustered into two categories A and B. Category A is further clustered into A1, A2 and A3. A1 includes sites S-3 and S-6 both of which are closed to industrial setup. A2 includes the sites S-4 and S-7 showing impact due to transportation of coal and presence of salt pans. A3 include s-1 and S-5 which have high human disturbances in the form of tourism activities.

Category B behaved distantly from Category A due to its location in the bay with low tidal activity.

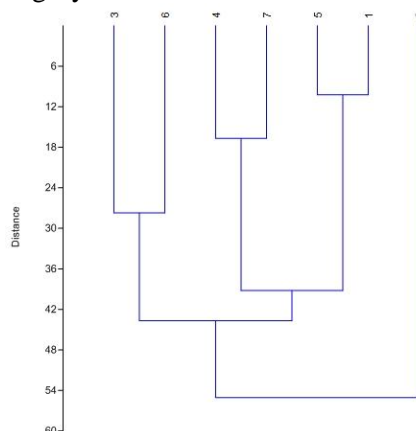


Figure 2: Dendrogram of the loctation

Table 2: Correlation among the parameters

	Correlations				
	CHL	POD	GSH	AA	MDA
CHL	1				
POD	-.144	1			
GSH	-.290	-.441	1		
AA	-.411	.731*	.048	1	
MDA	-.107	-.103	.141	-.198	1
*. Correlation is significant at the 0.05 level (1-tailed).					

CONCLUSION

From the above analysis, it is concluded that the mangrove at S-6, S-2 were under more stress as compared to that present at S-5 and S-1. The stress indicator MDA showed higher values at station S-6 and low levels of chlorophyll indicating damage to the cell membrane. The mangroves present at station with high anthropogenic activities and industrial showed more damages as compared to the other stations. Further, studies to relate the damages through the anatomical features of the leaves are required to understand the level of pollutant uptake.

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REFERENCES

[1] FSI, 2015. Mangrove cover, India state of forest cover, pp 64-67.

[2] Shah, D.G., Bahuguna, A., Deshmukh, .B, Nayak, S.R., Singh, H.S. and Patel, B.H., 2005. Zoning and monitoring dominant mangrove communities of a part of the marine national park, gulf of Kachchh. Journal of Indian society of remote sensing, vol.33 (1) 155-162.

[3] GEC, 2012. Mangrove Restoration through Public-Private Partnership, Gujarat. Gujarat Ecological Ccommisson, Gandhinagar. Pp. 86

- [4] Sawale, A.K. and Thivakaran, G.A., 2013. Structural characteristics of mangrove forest of Kachchh, Gujarat. *J. Mar. Biol. Ass. India*, 55 (1), 5-11.
- [5] Panda, A. K.; Sastry, V. R. B.; Kumar, A.; Saha, S. K., 2006. Quantification of karanjin, tannin and trypsin inhibitors in raw and detoxified expeller and solvent extracted karanj (*Pongamia glabra*) cake. *Asian-Aust. J. Anim. Sci.*, 19 (12): 1776 – 1783
- [6] Sivasankaramoorthy S., 2012. Salinity Tolerance in Some Mangrove Species from Pitchavaram (Tamil Nadu). *International Journal of Bioassays*, 01(10), pp.86–90.
- [7] Mitra A, Zaman S, Pramanick P, Bhattacharyya, S.B., and Raha, A.K.,2014. Stored Carbon in the dominant Seaweeds of Indian Sundarbans, *Pertanika. J. Trop Agric Sci.* 37 (2), pp.263-275.
- [8] Basak, U.C., Das, A.B. and Das, P., 1996. Chlorophylls, Carotenoids, Proteins and Secondary Metabolites in Leaves of 14 Species of Mangrove. *Bulletin of marine science* 58 (3), pp.654-659.
- [9] Harbison, P., 1986. Mangrove muds—A sink and a source for trace metals. *Mar. Pollut. Bull.* 17, pp.246–250.
- [10] Tam, N.F.Y., Wong, Y.S., 1999. Mangrove soils in removing pollutants from municipal wastewater of different salinities. *J. Environ. Qual.* 28, pp.556–564.
- [11] MacFarlane, G.R., Koller, C.E., Blomberg, S.P., 2007. Accumulation and partitioning of heavy metals in mangroves: a synthesis of field-based studies. *Chemosphere* 69, pp.1454–1464.
- [12] Hasanuzzaman M, Fujita M. 2013. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system *Ecotoxicology*, 22 (2) 7
- [13] Mantri, N., Patade, V., Penna, S., Ford, R., Pang, E., 2012. Abiotic stress responses in plants: present and future. In *Abiotic stress responses in plants: metabolism, productivity and sustainability*. Edited by: Ahmad P, Prasad MNV. New York: Springer; pp.1–19.
- [14] Hasanuzzaman, M., Nahar, K., Alam, M.M, Fujita M., 2012. Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. *Australian journal of crop sciences*, 6(8), pp.1314-132.
- [15] Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ Exp Bot* 32, pp.83–100
- [16] Roscoe, R.S. and George R. K. 1954. A Rapid, Simple Method for the Determination of reduced, dehydro- and total ascorbic acid in biological material *J. Biol. Chem.*, 212, pp.59-68.
- [17] Patterson, J.W. and Lazarow, A., 1955. *Methods of Biochemical Analysis*. Glick D. (ed). New York, Inter Science, 2, pp.259
- [18] Shannon, L.M., Key, E., Lew, J.Y. 1966. Peroxidase isozyme from Horseradish root. I. Isolation and physical properties. *J Biol Chem*, 249(9), pp. 2166–2172
- [19] Heath, R.L. and Packer, L., 1968. Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.* 125, pp. 189–198.
- [20] Yan, Z., Li X, Chen J, Yee Tamc NF. 2014. Combined toxicity of cadmium and copper in *Avicennia marina* seedlings and the regulation of exogenous jasmonic acid. *Ecotoxicology and Environmental Safety*, 113, pp. 124–132.
- [21] Kirankumar, M., Mounika, S.J., Ranjan, T.J.U., Rao, P.S. and Sandeep, B.V. 2014 Assessment of Biochemical, Phytochemical and Antioxidant Activities of Eight Mangrove Plant Leaf Extracts. *European academic research*, 2 (9), pp.11976-11991

- [22] Devi, V., Karthikeyan, K., Lekameera, R., Nandhagopal, Mehta, P.N. and Thivakaran, G.A. 2014 Water and sediment quality characteristics near an industrial vicinity, Vadinar, Gulf of Kachchh, Gujarat, India. *International Journal of Plant, Animal and Environmental Sciences*, 4 (3), pp. 219-226
- [23] Hameed, A., Gulzar, S., Aziz, I., Hussain, T., Gul B., Khan M.A. 2015. Effects of salinity and ascorbic acid on growth, water status and antioxidant system in a perennial halophyte. *Aob Plants* 7: plv004; doi:10.1093/aobpla/plv004
- [24] Cheeseman, J.M., Herendeen, L.B., Cheeseman, A.T., Clough BF1997. Photosynthesis and photoprotection in mangroves under field conditions. *Plant, cell and environment*, 20, pp. 579-588
- [25] Caregnato F.F., Koller, C.E., MacFarlane, G.G., Moreira, J.C., 2008. The glutathione antioxidant system as a biomarker suite for the assessment of heavy metal exposure and effect in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Marine Pollution Bulletin*, 56, pp.1119–1127.
- [26] Lewis, R. S., Ross, P. E. & Cahalan, M. D. (1993). Chloride channels activated by osmotic stress in T lymphocytes. *Journal of General Physiology* 101, pp. 801–826.
- [27] Passardi, F., Penel C. and Dunand C. (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends in Plant Science* 9, pp. 534-540
- [28] Halliwell B, Gutteridge JMC (1990). The antioxidants of human extracellular fluids. *Arch Biochem Biophys* 280, pp. 1–8
- [29] Bonnes, T.D., Guerin, M.C., Torreilles J (1992). Malonaldehyde available indicator of lipid peroxidation. *Biochem. Pharmacol.* 42, pp.985-988
- [30] Peng, Y.L., Wang, Y.S., Fei, J., Sun, C.C., Cheng, H. 2015. Ecophysiological differences between three mangrove seedlings (*Kandelia obovata*, *Aegiceras corniculatum*, and *Avicennia marina*) exposed to chilling stress. *Ecotoxicology*, vol. 24 (4). DOI 10.1007/s10646-015-1488-7

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