# Marine Environmental Monitoring using Biomarkers in Relevance to the Development of Ecological Indices of Gulf of Mannar Costal Region

N.Nagarani*, and M.Anand

***Abstract---***The purpose of this study was to explore a possible relationship between the distribution of inorganic metals and oxidative stress as an ecological index in the seaweeds of southeast coast of India. The distribution of elements and trace metals (phosphorus, nitrogen, lead, Manganese, Nickel, Mercury, Arsenic, Iron, Copper and Zinc) were determined in the marine plants and its surrounding seawater. An ICP-AES were used to determine the level of heavy metals. The impact of metals on the metabolism of the plants was determined by the level of antioxidant response. Mercury, lead and arsenic were below the detectable limit. Low levels of Ni, Cu were observed in the collected species. The TBARS, SOD, CAT, POX, GPx formation in the marine plants showed a positive correlation with the trace element accumulation in seaweeds. The result explored that these parameters can be used as biomarkers for the ecological indices in environmental monitoring.

***Keywords---***Biomarkers, Ecological indices, Gulf of Mannar, Heavy metals, ICPAES

## I. Introduction

OXIDATIVE stress biomarkers have been widely used for a fundamental approach in the assessment of ecosystem health and in the development of ecological indices. Biomarkers are early warning to exposure to contaminants in the environment which are result in long-term physiological disturbances. These pollutants induce reactive oxygen species and its accumulation can cause irreversible deleterious effects, including inactivation of antioxidant enzymes, depletion of nonenzymatic antioxidants, membrane lipid peroxidation, oxidation of DNA and other macromolecules, or even cellular death [1].

This study was, therefore, designed to investigate (i) the extent of heavy metals accumulated in seaweed species and (ii) to examine changes in anti-oxidant enzyme activities (SOD, CAT, GSH and GPX) and lipid peroxidation in relation to element accumulation. The ultimate focus of the present study is to examine if aquatic pollutants present in the selected coastal regions is generating biological responses in the marine algae. To accomplish this objective, biomarker responses were measured in the marine algal species collected along the coasts of Gulf of Mannar region.

Nagarani Nagarajan[1] is with the Department of Marine and Coastal Studies, Madurai Kamaraj University, Madurai-625021, Tamilnadu-India (corresponding author's phone: 91-9942502456 ; e-mail: nagaranikannan@yahoo.co.in).

Anand Muthusamy [2], was with Department of Marine and Coastal Studies,Madurai Kamaraj University, Madurai-625021. Tamilnadu-India. (e-mail: anandm21@yahoo.com.

## II. Materials and methods

### A. Sample Collection and Preparation

The fresh seaweeds Thalia was collected from Pudumadam coast, Gulf of Mannar, Tamilnadu, India during April 2014. Collected marine algal samples were immediately brought to the University Research laboratory in new plastic bags containing filtered sea water in ice (4°C). The thalli ($m$ = 15 ± 3 g wet phytomass, $N$ = 20) cleared from apparent epiphytes and epibionts with filtered seawater. Plants were washed thoroughly with tap water to remove epiphytes, sediments and shade dried for metal analysis stored at -80°C for further use.

### B. Determination of Metal concentrations of native marine algae

Perkin Elmer atomic absorption spectrophotometer model was used to analyse the concentration (lg/g) of heavy metals concentrations of lead, Manganese, Nickel, Mercury, Arsenic, Iron, Copper and Zinc were determined according to the standard double acid digestion methods analyzed using ICP-AES. Standards were made using certified solutions (Merck, UK) acidified with $HNO_3$ to the same pH as the samples. Results are expressed as the means ± S.E. of three replicate samples.

### C. Biochemical analysis

Frozen tissue was homogenized in ice-cold 0.1 M Phosphate buffer at pH 7.8 containing 1 mM EDTA, 1 mM β-mercaptoethanol and 5 ml of 4% polyvinylpyrrolidone/g FW. The homogenate was filtered through a nylon mesh and centrifuged at 20,000 g at 4°C. The supernatant was used for measuring enzyme activity. MDA activity was determined to indicate the level of lipid peroxidation of seagrass species as described by Buege and Aust [2]. The antioxidant enzyme response was measured using Double beam UV spectrophotometer (Model 2201; Systronics) following the methods viz., Catalase [3]; Superoxide dismutase [4]; Glutathione peroxidase [5]; Reduced glutathione [6] and Ascorbate peroxidase [7]. Data were analyzed through ANOVA followed by post-hoc mean comparisons test (Tukey's test). Significance level adopted was 95%. Results were expressed as mean ± standard error.

## III. Result and Discussion

The present paper deals with the ecological status of marine algae in the Gulf of Mannar region with reference to the anthropogenic activities of southeast coast of India. Physico-

http://dx.doi.org/10.15242/IICBE.C0115019 

chemical characteristics of water in study area are depicted in Table 1 and Figure 1. The surface water temperature varied from 30 to 32°C during the sample collection. Generally, surface water temperature is subjective to the natural parameters like, intensity of solar radiation, freshwater inflow etc [8]. The average dissolved oxygen level for polluted waters in the Gulf of Mannar was previously reported as 3.79 ml/l [8]. Phosphate and nitrate concentrations were less and nitrite was higher when compared to the previous report [9]. When this nitrite when enters the biological systems it can oxidize the antioxidants, which are significantly more toxic to aquatic life than nitrate [10].

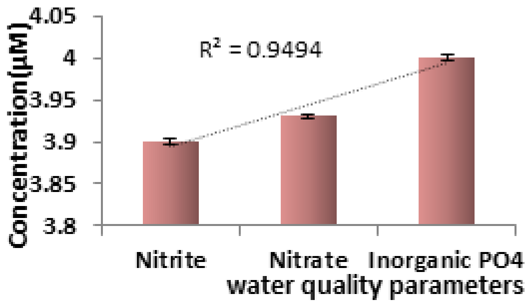


TABLE I
PHYSICO-CHEMICAL PARAMETERS OF PUDUMADAM COASTAL WATERS

| Temp (°C) | Salinity (ppt) | pH | DO (mg/L) | Turbidity |
|---|---|---|---|---|
| 32 | 32.9 | 7.85 | 3.93 | 6 |

The distribution of metal contents in the collected seaweed species reveals that seaweeds accumulates considerable amount of essential and non essential elements present in their environment (Table 2). It seems that the tendency of seaweed to accumulate the metals was exactly the same order in all the selected species, Fe > Mn > Cu > Zn > Ni > Pb > Hg/As. Cu and Zn concentrations of the seaweeds analyzed in the present study are less than maximum permissible limits prescribed for seafood for human consumption (10 mg/kg, 50 mg/kg respectively) in India (Food And Agriculture Organisation[11] .

Similar to the previous reports of Ragupathi Raja Kannan et al., [12] higher concentrations of biologically active or essential elements like Fe, Mn, Cu and Zn were noted than the level of biologically inactive or non-essential elements like Pb. Since, most of the metals play an important role in the biological metabolism, such as Fe (hemoglobin, SOD, CAT), Cu (respiratory pigments), Zn (SOD), Co (Vitamin B12), Mo and Mn (enzyme cofactor) [13]. Compared to the present study higher levels of Cu and lower level of Fe was reported in marine sponge *Haliclona tenuiramosa;* seaweed Cladophora *glomerata* [14] and higher concentration of Cu and Zn in *Amphiroa fragilissima*, low level of Fe and Mn than the present report [15]. The high Fe levels in the present study reveals the role of iron for normal growth of marine plants and biomagnifications or accumulation of iron from the surrounding environment.

Among the collected seaweed the accumulation of metals in the red algae was in the order of Fe > Ni > Cu; in green algae Mn > Zn and in brown algae Fe followed by Mn. Significant in the bioaccumulation of metals were observed among the three algae (P= 0.110939) Many metals are essential to living organisms but some of them are becoming toxic at high concentrations such as transition metals Fe, Cu, Co and Mn. In the present study the level of Hg, As and Pb were below the non-dectectabe range (< 0.01 ppm). Whereas the higher amount of Hg and Pb were reported in crab and shrimp [16]. Among the metals Pb, Hg, Ni, Se, Cr, As etc are generally not required for metabolic activity and produce reactive oxygen species which in turn are toxic to living organisms damaging lipid, DNA, protein at very low concentrations [1].

Usually plants encounters the oxidative stress upon exposure to pollutant or heavy metals. Which disturbs their cellular ionic homeostasis through their oxidative defense mechanisms such as enzymes, chelation etc. [13]. The metabolic activity of seaweeds in their native sites is given in table 3.

The reduced glutathione (GSH) was found to increase on Fe accumulation. Since GSH has a vital role in iron metabolism (Table 3). The increase in the reduced glutathione level in the present study may also due to the synthesis of metal chelator- phytochelations [17]. The increase in the levels of MDA as in *Dictyota dichotomo* and *Sargassum myriocytum* may be due to external physiological stress. Where as in Coral species the level of SOD was higher, GPx was lower and equal amount was reported earlier [18]. SOD increase in the seaweed was noted with higher concentration of Fe, Cu and Ni. Since Fe and Cu are cofactors of SOD isoforms. The occurrences of biochemical's changes in the seaweed species follow the order of SOD (Red > brown > Green). There are no significant changes in the levels of APX in the collected species.

TABLE II
DISTRIBUTION OF METALS IN SEAWEEDS (MG/KG)

| Seaweed species | Cu | Zn | Fe | Pb | Mn | Ni | Hg | As |
|---|---|---|---|---|---|---|---|---|
| **Brown algae** | | | | | | | | |
| *Sargassum wightii* | 0.06 | 0.112 | 87.1 | 0.1 | 0.278 | 0.02 | ND | ND |
| *Turbinaria conoides* | 0.01 | 0.12 | 102.2 | ND | 0.72 | 0.12 | ND | ND |
| *Sargassum myriocystum* | 0.12 | 0.13 | 65.23 | ND | 0.23 | 0.06 | ND | ND |
| *Dictyota dichotomo* | 0.11 | 0.01 | 45.32 | ND | 0.401 | 0.124 | ND | ND |
| *S. myriocytum* | 0.12 | 0.109 | 97.3 | ND | 0.192 | 0.08 | ND | ND |
| *Turbinaria conoides* | 0.09 | 0.1 | 99.23 | ND | 0.198 | 0.16 | ND | ND |
| **Green algae** | | | | | | | | |
| *Caulerpa taxifolia* | 0.23 | 0.09 | 92.3 | ND | 0.179 | 0.189 | ND | ND |
| *Codium arabicum* | 0.119 | 0.193 | 29.264 | ND | 0.984 | 0.254 | ND | ND |
| *Codium adhaerens* | 0.1339 | 0.185 | 13.891 | ND | 2.61 | 0.091 | ND | ND |
| *Chaetomorpha antenna* | 0.03 | 0.01 | 43.23 | ND | 1.92 | 0.05 | ND | ND |
| **Red algae** | | | | | | | | |
| *Gracilaria corticata Var. corticata* | 0.18 | 0.02 | 92.3 | ND | 1.02 | 0.12 | ND | ND |
| *Centroceras clavulatum* | 0.13 | 0.12 | 76.3 | ND | 0.99 | 0.76 | ND | ND |
| *G. edulis* | 0.212 | 0.065 | 87.02 | ND | 0.98 | 0.98 | ND | ND |
| *G.corticata* | 0.248 | 0.009 | 73.2 | ND | 1.22 | 0.75 | ND | ND |

TABLE III
BIOCHEMICAL RESPONSE OF SEAWEEDS IN PUDUMADAM COASTAL REGION

| Seaweed species | **LPO** (µmole/Cm) | **CAT** (U/mL) | **GSH** (µM) | **GPx** (U/mL) | **SOD** (U/mL) | **APX** (mM/cm) |
|---|---|---|---|---|---|---|
| **Brown algae** | | | | | | |
| *Sargassum wightii* | 6.22 ± 0.11 | 2.34 ± 0.21 | 33.1 ± 1.61 | 0.156 ± 0.01 | 1.26 ± 0.07 | 0.0049 ± 0.003 |
| *Turbinaria conoides* | 5.46 ± 0.79 | 2.57 ± 0.05 | 54.2 ± 1.93 | 0.348 ± 0.01 | 4.59 ± 0.04 | 0.044 ± 0.001 |
| *Sargassum myriocystum* | 11.62± 0.25 | 0.88 ± 1.4 | 39.9 ± 1.57 | 0.073 ± 0.005 | 1.78 ± 0.02 | 0.084 ± 0.012 |
| *Dictyota dichotomo* | 316.75 ± 0.46 | 2.26 ± 0.02 | 46.8 ± 0.72 | 0.054 ± 0.002 | 3.46 ± 0.015 | 0.089 ± 0.007 |
| *S. myriocytum* | 207.9 ± 0.53 | 0.23 ± .001 | 281.7 ± 0.87 | 0.056 ± 0.03 | 3.87 ± 0.09 | 0.21 ± 0.002 |
| *Turbinaria conoides* | 84.87 ± 0.02 | 1.88 ± 0.05 | 636.9 ± 0.44 | 0.059 ± 0.02 | 3.72 ± 0.013 | 0.29 ± 0.002 |
| **Green algae** | | | | | | |
| *Caulerpa taxifolia* | 52.75 0.07 | 2.16 ± 0.03 | 70.51 ± 0.01 | 0.539 ± 0.003 | 1.58 ± 0.005 | 0.013 ± 0.001 |
| *Codium arabicum* | 22.3 ± 0.8 | 1.52 ± 0.06 | 81.44 ± 0.89 | 0.225 ± 0.001 | 1.29 ± 0.02 | 0.14 ± 0.001 |
| *Codium adhaerens* | 35.99 ± 0.04 | 0.52 ± 0.08 | 356.9 ± 0.05 | 0.546 ± 0.03 | 0.34 ± 0.04 | 0.018 ± 0.011 |
| *Chaetomorpha antenna* | 34.2 ± 0.002 | 0.75 ± 0.01 | 229.1 ± 0.2 | 0.159 ± 0.01 | 3.49 ± 0.15 | 0.68 ± 0.002 |
| **Red algae** | | | | | | |
| *Gracilaria corticata Var. corticata* | 21.67 ± 0.02 | 4.96 ± 0.03 | 43.31 ± 0.002 | 0.29 ± 0.002 | 1.38 ± 0.19 | 0.032± 0.004 |
| *Centroceras clavulatum* | 34.92 ± 0.006 | 0.29 ± 0.01 | 143.6 ± 0.05 | 0.059 ± 0.02 | 4.56 ± 0.11 | 0.133 ± 0.012 |
| *G. edulis* | 34.96 ± 0.011 | 2.09 ± 0.03 | 168.9 ± 0.001 | 0.87 ± 0.01 | 3.57 ± 0.19 | 0.283 ± 0.005 |
| *G.corticata* | 89.26 ± 0.02 | 1.58 ± 0.05 | 24.03 ± 0.02 | 0.096 ± 0.02 | 4.04 ± 0.03 | 0.001176 ± 0.01 |

## IV. CONCLUSION

Seaweeds are nowadays widely used as in situ indicators of marine ecosystem health. There is a growing concern that the elements through the natural cycling process are being disturbed by anthropogenic activities, especially the exploitation of resources, the marine biodiversity of the Gulf of Mannar is facing the danger of shrinking. There are number of fisheries industries located at Mandapam, Ramnad which discharge the wastes into the sea. Ultimately, these growth of industrial, domestic and urban discharge of its effluents. From the present study, we conclude that SOD can be used as the bookmaker for Ni, Fe contamination. Red and brown algae can be used as bioindicators for pollution than green algae. At the same time due to over

studies must focus on measuring levels of pollution that may induce irreversible ecological changes to aquatic ecosystems Till now the level of toxicity were moderate and it was progressing towards the danger. Efforts can be made to control the activities that release pollutants unnaturally into the environment. Efforts can be made to maintain and control the activities that release pollutants unnaturally into the environment from both public and government so that the clean and clear environment can be maintained.

## ACKNOWLEDGMENT

The authors would like to thank the Council of Scientific and Industrial Research, India for financial support to complete the work.

## REFERENCE

[1] N.Nagarani, V. Janakidevi, C. Archana Devi, and A.K. Kumaraguru, Genotoxicity assessment of mercuric chloride in the marine fish *Therapon jarbua*. *Environment Asia* Vol. 2, 2009, 50–54.

[2] J.A.Buege, and S.D. Aust, S.D, Microsomal Lipid Peroxidation: *Methods in Enzymology* 52, 1978, 302–310. http://dx.doi.org/10.1016/S0076-6879(78)52032-6

[3] T. Matsumura, N. Tabayashi, Y. Kamagata, C. Soum, and H. Saruyama, Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress. *Physiol. Plant*. 116, 2002,317-327. http://dx.doi.org/10.1034/j.1399-3054.2002.1160306.x

[4] A. Calatayud, J.W. Ramirez, D.J. Iglesias, and E. Barreno, Effects of ozone on photosynthetic CO2 exchange, chlorophyll *a* fluorescence and antioxidant system in lettuce leaves. *Physiol. Plant.* 116, 2002, 308-316. http://dx.doi.org/10.1034/j.1399-3054.2002.1160305.x

[5] A.F.Boyne, and G.L. Ellman, "A Methodology for Analysis of Tissue Sulphydryl Components." *Analytical Biochemistry* 46, 1972, 639–653. http://dx.doi.org/10.1016/0003-2697(72)90335-1

[6] J.T.Rotruck,A.L. Pop, H.E.Ganthe, H.E, A.B. Swanso, D.G. Hafema, and W.G. Hoekstra, Selenium, biochemical role as a component of glutathione peroxidase. *Science* 22, 1973,588–590. http://dx.doi.org/10.1126/science.179.4073.588

[7] Y.Nakano, and K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 1981, 867-880.

[8] P.K.Bindu Sulochanan, D. Krishnakumar, P. Prema, K.K. Kaladharan, G.S. Valsala, G.S. Bhat and K. Muniyandi. Trace metal contamination of the marine environment in Palk Bay and Gulf of Mannar. *J. Mar. Biol. Ass. India*, 49 (1), 2007, 12 - 18.

[9] T. Thangaradjou, and L. Kannan, Nutrient characteristics and sediment texture of the seagrass beds of the Gulf of Mannar. *Journal of Environmental Biology*.28 (1), 2007, 29-33.

[10] WHO. Nitrate and Nitrite in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality. WHO Press, Switzerland. Page no 1- 23, 2011.

[11] FAO.World Food Security: a Reappraisal of the Concepts and Approaches. Director Generals Report, Rome, 1983.

[12] R. Ragupathi Raja Kannan, R. Arumugam, and P. Anantharaman, Chemometric Studies of Multielemental Composition of Few Seagrasses from Gulf of Mannar, *India. Biol Trace Elem Res*. 143, 2011, 1149–1158. http://dx.doi.org/10.1007/s12011-010-8911-y

[13] P.C.Nagajyoti,K.D. Lee, and T.V.M. Sreekanth, Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett*. 8, 2010, 199–216. http://dx.doi.org/10.1007/s10311-010-0297-8

[14] K. Murugasen, and S.R. Harish, Antioxidant modulation in response to heavy metal induced oxidative stress in *Cladophora glomerata*. *Indian J Exp Biol*. 45(11), 2007, 980-983.

[15] S.Narasimman, and K. Murugaiyan, Proximate and Elemental composition of *Amphiroa fragilissima* (Linnaeus) Lamouroux from Tharuvaikulam coast, Gulf of Mannar region, South-East coast of Tamil Nadu. *Int. J Rese. Bot*. 3(3), 2013, 44-47.

[16] M. Hosseini, M. Daryashekan, M. Kashefi, and F.A.Monikh, Level of Cd, Hg, Mn and Pb in sediment and invertebrate of North Persian Gulf. *Indian journal of Geo-marine Sciences*. Vol 43(4), 2014, 561-563.

[17] S.K.Yadav, Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S Afr J Bot*.76, 2010, 16–179. http://dx.doi.org/10.1016/j.sajb.2009.10.007

[18] R. Anithajothi, K. Duraikannu, G. Umagowsalya, and C. M. Ramakritinan The Presence of Biomarker Enzymes of Selected Scleractinian Corals of Palk Bay, Southeast Coast of India. *BioMed Research International* Volume 2014, , 6 pages