

## **Determination of Acetylcholinesterase activities in Marine gastropod (*Morula granulata*) as a Biomarker of neurotoxic contaminants along the Goan Coast**

**A. Sarkar, Priyanka M. Tegur, Sutapa Jana and D. P. Rao**

Marine Pollution Assessment and Eco-toxicology Laboratory,  
National Institute of Oceanography, Dona Paula, Goa.

### **Abstract**

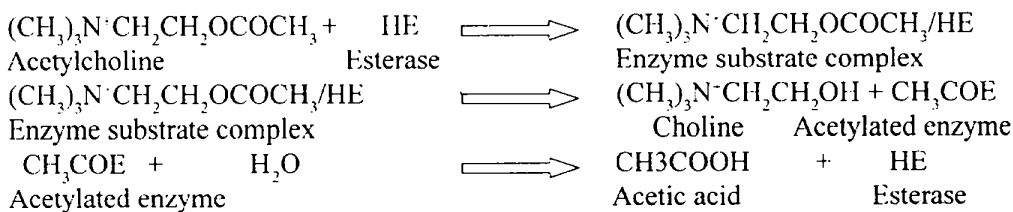
Acetylcholinesterase (AChE) is an enzyme that degrades the neurotransmitter acetylcholine, producing choline and acetate. group. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission. The presence of neurotoxic compounds result in measurable inhibition of this enzyme. As a result, acetylcholine is accumulated, there by nervous transmission is interrupted. Therefore, it leads to several diseases or even death. Hence it is considered as a suitable biomarker for detecting environmental pollution caused by the neurotoxic compounds. We have carried out experiments with marine gastropods (*Morula granulata*) at the selected sites, Arambol, Sinquerim, Velsao along the Goan coast. The variation of AChE activities along the Goa coast was assessed in comparison with the value of AChE at Betul which was considered as reference site with AChE activity (143.44 nmol/mg/min). The results showed that the highest AChE activity was observed at Sinquerim site (97.85 nmol/mg/min) and the lowest activity at Arambol site (63.66 nmol/mg/min) and the average AChE activity was found at Velsao site (73.22 nmol/mg/min). Thus on the basis of the variation of AChE activities in marine gastropods the contamination of the coastal environment can be evaluated for biomonitoring of pollution by neurotoxic compounds.

### **Introduction:**

The marine environment is under constant stress due to the contamination by highly persistent organic pollutants such as polychlorinated biphenyls, polychlorinated dibenzo dioxins, polyaromatic hydrocarbons, organochlorine pesticides (eg. DDT, hepox, endrine etc) because

of extensive shipping activity, riverline runoffs, industrial discharge, drainage of municipal sewage etc. There are countless number of anthropogenic chemicals and radiation that has become the main cause of marine pollution .As the marine organisms thrive in this polluted water, whose percentage of toxicity is increasing day by day,due to constant deposition of harmful chemicals.Because of this the organisms have slowly started accumulating these xenobiotic toxins and neurotoxic compounds in these tissues that are able cause damage to the physiological status of organisms,thus altering the population dynamics and it create trans generational effects. Thus, Biomarkers have been employed in ecotoxicological studies to assess the negative effects of contaminants in aquatic organisms, gastropods in particular and to define environmental quality. The gastropods are important components of many ecological communities, consuming vast amounts of invertebrates and in turn providing an abundant food source for many other predators, and thus it is necessary to assess the amount of contamination in the marine ecosystem that is causing damage to marine life. . Among the biomarkers ,the enzyme acetylcholinesterase(AChE) is considered as a suitable biomarker for detecting environmental pollution caused by the neurotoxic compounds such as organophosphates, carbamate and heavy metals(Galgani and Bocquenc, 2000).. Since AChE plays a important role in the functioning of the neuro-muscular system,also has a high catalytic activity and is responsible for signal termination at cholinergic synapse by rapid hydrolysis of neurotransmitter, acetylcholine. The presence of nucrotoxic compounds result in measurable inhibiton of this enzyme.As a result, acetylcholine is accumulated, the nervous transmission is interrupted, which leads to serious dysfunction tetany, paralysis and even death.(J.V. Rao et al 2005, S.Bonacci et al 2006, Engenheiro F.L et al., 2005)In different field of studies variations in AChE activity of marine gastropod have been observed.(Sarkar A et al.,2006).

The reaction of Mechanism: The mechanism of the interaction of acetylcholinesterase with acetylcholine can be explained by the following equation.



**Materials and Methods:**

In order to measure the seasonal variation of AChE activities in marine snails (*Morula granulata*), we collected the samples from selected sites Arambol, Sinqerim and Velsao along the Goa coast during monsoon season.The organisms are indentified with reference organisms from zoological survey of India. The selection of this particular species of marine snails is that they are considered as very good sentinel or indicator organisms and was available in all the sampling sites throughout the year.

### Taxonomy about *Morula granulata*

Kingdom: *Animalia*

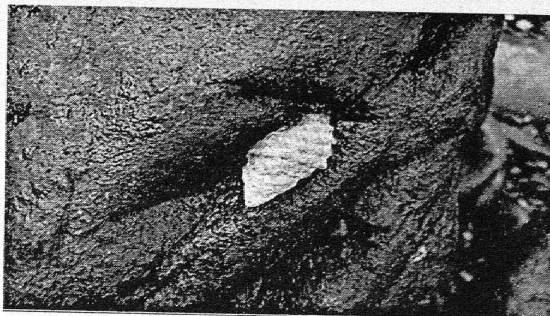
Phylum: *Mollusca*

Class: *Gastropoda*

Order: *Caenogastropoda*

Family: *Muricidae Rapaninae*

Genus: *Morula* Species: *granulata*



#### Isolation of AChE enzyme :

After collection of the snails (*Morula granulata*), they are kept in boxes containing their respective sea water from the respective sites. Enzymes are isolated by breaking the shells and whole tissues are weighed and kept in ice for maintaining the temperature at  $-4^{\circ}\text{C}$ . To each homogenate tube about 1g of tissue is added along with 1ml of phosphate extraction buffer (0.01M, pH 7.4), and 1ml of sucrose (0.25), followed by  $50\mu\text{L}$  of Triton X-100 (0.2%). The phosphate buffer and sucrose aim to extract enzyme from tissue and the Triton X-100 is used for the cell lysis. Each tube is subjected to homogenization at an interval of 30 seconds (Homoginizer-Ultra Durax). Then, the whole homogenate are centrifuged by refrigerated centrifuge (RC 4100 D) at  $-4^{\circ}\text{C}$  and for 30min at a speed of 8000rpm. After the process of centrifugation, the supernatant is transferred in to the vials and stored at  $-20^{\circ}\text{C}$ . The protein concentration of each of the sample extract was determined according to Lowry's method (1951) using bovine serum albumin as the standard.

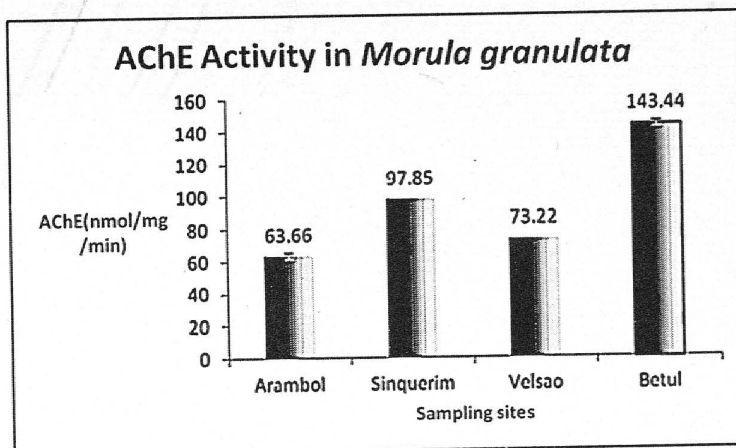
#### Determination of AChE activity:

The acetylcholinesterase (AChE) enzyme activity induces an acetic acid release in the solution; variations in the pH are directly related to its activity. AChE activity was measured by the delta-pH metric method (Sarkar et al, 1992) in which 0.1 ml of enzyme sample was incubated with 0.2 ml of acetylcholine bromide substrate at room temperature for one hour in a phosphate buffer medium solution (0.01M, pH  $8.00 \pm 0.20$ ). This last solution was also used as an indicator (bromothymol blue) in order to see the kinetics of enzymatic activity (yellow below pH 6.0 and blue above 7.6). The pH was recorded every 10 minutes for one-hour period. The difference of pH between the last and the first values is directly related to the acetic acid amount due to AChE activity. AChE activity is expressed in nmol of acetic acid released per minute per milligram of protein.

#### Results:

The following table shows the results of AChE enzyme activity measured in terms of variation in pH values ( $\Delta\text{pH}$ ) as the time increases. The enzyme activity is given in nmol/mg/min

Sl.no	Site	Sample	AChE(nmol/mg/min)	Std dev
1	Arambol	<i>Morula granulata</i>	63.65562	1.818732
2	Sinquerim	<i>Morula granulata</i>	97.85309	0.161385
3	Velsao	<i>Morula granulata</i>	73.21859	0.089619
4	Betul	<i>Morula granulata</i>	143.441	1.933168



Above graph it clearly shows that the AChE activity in *Morula granulata* varies accordingly along the goa coast. The AChE activity was found to be highest in *Sinquerim* site (97.85nmol/mg/min) and lowest in Arambol site (63.66nmol/mg/min). and average activity is seen velsao site(73.22nmol/mg/min) as compared to the standard site Betul (143.44nmol/mg/min). Betul is considered as standard due to its pristine environment. Hence it shows clearly that Arambol site is highly polluted & Velsao is moderately polluted and sinquerim site is less polluted according to the standard, hence it clearly substantiate the fact that the coastal regions are highly contaminated.

#### Conclusion:

The results obtained clearly shows that the coastal regions are polluted at a different levels and the effects can be seen by the molecular biomarker studies. The variation in the AChE activity in marine organisms at the selected sites clearly indicates the impact of pollutants on the marine environment. This provides an insight into the state of pollution along the coastal regions.

#### Acknowledgement:

We are thankful to the Director, NIO for his encouragement and interest in this ecotoxicological research and to the CSIR for all sorts of financial and infrastructural facilities and i acknowledge the help and cooperation of the technical staff of NIO at the DTP section.

## References:

1. Sarkar, A., 1992. Evaluation of the toxicity of organic matter in marine sediments. *Water science and technology* 25: 3, 255-257.
2. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951. *J. Biol.Chem* 193, 265.
3. Sarkar, A (2006a) Biomarkers of marine pollution and bioremediation. Guest editorial , *Ecotoxicology*, vol. 15, 331 – 332.
4. Engenheiro E.L., Hankard P.K., Sousa J.P., Lemos M.F., Weeks J.M., Soares A.M.V.M. Influence of dimethoate on acetylcholinesterase activity and locomotor function in terrestrial isopods. *Environmental Toxicology and Chemistry*, 2005, Vol. 24, No. 3, pp. 603–609.
5. Rao J.V., Begum G., Pallela R., Usman P. K., Rao R.N. Changes in Behavior and Brain Acetylcholinesterase Activity in Mosquito Fish, *Gambusia affinis* in Response to the Sub-Lethal Exposure to Chlorpyrifos. *Int. J. Environ. Res. Public Health*, 2005, 2(3), 478-483
5. Bonacci Stefano, Corsi Ilaria, Focardi Silvano. Cholinesterase activities in the adductor muscle of the Antarctic scallop *Adamussium colbecki*. *Antarctic Science* 2006, 18 (1), 15–22.
6. Bocquene, G.;Galgani,F.andTruquet, P.:Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. *Marine Environmental Research*(1990), Volume30,