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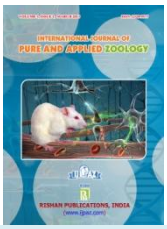

RESEARCH ARTICLE **OPEN ACCESS**

SEASONAL CHANGES ON GLUTATHIONE S-TRANSFERASE ACTIVITIES IN *PERNA VIRIDIS* ON DIFFERENTLY POLLUTED AREAS ALONG THE SOUTH EAST COST OF INDIA

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ABSTRACT

The health of an organism depends on its self protective ability. The regulatory enzyme, biomarkers measurable changes have been used as an effective early warning tool in ecological risk assessment. In order to develop an integrated risk assessment strategy for the South east coast of India. The green mussels Perna viridis were collected from three sites along the southern east coast with different Polyaromatic aromatic hydrocarbons (PAHs) contaminant characteristics. PAHs contents associated responses of detoxification mechanisms evaluated by measuring GST. GST was determined on different tissues (gills, digestive gland, foot) of Perna viridis. The summer showed higher enzyme activity of GST.In the organs wise reaction, the liver showed higher enzyme activity when compared to gill and foot in the enzyme of GST. In the case of three stations from the east coast, The station -1 (Rayapuram harbor) showed a higher enzyme elevation when compared to station-2 (Rayapuram off the coast) and 3 (Parangipettai).

Keywords: GST, PAHs, seasonal changes, *Perna viridis*, microsome, green mussel, East coast of India.

INTRODUCTION

Polycyclic aromatic hydrocarbons(PAHs) are a group of chemicals that are formed during the incomplete burning of coal, oil and gas, garbage or other organic substances.Glutathione S-transferase represent a complex grouping of proteins . GST may play an important role in detoxifying strong GST isozymes posses the various activities and participate in several types of reactions . Glutathione S-transferase (GST) catalyze the conjugation of glutathione with xenobiotic compounds containing electrophilic centers. Arene oxides produced by the action of cytochrome P-450 systems on aromatic compounds can be conjugated to glutathione by GST. Most of this enzyme can catalyse the conjugation of reduced glutathione with compounds that contain an electrophilic centre through the formation of thioethar bond between the sulphur atom of GSH and the substrate (Chasseaurd, 1979; Mannervik, 1985) in the cytosolic foreign compounds like carcinogen, PAHs, etc.,. The glutathione S-transferases (GST) represent a major group of detoxification enzymes (Hayes and Pulford, 1995). Glutathione-S-transferases (GSTs) are a multigene family of dimeric, polyfunctional enzymes that primarily catalyze the conjugation between electrophilic compounds and the tripeptide glutathione (GSH). GSTs have a wide distribution from bacteria to vertebrates (Stenersen *et al*., 1987) and have been classified in 7 different classes: mu (μ), pi (π), alpha (α), theta (θ), sigma (σ), kappa (κ), and xi (ξ).This

classification is based on substrate specificity, immunological cross-reactivity and protein sequences (Tomarev *et al*., 1993, Pemble *et al.*, 1996).The glutathione S-transferases (GST) represent a major group of detoxification enzymes. All eukaryotic species possess multiple cytosolic and membrane-bound GST isoenzymes, each of which displays distinct catalytic as well as noncatalytic binding properties. The catalytic foreign metabolites formed by phase I biotransformation are conjugated via phase II enzymes (e.g. GST) before the elimination and excretion. The aim of the present study represented the seasonal changes with GST response in *Perna viridis* on differently polluted areas along the southern east coast of India.

MATERIALS AND METHODS

Animal Selection and Collection in different seasons

During the study period (2005-2007), samples of water and biota were collected fortnightly, the data were pooled seasonally to understand the seasonal effect. The four distinct seasons were monsoon (October to December) post-monsoon (January to March), summer (April to June) and pre-monsoon (July to September) periods. Site Selection Sampling sites surveyed along the Southern east coast of India represented different oil contamination scenarios. Two sites were located in the Tamilnadu state capital city Chennai; (Station-1, Kasimedu fishing harbor, Rayapuram, at Chennai is heavily oil polluted and Station-2, about 3km offshore from the harbor, is moderately polluted). The least oil polluted site is Station-3, Vellar estuary, Paragipettai, Cuddalore District, which also supports a fishing harbor and was selected as the reference site. The experimental animals, the green mussel *Perna viridis* were collected fortnightly between January 2005 and December 2007, from each Station. Immediately after collection,mussels' length (mean 10.086±0.77 cm) and weight (mean 104±20.38 g) were measured. At least three animals were sacrificed for organ collection. Liver (hepatopancreas), gill (ctenidium) and foot (muscle) were dissected out, stored in Cryocane (liquid nitrogen), taken to the laboratory and analyzed within 24 h.

Microsomal preparation

The maximum of 1500 mg each of liver, gill and foot tissues were homogenized in two volumes of 0.1 M sodium phosphate buffer containing 2 mM glutathione and 1mM Ethylene-Diamine-Tetra Acetic acid (EDTA) tetrasodium salt, pH 6.5, using Polytron homogenizer (Mini Polytron, Switzerland). The homogenates were centrifuged at 12500 g for 20 min to remove cell debris and mitochondria. The supernatants were centrifuged for 75 min at 135000 g to sediment the microsomes. The microsomal pellets were resuspended with two volumes of 0.1 M phosphate buffer (pH 8.0) containing 0.15 M potassium chloride and 20% glycerol, then stored in liquid nitrogen container until analysis. Generally, GST was measured in freshly prepared samples. The cellular fraction protein concentration was determined by the method of Bradford (1976),all operations were performed at 4°C.

GST Assay

The standard assay mixute contained 0.1 M phosphate buffer pH 6.5 1 mM 1-chloro-2, 4, dinitrobenzene and 50µl of enzyme sources. The complete assay mixure without enzyme was used as a control. The rate of reaction was measured as the increase in absorbance at 340nm. The chemical rate of reaction determined in the absence of sample was subtracted from total rate. 1 µmol of GSH conjugated min^{-1} mg^{-1} protein was defined as one unit of GST glutathione used in scavenging hydroxyl radicals stimulated glutathione reductase activity (Habig *et al.*, 1974)

RESULT

Season depended activity of GST

The GST activity is higher during the summer seasons, moderate in pre and post monsoon and lower in monsoon period. The high enzyme elevation shown in liver than foot and gill. The GST activity also a tissue specific reaction. The higher concentration shown the higher enzyme level in liver than others, the foot and gill, within the seasons, the GST enzyme level is increased during summer (Figure 1) and very minimal activity monsoon (Figure 2) periods and moderate activity in during post and or pre monsoon period (Figures 3 and 4).

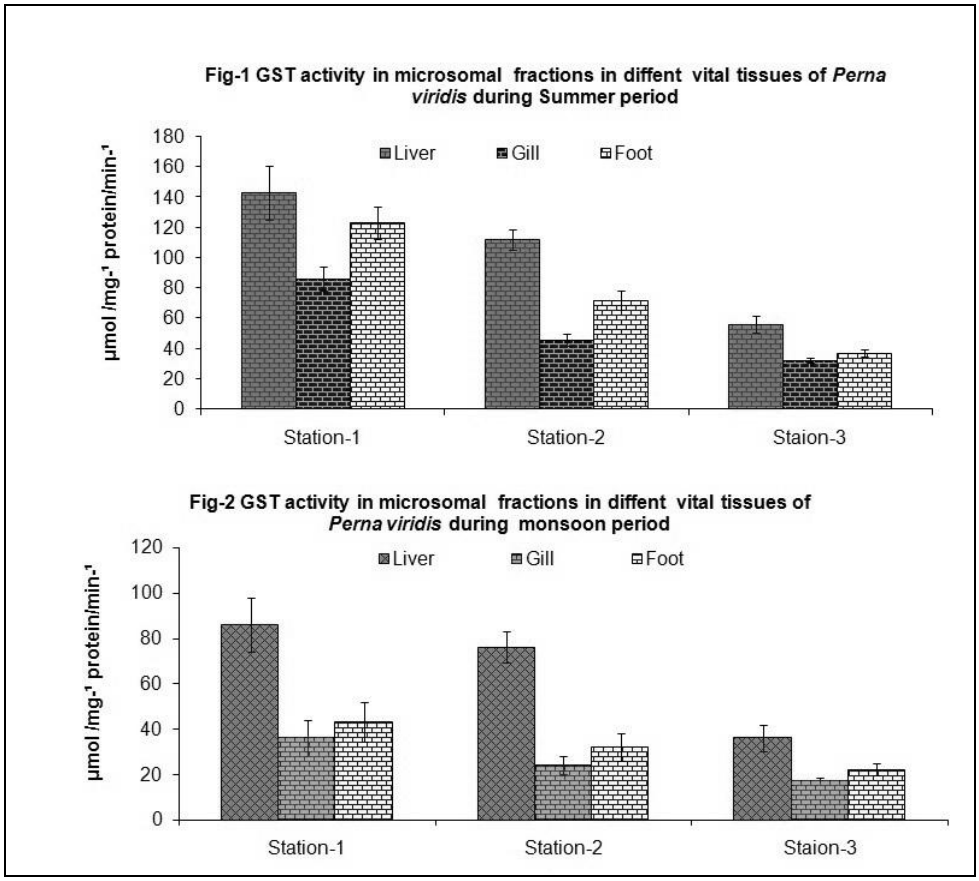
Fig-1 GST activity in microsomal fractions in diffent vital tissues of *Perna viridis* during Summer period
µmol /mg-¹ protein/min-¹
180
160
140
120
100
80
60
40
20
0
Liver
Gill
Foot
Station-1
Station-2
Staion-3
Fig-2 GST activity in microsomal fractions in diffent vital tissues of *Perna viridis* during monsoon period
µmol /mg-¹ protein/min-¹
120
100
80
60
40
20
0
Liver
Gill
Foot
Station-1
Station-2
Staion-3

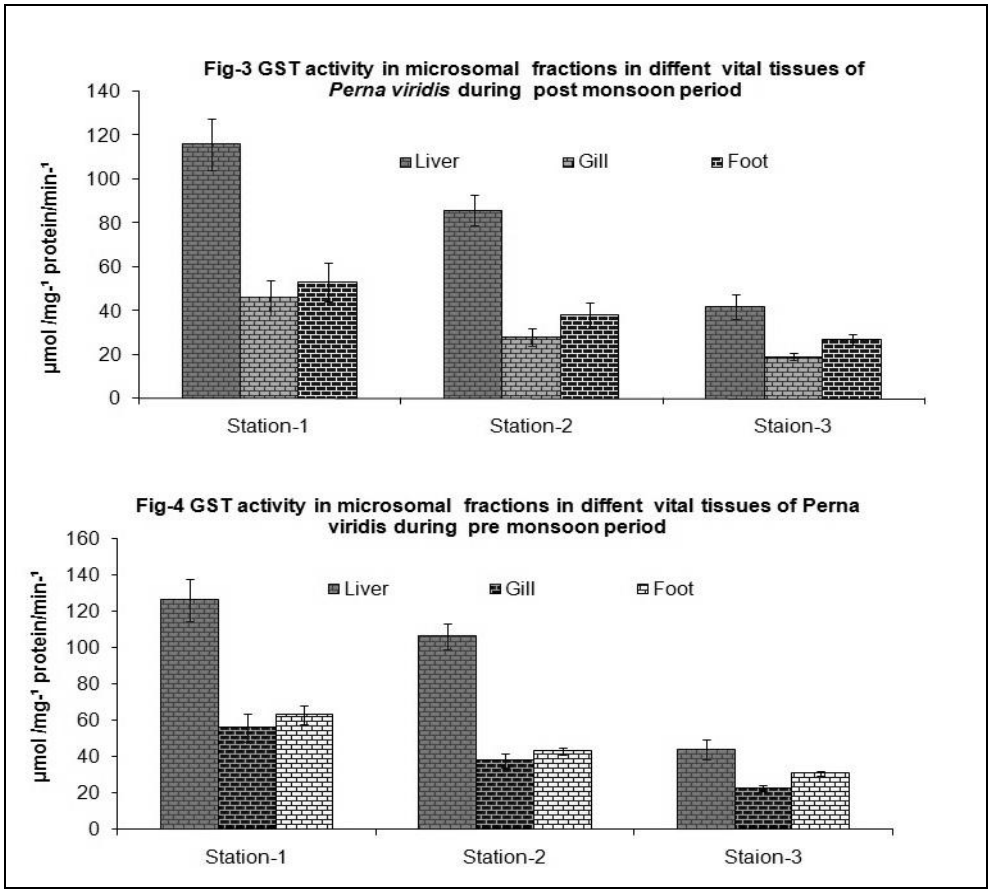
Fig-3 GST activity in microsomal fractions in diffent vital tissues of *Perna viridis* during post monsoon period
µmol /mg-¹ protein/min-¹
140
120
100
80
60
40
20
0
Liver
Gill
Foot
Station-1
Station-2
Staion-3
Fig-4 GST activity in microsomal fractions in diffent vital tissues of Perna viridis during pre monsoon period
µmol /mg- protein/min-¹
160
140
120
100
80
60
40
20
0
Liver
Gill
Foot
Station-1
Station-2
Staion-3

DISCUSSION

GSTs are more abundant than GPxs in fish liver (Stephensen *et al*., 2002). GST activity was found in cytosol from digestive glands of *Littorina littorea* and *Mytilus edulis* and the hepatopancreas of *Carcinus maenas*, since these cells have extensive endoplasmic reticulum and Golgi network, which is characteristic of cells involved in protein synthesis (Merdsoy and Farley, 1973; Mason *et al*., 1984; Pipe 1986; Pipe and Moore, 1986). The GST activity increased during summer seasons in station-1 than station-2 and station-3 that reflect the increased xenobiotic levels. The digestive gland showed a very higher enzyme elevation than gill and foot. Rayapuram harbor (station-1) is the highly contaminated area, offshore (station-2) is the moderate contaminated area and Parangipettai (station-3) is the least contaminated area. All the Phase I (Amutha *et al*., 2009), antioxidant (Amutha and Subramanian, 2012) and Phase II metabolic enzymes are elevated in Rayapuram harbour area *P. viridis* digestive gland, foot and gill respectively and Vellar estuary, Parangipettai is the least enzyme activity when compared to others. The GST activities were closely correlated with *Mytilus galloprovincialis* in the collected in front of an oil refinery from the Mediterranean coastal areas (Trisciani *et al*., 2012).

Conclusion

In the present study, the GST act as a biomarker as well as its contaminant range against PAHs.

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