IMPACT OF HEAVY METALS ON ANTIOXIDANT ACTIVITY IN DIFFERENT TISSUE OF MILK FISH *Chanos chanos*.

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ABSTRACT : The impact of heavy metal accumulation on antioxidant activity in *Chanos chanos*, (Milk fish) was studied in two different locations polluted sites (Kaattuppalli Island) and less polluted sites (Kovalam estuary). Accumulation of heavy metals in the gills, liver and muscles were observed Zn >Fe >Cu >Pb >Mn >Cd >Ni. The results reveal that highest concentration of metals in muscle, gills and liver were observed in Kaattuppalli Island when compared to Kovalam estuary. The antioxidant activity showed significant increased in lipid peroxidase (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-tranferese (GST) and reduced glutathione (GSH) in different tissues of *Chanos chanos* collected Kaattuppalli Island. Among the studied enzymes, total glutathione peroxidase, catalase and glutathione S-transferase appeared to be the most responsive biomarkers of oxidative stress biomarkers and membrane disruption as the sensitive parameters of environmental pollutant contamination and their importance in biomonitoring of aquatic ecosystems. This is also the first such attempt reported at the tissue level from South India stressing the importance of biomarkers in biomonitoring programmes using fish muscle, gills and liver as the model system.
Key words: Heavy metals, Antioxidant enzymes, Environmental contamination, *C. chanos*

INTRODUCTION

Estuaries are highly sensitive zones and regarded as the natural channel for the transfer to agricultural, industrial and urban pollution (Roast *et al.,* 2001). Recently industrial sector produced large quantities of effluents (Khurana *et al*., 2003). Environmental pollutants like heavy metals serve as the major contributors to aquatic ecosystems (Sanders, 1997). Trace elements are environmental toxic compounds capable of causing physiological damage to organisms (Flower, 1975). However, heavy metals may enter aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbour activities and atmospheric deposits (Nair *et al.,* 2006). Many investigators has been studied the trace elements contaminated by different organisms and fishes (Ahmad *et al.,* 2006). The coastal development of heavy industrial facilities, particularly those associated with oil refineries and petrochemical manufactures significant effect on environmental health. The installation, operation of major refining, petrochemical zones may cause loss of habitat changes in sediment dynamics, increased input of hydrocarbons and heavy metals (Croudace and Cundy, 1995).

The anthropogenic input of trace metals and heavy metals are adsorption in fish that takes place primarily through gills. Heavy metals can interact with cell membrane and alter normal physiology by stimulating LPO (Evans, 1987). Lipid peroxidative damage to gill membrane may resulting from oxidative deterioration of polyunsaturated fatty acids thus impacting solute and water transport and osmoregulatory functions of gills (Athikesavan *et al.,* 2004). Iron (Fe) and copper (Cu) are naturally occurring metals in food and drinking water. They provide essential trace metals only at low concentrations and cause risks when levels are high. Iron is discharged from industries like oil washer and petroleum refinery etc., whereas chromium important metallic that is liberated from chrome plating, welding, painting, metal finishes, and steel manufacturing industries.

Fish are at threat from aquatic pollution and together with their long-term exposure in natural habitat provide suitable biomarkers for environmental pollution (Padmini *et al.,* 2004). These metals are known to generate 'reactive oxygen species' (ROS). ROS homeostasis have been altered, a first level of cellular responses against these free radicals, the antioxidant defense and repair systems minimize the damage that actually occurs (Fedorovich, 1995).

The number of industries, establishment of river, as well as developmental activities along the Coast of Kaattuppalli Island, renders this coastal zone to highly polluted and vulnerable (Rajeshkumar, 2010). In previous our study described histological alteration as well as expression of heat shock protein (HSP70) in different tissues of the milk fish (*C.chanos)* collected from Kaattuppalli Island (Rajeshkumar and Munuswamy, 2011). The other site like Kovalam coast which is relatively free from pollution because the contaminants in aquatic environments rarely occur as single chemicals but rather as *'cocktails'* of heavy metals and other contaminants and only few studies have assessed the consequence of environmental pollution on fish cells (Iwama *et al.,* 1998). In the present study was to investigate the cumulative effect of aquatic contaminants on oxidative stress biomarker responses at the tissue level. Hence, a correlation between the environmental contaminants in Island estuarine water, and their bioaccumulation with reference to heavy metals was assessed in different tissues of *C.chanos* (Milk fish) responses at the structural level were also studied.

MATERIALS AND METHODS

DESCRIPTION OF STUDY AREA

Kovalam coast (12°49′N, 80°5′E) is situated on the east coast of Tamil Nadu, India and is about 35 km South, Chennai. It runs parallel to the sea coast and extends to a distance of 20 km. It was chosen as less polluted site for the present investigation as it is surrounded by high vegetation and it is free from industrial or urban pollution. Kaattuppalli Island (13º21'N, 30º20'E), is a narrow longitudinal island, situated in the eastern coastal plain, North, Chennai, separated from the mainland by the backwaters on the eastern aspect, extending from Pulicat Lake, North, Buckingham Canal West, Ennore Creek, South and Bay of Bengal, East. Ecosystem was chosen as the polluted site as in its immediate coastal neighbourhood a number of industries are situated which include desalination parts, petrochemicals, fertilizers, pesticides, oil refineries, rubber factory and thermal power station that discharge effluents into the marine Island ecosystems (Fig.1).

Figure 1. Map showing the study area and sampling sites
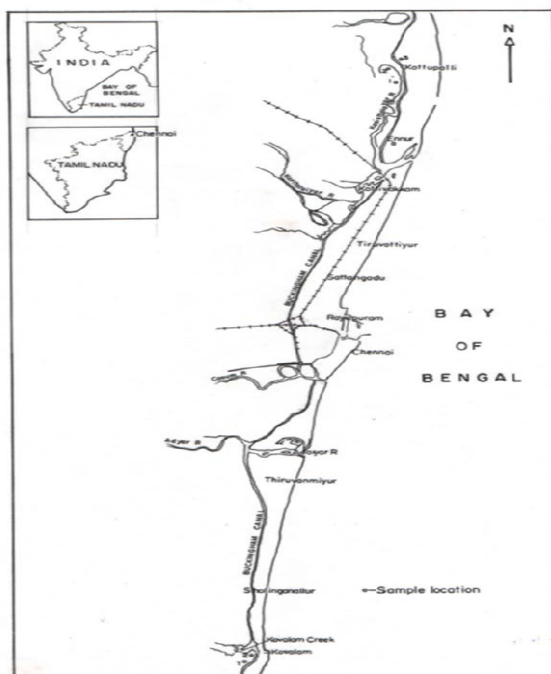
EXPERIMENTAL ANIMAL

C.chanos (Milk fish) were collected by fishermen using multifilament, nylon gill net of mesh sizes ranging from 30 mm. After collection, samples were kept in ice pack and brought to the laboratory on the same day and then frozen at -20°C until dissection, according to standard FAO methods. Simultaneously surface water samples were collected from both less polluted (Kovalam coast) and polluted sites (Kaattuppalli Island) using a non-metallic aqua-trap water sample.

HEAVY METAL ANALYSIS

One gram of muscle, liver, intestine and gill racers from each sample was dissected for analysis. Dissected samples were transferred to a Teflon beaker and digested in an acid solution to prepare the sample for heavy metal analysis (Kenstar closed vessel microwave digestion) using the microwave digestion program. The samples were digested with 5 ml of nitric acid (65%). After complete digestion the samples were cooled down to room temperature and diluted to 25 ml with double distilled water. All the digested samples were analysed three times for metals like Cu, Cd, Pb, Zn, Mn Ni and Fe using Atomic Absorption Spectrophotometer (Perkin-Elmer AA 700). The instrument was calibrated with standard solutions prepared from commercially available chemicals procured from Merck, Germany (Kingston and Jassie, 1988).

ESTIMATION OF PROTEIN

Tissue samples were homogenized in 10 % 0.1M Tris-HCl buffer (pH 7.2) and centrifuged at 12,000 g for 30 min at 4 $^{\circ}$C. The supernatant obtained was used for the analysis of enzymatic as well as non-enzymatic antioxidants. Protein in each sample was estimated with Coomassie brilliant blue G-250 using bovine serum albumin as a standard (Bradford, 1976).

ANTIOXIDANT ENZYMES

Levels of lipid peroxide were determined by the method (Ohkawa *et al.,* 1979) SOD activity was determined by the method of (Marklund and Marklund, 1974). Catalase activity was determined by the method of (Sinha, 1972). Glutathione peroxidase activity was determined essentially as described by (Rotruck *et al.,* 1973). The GST activity was determined by the method of (Habig *et al.,* 1874). The reduced glutathione content was estimate by the method of (Mron *et al.,* 1979).

STATISTICAL ANALYSIS

All the grouped data were analysed using SPSS/10.0 software. Hypothesis testing method included one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test. P<0.05 was considered to indicate statistical significance. All the results were expressed as mean ± S.D in each tissue.

RESULTS

METAL ACCUMULATION IN FISH TISSUE

Bioaccumulation of heavy metals in milk fish *C.chanos* showed marked differences in the accumulation patterns (Fig. 2). The metals studied, Pb, and Cd concentrations were low whereas Zn, Cd and Fe were high in all tissues. Studies clearly showed that heavy metal accumulation in the water as well as in sediment sample was high in Kaattuppalli Island when compared to Kovalam coast. (*P*<0.05).
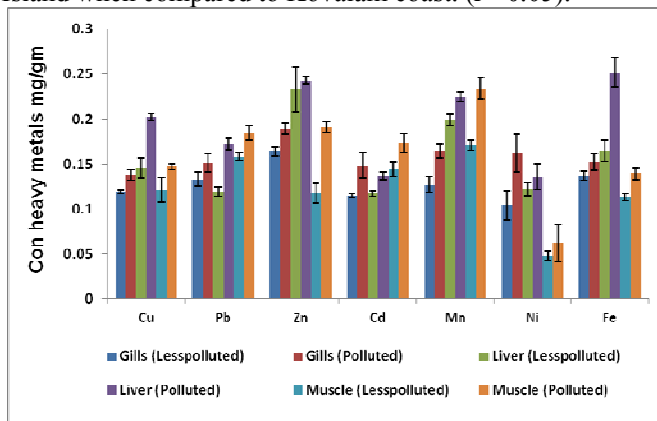

Figure 2. Heavy Metal concentration in gills, liver and muscle (µg/ g $^{-1}$) of *C. chanos* from Kaattuppalli Island (Polluted) and Kovalam coast (less polluted)

ANTIOXIDANT ENZYME ACTIVITY IN TISSUES

The levels of lipid peroxidase were significantly increased in Kaattuppalli Island when compared to Kovalam coast ($P <0.01$; Fig. 3). The increased level of superoxide dismutase catalase, glutathione peroxidase, glutathione-*S*-transferees and reduced glutathione in Kaattuppalli Island, whereas, the decreased level of superoxide dismutase, catalase, glutathione peroxidase, glutathione-*S*- transferees and reduced glutathione in Kovalam coast. ($P <0.05$; Fig. 4-8).
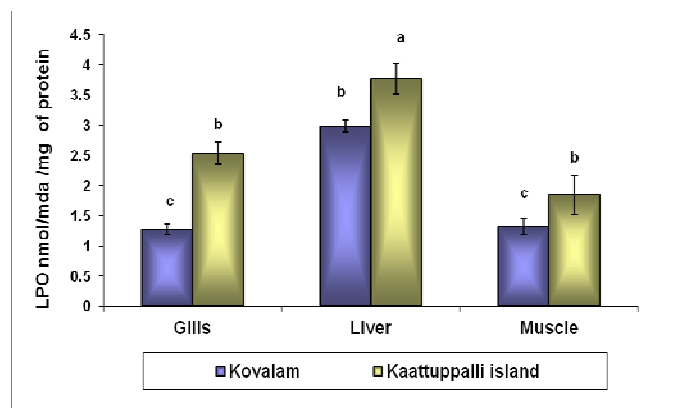

Figure 3. Quantitative analysis of lipid peroxidation (LPO) in the gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.
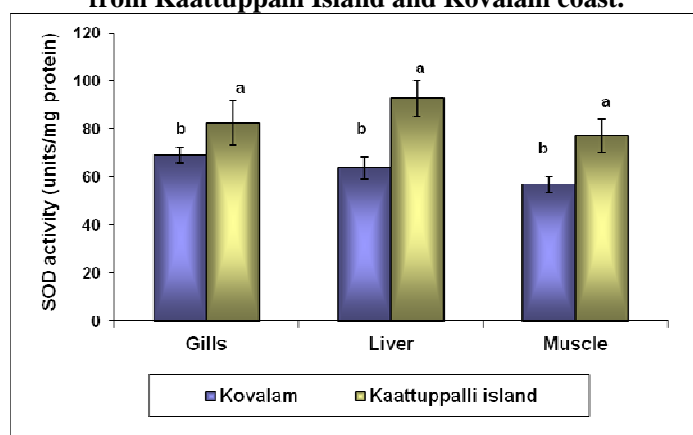

Figure 4. Superoxide dismutase (SOD) in gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.
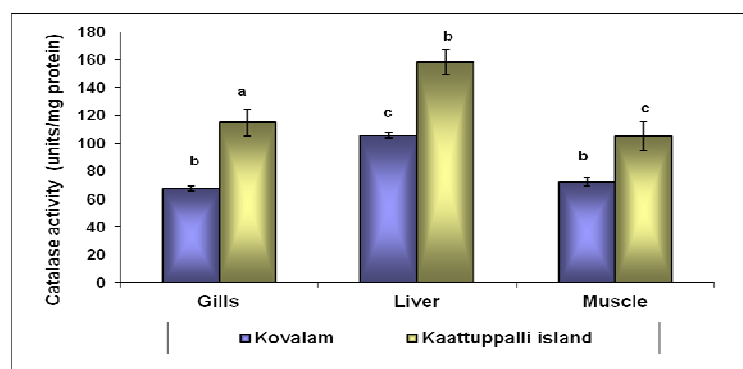

Figure 5. Catalase (CAT) in gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.
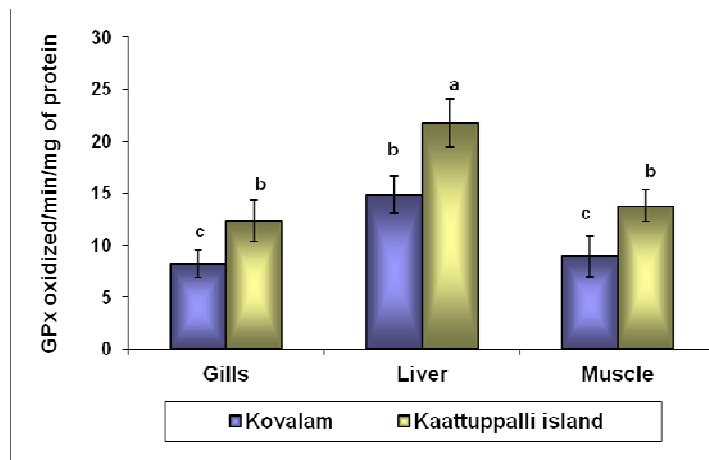
Figure 6. Glutathione peroxidase (GPx) in gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.
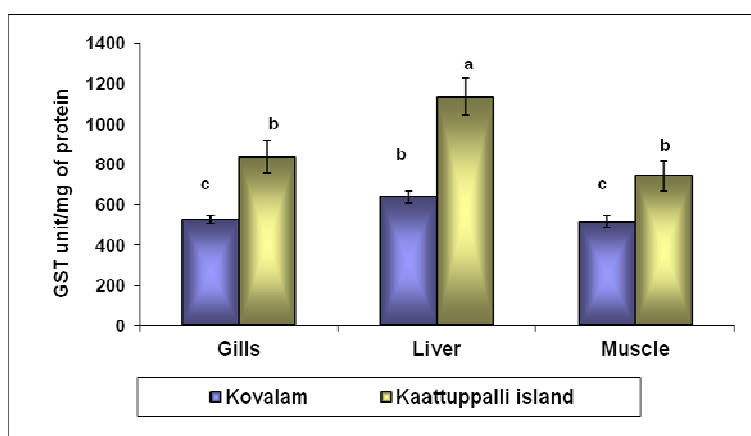

Figure 7. Glutathione -*S*- transferase (GST) in gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.
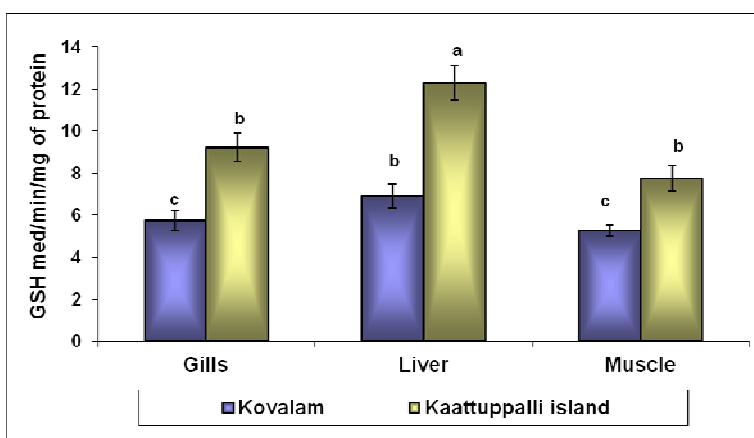

Figure 8. Reduced glutathione (GSH) in gills, liver and muscle of *C. chanos* from Kaattuppalli Island and Kovalam coast.

DISCUSSION

Heavy metals are widespread pollutants of great environmental concern particularly in estuaries as they are non-biodegradable (Ragunathan and Srinivasan, 1983). In the aquatic environment, despite the presence of constitutive or enhanced antioxidant defence systems, increased levels of oxidative damage occur in organisms exposed to contaminants which stimulate the production of ROS increased production of ROS and subsequent oxidative damage has been associated with pollutant-mediated mechanisms of toxicity in fish liver (Livingstone, 2001). Proteins constitute also a target for oxidative damage with subsequent alteration of their functions. In flounders, living in contaminated waters with xenobiotics, increased levels of oxidised proteins were reported from polluted sites (Padmini and Geetha, 2007).

A significant increase in lipid oxidation markers may indicate the susceptibility of lipid molecules to reactive oxygen species and the extend of oxidative damage imposed on these molecules. Lipid peroxidation levels significant increase in different tissue of *C. chanos* collected from Kaattuppalli Island may be due to antioxidant enzyme activities that are up regulated upon oxidative stress in different tissues of the fish in increasing order. Similar observations of antioxidant enzyme levels in fish such as *Geophagus brasiliensis* in response to oxidative stress were made earlier (Lenartova *et al.,* 1970). Maintenance of high constitutive levels of antioxidant enzymes like superoxide dismutase and catalase is essential to prevent oxyradical-mediated lipid peroxidation (Lushchak *et al.,* 2001).

Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2). Decreased activity of this enzyme leads to the accumulation of (O_2^-) which in turn accelerates the conversion of Fe^{3+} to Fe^{2+}. The latter serves as a substrate for hydroxyl radicals (Halliwel and Gutteridge, 1985). Previous studies describe SOD as a scavenger of superoxide radicals generated by normal physiological activity and accumulation of the xenobiotic compounds present in any environmental situation (Halliwel and Gutteridge, 2001). In the current study, the induction of SOD in different tissues such as gills, liver and muscle is likely indicative of superoxide radical production resulting from contaminants. Consistent with our results, increased SOD activity in the tissue of *Mugil cephalus* was reported by (Padmini and Usharani, 2009) in polluted waters. Catalase plays an important role in the decomposition of H_2O_2 to water. Due to altered glutathione redox ratio and a subsequent increased in reduced glutathione levels, the glutathione mediated detoxification process may also be affected. However, the activity of CAT increased in all the tissues of the fish *C. chanos.* CAT activity may be due to a flux of superoxide radicals, which have been shown to inhibit CAT activity (Wilhelmfilho, 1996). Under acute oxidative stress, toxic effects of pollutants may overwhelm antioxidant defense (Bebianno, 1998). Furthermore, the observed increased in the glutathione detoxification system in the gill at the first point of contact with environmental xenobiotics indicates that the system provide a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982). These results are comparable to those found in other studies, where CAT and GPX activity increases at sites contaminated with metals and petrochemicals (Lima et al, 2006). Heavy metals such as Cd and Pb (Almeida *et al.,* 2004) are well-studied heavy metals, which increased CAT and GPx activity in bivalves. Elevated GPx activity is also observed in molluscs exposed to petrochemical products (Pan *et al.,* 2006).

The results reported in the present and in other studies indicate that antioxidant enzyme responses are transient and variable for different species, (Livingstone, 2001). Indeed, in field studies, higher, equal or lower activities of various antioxidant enzymes have been observed in polluted sites compared to less polluted sites. This might be a factor responsible for the lack of elimination of toxic compounds that enter the fish and thus result in their accumulation, aggravating oxidative stress. Various tissues of *Liza macrolepis,* inhabiting the Ennore estuary, have shown that the concentration of certain heavy metals such as mercury, cadmium, zinc and iron exceeds the permissible safe levels proposed by the Industrial Toxicology Research Centre, Lucknow, India (Porte *et al.,* 2000).

An increased activity of GPx was observed in different tissue samples of milk fish from Kaattuppalli Island compared to the Kovalam estuary which might be due to a consequent depletion of GSH. Fish inhabiting the highly polluted sites developed an enhanced state of oxidative stress characterized by increased levels of lipid peroxidation markers such as conjugated, lipid hydroperoxide and lipid peroxidase, similar to a response observed in fish under the same conditions. The observed reduction in GSH levels with a confirmation by the decreased glutathione redox ratio levels may be the likely reason for the inhibition of activity of GST. The increased GST activity favours defective detoxification processes leading to further accumulation of metals in fish tissues, aggravating the oxidative stress situation (Vander Oost *et al.,* 2003).

In conclusion, estimation of oxidative stress biomarkers in fish could provide a useful indicator of pollution of water bodies. The results also indicated that the antioxidant defense components namely, LPO, SOD, CAT, GPx, GST and GSH are sensitive parameters that could provide useful biomarkers for the evaluation of contaminated aquatic ecosystems. The relationship between the degree of deficiency of antioxidant defense and lipid and protein oxidation suggests that these parameters could also be used as biomarkers. Hence, as part of an effort to continue monitoring the ecological health of the estuary Island and their inhabitants and to ensure environmental safety, this type of study represents an important tool because of its unique approach to establish the correlations between metal concentration in water and their bioaccumulation in aquatic species along with the assessment of contaminant impact on stress biomarkers.

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