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Bioaccumulation of heavy metals and oxidative stress in *Mugil cephalusin* Adyar estuary

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***Abstract*— Heavy metals in the marine environment are of considerable interest because they remain in the organism and may accumulate and magnify in body tissue. The presence of toxic metals in food chain accumulates in the body tissue of aquatic organisms. Fish is a major component of the aquatic habitat and serve as bio-indicator of heavy metals levels in its habitat. Mugilcephalushas been recognized as a good bio-accumulator of organic and inorganic pollutants. The accumulations of Arsenic (As), Lead (Pb) and Mercury (Hg) in Mugilcephalus from adyar estuary located in chennai, Tamilnadu state, India were estimated. The results revealed that the Pb and Hg concentration was higher during pre-monsoon season and As was higher during monsoon season. The order of accumulation is Pb>As>Hg. Oxidative stress is a common pathway of toxicity and Mugilcephalus posses the well developed antioxidant defense systems for neutralizing the toxic effects of Reactive oxygen species. The estimation of anti oxidant activity shows seasonal variation in SOD, CAT, GST and GSH.**

***Keywords*: Heavy metals, SOD, CAT, GSH, GST, Protein, Mugilcephalus**

I. INTRODUCTION

Heavy metals usually occur in small amounts in groundwater around industrial areas that use variety of chemicals in the manufacture of batteries, paints, pharmaceutical products, leather processing and agrochemicals. These industries dispose the treated/partially treated wastewater that do/do not meet the standards in the surface water bodies such as rivers, lakes, ponds and into the sea in coastal areas. Contamination due to suite of heavy metals is also commonly reported around landfills. [1]; [2].Arsenic (As), Lead(Pb), Mercury(Hg), are highly toxic to the environment and humans. The prediction of bioavailability of metals is of crucial importance for the assessment of environmental quality of contaminated soil [3]. The distribution and abundance of total metal concentrations are useful indicators of the extent of soil contamination [4] , but risk from metals depends on their bioavailability[5]. Heavy metals may affect organisms by accumulating in their bodies or by transferring to the next trophic level of the food chain. Accumulated heavy metals in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level [6]. Aquatic organisms exposed to a higher concentration of trace metals in water may take up substantial quantities of these metals[7]. *Mugilcephalus*Linnaeus (1758)the common name is striped mullet, order-Mugiliformes, family-Mugilidae. The flathead grey mullet(*Mugilcephalus*) is an important edible fish species in coastaltropicalandsubtropicalwaters worldwide.There are nine species of mullet found in the western central atlantic, the most common species is striped mullet. Environmental stressors can alter the physiological and biochemical parameters in fish, including morphological indices, antioxidant responses and energy metabolic parameters [8]. Some toxic contaminants affect the production of an ROS (Reactive Oxygen Species), which can induce oxidative damage and maybe a mechanism of toxicity for aquatic organisms living in polluted areas [9]; [10]. Many waterborne organic contaminants can stimulate production of ROS and causes oxidative damage to aquatic organisms. [11]

II. MATERIAL AND METHODS

STUDY AREA

In 1798 Adyar River finds its position in a British map as a suburb and it was one of the ecological heritage centers in chennai. Fisheries department , Government of Tamil Nadu taken control of the creek area and sets up fish farms and

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related institutions in 1950 , In 1993 a city based NGO , filed a public interest litigation to maintain Adyar creek as a sanctuary, leading to the formation of Adyar poonga trust in 2006 with the aim of restoring the ecological balance. Since 1997, the Government of Tamil Nadu had been envisaging to protect the Creek and creation of an Eco Park to restore the ecological balance and raise public awareness on environmental issues. The Adyar Creek has several threats such as heavy accumulation of silt over a period of time. Slums have encroached upon more than half the footbridge. The remaining portions of debris impede the flow of water and now the river is used as a garbage dumping area. A number of cattle sheds, which are set up along the creek, not only reduce the width of the creek but also pollute it. The other sources of pollution are the raw sewage let in at various points from the encroachments, storm water drains and other upstream sources such as industries, hospitals and sewage pumping stations. Heavy encroachment along the creek has also resulted in gross pollution of the wetland, turning it into a health hazard [12].

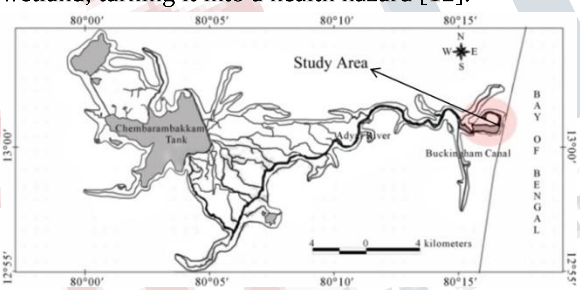

Fig (a): Location of study area

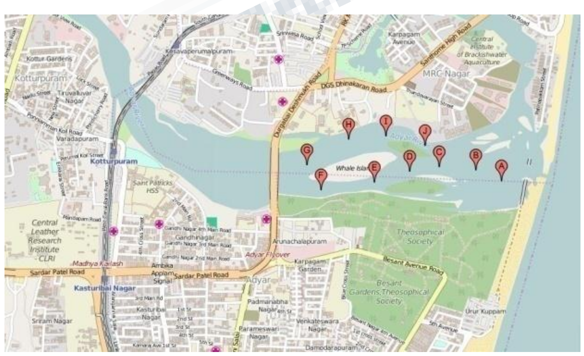

Fig (b):Location sampling sites in adyar estuary

III SAMPLING

The samples were collected during the period of 2014 to 2015 in monsoon, post monsoon and pre monsoon from 10 sites from Adyar estuary in similar GPS location. Samples collected by local fishermen using dug-out canoes and nets throughout the period of time. The fish were kept in containers with water from Adyar estuary. The samples are immediately transfer to lab and muscle tissue were removed and stored in the deep freezer for further analysis [13].

IV HEAVY METAL ANALYSIS

The samples were prepared for laboratory analysis of their heavy metals levels. The specimen samples were cleaned and transferred whole into an electric oven at 40^{o}C. They were dried in the oven at this temperature for 24hours and then pulverized in a clean dry porcelain mortar [14]. The pulverized samples were dried further for 1hour at a reduced temperature of 20^{o}C and put into clean dried bottles. Then, 3.0g each of the pulverized and dried samples was weighed into a silica crucible and ashed in a muffle furnace at a temperature of 550^{o}C for 5hours [15]. The ashes were cooled to room temperature and sieved to remove bigger particles and then transferred into a 250ml conical flask. Thereafter, 20ml of concentrated HNO_3 was added and diluted to 50ml with deionised water and swirled gently after which the volume was made up to 100ml with deionised water and analyzed for heavy metals using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Model ELAN DRC II, Perkin-Elmer Sciex Instrument, USA.

V ANTIOXIDANT ENZYME ACTIVITY

Assays are performed to analysis SOD (Superoxide Dismutase), CAT (Catalase), GSH (Glutathione), GST (glutathione-s-transferase)actives in tissue samples. SOD activity was determined by TrisHcl buffer (0.1M, PH-8.2). The pyrogallol inhibits the auto oxidation in the sample. It's used to quantify the SOD activity and its measured at 420 nm

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[16]. The catalase (CAT) activity is based on the fact that dichromate in acetic acid is reduced to chromic acetate, when heated in the presences of hydrogen peroxide [17]. For the determination of GST, the tissue samples are homogenized in phosphate buffer (0.1M, PH 6.5). Homogenized samples are centrifuged. The supernatant was used to determine the enzyme activity [18]. For Reduced glutathione (GSH) determination the DTNB 5-5 di thiobis 2-nitro benzoic acid reacts with aliphatic thiol compound at PH 8 to produce 1 mole of p nitro thiophenol of thiol. The thiol concentration measured at 412nm [19]. The protein content in the tissue was estimated according to the dye binding method[20].

VI. RESULT

Heavy metal analysis:

The concentration of Arsenic (As)was maximum (1.6620±0.068 µg/g) during monsoon and minimum during (1.3610±0.069 µg/g) post-monsoon. The concentration of lead (Pb)found to be maximum (3.9080±0.172µg/g) during pre-monsoon and minimum (3.2140±0.124µg/g) during post-monsoon. Mercury (Hg) was found maximum (1.0570±0.096 µg/g) during pre-monsoon and minimum (0.6660±0.046 µg/g) during post-monsoon.

Estimation of antioxidant enzyme activity:

In MugilcephalusSOD was higher (10.6220±1.1399) in during monsoon season.GST values are higher (68.0430±7.7020) during monsoon season.CAT was higher (304.0710±77.4037) during monsoon than in pre and post monsoon. GSH was higher during post monsoon(301.8880±97.4339). The estimated protein was higher (3.2260±0.141) during pre monsoon season.

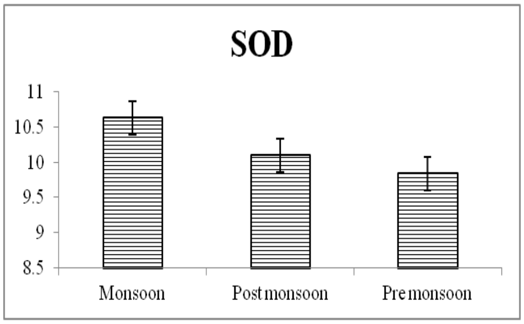

Fig (3) Seasonal variation of SOD

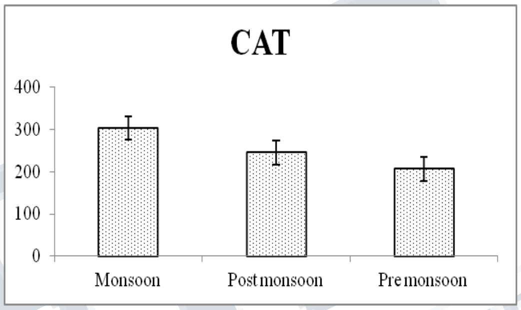

Fig (4) Seasonal variation of CAT

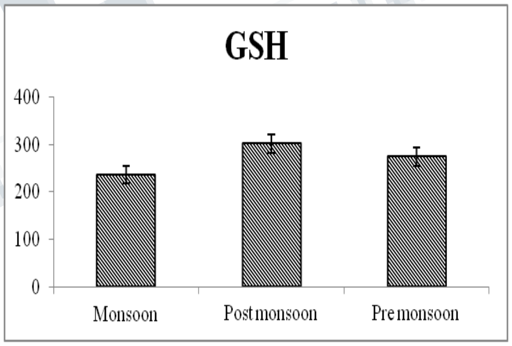

Fig (5): Seasonal variation of GSH

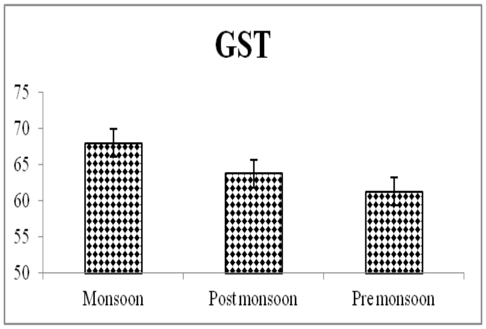

Fig (6): Seasonal variation of GST

Tables I: The mean and S.D of heavy metals present in Mugilcephalus

Seasons	As (µg/g)	Pb (µg/g)	Hg (µg/g)
Monsoon	1.6620±0.068	3.5140±0.077	0.7060±0.020
Post monsoon	1.3610±0.069	3.2140±0.124	0.6660±0.046
Pre monsoon	1.6020±0.196	3.9080±0.172	1.0570±0.096

Tables II: The mean and S.D of Antioxidant enzymes activity in Mugilcephalus

Seasons	CAT	SOD	GSH	GST	Protein
Monsoon	304.0710±77.4037	10.6220±1.1399	235.8500±70.3168	68.0430±7.7020	3.0020±0.215
Post monsoon	245.9360±49.4551	10.0920±0.9236	301.8880±97.4339	63.7770±7.8413	3.1520±0.191
Pre monsoon	206.5730±32.5207	9.8380±0.6840	273.5860±82.6036	61.2970±5.6711	3.2260±0.141

VII DISCUSSION

Fishes are on the top of the aquatic food chain and accumulate large amounts of metals from water and sediment. Marine organisms, including fish, accumulate heavymetals through direct absorption, or via food chain, and transfer them to human being by consumption, causing acute chronic or disorders.[21] The antioxidant activity system provides the first defense against oxidative toxicity at a cellular level. In this study there is a seasonal variation in the antioxidant activity of littoral biota of Adyar estuary. SOD is the first enzyme deals with oxygen radicals and responsible for catalyzing the dismutation of high superoxide radical O^-_2 to O_2 and H_2 O_2.Metal induce antioxidant enzyme activity in fishes hence, Arsenic, Lead and Mercury increased in pre-monsoon and monsoon season.The heavy metals induce antioxidant enzyme activity results in increased activity of SOD, CAT, GSH and GST. The SOD enzymes are enzymes that catalyze the dismutation of superoxide into hydrogen peroxide and oxygen, whereas CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen. The increased SOD and CAT activities in fishes exposed to heavy metals biochemical responses to over production of superoxide radicals and H_2O_2 in fish *Mugilcephalus*.GST catalyzes the transformation of a wide variety of electrophilic compounds to less toxic substances by conjugation to GSH [22]; [23]. High levels of stress such as high doses of compounds, induces a quick increase in the activity of GST and later decrease in GST activity may be due to the depletion of the inducible molecules. The majority of GST enzyme substrates are xenobiotics or products of oxidative stress[24]; [25]. When in contact with some pollutants, such as metals, fish cells usually try to remove them by direct conjugation with GSH. The consumption of GSH due to the direct scavenging of ROS or as a co-factor for GST activity may decrease GSH levels and GSH: GSSG ratio leading to a disruption of redox homeostasis. ROS directly and is involved in numerous processes essential to normal biological function, such as DNA and protein synthesis [26]. The water quality was lower during the dry season. This observation can be explained by the effect of pollutant concentration caused by lower volumes of water. In contrast, the input of additional water during the dry season dilutes toxic substances.[27].

CONCLUSION

This study is helps to understand the heavy metal pollution in Adyar estuary. It gives a clear knowledge about the accumulation of heavy metals(As, Pb and Hg) in *Mugilcephalus.* The relatively higher concentrations of metals in *Mugilcephalus*is directly or indirectly related to unremitting industrialization or urbanization in and around Adyar estuary. The increased concentration of heavy metals in fish, induce the antioxidant enzyme activity leads to the depletion of protein. Moreover, these results can also be used to understand the metal pollution, enzyme activity and protein content in *Mugilcephalus* to evaluate the possible risk associated with their human consumption.

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