Biochemical responses of Asian sea bass, *Lates calcarifer* (Bloch) sublethal mercury exposure

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ABSTRACT

Mercury is an important group of estuarine pollutants. It is known to be able to disturb the integrity of biochemical and physiological mechanisms in aquatic organisms, including estuarine fish. Biochemical changes occurring in the metabolically active tissues of gills (GL), liver (LI) and muscles (MU) of the fingerlings of Asian sea bass, *Lates calcarifer* on exposure to two sub-lethal doses (0.127 and 0.254 ppm) of mercury were studied for 28 days of exposure (DoE). Sub-lethal doses of mercury significantly (P<0.05) altered the levels of the total protein (TP), carbohydrate (TFS), and lipid contents (TL) in test fishes. Percentage decrease in all biochemical components increased with the progressing DoE, irrespective of the exposure concentrations. The order of percent decrease in the concentrations of the TP, TFS and TL in different tissues at the end of 28 DoE was found to be MU>GL>LI, MU>LI>GL and LI>MU>GL. Results of this study revealed that sub-lethal doses of mercury significantly alter the proximate composition of major tissues, particularly the TP levels in the MU tissues and thereby reducing the nutritive value of this economically important sea bass.

Index Items: *Mercury, Sea bass, Biochemistry*

INTRODUCTION

The effects of environmental pollutants on the mortality of aquatic animals have been studied by many workers, but very little is known about the disturbed physiological and biochemical processes within the organism following exposure to environmental pollutants. This is of great importance as contamination of natural water resources by heavy metals threatens fish culture and population (Christensen, 1975). When heavy metal ions exceed a threshold concentration in the aquatic ecosystem, they act as pollutants and create stress in fish. Environmental pollution is reported as one of the major factors causing hypoxemia in animals (Black *et al.*, 1962). The respiratory potential of an animal is an important physiological parameter to assess the toxic stress because it is a valuable indicator of energy expenditure in particular and metabolism in general.

The biochemical and physiological adaptations made by fish in response to change in environmental oxygen levels can be correlated to the ecology of the species. Species such as the European carp, *Cyprinus carpio*, which has been shown to live for several months in water of very low oxygen content (Blazka, 1958) are able to survive by reducing their oxygen uptake and changing to anaerobic metabolism (Johnston, 1975). Other species such as salmonids, adapted to environments of high oxygen tension, are less able to survive hypoxia (Itazawa, 1971). Since different fishes react differently to hypoxic situation, the procedure of using oxygen consumption as a yard stick to measure the metabolic activities of the body may not produce satisfactory results. Although a number of methods are now available to measure sublethal effects of pollutants (Sprague, 1971) most of them are long term and are not suitable for routine monitory programmes.

Extensive investigations have revealed that different tissues of fish can sustain varying levels of anaerobic metabolism. In most teleosts fermentation of glucose to lactate provided the main source of energy under hypoxic conditions (Health and Pritchard, 1965). Black *et al.*, (l96l) found that the endurance of fast swimming fish is limited by the anaerobic energy released when stored glycogen is transformed by the Emden – Meyer of cycle to form lactic acid within muscle cells. In almost all these circumstances, the major share of stored energy comes from the carbohydrate or glycogen reserves. Thus, carbohydrates form the central point in energy production because of great mobility in the living systems, together with its capacity to get compartmentalised within cells and tissues. The mobility is provided by glucose and compartmentalisation by glycogen and g1ucose – 6 - phosphate. It is widely accepted that carbohydrate deposits in the form of glycogen in tissues like liver and muscle provided the immediate energy requirements in teleost fishes under a variety of stressors including exercise (Black *et al.*, 1962) physical disturbance (Nakano and Tomlinson, 1967), starvation (Black *et al.*, 1966). From a biochemical point of view, life is uniquely characterised by its association with protein. It has been demonstrated by several investigators (Idler and Clemens, 1959) that tissue proteins are energy sources for fishes during stress, spawning and muscular exercise. Though considerable information is available dealing with the determination of acute toxic levels of several pollutants and their influence on oxidative metabolism, studies on the tissue energy sources are relatively very few.

MATERIALS AND METHODS

Collection of Experimental animal

Healthy hatchery reared juvenile Asian sea bass, *L. calcarifer* with mean total length of 6.03 ± 0.59 cm and mean total weight of 8.76 ± 1.24gm were obtained from the Rajiv Gandhi Centre for Aquaculture, Thirumullaivasal near Sirkali, Nagapattinam Dist, Tamil Nadu, India. Fishes were acclimatized for 2 weeks in stock tank to the experimental glass aquaria (120x50x50 cm) filled with 250 l of water with a salinity of 27±2 ppt, under a natural photoperiod 12 h:12 h (light:dark) cycle. The water in the tanks was passed through a 1µm filter, UV-sterilized, and refilled daily. Fish were fed twice daily with chopped fresh fish. They were starved for 24 h before and during the experiment.

Experimental Procedure

Test concentration

Fish were exposed to nominal 0.127 and 0.254ppm as mercury. Doses were theoretically sublethal, 10% and 20%, respectively, of the maximum acceptable toxicant concentration (MATC), which was 1.27 ppm. The MATC was represented as no observed effect concentration (NOEC) < MATC< LOEC (lowest observed effect concentration). The test concentration was estimated using the application factor (AF) concept, by dividing the limits (NOEC and LOEC) of the MATC by the 96-h LC_{50} (AF= $MATC/LC_{50}$ = $(NOEC–LOEC)/LC_{50}$).

System design

A recirculation closed system was set up according to Muthuwan (1998). The experiment was carried out in 360 L glass aquarium (120 x 60 x 50 cm), in which one compartment (50 x 50 x 40 cm) was partitioned by a plastic gauze (mesh size 1.5 mm) to contain a biofilter. Each aquarium was filled with 300 L of natural sea water (salinity of 26 ±2 ppt), which was pumped continuously over a biofilter column at a rate of 4 l/min. The water was continuously aerated throughout the experiment.

Test procedure

After 2 weeks of acclimatisation in a holding tank, ten healthy fish (7.76 ± 0.19cm in length and 10.69 ± 0.84gm in weight) were transferred to each aquarium at a loading density of 0.71 g/L. Three replicates were performed for test concentration and control. Fishes were fed twice daily with chopped fresh fish at 10:00 and 14:00 h. Uneaten food was quickly removed from the system. Fishes were starved for 24 h before sampling. The experimental water (50%) was changed every 2 weeks to keep the water quality within acceptable limits according to APHA (1995); water quality (dissolved oxygen, temperature, pH and salinity) was measured everyday and water chemistry (ammonia nitrogen, nitrite nitrogen, nitrate nitrogen) was measured twice weekly. All chemical parameters were determined following the techniques of APHA (1995) using analytical grade reagents. The actual concentration of mercury was measured weekly before and after its addition to maintain concentrations at the designed level. Water characteristics and the actual mercury concentrations are shown in Table 1. Mortality and behaviour were observed everyday in each concentration. Two fishes from each aquarium were sampled at 0, 7,14 21 and 28 days post-exposure.

Tissue samples and biochemical analysis

The fishes were exposed to 0.127 ppm and 0.254 ppm concentrations of mercury for 28 days. After 0,7,14,21 and 28 days, the fishes were sacrificed. Muscle, gills and liver were excised out and analyzed for biochemical composition. Total protein was estimated in UV visible double beam spectrophotometer by Biuret method using bovine serum albumin as standard as suggested by (Lowry *et al.*, 1951). Total free sugar was estimated by Phenol - Sulphuric acid method of Roe, 1955. Total lipids were estimated by gravimetric methanol - chloroform extraction method suggested by Folch *et al.* (1957) . Accuracy of the analytical methods was tested against prepared standards and deviations from real standard values are expressed as coefficient of variation. Fluctuations in concentrations of biochemical components in different treatment groups and organs were assessed by analysis of variance (ANOVA).

RESULTS

Changes in the Total Protein (TP) Levels

Levels of the TP in different tissues of control and exposed Asian sea bass during the exposure period are depicted in Fig.1, 2 & 3. The TP concentrations were significantly lower in test Asian sea bass than those of controls on all DoE (P<0.05). The rate of depletion was found to be highly time and tissue dependent. The order of percent decrease of the TP concentrations in different tissues at the end of 28 DoE was observed to be MU> GL>LI. A progressive depletion in the TP levels of test Asian sea bass was recorded in the tissues of LI and MU during the exposure period. Significant variation in the TP content between exposure concentrations of 0.127 ppm and 0.254 ppm was noticed (P<0.05). The levels of hepatic protein of test Asian sea bass were found to be almost similar to that of control Asian sea bass on 0 and 7 DoE but depletion was more prominent on 14, 21 and 28 DoE. The magnitude of depletion in the hepatic protein was directly proportional to the concentration of mercury. Higher percent depletion in the hepatic protein was observed in test Asian sea bass exposed to 0.254ppm compared to those exposed to 0.127ppm of mercury (P<0.05).

Changes in the Total Free Sugar (TFS) Levels

Levels of the TFS in different tissues of test Asian sea bass and controls during the exposure period are shown in Fig. 4, 5 & 6. The TFS concentrations were significantly lower in test Asian sea bass than those of controls on all DoE. The depletion in the TFS levels in the MU of test Asian sea bass was significant with the progress in the period of exposure. Concentrations of hepatic total free sugar in the test Asian sea bass ranged from 24.63±0.85 (0 DoE) to 25.59±0.03 mg/100mg (28 DoE) over control Asian sea bass (100%). The levels of the TFS in the LI of test Asian sea bass exhibited a biphasic pattern: higher concentrations on 0 DoE and 7 DoE and lower on 14 DoE and 21 DoE and 28 DoE. The order of percent decrease in the TFS levels in the studied tissues on the last day of exposure (28 DoE) was found to be MU>LI>GL.

Changes in the Total Lipid (TL) Levels

Levels of the TL in different tissues of the test Asian sea bass and controls during the exposure period are depicted in Fig.7, 8 & 9. In general, the TL concentrations in all the studied tissues of Asian sea bass exposed to sub-lethal doses of copper were significantly lower than those in controls (P<0.05). The percent decrease in the hepatic lipid was higher in the LI than in the tissues of MU and GL and the order of percent decrease on 28 DoE was found to be LI>MU>GL.

DISCUSSION

In the present study, there was a significant depletion of glycogen in the liver and muscles of fishes exposed to mercury. Reactions of liver glycogen were more significant than those of the muscle. In the present study, similar to the observations by Dange (1986) the least extensive changes were seen in the copper dosed fishes. When subjected to sublethal exposure, the energy reserves (glycogen and Protein) in the major reservoir (liver) were observed to be depleted in different proportions. Besides liver, the glycogen reserves of the muscle too, were found to be depleted on exposure of fingerlings to mercury although this source was depended upon only after 7days of exposure. The glycogen and the protein reserves were relatively less depleted.

Ram and Sathyanesen (1987) reported that even in small concentrations pollutant like mercury was capable of biochemical alterations, which could lead to severe physio metabolic dysfunction and death. It was further pointed out that young fishes are generally more susceptible to emission toxicity than the adults. Literature reports depletion of reserves because of pollutants, such as a significant decrease in the glycogen reserves of both the liver and muscle has been reported in *Heteropneustes fossilis* in response to 25 and 50 ppm of mercury (Qayyam and Shaffi, 1977). However, there are also reports of various toxicants caused an increase in the glycogen level of various tissues of different fishes (Nath and Kumar, 1987). The increased concentration of protein in *Apanius disper* expososed to mercury is supported by the work of Hilmy *et al*. (1980). The transport and storage of these metal ions in an effort to detoxify, required buffering of the metal concentrations resulting in protein synthesis. A majority of proteins are continually degraded and resynthesised quickly. Accordingly to Farman Farmaian *et al*., (1980), heavy metals inhibit amino acid absorption by the organism, by acting on the luminal surface of microvillae membrane of the gut. This is more probable due to the specific binding of the Hg to the exposed luminal sulphydryl groups of the carrier protein of the neutral aminoacids. Rai (1987) also observed similar changes in the quantity of serum protein of a fresh water teleost, *Catla catla* after mercury treatment. The present study, found that the fish became irritated after exposure to mercury. They get irritated at the slightest provocation and were hyperactive and hyperactivity depletes the stored food materials present in the muscle and liver. Toxicants are known to oxidise glutathione, haemoglobin etc. damage cell membranes and organelles by lipid peroxidation, inhibit many enzymes and thus disrupt important physiological functions of the body. The body needs enough energy to produce glutathione, metallothionine, glucuronic acid and other substances to remove toxicants by activation, inactivation or conjugation and to repair damaged organelles and replace lost cell constituents.

Diffusing capacity of the gills is reduced subsequent to the irritating action of pollutants which are responsible for the secretion of mucus on the surface of the gills (Shaffi, 1980). Interference with gas exchange reduces oxygen levels within the blood circulating to the brain where responses are initiated by the respiratory centre medula oblongata. The respiratory centre may coordinate cardiovascular changes and stimulate the hormonal system and erythropoietic tissue compensate for the decreased oxygen supply to the tissues. A decreased glycogen level in the body may be a step in that line. Increase in anaerobic metabolism has been shown to be a rapid and clear response against depletion of energy caused by lack of oxygen (Van den Thillart, 1982). Basha *et al*., (1984) suggested the prevalence of hypoxic conditions in the tissue and a reduction in the rate of oxidative metabolism at the mitochondrial level in *Tilapia mossambica* exposed to toxicants. In the present study also, mercury may create such a condition in the fish, *L.calcarifer.* The significant decrease in the protein content of liver and muscles of metal dosed *L.calcarifer* occurred at the end of the exposure period, mainly at 21 and 28 days. This clearly indicates that the body utilises the glycogen stores first to meet the increased energy demand. When the glycogen stores decrease, the body utilizes the protein for energy production. This is manifested as decrease in the protein content in different tissues and sera. The decline in the liver and muscle protein would suggest an intensive proteolysis contributing to the increase in free amino acids to be fed into FCA cycle as keto acids. Such a possibility is further strengthened by the investigation of Shakoori *et al.,* (1976) which revealed both qualitative and quantitative variations in the tissue amino acids of fishes exposed to toxicants.

Decreased protein content may be possibly due to protein breakdown which increases the aminoacid pool in the tissue. It is also reported that decreased protein may damage the hepatic tissue and an intensive proteolysis (Kurpad., 2006) result in increased amounts of free aminoacid to be fed into TCA cycle as keto acids. The decrease in protein following exposure to Mercury suggests a possible degradation by increased proteolysis. The increased proteolysis could be attributed to the damage caused to lysosomal membranes, permitting the leakage of lysosomal enzyme into the cystol. The lack of alteration in protein levels in the liver and muscle of *L.calcarifer* exposed mercury at 7 and 14 days (except in the liver of fishes exposed to higher concentrations of mercury) could be because the body utilises the glycogen of these tissues during the initial period of exposure. The depletion of glycogen in the tissues of *L.calcarifer* after metal exposure proves this. These findings support the concept of Fry (1971) that fishes tend to resist a changed situation for a specific period, but will eventually succumb as a result of their inability to adapt.

A defect in protein synthesis by the action of toxicant can also decrease the protein content in different tissues. An altered relationship between the ribosomes and the membranes of the endoplasmic reticulum may also produce a defect in protein synthesis. Rath and Misra (1980) examined the changes in nucleic acids and protein content in the liver, muscle and brain of *Tilapia mossambica* exposed to the insecticide, dichlorvos. Post exposure studies revealed a significant decline in DNA and RNA contents of the liver, muscle and brain. They observed that the liver showed a greater loss of protein than the muscle.

CONCLUSION

Similarly in the present study in *L.calcarifer* exposed to mercury, the liver showed a greater loss of protein than the muscle. The results of the present study show a decrease in protein contents in the liver and muscle of *L.calcarifer* exposed to mercury and this may be due to the decreased protein synthesis and an increased proteolytic activity.

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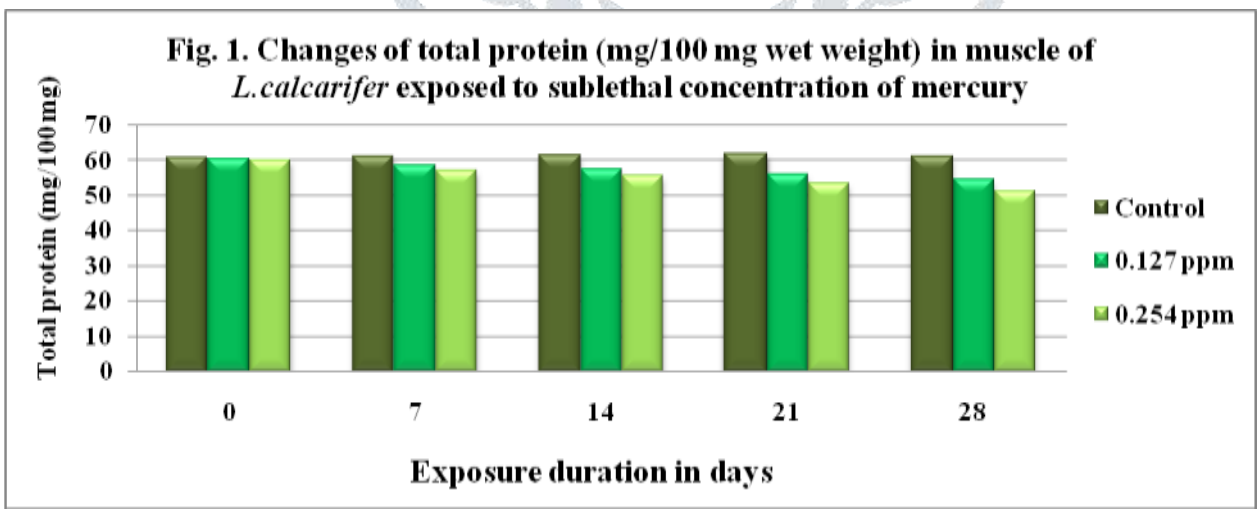

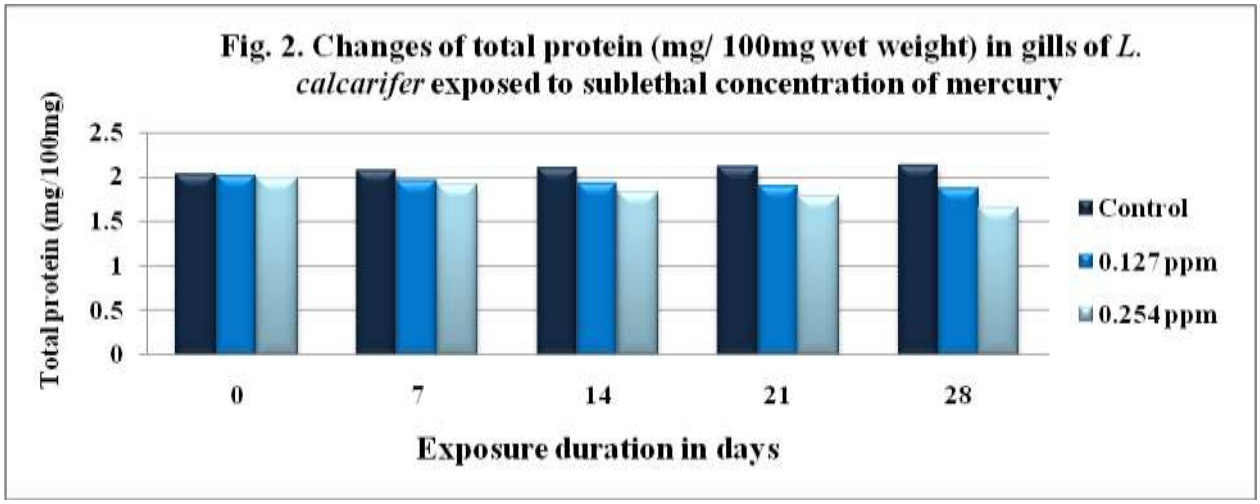
Fig. 2. Changes of total protein (mg/ 100mg wet weight) in gills of L. calcarifer exposed to sublethal concentration of mercury
Total protein (mg/100mg)
2.5
2
1.5
1
0.5
0
0
7
14
21
28
Exposure duration in days
Control
0.127 ppm
0.254 ppm

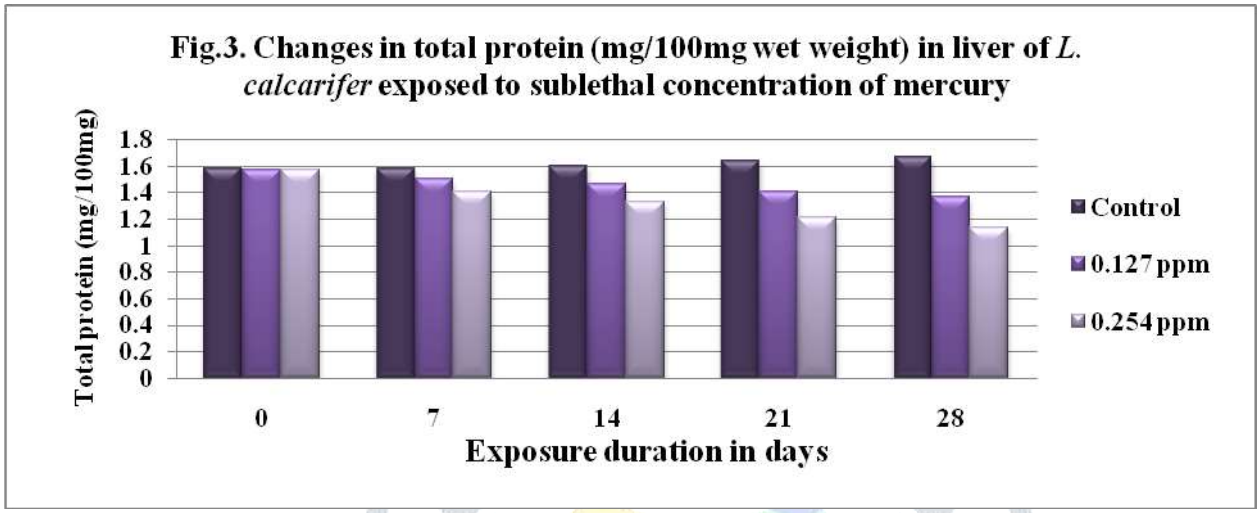
Fig.3. Changes in total protein (mg/100mg wet weight) in liver of L. calcarifer exposed to sublethal concentration of mercury
Total protein (mg/100mg)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
7
14
21
28
Exposure duration in days
Control
0.127 ppm
0.254 ppm

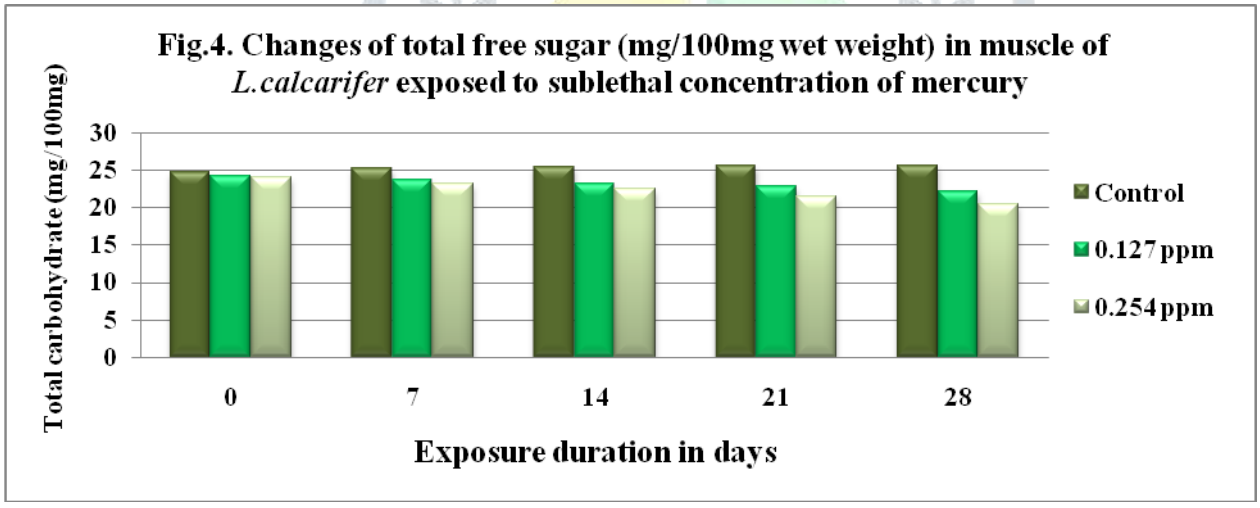
Fig.4. Changes of total free sugar (mg/100mg wet weight) in muscle of L.calcarifer exposed to sublethal concentration of mercury
Total carbohydrate (mg/100mg)
30
25
20
15
10
5
0
0
7
14
21
28
Exposure duration in days
Control
0.127 ppm
0.254 ppm

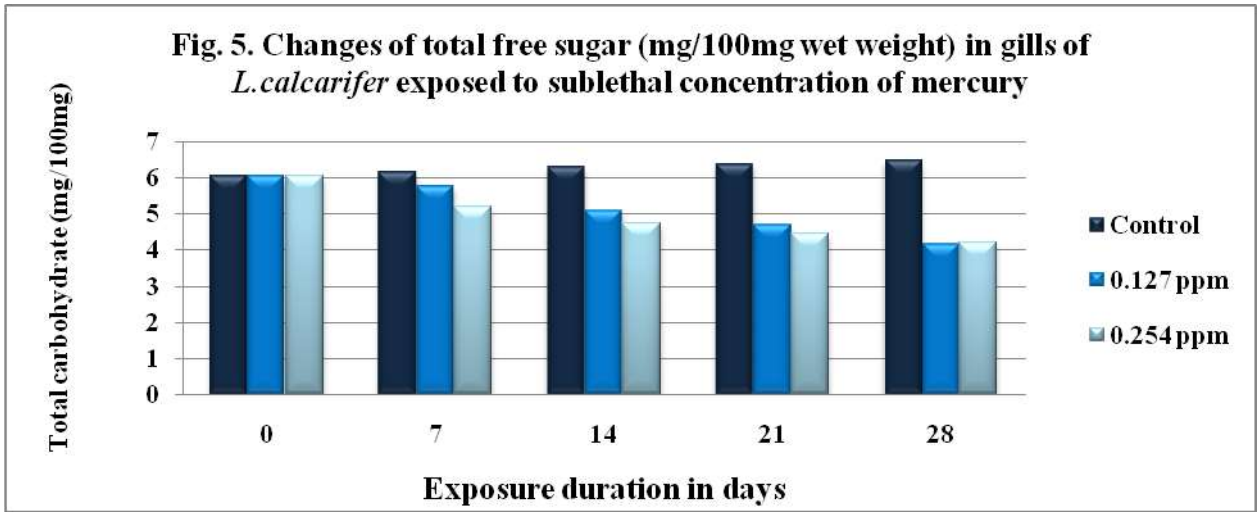

Fig. 5. Changes of total free sugar (mg/100mg wet weight) in gills of *L.calcarifer* exposed to sublethal concentration of mercury

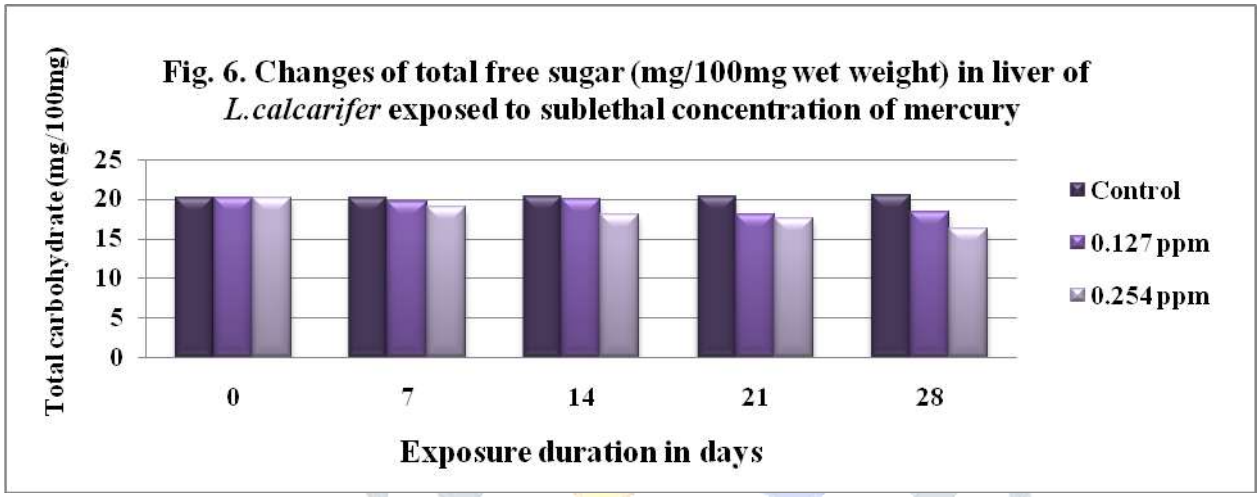

Fig. 6. Changes of total free sugar (mg/100mg wet weight) in liver of *L.calcarifer* exposed to sublethal concentration of mercury

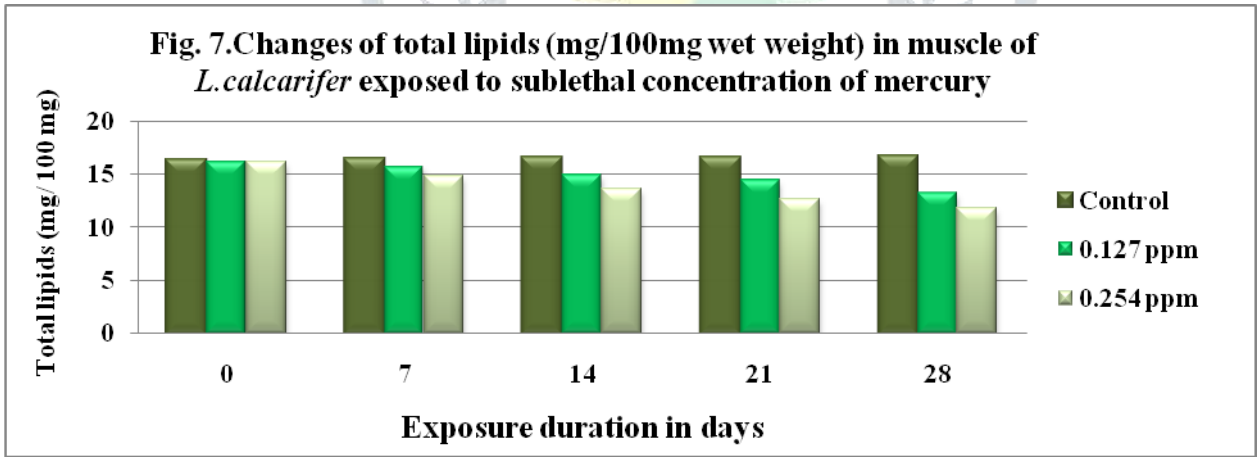

Fig. 7.Changes of total lipids (mg/100mg wet weight) in muscle of *L.calcarifer* exposed to sublethal concentration of mercury

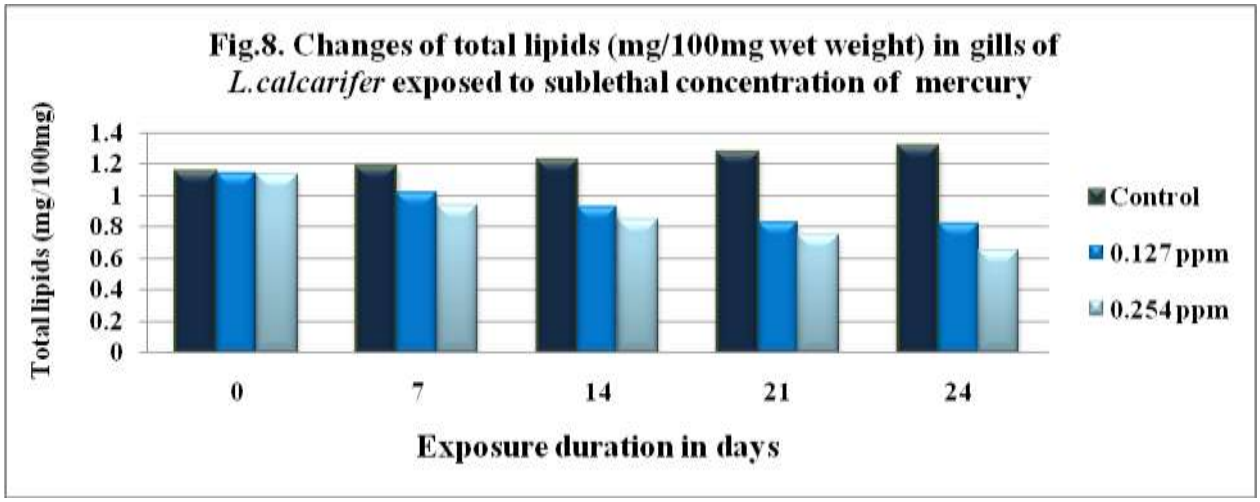
Fig.8. Changes of total lipids (mg/100mg wet weight) in gills of
L.calcarifer exposed to sublethal concentration of mercury
Total lipids (mg/100mg)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
7
14
21
24
Exposure duration in days
Control
0.127 ppm
0.254 ppm

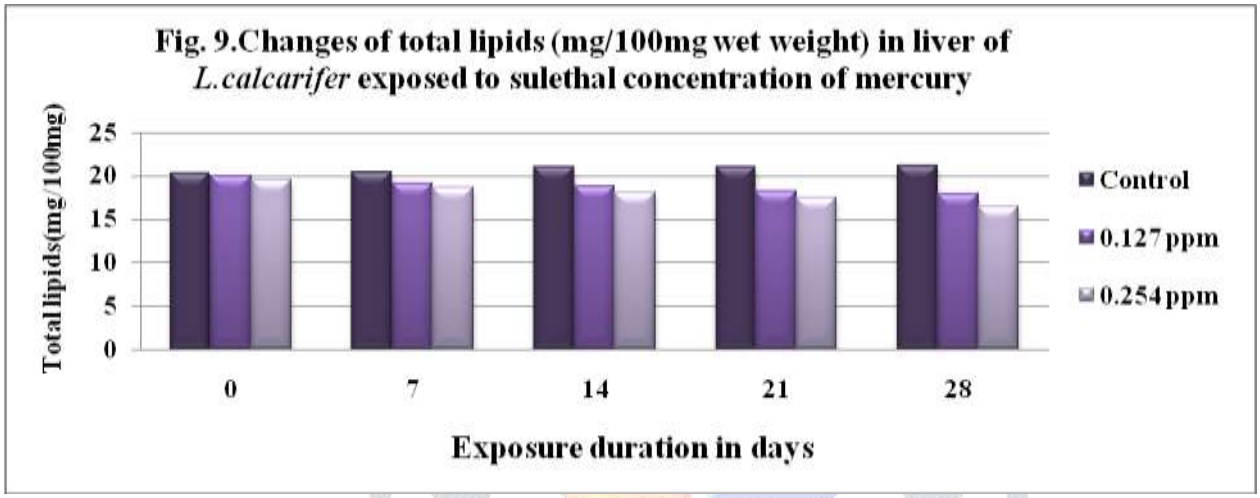
Fig. 9.Changes of total lipids (mg/100mg wet weight) in liver of
L.calcarifer exposed to sulethal concentration of mercury
Total lipids(mg/100mg)
25
20
15
10
5
0
0
7
14
21
28
Exposure duration in days
Control
0.127 ppm
0.254 ppm