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Research Article

BIOCHEMICAL RESPONSES OF ASIAN SEA BASS, *LATES CALCARIFER* (BLOCH) SUBLETHAL COPPER EXPOSURE

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ABSTRACT

Copper 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 (6.83 ppm and 13.66 ppm) of copper were studied for 28 days of exposure (DoE). Sub-lethal doses of copper significantly (P<0.05) altered the levels of the total protein (TP), carbohydrate (TC), 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, TC 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 copper 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.

Keywords: Copper, Toxicity, Sea bass, Biochemistry.

INTRODUCTION

The nutritional value of different species of fishes depends on their biochemical components such as protein, carbohydrate and lipids. These proximate components could serve as sensitive indicators for detecting potential adverse effects, particularly the early events of pollutant damage because their alterations appear before the clinical symptoms produced by the toxicant (Rao, 2006). It is therefore important that potential effects of acute and chronic concentrations of pollutant on proximate composition are determined and interpreted to delineate mechanisms of pollutant action and possible ways to mitigate adverse effects (Matos *et al*., 2007).

Copper 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. Among the different heavy metals, copper is special concern, since the metal is considerably toxic to aquatic animals at ecologically relevant concentrations (Mzimela *et al.*, 2002). Sub lethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism by reacting with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival (EIFAC, 1975 and Waldichuk, 1979). Copper is a trace element that plays a fundamental role in the biochemistry of organisms, including aquatic organisms that can take it up directly from water (Grosell *et al.*, 2003). However, it can become toxic at high concentrations (Lam *et al.*, 1998).

The protein content in the tissues of animals plays a role in the metabolism (Palanivelu *et al.*, 2005). Moorthy and Priyamvada, (1982) stated that the protein content of the cell may be considered as an important tool for evaluation of physiological standards. The soluble protein fraction represents the activity level of enzymes in general. The structural protein fraction forms the structural moiety of a cell (Lehninger, 1978). Begam and Vijayaraghavan, (1996) observed protein depletion in the fish indicates the physiological strategy in order to meet the energy demand and to adapt itself to the changed metabolic system which may lead to the stimulation of degradative processes like proteolysis and utilization of degraded products for increased energy metabolism. When any aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome the toxic stress (Maharajan *et al.*, 2012a, 2014; Paruruckumani *et al*., 2015a, b, c & d). Verma *et al*. (1981) reported on the toxic effects of sublethal concentration of copper sulphate, on certain biologically important enzymes in *Saccobranchus fossilis.*

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In the tissue protein, carbohydrate and lipids play a major role as energy precursors for aquatic organisms exposed to stress conditions (Ramalingam, 1980). An alteration in biochemical and physiological changes in the crab *Portunus pelagicus* due to copper and zinc have been reported by Hilmy *et al.* (1988). Similarly, Katticaran *et al.* (1995) reported the variations of carbohydrate and protein contents in the clam, *Sunetta scripta* during its exposure to copper. Villalan *et al.* (1988) observed that heavy metals altered protein, lipid and carbohydrate levels in the crab, *Thalamita crenata.* Baden *et al.* (1994) also reported similar changes in the distribution of glycogen in the tissues of Norway lobster, *Nephrops norvegicus* exposed to copper. Maharajan *et al*., (2012b & c) observed the biochemical changes of various tissues and haemolymph of spiny lobster, *P.hoamrus homarus* and the fresh water crab, *Paratelphusa jacquemontii* when exposed to sub-lethal doses of copper. Holland *et al*. (1960) reported some of the effects copper has on fish. Copper salts combine with proteins present in the mucus of the fish's mouth, gills, and skin, preventing aeration of the blood.

The copper is known for instantaneous physiological disorders and alteration in the pathways of protein metabolism in tissues and organs. Therefore the biochemical parameters are the best indicators of stress situations caused by copper as one of the heavy metals. Toxicity testing is an essential tool for assessing the effect and fate of toxicant. Thus, this study was planned to estimate the toxicity and variations in the protein, total free sugar and lipid levels in various tissues of Asian sea bass, *L. calcarifer.*

MATERIALS AND METHODS

Experimental fish

Healthy hatchery reared three month old juvenile Asian sea bass, *L. calcarifer* with mean total length of 7.06 ± 0.15cm and a mean total weight of 10.18 ± 0.24gm were obtained from the Rajiv Gandhi Centre for Aquaculture, Thirumullaivasal near Sirkali, Nagapattinam Dist, Tamil Nadu, India. Fish samples were acclimatized for 2 weeks in a 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 commercially prepared sea bass pettet feed. They were starved for 24 h before and during experiment.

Chemical

For preparation of stock solution 3.9 gram of Copper ll sulphate pentahydrate ($CuSo_4$ 5 H_2O) (Merck) was dissolved in one litre of double distilled water and used as stock solution. It was stored in a clean standard flask at room temperature, in the laboratory.

Experimental Procedures

Test Concentration: Fish were exposed to nominal 6.83ppm and 13.66 ppm as copper. Doses were theoretically sublethal, 10% and 20% respectively, of the Maximum Acceptable Toxicant Concentration (MATC), which was 68.3ppm.

Experimental design: A recirculation closed system was set up according to Muthuwan (1998). The experiment was carried out in 360 l glass aquarium (120x60x50 cm), in which one compartment (50x50x40 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 27±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 acclimatization in a holding tank, ten healthy fish (8.06 ± 0.19cm in length and 11.18 ± 0.67gm in weight) were transferred to each aquarium at a loading density of 0.69 g/l. Three replicates were performed for test concentration and control. Fish were fed twice daily with chopped fresh fish at 10:00 and 14:00. Uneaten food was quickly removed from the system. Fish 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. The ammonia nitrogen and nitrite nitrogen levels were controlled and kept within 0.2 mg/l for exchanging the water in 25%. The actual concentration of copper was measured weekly before and after its addition to maintain copper concentrations at the designed level. Mortality and behaviour were observed daily in each concentration. Two fish from each aquarium were sampled at 0, 7, 14, 21 and 28 days post-exposure.

Tissue samples and biochemical analysis: Sample was extracted from the tissues of muscle (MU), gills (GL) and liver (Li) at different concentration and different duration. Concentrations of biochemical constituents in different tissues were estimated by following standard procedures. The total protein (TP) and the total carbohydrate (TC) concentrations in different tissues were determined according to the methods of Lowry *et al*. (1951) and Roe (1955). The total lipid (TL) content was estimated by the method of Barnes and Blackstock (1965). 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

Copper induced changes in proximate composition

Changes in the TP Levels: Levels of the TP in different tissues of control and exposed Asian sea bass during the exposure period are depicted in Figure 1, 2 & 3. The TP concentrations were significantly lower in test Asian sea bass than those of controls on all DoE (P<0.01). 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 6.83ppm and 13.66 ppm was noticed (P>0.01). 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 copper. Higher percent depletion in the hepatic protein was observed in test Asian sea bass exposed to 13.66ppm compared to those exposed to 6.83 ppm of copper (P<0.05).

Changes in the TC Levels: Levels of the TC in different tissues of test Asian sea bass and controls during the exposure period are shown in Figure 4,5 & 6. The TC concentrations were significantly lower in test Asian sea bass than those of controls on all DoE. The depletion in the TC levels in the MU of test Asian sea bass was significant with the progress in the period of exposure. Concentrations of hepatic carbohydrate in the test Asian sea bass ranged from 25.48±0.45% (0 DoE) to 22.01±0.61% (28 DoE) over control Asian sea bass (100%). The levels of the TC 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 TC levels in the studied tissues on the last day of exposure (28 DoE) was found to be MU>LI>GL.

Changes in the TL Levels: Levels of the TL in different tissues of the test Asian sea bass and controls during the exposure period are depicted in Figure 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.

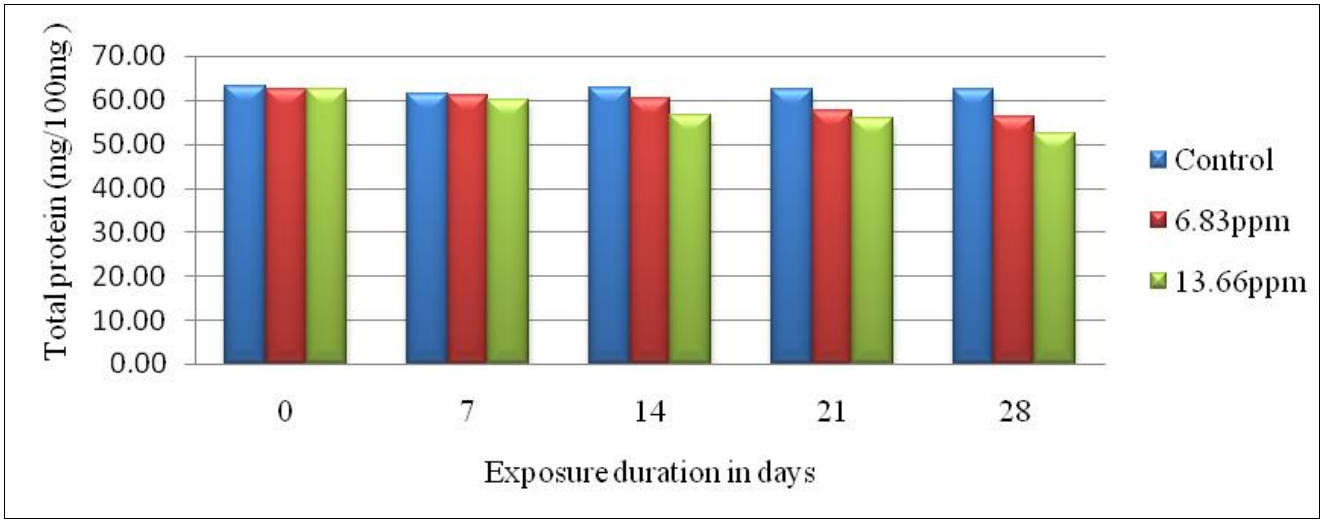

Figure 1. Changes of total protein (mg/100 mg wet weight) in muscle of *L. calcarifer* exposed to sublethal concentrations of copper.

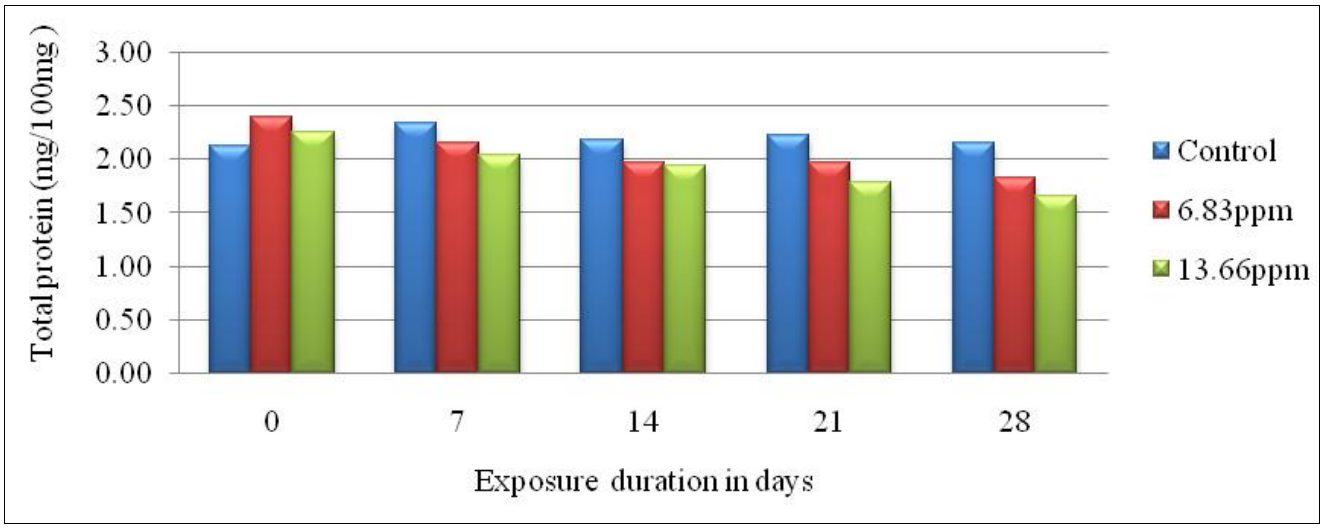

Figure 2. Changes of total protein (mg/100 mg wet weight) in gills of *L. calcarifer* exposed to sublethal concentrations of copper.

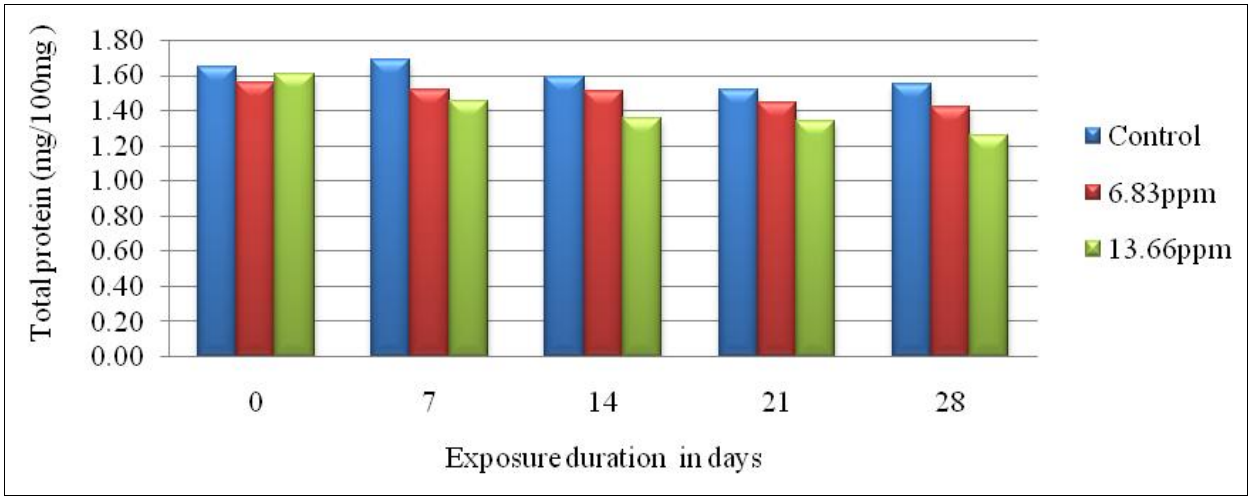

Figure 3. Changes of total protein (mg/100 mg wet weight) in liver of *L. calcarifer* exposed to sublethal concentrations of copper.

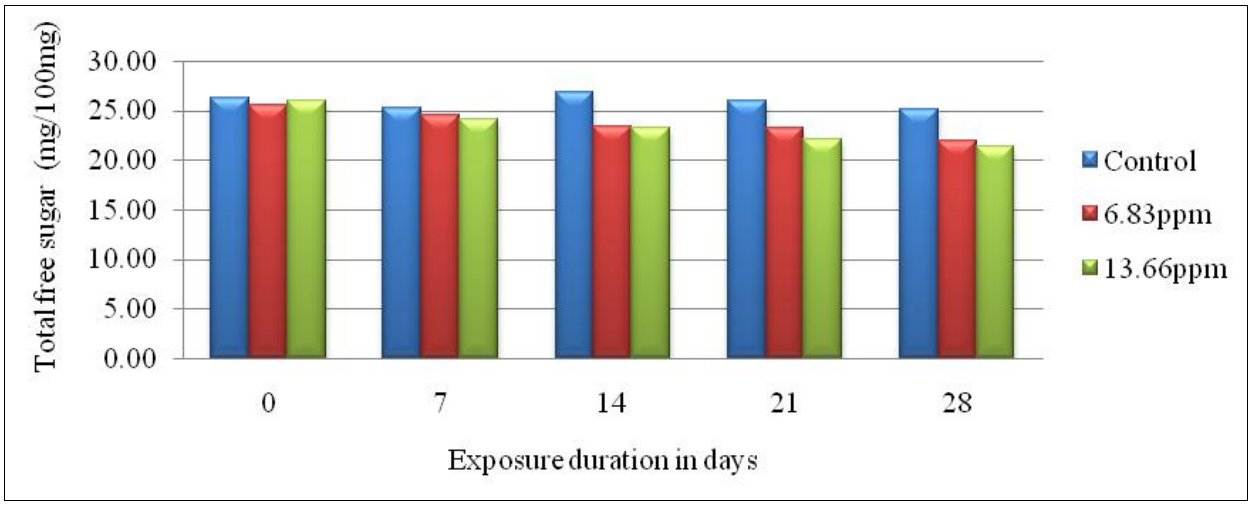

Figure 4. Changes of total free sugar (mg/100 mg wet weight) in muscle of *L. calcarifer* exposed to sublethal concentrations of copper.

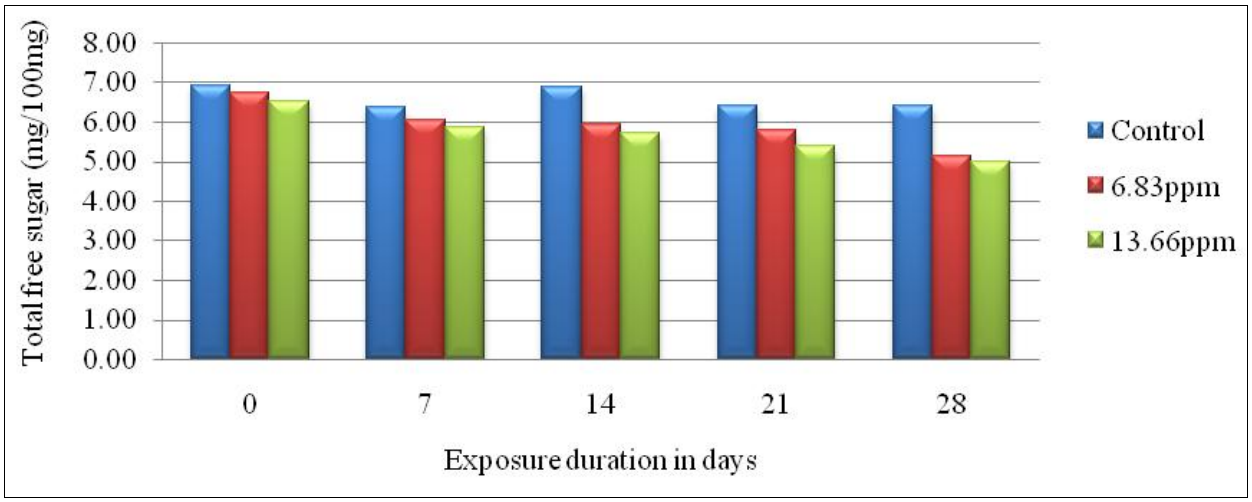

Figure 5. Changes of total free sugar (mg/100 mg wet weight) in gills of *L. calcarifer* exposed to sublethal concentrations of copper.

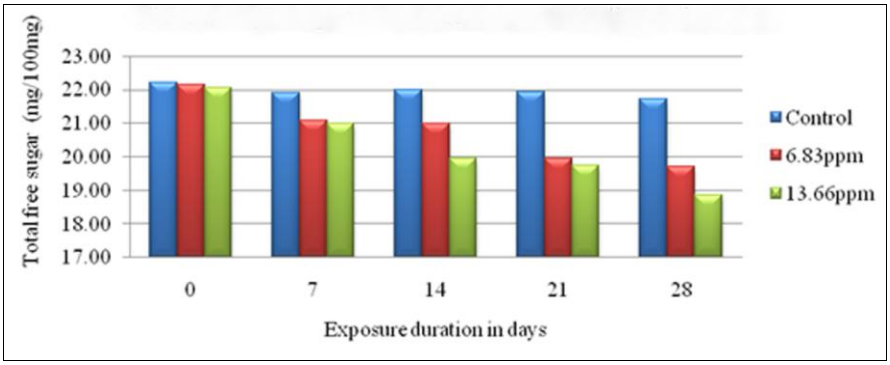

Figure 6. Changes of total free lipids (mg/100 mg wet weight) in liver of *L. calcarifer* exposed to sublethal concentrations of copper.

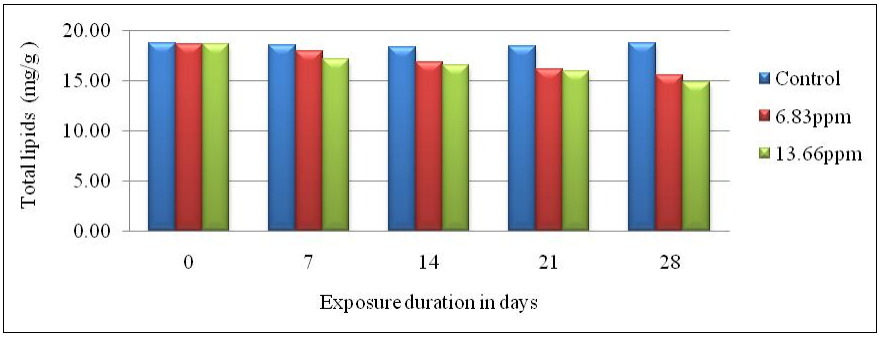

Figure 7. Changes of total lipids (mg/100 mg wet weight) in muscle of *L. calcarifer* exposed to sublethal concentrations of copper.

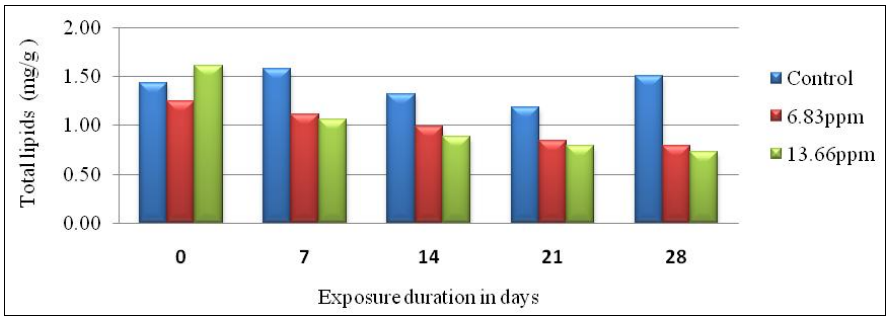

Figure 8. Changes of total lipids (mg/100 mg wet weight) in gills of *L. calcarifer* exposed to sublethal concentrations of copper.

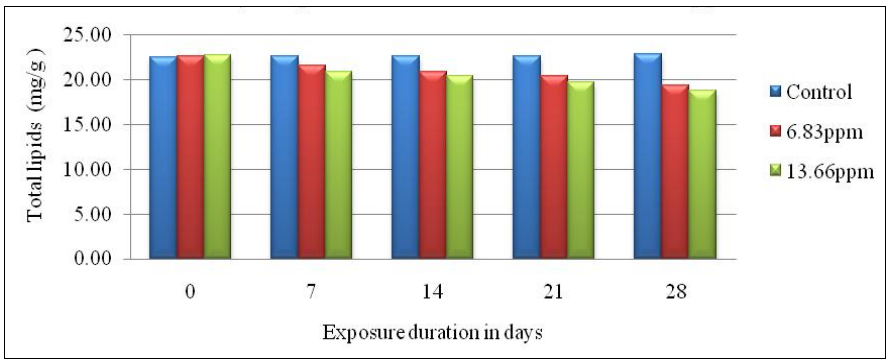

Figure 9. Changes of total lipids (mg/100 mg wet weight) in liver of *L. calcarifer* exposed to sublethal concentrations of copper.

DISCUSSION

Heavy metals are recognized as one of the most hazardous environmental pollutants and are toxic to many living organisms (Sontakke and Jadhav, 1997). Metal ions once absorbed into the body are capable of reacting with a variety of active binding sites and then disturbing the normal physiology of the organism which may lead to the death of organism. Fishes are responding to various stressors by a series of biochemical and physiological stress reactions, so called secondary stress responses comparable to those of higher vertebrates (Mazeaud and Mazeaud, 1981).

The total protein content in muscle, gills and liver was altered; the changes in protein levels were insignificant when compared to the control fish. In the present study, the total protein in the muscle, gills and liver of *L. calcarifer* showed decreasing trend as the duration of exposure to copper increased (muscle: 62.59±0.59 mg/100mg to 52.14±0.63 mg/100mg, gills: 2.42±0.09 mg/100mg to 1.72±0.06 mg/100mg and liver: 1.75±0.07 mg/100mg to 1.27±0.08 mg/100mg). It is likely that the observed reduction in total protein of *L. calcarifer* is due to a direct consequence of the stress imposed by copper. The alterations in the protein levels might be due to the adaptation of the animals to metal stress. The present study supported by previous reports (Vutukuru, 2003) has shown the decrease of total protein content in fish exposed to Copper. Decreased rate of protein synthesis, utilization for energy or secreted mucous proteins could alter the protein levels in animals under metallic stress (Vutukuru, 2005). Also, the depletion of total protein suggests an increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. The decreased protein level during exposure to pollutants may be due to increased catabolism and decreased anabolism of proteins as reported in freshwater bivalve *Parrysia corrugata* (Deshmukh and Lomte, 1998).

The initial declining levels of protein may be attributed to the higher rate of energy production at the onset of various enzymatic blockages. It is known that copper blocks the mRNA synthesis and thereby the protein synthesis at the level of transcription. However, copper inhibits the action of enzyme protease reflecting a steady decline in the total percentage of protein.

Total free sugars are very important biological compounds as they are the chief source of energy and also structural constituents of protoplasm. In the present study the total free sugar in the muscle, gills and liver of *L. calcarifer* showed decreasing trend with increasing concentration of copper (muscle: 26.54±0.30 mg/100mg to 22.01±0.61 mg/100mg, gills: 6.89±0.08 mg/100mg to 5.01±0.12 mg/100mg and liver: 22.35±0.49 mg/100mg to19.05±0.22 mg/100mg). It may be due to the breakdown of glycogen to cope with the high energy demand for the detoxification process, since carbohydrate forms major source of energy under toxicity (Hochachka and Somero, 1973).

Glycogen plays an important role as a readily mobilized storage form of total free sugar in muscle (Stryer, 1988), which decreases during toxicity as evidenced in *L. calcarifer* also. Similarly, the total free sugar declines in the gills and it deposit as granules on the lamellae interrupting the uptake of oxygen. This is consistent with the earlier studies in Norway lobster, *Nephrops norvegicus* resulting in hypoxic condition (Baden *et al.*, 1994), where the binding of oxygen with haemoglobin decreases with the increase in concentration of copper, thereby resulting in hypoxia (Depledge and Bjerregaard, 1989). The total free sugar may have been used to fuel detoxification mechanism operating within the animal as reported in *Panulirus longipes* and *Jasus lalandii* (Cockcroft, 1997).

The total lipids in the muscle, gills and liver of *L. calcarifer* showed decreasing trend as the duration of exposure in each concentration of copper increased (muscle: 18.92±0.62 mg/g to 15.13±0.48 mg/g, gills: 1.64±0.06 mg/g to 0.76±0.04 mg/g and liver: 22.92±0.67 mg/g to 19.21±0.42 mg/g). A significant decrease in muscle and liver may be due to its utilization for energy during detoxification mechanism.

In the sea bass, *L. calcarifer* the liver is the most sensitive indicator of physiological stress than the muscle tissue (Trendal and Prescott, 1989). The finding is in accord with the results obtained in *L. calcarifer*, although muscle and liver are the major energy stores. Lipids were found to be the primary source of energy under stress condition in *Penaeus duorarum* (Schafer, 1968). An increase in metal concentration and exposure duration resulted in the reduced level of lipid in *Oreochromis mossambicus* (Overstreet, 1988). Similar results are reported in bivalve *Sunetta scripta* (Katticaran *et al.*, 1995).

Copper has been associated with lipid peroxidation. Beckman and Zaugg (1988) reports that cuprus- ions may potentiate lipid peroxidation by a metal- metal reaction reducing the ferric ions rather than by promoting propagation reaction in the fish Chinook salmon *Oncorhynchus tshawytscha*. As the lipid reserves are ultimately transferred to the detoxification process in *L. calcarifer*, the total percentage of lipids decline severally in liver followed by muscle and gills. These results suggest the important role of liver in storage and mobilization of energy during detoxification of copper in *L. calcarifer*.

CONCLUSION

Results of this study revealed that sub-lethal doses of copper significantly alter the proximate composition of major tissues, particularly the total protein levels in the muscle tissues and thereby reducing the nutritive value of this economically important sea bass.

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