Heavy metal induced biochemical effects in an estuarine teleost

K B Veena, C K Radhakrishnan & J Chacko

School of Marine Sciences,
Cochin University of Science and Technology, Cochin 682 016, India

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Etroplus maculatus (Bloch), an estuarine teleost was exposed to lethal and sublethal concentrations of copper, mercury and selenium. At lethal concentrations, copper was found to be most toxic. Three stages of maturity selected for the experiments were observed to have been effected by the sublethal concentration of each metals differently. The changes caused in the biochemical constituents of various tissues has been presented and discussed.

Heavy metals are regarded as serious pollutants of the aquatic ecosystem because of their environmental persistence and their ability to be concentrated by aquatic organisms. Their toxicity, even at low concentrations, has been found to induce serious health hazards.

Copper, selenium and mercury are the three metals selected for the present study. Copper and selenium are essential dietary trace elements in fish. However, they could function as potential water borne toxicants at elevated concentrations. Copper, widely used as an algicide and in the treatment of fungal and parasitic disorders in fishes, finds its way into the estuary from the various industrial units in the Greater Cochin industrial belt. Significant input of selenium in the aquatic environment results from geochemical processes as well as from the combustion of fossil fuels. Eventhough aquatic levels of this metal has not been reported from the Cochin backwaters, its occurrence in the bivalve Villorita cyprinoides var cochinensis sampled from these waters makes its presence evident. Mercury concentrations reported from the aquatic system around Greater Cochin has only served to induce more rigorous and systematic investigation.

A realisation of the potential hazard that these metals pose to fishes, prompted the initiation of this study of the toxic effects of these metals (manifested both at the lethal and sublethal levels) on the estuarine teleost Etroplus maculatus. The emphasis has been to study the biochemical changes undergone at different stages of maturity by the three metals. Estimation of sialic acid, a constituent of glycoprotein hormone and cholesterol, a precursor of steroid hormone gives a measure of the respective hormones in the tissues examined. The variation in the concentrations of various energy reserves (glycogen, lipid and protein) in the muscle and liver were estimated. Lactic acid has been estimated to study the anaerobic respiration aspects of the fish during metal stress.

Materials and Methods

The fishes were collected from a brackish water canal of the Cochin backwater system. Animals for toxicity studies were maintained in the laboratory in well-aerated, dechlorinated, tap water. For sublethal studies, only the females were selected.

The three metals used were copper, mercury and selenium. Metallic copper was washed with concentrated nitric acid to remove traces of oxide film formed. Nitric acid was washed off with acetone. One gram of copper (dissolved in the minimum quantity of dil. nitric acid), 1.3539 g of mercuric chloride and 1.406 g of selenium dioxide were weighed out and made up to 1000 ml each in deionised distilled water to obtain 1000 ppm stock solutions of each metal. The test concentrations were prepared by appropriately diluting the stock solutions.

All tests were conducted in glass troughs, by static renewal bioassays. The physico-chemical parameters of the test medium were - temperature: 28°C ± 2; pH: 6.8 ± 0.1; dissolved oxygen: ≤ 8.2 ml l⁻¹.

A range of test concentrations (ppm), as indicated by preliminary toxicity tests were prepared (0.04, 0.05, 0.06, 0.07 for copper, 0.12, 0.13, 0.14, 0.15 for...
mercury and 4.00, 5.00, 6.00, 7.00 for selenium). Duplicates were maintained for each of the test concentrations and for the controls. Mortality was recorded at 24h intervals during the 96h period. The LC50 values were calculated from the cumulative percentage mortalities using the log-probit method of Litchfield and Wilcoxon.

Metal induced changes in the biochemical functions of fishes were assessed through sublethal toxicity studies. The metal concentrations corresponding to 1/10 of the LC50 value was chosen as the respective sublethal test concentrations.

The acclimatised fishes were maintained in three different troughs: the first trough containing fishes meant to be withdrawn immediately prior to the commencement of the experiment, the second trough containing fishes meant to be withdrawn after 48h of the metal exposure and the third trough with fishes to be withdrawn after 96h of exposure. Tissues from six fishes of the same maturity stage were homogenised. Approximately 1g was weighed to make a sample. Three such samples were taken for the biochemical analyses.

The different stages identified were, stage I (immature virgins - 5-9mm body length; 0.06 to 0.16mg body weight), stage II (maturing - 10-12mm body length; average body weight 0.34mg) and stage III (ripening - body length of about 13-14mm and weight about 0.4mg).

The biochemical constituents analysed from different tissues were, sialic acid from pituitary gland and blood, cholesterol from blood and ovary, glycogen, lipid, protein and lactic acid from muscle and liver.

Thiobarbituric acid assay, as proposed by Warren, was adopted for the estimation of sialic acid. Cholesterol estimation was based on Liberman-Burchard reaction. The quantitative determination of lipid was performed according to the procedure suggested by Barnes & Blackstock. Protein was estimated by the method given by Lowry et al. The modified Pfuger method as given by Hassid & Abraham, was used for the estimation of glycogen. Glycogen was hydrolysed to glucose and glucose was then estimated by Heath & Barnes method. Lactic acid was estimated by the procedure given by Barker. All biochemical estimations were conducted by using Hitachi 150-20 UV-VIS spectrophotometer. The data obtained was analysed statistically using ANOVA.

Results and Discussion

Results of the acute static bioassays have been presented in Table 1. Copper was found to be 92 times and mercury 35 times more toxic than selenium. Thus the order of toxicity to E. maculatus, was Cu>Hg>Se. The fishes progressively exhibited irregular erratic swimming, frequent surfacing, gulping of air, revolving, convulsions, extension of fins, accelerated ventilation with rapid arhythmic opercular and mouth movements etc. Dead animals had blood clots on the gill surfaces with widely opened mouth and stretched gills. Air bubbles were seen trapped within the mucus. Abnormalities exhibited have been attributed to nervous impairment and blockage of nervous transmission between nervous system and various effector sites as a result of heavy metal treatment. The sub-lethal concentrations used for the three metals were - Cu, 0.005 ppm; Hg, 0.013 ppm and Se, 0.462 ppm.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Concentrations (ppm)</th>
<th>CPM</th>
<th>96h LC50 values (95% confidence limits)</th>
<th>Relative toxicity comparison between <em>96h LC50</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>0.04</td>
<td>20</td>
<td>0.05</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>40</td>
<td>0.05-0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>80</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.07</td>
<td>80</td>
<td></td>
<td></td>
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<tr>
<td>Mercury</td>
<td>0.12</td>
<td>40</td>
<td>0.13</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>40</td>
<td>0.12-0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>80</td>
<td></td>
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<tr>
<td></td>
<td>0.15</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>4.00</td>
<td>20</td>
<td>4.62</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>60</td>
<td>3.97-5.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.00</td>
<td>80</td>
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*no of times more toxic vis-a-vis selenium
CPM—Cumulative percentage mortality
During this period, the animals did not exhibit any signs of stress. Figures 1 to 3 represent the toxic effects of pollutants on the various biochemical constituents in different tissues in the three female maturity stages analysed during 0, 48 and 96h of exposure.

Analyses of the biochemical constituents revealed that these metals affected the different maturity stages of the teleost adversely. Copper and mercury induced variations in concentrations of sialic acid in the pituitary gland. When the animals were exposed to selenium, significant variation was observed both between stages and between different time intervals in the sialic acid concentrations of the pituitary gland. The increase in the sialic acid content of the pituitary gland after 96h exposure to copper (Fig. 1a) suggested activation of the gonadotrophs by this metal. Contrary to the action of copper, mercury exposure resulted in a decrease in the sialic acid content of the pituitary gland, after an initial increase in concentrations at 48h. Very high concentrations were recorded in stage I animals. This abnormality has been attributed to a shock on the central nervous system triggering off over-activation of the pituitary gland which resulted in the production of high concentration of glycoprotein hormone\(^\text{12}\). Mercury compounds react directly or catalytically with a group of lipids in the

![Fig. 1: Metal induced variations of sialic acid and cholesterol concentrations (mg g\(^{-1}\)) in different tissues at the three stages of maturity (I,II,III) of Etroplus maculatus.](image)

![Fig. 2: Metal induced variations of protein and lipid concentrations (Mg g\(^{-1}\)) in different tissues at the three stages of maturity (I,II,III) of Etroplus maculatus.](image)
membranes of cells of the central nervous system to promote hydrolysis and hydrolytic decomposition. Selenium exposure resulted in a very slight decrease in the sialic acid content of pituitary gland with its concentration being very close to the control values.

A statistically significant variation was observed in the sialic acid content of blood in the case of the copper and mercury exposed animals both with time and between different stages. Observed trend in the sialic acid content of the blood after exposure to the metals has been related to the mobilization of the glycoprotein hormones from the pituitary gland to the blood (Fig. 1b).

A significant variation in ovary cholesterol concentration was observed between different time intervals on exposure to copper. Mercury, selenium and copper produced significant variations in the concentration of blood cholesterol between different stages of maturity. Cholesterol concentrations in the ovary and blood were considerably altered from control values on exposure to all the three metals (Fig. 1c, d). The changes in the cholesterol concentrations on exposure to copper and mercury are indicative of an initial mobilization of cholesterol from blood to ovary, generally for use in the production of steroid hormone and other macromolecules required for ovarian development. Increase in concentrations in the cholesterol content of the ovary (Fig. 1c) is due to its accumulation followed by a suppression of the steroid hormone production. The elevated blood cholesterol concentration was also indicative of the damage and the disruption of the normal functioning of the liver which is the major site of cholesterol metabolism. Selenium exposure did not resemble the effects of copper and mercury exposure, as an increase in the cholesterol content of the ovary and blood was observed at the end of the exposure period. The route of entry of selenium compounds into the ovary was by incorporation into protein and lipoprotein that were used up by the ovary during vitellogenesis at the stimulation of estrogen.

When subjected to sublethal exposure, the energy reserves (glycogen, protein, and lipid) in the major reservoir (liver), were observed to have been depleted in different proportions. Copper exposure resulted in a marked depletion of glycogen reserves (Fig. 3a, b). A statistically significant variation was found between stages and also between different time intervals in the liver glycogen content after exposure to copper. Statistically significant variations were also observed in the case of liver lipid (between time interval) and protein. Lipid (Fig. 2c, d) and protein (Fig. 2a, b) were observed to have been used up only after 48h of exposure. Mercury and selenium exposure also, induced high depletion of glycogen (Fig. 3a, b). The variations were statistically significant between different stages of maturity. Lipid and protein were also used up simultaneously, with the lipids being preferred to proteins. Mercury induced changes in liver lipid concentrations were found to be statistically

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**Fig. 3**—Metal induced variations of glycogen and lactic acid concentrations (mg g⁻¹) in different tissues at the three stages of maturity (I, II, III) of *Eriohipus maculatus.*
different both at different time intervals and maturity stages.

The depleted glycogen concentration was reflected in elevated concentrations of lactic acid in the liver (statistically significant variation was observed, both between periods and stages on exposure to all metals) when exposed to all three metals.

Besides liver, the glycogen reserves of the muscle too, was depleted on 48h exposure to copper and selenium although. A statistically significant variation between different time intervals was observed in copper dosed animals. Significant variation was also seen between stages on exposure to mercury and selenium. The lipid and the protein reserves were comparatively less depleted. In selenium exposed animals, significant variation in muscle lipid content was observed both between stages and with time. Variation between period was also significant in the copper dosed animals. Unlike the lactic acid content of the liver (Fig. 3c), elevation in muscle (Fig. 3d) was rather low except in the case of copper and mercury. Statistically significant variations could be seen in muscle lactic acid concentration between time intervals on exposure to copper, mercury and selenium. Variations were found to be significant between stages only on exposure to copper and selenium.

The increased concentration of protein after 48h exposure to copper may be due to the transport and storage of these metal ions in an effort to detoxify it.

It is suggested that the increased lactic acid concentrations observed after exposure to the metals reduces oxygen consumption and high activity levels of succinate dehydrogenase with significant increase in lactate dehydrogenase activity. This indicated that the energy requirements in fish were being met through anaerobic oxidation due to impaired oxidative and transphosphorylative activities. A similar observation was made by Naidu et al. Lactic acid increases concomitant with glycogen decrease in the muscle and liver indicated that one of the main sites of metal-action was the respiratory system. This increase in lactic acid content indicated severe hypoxia which amply confirmed Skidmore's hypothesis, that gill damage resulted from lethal metal toxicity by modifying gas exchange and by creating a hypoxic condition at the tissue level.

In conclusion, it could be inferred that although, based on the LC50 values, copper was seen to be the most toxic metal, sublethal exposure resulted in mercury to be the most toxic of the three metals.

Acknowledgement

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References