

### **Histopathology-Liver**

When the fishes treated with 25 mg L<sup>-1</sup> lead nitrate for 10 and 20 days, space between hepatocytes was wider, due to disintegration of some hepatic cells. Large blood spaces were formed in the tissue mass. Vacuoles were more frequent and larger. After treated to 28 mg L<sup>-1</sup>, the nucleus was either absent or shifted to the margins of the hepatic cells. When the fishes treated with 30 mg L<sup>-1</sup> concentration of lead nitrate for both durations liver showed wider intrahepatic spaces with haemorrhages and cells were shrunk and no nucleus was observed.

### **Kidney**

Slight changes in renal histology were observed after the treatment of sublethal concentration of 25 mg L<sup>-1</sup> lead nitrate for 10 and 20 days. Interstitial haemopoietic tissue reduced and the lumen of the tubules was also wider in comparison to the control and the glomeruli, neck segment, shows slight necrosis. While after treated to 28 mg L<sup>-1</sup>, showed strong changes in haemopoietic tissue. Many spaces appeared between the cells. The tubules shrunk a lot. Blood spaces were frequently seen.

When the fishes were treated to 30 mg L<sup>-1</sup> concentration of lead nitrate for 10 days, necrosis of the tissues were clearly seen with shrunken tubules, wider blood spaces and more signs of haemorrhage. After 20 days, blood vessels showed clumps of erythrocytes and the glomeruli was seen spongy mass and completely deteriorated

### **Kidney**

After exposure to 25 mg L lead nitrate for 10 and 20 days, not much variation was observed in the kidney tissues, except that the glomeruli, proximal and distal tubules showed positive reactions. After exposure to 28 mg L<sup>-1</sup>, the carbohydrates in kidney of *Mystus cavasius* showed strong positive reaction in glomeruli. Blood cells also showed positive reactions. When the fishes were exposed to 30 mg L<sup>-1</sup> concentration interstitial haemopoietic tissue showed strong positive reaction with PA/S stain.

### **Bromophenol blue test for protein**

In order to see the changes in protein content in liver and kidney of *Mystus cavasius* the tissues were stained with Bromophenol Blue (BB) stain, where proteins are stained as blue colour.

### **Liver**

In 25 mg L<sup>-1</sup> lead nitrate intoxication for 10 and 20 days, nuclei of the hepatic cells showed weak reactions in comparison to control. Cytoplasmic inclusions showed weak positive BB stain. After exposure to 28 mg L<sup>-1</sup> lead nitrate. The hepatic cells started

breaking and the cell aggregates showed strong positive reactions. After exposure 30 mg L-1 concentration of lead nitrate for both durations liver showed wider intrahepatic spaces and aggregated broken cells that showed strong positive reactions with BB stain.

Fish treated with 25 mg L-1 lead nitrate for 10 and 20 days interstitial haemopoietic tissue and blood cells showed positive reactions. The glomeruli and distal tubules also showed positive reactions. After exposure to 28 mg L-1, the proteins in kidney of *Mystus cavasius* showed strong positive reaction in glomeruli and Blood cells. The cytoplasm showed negative reactions. After exposure to 30 mg L-1 concentration of lead nitrate for 20 days, the interstitial haemopoietic tissue showed positive reaction with BB stain. Positive reaction was seen at few places, but rest of the glomeruli showed weak positive reaction.

It may be concluded that the results of this study are highly useful in evaluating the heavy metal toxicity by uncontrolled discharge of polluted materials in the aquatic environment. On the whole, with the knowledge of acute and subacute heavy metals toxicity studies, it could be possible to establish limits of tolerance and susceptibility of a particular fish to the toxicity of heavy metals in the aquatic ecosystem.



## **D. Summary of the findings (in 500 words)**

The aim of the present study was to throw more light on the effect of acute and chronic toxicity of lead nitrate on health of *Mystus cavasius*.

### **Determination of LC<sub>50</sub> and acute toxicity**

When the fishes were exposed to the different concentrations of lead nitrate, the mortality of the fishes were observed every 24 hours and recorded up to 96 h. The fish mortality with 35.0 mg L<sup>-1</sup> Pb(NO<sub>3</sub>)<sub>2</sub> was 8.33% and the highest mortality was observed with 65.0 mg L<sup>-1</sup> Pb(NO<sub>3</sub>)<sub>2</sub> as 100%. A dose response curve was plotted for the log of does versus the probits to determine the LC<sub>50</sub> value. The 96 h LC<sub>50</sub> value was obtained as 55.00 mg L<sup>-1</sup> lead nitrate with a range of 50.29 to 62.77 mg L<sup>-1</sup> ( $R^2 = 0.902$ ).

### **Biochemical Estimation**

#### **Liver**

The control liver showed total glycogen as  $13.045 \pm 0.593$  mg of glycogen per gram of tissue wet weight. After 10 days, the total glycogen level decreased significantly in all concentrations of lead nitrate with respect to the control. After 20 days of exposure, the total glycogen content for all concentrations did not improved when compared against control.


#### **Kidney**

The kidney from control fishes showed total glycogen as  $2.76 \pm 0.27$  mg of glycogen per gram of tissue wet weight. After 10 days, the total glycogen increased significant as the concentration increases and with 30 mg of lead nitrate, the total glycogen was  $3.44 \pm 0.13$  mg g<sup>-1</sup> wet tissue weight. After 20 days of exposure, the total glycogen content was  $3.53 \pm 0.14$  raised in comparison to control.

15. Whether any Ph.D. enrolled/produced out of the project - Yes

16. No. of publications out of the project (please attach) – 2 Paper Published attached with bound copy.

  
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