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STUDIES ON THE EFFECTS OF COPPER SULPHATE INDUCED CHANGES ON SOME ORGANS OF AN AIR BREATHING FISH *Clarias batrachus* (Linn.)

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ABSTRACT

The present study was to evaluate heavy metal toxicity stress symptoms in fish blood during long-term exposure of sublethal concentration of copper sulphate (CuSO_4). The effects of copper on various haematological parameters were evaluated exposing Indian Air breathing fish, *Clarias Batrachus* (Linn.) to a sub-lethal concentration of copper sulphate (0.36 mg/l) for different periods. Exposure of fish to copper showed a significant decrease in the haemoglobin (Hb) content from 10.73 to 6.60%, red blood cells (RBC) from 2.86 to $1.84 \times 10^6/\text{mm}^3$ and packed cell volume (PCV) from 31.00 to 23.33% at the end of 45th day as compared to control. Histology of liver exposed to 36 hour LC_{50} concentration of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with varying water pH (4 ± 0.5 , 7 ± 0.5 and 8 ± 0.5) was studied in an air-breathing fish (*Clarias Batrachus* Linn.). The histopathological changes observed in the liver tissue post exposure included necrosis, degradation of hepatocytes, degeneration of blood vessels, distended sinusoids with pyknotic nuclei and vacuolation of cells. The degree of damage to the liver tissue was proportional to the nominal concentrations of the metals used. Further the pH of diluent water affected the alteration more acutely signifying a synergistic effect.



KEYWORDS: *haematological parameters , synergistic effect , Intensive industrial developments.*

INTRODUCTION

Intensive industrial developments in last few decades have increased the concentration of copper Sulphate in river and ponds, affected fishes and deplete natural resources. Heavy metals have become major environmental hazards, although they have great biological significance as micronutrients. Major sources of Cu in aquatic environment are sewage and industrial effluents. Cu toxicity to fishes had already been reported by many workers (Eriksen et al., 2001; Wepener et al., 2001; Dhanapakiam et al., 2006; Lodhi et al., 2006). Copper sulphate is widely used as an algaecide for controlling phytoplankton in fish ponds as well as a herbicide, used in aquatic weed control since 1882 (Effler et al., 1980; Carbonell and Tarazona, 1993). Copper sulphate has many industrial applications including the preparation of Bordeaux mixture (a fungicide) and the manufacturing of other copper compounds.

Fishes are the simple and reliable biomarker of copper pollution of aquatic bodies (Lodhi et al., 2006). The metallic ion present in water enters the fish body and gets accumulated in various organs like liver and kidney (Shukla et al., 2007). The blood parameters have been used as sensitive indicator of stress in

fish exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, etc. These metallic ions are the probable major cause of the physiological abnormalities in fish.

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals such as copper (Cu) have gained wide interest in the scientific community in recent years due to its potential human health hazards. Cu is an essential trace nutrient which is discharged into freshwater environments in large concentrations as an industrial effluent and severely affects the freshwater fauna, especially fishes. Elevated levels of Cu may become acutely or chronically toxic to aquatic lives. Although hepatic Cu levels vary greatly between teleost species, the liver is the main area of Cu storage in almost all species studied. In fish, Cd has been shown to alter the structure and to cause morphological changes of varying severity in various organs.

Aquatic organisms, including fishes, accumulate pollutants directly from contaminated water and indirectly via the food chain. The organ most associated with the detoxification and biotransformation process of toxicants including metals is the liver, because the liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health and exhibit the effects of a variety of environmental pollutants. Histopathological changes in different tissues of fish assess the extent of damage caused by pollutants and is recognized to be a reliable biomarkers of stress in fish. Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect effects of irritants in various tissues and organs. Another important parameter influencing the toxicity of metals is pH, which shifts speciation to or away from toxic effect depending upon the ions formed. In general a lower pH will increase free metal ions, while more alkaline pH will result in more carbonate complexes and fewer toxic free metal ions. Therefore, the present study is aimed to analyze the histopathological changes in the liver tissue of the fish *Clarias Batrachus* (Linn.) exposed to sub lethal concentration of copper sulfate (CuSO_4) at varying water pH.

MATERIALS AND METHODS:

The experimental fish *Clarias Batrachus* (Linn.) was purchased from the local landing sites and care was taken to minimize stress incurred by the fish during transportation and were maintained in glass aquaria containing tap water and acclimatized to laboratory conditions. All the necessary precautions for maintaining the fish were laid down as per the recommendations of APHA. Different water quality parameters viz., Dissolved Oxygen (DO), Specific Conductivity, alkalinity, hardness, pH and temperature were analyzed and recorded. Healthy fishes were selected for experimentation. The experiment was carried out using 3 ppm (48 hour LC_{50} value at pH7) of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 24 ppm (48 hour LC_{50} value at pH 7) in tap water. The pH was adjusted using ultra-pure HCl and/or NaOH and was continuously monitored during the toxicity test, with adjustments made as required. A control was run without the addition of metals for each of the three pH treatments. 36 hour post treatment fishes from both control and experimental groups were sacrificed and the liver tissue was excised out.

Table: I
Water quality parameters at different pH

pH	Water Temperature ($^{\circ}\text{C}$)	Specific Conductivity (S/m)	Dissolved Oxygen (mg l^{-1})	Total Hardness (mg l^{-1})	Total Alkalinity (mg l^{-1})
4 \pm 0.5	17 \pm 1	0.13 \pm 0.01	6.14 \pm 0.00	25 \pm 1.00	4 \pm 1.00
7 \pm 0.5	17 \pm 1	0.12 \pm 0.01	6.14 \pm 1.00	32 \pm 1.00	35.5 \pm 1.00
8 \pm 0.5	17 \pm 1	0.13 \pm 0.01	6.14 \pm 1.00	39 \pm 1.00	48.2 \pm 100

RESULTS AND DISCUSSION :

The morphological section of liver of control fish showed the normal hepatocytes and exhibits a homogenous cytoplasm around the spherical nucleus. There was no clear division of hepatic cells into lobules. Normal hepatocytes and sinusoids with prominent nuclei were observed in control liver tissue of *Clarias Batrachus (Linn.)* at pH 7 ± 0.5 while at pH $4=0.5$ there was moderate degradation of cellular hepatocytes in comparison to the control liver tissue at pH 8 ± 0.5 which showed slight degradation of the same. Liver tissue treated with Cu at pH 4 ± 0.5 , showed severe necrosis, hemorrhage and degeneration of blood vessels. Pyknotic nuclei were more prominent with indistinct cells. On treatment with Cu at pH 8 ± 0.5 , hepatocytes exhibited an appearance of some small vascular structures, probably due to the presence of lipids. Major structural changes in the hepatocytes such as focal necrosis, severe congestion in sinusoids were also observed. Hypertrophy of hepatocytes with pyknotic nuclei was quite evident in liver tissue exposed to Cu. On treatment, it was seen that the liver cells degenerated; the normal architecture of the liver was markedly disorganized. In addition, dilated sinusoids with congestion were noticed. First signs of pathological processes such as necrosis and lipid infiltration around the blood vessels, regarded as a sign for toxic liver injury, were noticed at this stage. Histopathological alterations result depending upon the metal type and concentrations, length of exposure, fish species, and other physico-chemical factors. It is brought about due to either increase or decrease in hepatic enzyme activities. Hepatocytes may thus be expected to be the primary targets of toxic substances, providing an excellent biomarker of aquatic pollution. The acidic pH of water magnifies the toxic effect of certain environmental pollutants including heavy metals. The alterations of the liver parenchyma, such as vacuolation and necrosis are often associated with acid water. The present findings are in accordance with above studies. The liver of fish exposed to 3 ppm and 24 ppm of copper sulfate for 36 hr at different pH exhibited several histological alterations like degradation of hepatocytes, distended sinusoids with pyknotic nuclei, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue. The acute toxic injury usually includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei.

CONCLUSION :

Histopathological alterations in air-breathing catfish, *Clarias Batrachus (Linn.)* under the influence of heavy metals such as Cu at different pH can be used as a sensitive model to monitor the aquatic pollution. The severity of damage is more in liver tissue exposed to Cu acidic pH of the medium showed the highest toxicity.

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