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Impact of Copper Sulphate on Juvenile Mahseer Fish

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ABSTRACT *The current study was conducted to investigate the potential hazard of copper sulphate in aquatic environments. Young Mahseer fish fry belonging to genus Tor were exposed both continuously and intermittently to 10ppm copper sulphate. Fishes subjected to continuous exposure demonstrated abnormal behaviour, swam with jerky movements and died after 48 hours where as fishes given dips lived up to 4 days. All fishes showed signs of respiratory distress. Histopathology report of both groups showed dilatation of liver sinusoids. Our experimental data highlights the toxicity of copper sulphate when present as a pollutant towards Mahseer fish and advocates judicial use of this compound for controlling infections in aquaculture.*

KEYWORDS : Mahseer, copper sulphate, liver, toxicity.

Introduction

Wastewater from industries contains a high concentration of contaminants such as heavy metals and organic materials which are hazardous to the environment. These contaminants include copper, which in the ionic forms Cu^{2+}, $Cu_2OH_2^{2+}$, and $CuOH^+$, is toxic to fishes (Moore JW, 1991). When discharged in large quantities from sewage or agricultural runoffs, fishes are most vulnerable and cannot escape the detrimental effects of these pollutants (Vinodhini R et al, 2008). Low concentration of heavy metals may cause chronic stress that might not kill the fish by itself but decrease its size and body weight, therefore reducing their capability to fight for food and habitat. Most of the heavy metal ions exhibit toxicity through the formation of coordination complexes and clusters in animal cells. Though copper, is an essential element for organisms, it may become extremely toxic for aquatic animals as its concentration in water increases [B. J. Shaw et al, 2012; D. Lapointe, 2011). Fishes have a tendency to bio-accumulate heavy metals and human beings can be at serious risk through contamination of the food chain (Rajamanickam V., 2008).

Copper sulphate ($CuSO_4$) is deliberately employed to control diseases and algae in aquaculture facilities [Y. H. Lin, et al, 2008). Even at recommended rates of application, this material may be poisonous to trout and other fish. Young fish fry are more susceptible than fish eggs to toxic effects of copper sulfate (Gangstad, 1986).

Most locals in countries like India and Pakistan employ low cost destructive methods of fishing by using easily available chemical pesticides such as cyclomethrin and copper sulphate .Though larger fish are targeted, the killing is indiscriminate. Juvenile fish are highly susceptible to chemical poisoning and get wiped out almost immediately. Such large scale mortalities of juvenile and reproducing adults can greatly alter fish population. A particularly devastating incident was witnessed where local people had used chemical poisons to collect over 70Kg of Mahseer from a small stretch of Girihole which is at the border of Hassan and Coorg districts.

The toxicity of copper sulfate to fish varies with the species and the physical and chemical characteristics of the water (R. Jagadeshwarlu, 2015) and little attention has been given so far to teleosts like Mahseer. Mahseer belonging to the genus *Tor* are commercially important game fish, as well as highly esteemed food fish. They fetch a high market price, and are potential candidate species for aquaculture (Ogale, 2002). Several of the larger species have suffered severe declines in recent years and are now considered threatened due to overfishing and pollution.

Considering the importance of this species for aquaculture, the present study was conducted to evaluate heavy metal toxicity stress symptoms in young *Mahseer* fish fry exposed for different time periods to copper sulphate (CuSO4). It is hoped that results of such a study would provide an insight into the toxicity of $CuSO_4$, aiming to propose margins for the safe use of $CuSO_4$ during Mahseer aquaculture in the future.

METHOD AND MATERIALS

Experimental design

Young *Mahseer* fish fry were obtained from Mahseer Fishery and Hatchery, Tata Power Company (Lonavala, Maharashtra) and transferred to indoor tanks for 5 days to acclimate prior to the toxicity assay. During the acclimation period, the juveniles were fed once daily. Continuous aeration was used to ensure dissolved oxygen. The photoperiod was 12 h light:

12 h dark. Selected healthy juveniles were randomly divided into glass tanks (5 fish per tank) containing 1 L of fresh dechlorinated water. Three tanks per treatment were randomly allocated and fish were exposed in triplicate to control (no added copper), 10 mg/l copper sulphate for 5 days using dip method (45 seconds once a day) and continuous exposure. Dosing in case of continuous exposure was carried out following daily water change.

Fish Sampling

Fish subjected to dips were randomly taken from each tank and dissected on an ice tray. The whole liver was removed and fixed in 10% v/v buffered formal saline (100 mL 40% v/v formaldehyde, 6.5 g NaH_2PO_4 (anhydrous), 4 g $NaH_2PO_4{\cdot}H_2O$, diluted to 1 L with distilled water, pH 7.2).

Histology

Histological analyses followed the standard techniques (G. L. Humason, 1979). Briefly, the samples were dehydrated in rising concentrations of ethanol, cleared in xylene, infiltrated with rising concentrations of liquid paraffin wax at 58°C, and later embedded in paraffin blocks. The sections were cut at 7-µm-thick with a Rotary microtome and stained using hematoxylin and eosin (H&E). Stained sections were observed by light microscopy.

RESULTS AND DISCUSSION

The test animals' abnormal behaviour varied according to the time of exposure to the test solution. Control fishes remained healthy throughout the experimental period. Mahseer fry which were given dips in test solution remained healthy for two days after which they started showing some signs of abnormal swimming behaviour. The fish tended to gather at the surface and died after four days. Mahseer exposed continuously to high concentrations of CuSO4 (10mg/L) also demonstrated abnormal behaviour. They tried to avoid the toxic water with fast swimming; these fishes were observed to have breathing difficulties and tried to breathe air from the surface water. They tried to avoid the toxic water with fast swimming and jumping; and showed jerky movements. Lastly, they settled on the bottom of aquarium; and after some time their bellies turned upwards and the fish died. The major cause of mortality might be respiratory epithe-

lium damage by oxygen culminating in the formation of a mucus film over the gills of fish. It was observed that continuous exposure to copper sulphate hastened fish mortality, indicating a direct proportional relationship between mortality and the time of exposure to copper sulphate.

Histological alterations observed in liver were indicative of the fish physiological status, revealing the mechanisms of Cu exposure. Liver is one of the top organs for copper accumulation in fishes and it has been used as a reference for analysis of tissue damage caused by environmental pollutants. Liver from the treatment with $CuSO_4$ **showed** dilatation of sinusoids, with the sinusoids becoming irregular in shape (Figure 1). These injuries were almost equivalent as seen by Tao Wang et.al, 2015.

CONCLUSION

Metals like copper are highly hazardous xenobiotics. Results of this study confirm that both continuous and intermittent exposure to $CuSO_4$ had obvious toxicity to juvenile Mahseer, with injuries being found in the liver of all fishes. A positive relationship was found between the mortality and exposure time; also, behavioural changes increased with increased exposure. Results obtained in this study clearly reveal the fact that it is necessary to control the use of a

heavy metal such as copper in aquaculture and that environmental contamination with this metal can represent a great threat to Mahseer fish. Lastly, considering the importance of this species for fish culture, genomics and proteomics studies can provide further insight into the detailed changes occurring in Mahseer after copper exposure.

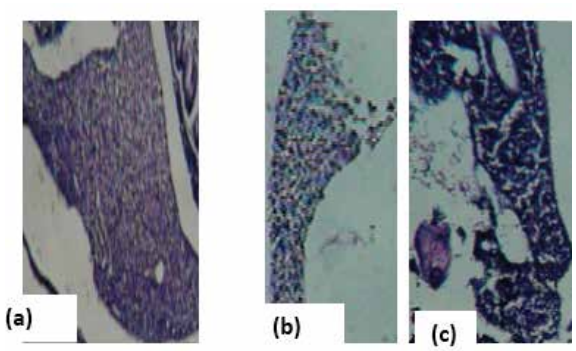

Fig.1: Effect of $CuSO_4$ on histopathology of Mahseer liver

Without exposure (b) After continuous exposure (c)After intermittent exposure

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REFERENCES

1. Moore J. W. (1991). "Inorganic contaminants of surface water: research and monitoring priorities" Springer-Verlag, New York.
2. Shaw, B. J., Al-Bairuty, G., Handy R. D. (2012). "Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (Oncorhynchus mykiss): physiology and accumulation," *Aquat. Toxicol.,* **116-117**:90–101.
3. Lapointe, D., Pierron, Couture P. (2011). "Individual and combined effects of heat stress and aqueous or dietary copper exposure in fathead minnows (Pimephales promelas)," *Aquat. Toxicol.* **104**: no. 1-2, 80–85.
4. Vinodhini R, Narayanan M (2008). Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio*(Common carp). *Int J Environ Sci Tech*. **5(2)**:179-82.
5. Rajamanickam V (2008). Effect of heavy metals on the level of vitamin E, total lipid and glycogen reserves in the liver of common carp (*Cyprinus carpio L.*). *Maejo Int. J. Sci. Technol*.**2**:391-399.
6. Lin, Y. H., Shie, Y. Y., Shiau S. Y., (2008). "Dietary copper requirements of juvenile grouper, Epinephelus malabaricus".*Aquacult*. **274 (1)**:161–165.
7. Humason, G. L., Freeman W. H., (1979). *Animal Tissue Techniques*, New York, NY, USA.
8. Wang,T., Xiaohua Long, Yongzhou Cheng, Zhaopu Liu, and Shaohua Yan (2015). "A Comparison Effect of Copper
9. Nanoparticles versus Copper Sulphate on Juvenile Epinephelus coioides: Growth Parameters, Digestive Enzymes, Body Composition, and Histology as Biomarkers". *Int.J. Genomics*, Article ID 783021.
10. Gangstad, E., Fresno, C.A. (1986). "Freshwater vegetation management", Thomson Publications.
11. Jagadeshwarlu, R., Pandari Reddy P., Sunitha Devi. G. (2015). Toxicity of Copper sulphate ($CuSO_4.5H_2O$) to *Oreochromis mossambicus* (Tilapia). *Biolife*, **3(3)**: 657-661.
12. Ogale, S.N. (2002). Mahseer breeding and conservation and possibilities of commercial culture. The Indian experience. In T. Petr and D.B. Swar (eds.) Cold Water Fisheries in the Trans-Himalayan Countries. FAO Fish. Tech. Pap. 43.