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Copper Toxicity and Bioassay Studies on Freshwater Fish "Cyprinus Carpio"

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Abstract: ***Copper is one of the most toxic heavy metal to fish and consumption of fish after copper treatment in water may pose a serious risk to human health. Fishes are generally used as pollution indicators in water quality management. The use of biological test system for monitoring pollution is gaining importance worldwide by employing toxicity test model Elevated levels of copper may become acutely or chronically toxic to aquatic lives. Chronic effects include reduced growth, shorter lifespan, reproductive problems, reduced fertility and behavioral changes.***

Keywords: Copper, *Cyprinus carpio*,Physico-chemical parameters, Bioassay, LC_{50}

1. Introduction

The aquatic environment subjected to different types of pollutants of industrial, domestic and agricultural wastes and severely affect the aquatic organisms. (Beaumont *et al*., 2000), reported that rapid industrialization in India has resulted into a substantial increase in the liquid waste (effluent), which is traditionally being discharged into open land or in nearby natural water, causing a number of problems like threat to plant and animal lives, surface water logging, ground water contamination and salinizing of land. The problems of environmental pollution and its deleterious effects on aquatic biota, including fish is receiving focus during the last few decades. Industrial discharges containing toxic and hazardous substances, including heavy metals contribute tremendously to aquatic ecosystem. Heavy metals are natural trace components of the aquatic environment, but their levels have been increased due to domestic, industrial and agricultural activities. It causes greatest threat to the health of Indian ecosystem. Level of trace elements in water and fish has been studied (Ikem *et al*., 2003). Discharge of heavy metals into the aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behaviour.

Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among all types of aquatic pollutants, heavy metals are of greatest concern. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna. Heavy metals are considered as the main cause of aquatic pollution and the magnitude of environmental degradation they caused is far beyond the recommended threshold limit values (Abdel-Meguid *et al*., 2002). Environmental pollution by heavy metals has increased as a result of rapid industrialization and uses of chemicals as fungicides, algaecides and insecticides. The metal contamination of freshwater bodies is a matter of serious concern from human health. From the point of view, many aquatic organisms particularly fish, forms an integral part of human diet. Among the various kinds of metals, copper is found to deteriorate the water quality by decreasing the available nitrogen and phosphorus, leading to loss of fertility and primary productivity of water bodies. Copper sulphate is one of the most widely used chemical as algaecide or herbicide for the control of phytoplankton or weeds respectively, in lakes, reservoirs, ponds and in municipal water treatment systems. The problem with use of copper is that there is only a narrow line that separates effective treatment levels from overdoses which kills fish (Watson and Yanong, 2002).

Copper is an essential heavy metal plays an important role in various biological processes including oxidative phosphorylation, gene regulation and free radical homeostasis as essential cofactor. However, when its concentration exceeds metabolic requirements, it becomes harmful and play a major role among pollutants. Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metals are regarded as serious pollutants of the aquatic environment because of their persistence and tendency to be concentrated in aquatic organisms. Most heavy metals released into the environment find their way into the aquatic phase as a direct input by various anthropogenic processes, atmospheric deposition and erosion due to rainwater (Singer *et al*, 2005). Copper plays a protective role against oxidative damage caused by variety of xenobiotic. The antioxidant effects of ceruloplasmin and metallothionein seems to be the mechanism by which copper protects under these conditions. Ceruloplasmin serves as a transport protein of copper in plasma. Parvez *et al*., (2003) reported that copper pre-exposure increases the activity of ceruloplasmin in fish serum. Ceruloplasmin, through ferroxidase activity, is involved in iron homeostasis and acts as an antioxidant in plasma. Copper is able to induce the biosynthesis of metallothionein. The metallothionein induction plays a role in the oxidative defence against chronic copper exposure in the liver of a freshwater fish. Elevated levels of copper may become acutely or chronically toxic to aquatic lives. Chronic effects include reduced growth, shorter lifespan, reproductive problems, reduced fertility and behavioral changes. Copper deficiency leads to physiological disturbances, symptoms which include depression of growth, anemia, bowing of legs, spontaneous fractures, ataxia of new born, cardiac and vascular disorders, depigmentation, decrease in some organs weight, depressed reproductive performance including egg production and tissue accumulation.

The toxicity of any pollution is either acute or chronic. Although the toxicant impairs the metabolic and

physiological activities of the organisms. Physiological studies alone do not satisfy and complete under toxic stress. Acute toxicity test in used to determine the concentration of a deleterious effect on a group of tests organism during a short-term exposure under controlled conditions. All toxicants are capable of severally interfering with the biological systems that producing damage to the structure and function of particular organism and ultimately to its survival. Acute toxicity test constitute only one of the many tools available to the aquatic toxicologists but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material. The 96-h LC_{50} sublethal toxicity tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metal. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms. And copper is a very important element, Which could influence the metabolism of the human body and it is also a nutritional element for living being. But if the intake is too much, it cause toxicity (Luoma and Rainbow, 2008).

Most of the heavy metals are micronutrients and they exert a prominent role in environmental deterioration. The heavy metal and pesticide contamination of aquatic ecosystems has increased manifold in the last few decades due to their extensive use in agricultural, chemical and industrial processes and is a real threat to the aquatic fauna. Fishes are rather more vulnerable since they are frequently exposed to aquatic pollutants. Fishes occupy the top of aquatic food chain, they are suitable bio indicators of metal contamination. Metals are well known inducers of oxidative stress, and assessment of oxidative damage in fishes can reflect metal contamination of the aquatic environment. Among metals, copper is used in industries manufacturing organic chemicals, fertilizers, iron and steel works, electrical works, antifouling paints, pulp and paper industries, pesticides, fungicides and automobile accessories (Singer et al., 2005). Even though copper is an essential trace element required in low concentrations, it is discharged into freshwater environments in large concentrations as an industrial effluent and severely affect the freshwater fauna, especially fishes.

Copper, is a trace metal for cellular metabolism, may become extremely toxic for aquatic animals as its concentration in water increases. The important natural sources of copper are the multiple human activities (industries, agriculture, and harbors) have considerably increased the input of this metal in estuarine and marine environments around the world (D'Adamo 2008). At equilibrium, there are few free copper ions in natural waters since most copper is associated with inorganic ions or organic substances. However, $CuSO_4$ has been widely used to control algae and some pathogens in fish culture ponds, increasing copper concentration in water. $CuSO_4$ is highly toxic to fish, so the concentrations required to control algae or pathogen agents must be below the toxicity threshold for fish. The effect of $CuSO_4$ on fish has been studied exhaustively and, as expected, some species have been found to be more susceptible to copper than others. This implies previous knowledge of the susceptibility of a given cultured fish species before using $CuSO_4$.

Table 1: Physico-chemical parameters of water used for the present investigation

Physico-Chemical Parameters	Valves
Temperature	27.2 ± 1.2^0C
pH	7.1 ± 0.08
Dissolved oxygen	6.4 ± 0.04 mg/L
Total hardness	18.2 ± 0.08 mg/L
Salinity	0.3 ± 0.02 ppt
Calcium	4.3 ± 0.3 mg/L
Magnesium	2.5 ± 0.06 mg/L

Values are mean ± S.E. of five individual observations

2. Method

Bioassay

Of the two types of bioassay experiment viz., Continuous flow (Mount and Warner, 1965) and static method (APHA, 1998) the static bioassay method was chosen considering the limitation of laboratory facilities.

Copper ($CuSo_4$) was used as a toxicant in the present investigation. Analytical grade of copper was obtained from (CAS no. 7632-00-0, purity> 99%) Loba chemie Pvt.Ltd.,Mumbai,india.

3. Result

Calculation of log concentration/probit regression line for 24h experiments in which Cyprinus carpio var communis were exposed to different concentration of copper

S. No	*Conc of Copper*	*No of Fishes used*	*% Mortality*	*Log Conc*	*Emprical probit*	*Expected probit*	*Working probit*	*Weighing Co-efficient*	*W*			
									w	wx	wy	Wxy
1	2	10	36	1.792	4.64	4.64	4.639	0.601	6.01	10.76	22.88	49.96
2	4	10	42	1.806	4.80	4.80	4.795	0.627	6.27	11.32	30.64	54.29
3	6	10	48	1.819	4.95	4.95	4.949	0.634	6.34	11.53	31.37	57.07
4	8	10	54	1.832	5.10	5.10	5.100	0.634	6.34	11.61	32.33	59.23
5	10	10	60	1.845	5.25	5.24	5.256	0.627	6.27	11.56	32.95	60.80
									31.23	56.80	154.60	281.37
									Sw	Swx	Swy	Swxy

4. Discussion

Aquatic organisms can take up copper directly from the water and elevated ambient copper concentrations can lead to excess copper accumulation in several tissues (Kamunde *et al*., 2002). Oxidative stress is generally accepted as one of the major effects of excessive cellular copper concentrations but other effects occur in osmoregulatory organs of aquatic organisms. In freshwater organisms osmoregulatory effects of acute copper exposure occur in the gills and can be related to the copper concentration in the gill tissue which in turn can be related to ambient copper concentration and water chemistry (Santore *et al*., 2001).Heavy metal contamination in the aquatic environment is a potential threat for aquatic organisms, when exposed to significant amount of metals as consequences of industrial, agricultural and anthropological activities. Heavy metals at high concentrations can cause harmful effects on metabolic, physiological, and biochemical systems of fishes (Atli and Canli, 2007) and it causes long-term eco-toxicological effects. Fish have been largely used as a bio-indicators for environmental pollutants used to estimate the influence of pollution due to the sensitivity of their biochemical and haematological parameters (Lopes *et al*, 2009). Industrial effluents contributing to aquatic pollution contain a vast array of toxic substances, which include heavy metals. It leads to alteration in physical, chemical and biochemical properties of water bodies in the aquatic organisms in environment.

Fresh water is contaminated with a wide range of heavy metals released from domestic, industrial and other man made activities has become a matter of concern over the last few decades, which may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment. The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal pollution in natural water. The effect of copper toxicity has been widely studied in aquatic animals. Deane and Woo, (2007) reported that all aquatic organisms maintain an internal environment which is different from their external environment and they regulate the passage of water and solutes between their interior and the exterior. Freshwater fish can be used as selective bioindicators of trace metals in freshwater reservoirs (Dobrowolski *et al*., 2006) since they not only accumulate metals in their bodies but also react to water contamination with alterations of various vital functions.

Considering the hazard of accumulation of copper in fish flesh the frequent use of copper sulphate is not recommended in fish farms. Copper is one of the most toxic heavy metal to fish and consumption of fish after copper treatment in water may pose a serious risk to human health. Fishes are generally used as pollution indicators in water quality management. The use of biological test system for monitoring pollution is gaining importance worldwide by employing toxicity test model with use of a “key species” of fish. In aquatic toxicology, the traditional LC50 test is often used to measure the potential risk of a chemical. Generally, the polluted waters are tested for their toxicity employing toxicity test in fish *Cyprinus carpio* and is still more commonly used by environmental lists and environmental protection agencies. Heavy metal contamination severely interfere with ecological balances of an ecosystem and produces devastating effects on environment quality anthropogenic inputs like waste disposal directly adds to the burden environmental degradation. Water is easily contaminated by heavy metals due to human activities with heavy metal contamination reported in aquatic organisms. The problem has become more serious for aquatic live close to the coastline where heavy metals tend to accumulate. The fact that increasing use of contaminating chemicals in many industrialized parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity. Assessment of toxicity on particular organism exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. Toxicity tests will reveal the organisms sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem (Figueiredo –Fernandes *et al*., 2007).

Widespread and release of toxicants in aquatic systems long and frequent use of metals by mankind. As the exposed organisms try to cope with this pollution, they develop species-specific defence mechanisms, which are rarely fully apprehended. For instance, fish are able to develop a copper (Cu) tolerance after sublethal Cu exposure in laboratory settings as well as in their natural habitat. However, not all fish species handle elevated Cu concentrations equally adequate. *Rainbow trout* appeared to be three times more sensitive to Cu exposure than *common carp*, and almost seven times more sensitive than *gibel carp*. Cu is an essential element, organisms build up homeostatic mechanisms and strictly regulate free Cu concentrations. When Cu concentration augments to a toxic range, oxidative stress, DNA damage and a disrupted ion osmohomeostasis occur (Bopp *et al*., 2008). Fishes are really susceptible to severe ion regulatory failure, found that when exposed to 50 µg/L Cu, calcium concentration in plasma of *rainbow trout* is also affected in the beginning of the exposure. With compensatory mechanisms, such as stimulation of Na^+/K^+-ATPase synthesis and enhanced chloride cell turnover, fish can compensate for the damage caused by Cu exposure, thus following a damage–repair model. Three phases are being recognized in this model: an initial shock phase, a recovery phase and an acclimation phase (McGeer *et al*., 2002). This temporary nature of Cu-induced osmoregulatory disturbance with return to control levels. In *Cyprinus carpio*, ion losses were reversed subsequent to the initial shock phase; however *common carp* and *gibel carp* did not re-obtain their control levels after one month of exposure. It might be that *rainbow trout*, after it survives the initial shock phase, is more capable of restoring homeostasis whereas *common carp* and *gibel carp* are better equipped to survive and acclimate to their reduced plasma ion levels.

Despite the essential role of copper in a number of vital biochemical processes, the metal is known to induce several histopathological changes in gills when present in higher

concentration in water. Copper accumulation in organs of animals of polluted water bodies leads to generation of free radicals which causes the biochemical and morphological alterations in them.Copper, one of the most toxic heavy metals is often found in natural water at concentrations which are detrimental to aquatic organisms. The main source of freshwater pollution is the discharge of untreated waste, dumping of industrial effluent and run-off from agricultural fields. Stress response is characterized by physiological changes and effect of pollutants on fish is assessed by acute and chronic toxicity tests. Copper is an essential trace metal for several fish metabolic functions.. The effect of copper sulphate on fish has been studied comprehensively and some species have been found to be more susceptible to copper (Wani *et al*., 2011).

5. Conclusion

Growth rate of fish is highly variable, and very sensitive to environmental factors. Thus, measurements of growth can be used to provide information on fish performance. The growth of fish is associated with changes in morphometric traits, body shape, and in chemical and biochemical body composition. Fish growth depends on water physio-chemical characteristics, and in polluted waters usually decreases. Reduction in growth can be caused by physiological or behavioural stress during exposure to toxicants. Any disturbances causes production induced by toxicants are reflected in fish growth rates. Early life stages are known to be very sensitive to intoxication. The bioassay of copper toxicity studies on fish *Cyprinus carpio* during the acute and sublethal treatment showed various behavioural changes like hyper excitability, loss of balance, asphyxiation, excess of mucous all over the body surface.

References

[1] BeaumontMW, ButlerPJ, TaylorEW, Exposure of *Brown trout, Salmo trutta*, to a sublethal concentration of copper in soft acidic water : Effects upon muscle metabolism and membrane potential .Aquat.Toxicol. 51:259-272. 2000.

[2] IkemAN, EgieborK, Nyavor, Trace elements in water, fish and sediment FromTuskegee Lake, Southeastern USA. Water, Air and Soil Pollut. 149:51-75. 2003.

[3] Abdel-MeguidNAM, Kheiralleh, Abu-ShabanK, AdhamA, Abdel-Moneim. Histochemical and biochemical changes in liver of *Tilapia Zilli*: as a consequence of water pollution. J. Biol. Sci. 2: 224 – 229. 2002.

[4] WatsonCE, Yanong, *Use of Copper in Freshwater Aquaculture and Farm Ponds.* Fact Sheet FA-13, Fish. and Aquat. Science, Institute of Food and Agric. Science, University of Florida. 10: 246-249. 2002.

[5] SingerC, ZimmersannS, Induction of heat shock proteins in the zebra muscle (Dreissena polymorpha) following exposure to platinum group meyals (platinum, palladium and rhodium): Comparison with lead and cadmium cadmium exposures. Aquatic Toxicology. 75: 65-75. 2005.

[6] ParvezS, SayeedI, PandeyS, AhmadA, Bin-HafeezB, HaqueR, AhmadI, RaisuddinS. Modulatory effect of copper on non-enzymatic Antioxidants in freshwater fish *Channa punctatus* (*Bloch*). Biological Trace Element Research 93, 237–248. 2003.

[7] LuomaSN, RainbowPS, Sources and cycles of trace matals. In: Metal Contamination in Aquatic Environments: Science and Lateral Management. Cambridge University Press, Cambridge. 47-66. 2008a.

[8] D'AdamoRM, Di StasioA, FabbrociniF, PetittoL, RoselliMG,Volpe, Migratory crustaceans as biomonitors of metal pollution in their nursery areas, The Lesina lagoon (SE Italy) as a case stud. Environmental Monitoring and Assessment, 143: 15-24. 2008.

[9] MountDI, WarnerRE, A serial-Dilaion Apparatus for Contonous Delicery of Various Concentration of Material in Water. U.S Public Health Serv.Publ No.999-WP-23.

[10] APHA (American Public Health Association). Standard method for the examination of water and waste water (20th Eds). 1998.

[11] KamundeC, GrosellM, HiggsD, WoodCM, Copper metabolism in actively growing *rainbow trout (Oncorhynchus mykiss).* Interactions between dietary and waterborne copper uptake. J Exp Biol 205: 279–290. 2002.

[12] SantoreRC, Di ToroDM, PaquinPR, AllenHE, MeyerJS, Biotic ligand model on the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and daphnia. Envron. Tox. Chem. 20, 2397–2402. 2001.

[13] AtliG, CanliM, Enzymatic responses to metal exposures in freshwater fish Oreochromis niloticus. 145: 282-287. 2007.

[14] LopesPA, PinheiroT, SantosMC., MathiasM, Collares-PereiraMJ, Viegas-CrespoAM, Response of antioxidant enzymes in freshwater fish populations *(Leuciscus alburnoides complex)* to inorganic pollutions exposure. Sci. Total. Environ., 280, 153-163. 2009.

[15] DeaneEE, WooNYS, Impact of copper exposure on endocrine, osmoregulatory and cytoprotective functions in the marine teleost, Sparus sarba, Aquat. Toxicol. 82: 85-93. 2007.

[16] DobrowolskiR, SkowronskaM, The study of trace metal levels in select environmental components of the Zemborzyce Reservoir. Pol. J. of Environ. Stud. 4, 537, 2006.

[17] Figueiredo-FernandesA, Ferreira-CardosoJV, Garcia-SantosS, MonteiroSM, CarrolaJ, MatosP, Fontainhas-FernandesA, Histopathological Changes in liver and gill epithelium of *Nile tilapia*, *Oreochromis niloticus*, exposed to waterborne copper. Pesquisa Veterinária Brasileira , Rio de Janeiro, 27 : 103-109. 2007.

[18] BoppSK, AbichtHK, KnauerK, Copper-induced oxidative stress in *rainbow trout* gill cells. Aquat. Toxicol. 86, 197–204. 2008.

[19] WaniI, Histopathological alterations induced in gill epithelium of African catfish, *clarias gariepinus*, Exposed to copper sulphate. Asian J.Exp.BIO. SCI.VOL 2(2): 278-282. 2011.