

## TO STUDY THE DETERMINATION OF MEDIAN TOLERANCE LIMIT (LC50) OF CLARIAS BATRACHUS FOR ZINC SULPHATE AND COPPER SULPHATE

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### ABSTRACT

Industries are the major sources of heavy metal pollution and it is released into water and soil. Heavy metals cause several ill effects to aquatic living organisms and environment. The acute toxicity of chosen metals (zinc sulphate and copper sulphate) against fresh water *Clarias batrachus* (Cat fish) and the chronic toxicity of sub lethal concentrations of heavy metals on the different organs such as liver, gill, muscle, kidney and brain of the chosen fresh water fish. Adult cat fish treated separately with zinc sulphate and copper sulphate) for 24, 48, 72, and 96hr at different dose levels showed significant behavioural changes in the Lethal concentration were determined by probit analysis method. The LC 50 values for ZnSo<sub>4</sub>,

CuSo<sub>4</sub> were found as 8.21ppm, 1.85ppm; respectively. Among the toxicants selected, CuSo<sub>4</sub> is more toxic than ZnSo<sub>4</sub> in cat fish.

**KEYWORDS:** Lethal concentration, *Clarias batrachus*, Zinc sulphate and copper sulphate.

### INTRODUCTION

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 as well as that of environment. The problem of pollution of the water where the wastes are usually discharged has increased to a great extent in recent years.

Aquatic life is strongly influenced by physical properties of a water body. It is known that heavy metals as well as agro-pollutants are potentially harmful to the aquatic lives. All pesticides applied for the pest control eventually pollute the water resources either in their original chemical form or in some degraded variety. On the other hand, all industries discharge their effluents indiscriminately in the adjoining water areas and frequently cause serious hazards to aquatic life. Among the aqua fauna, fishes are affected to a significant extent. There are ample reports dealing with mortality and pollution.<sup>[1]</sup> Short term toxicity tests had been designed for the estimation of lethality of the xenobiotics to the test specimens. The most widely adapted technique for short term bio assay is the evaluation of a Medium Tolerance Limit (TLM). Determination of 24, 48, 72 and 96h TLM value by probit analysis (static bio assay) procedure is generally applied to determine the toxicity levels of the xenophobic materials to various aquatic organisms under laboratory conditions.<sup>[2]</sup> These toxicity tests are necessary to predict the safe concentration of the chemicals in the environment.<sup>[3]</sup> TLM or Lethal Concentration<sub>50</sub> (LC<sub>50</sub>) is the particular concentration of a chemical in the experimental water at which just 50% of the test organisms are able to survive for a specific period of exposure.<sup>[4,5]</sup> Many investigators had accepted this method for the determination of acute toxicity<sup>[6, 7, 8]</sup> even in recent years for scientific, economic and ethical reasons the TLM test have been prescribed as one of the toxicity tests. APHA<sup>[4]</sup> suggested that the preferable duration for a toxicity test should be at least 24, 48, 72 and 96h. For the present study, the selected exposure period was 96 in the fish *Clarias batrachus*.<sup>[9]</sup>

## MATERIALS AND METHODS

Adult and live fish *Clarias batrachus* were collected from the fish farm Patra and Bhadbhada Bhopal (M.P.) brought to the laboratory, cleaned by using 0.1% KMnO<sub>4</sub> to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. Two heavy metals zinc sulphate (ZnSO<sub>4</sub>), copper sulphate (CuSO<sub>4</sub>) were chosen as xenobiotics for estimation of their lethality on *C. batrachus*. A batch of 10 adults, healthy disease - free laboratory acclimated individual of some age irrespective of sex, were released in plastic aquaria measuring 5L diluted water. The test fish were starved for 24, 48, 72, and 96h; Test period. Feeding tends to increase the metabolic activities such as rate of respiration; excretion and production of other waste products altogether affect the toxicity of the test solution, so

food supply to the test specimen was avoided. For the determination of 96h TLM for ZnSO<sub>4</sub>, CuSO<sub>4</sub> a set of 4 plastic aquaria were arranged for each chemical, containing 5L of water. After releasing the test individuals, different concentrations of each chemical were added to respective aquaria very carefully and mixed thoroughly. Any change in behaviour and other responses were carefully noted. The specific doses had been determined by trial and error method. The individuals were considered dead when they failed to respond to touch stimulus. After the death of each fish, its body was removed immediately from the containers along with water allotted for each fish. The aquaria water containing the compounds was renewed after each 24h keeping the concentration constant throughout the experiment. After 96h (the end of the test), survived individuals in each container were counted. Determination of 96h TLM values of ZnSO<sub>4</sub>, CuSO<sub>4</sub>, in adult *C. batrachus* had been done by the Finney<sup>[8]</sup> method of probit regression analysis. Percentage of mortality in static bio assay was converted into probit mortality following the method of Doudoroff *et. Al.*<sup>[10]</sup>

Fish exposed to different doses of pollutants displayed marked behavioural changes. Fish exposed to sub lethal doses of the test chemicals exhibit cessation of all movements and became very sluggish. Toxicity is a relative property of a chemical which refers to its potency to induce harmful effects on an organism. It is a function of concentration of the toxicant and the length of exposure of the animal.<sup>[11]</sup> Fish exposed to lethal doses of the xenobiotics showed drastic changes in the behavior. Respiratory upset, insensitivity to external stimuli, erratic and jerky movements along with a tendency to jump outside the convulsion were the general responses in all the treatments. The toxic effects may include both lethal and sublethal concentration which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behaviour.<sup>[12]</sup> The determined 96h TLM values for ZnSO<sub>4</sub>, CuSO<sub>4</sub> were 8.51 ppm and 1.81 ppm, respectively (Table 1). The point of Intersection with the calculated 50% mortality was determined as the TLM value Fig.1.

## RESULT AND DISCUSSION

**Table: 1 Acute Toxicity Test showing tolerance of *Clarias batrachus* to Zinc Sulphate**

Exposure period(h.)	LC20 (ppm)	LC50 (ppm)	LC90 (ppm)	Regression equation	Slope "S"	95% confidence Limit		Chi-square value	
						Lower Limit	upper Limit	Calculated value	Table value
24	8.31	8.56	8.95	Y=3.330+ (-28.53) x	5.130	8.25	8.35	0.345	5.99
48	7.97	8.29	8.79	Y = 2.85+ (-21.45) x	4.485	8.22	8.34	0.006	5.99
72	7.90	8.20	8.66	Y =2.784 +(-22.84) x	4.673	8.09	8.26	0.060	5.99
96	7.88	8.13	8.51	Y = 3.330+(-27.88) x	5.130	8.45	8.64	0.345	5.99

**Table: 2 Acute Toxicity Test showing tolerance of *Clarias batrachus* to Copper sulphate**

Exposure period(h.)	LC20 (ppm)	LC50 (ppm)	LC90 (ppm)	Regression equation function	Slope "S"	95% confidence Limit			Chi-square value
						Lower Limit	upper Limit	Calculated value	Table value
24	1.61	1.86	2.25	$Y=3.330+(-6.21)x$	5.130	1.802	1.997	0.345	5.99
48	1.37	1.70	2.19	$Y=2.85+(-4.39)x$	4.480	1.652	1.771	0.006	5.99
72	1.27	1.59	2.09	$Y=2.784+(-4.13)x$	4.480	1.647	1.647	0.006	5.99
96	1.18	1.43	1.81	$Y=3.330+(-4.77)x$	5.13	1.497	1.647	0.345	5.99

In present investigation, it has been endeavored to evaluate the environmental risk of man made chemicals mostly focusing on Fish due to their infusion of trial and error methods applying acute and sub lethal exposures of ZnSO<sub>4</sub> and CuSO<sub>4</sub> may be explained unsuitable stress. Further, metal pollution may make copper shift in the community structure to metal tolerant organisms, which are often capable of accumulating large amounts of metals.<sup>[13]</sup> The changes in behavior might be due to hyperactivity of the neural mechanism, as well as, for hypersecretion of stress hormones secretory endocrine glands. Studies on the median tolerance limits usually provide measures of relative toxicity of different chemicals and also the sensitivity of fish species, but not throw right on the maximum concentration to toxic materials which would not affect the metabolism of the fish. Tolerance of an organism is often measured in terms and of the level of lethal factor and length of time the animal survives. The relationship between the logarithm of the water concentrations of the test toxicants (ZnSO<sub>4</sub>, CuSO<sub>4</sub>) and the logarithm of fish mortality<sup>[8]</sup> appear to be linear for all the treatments and accordingly the regression lines have been drawn detecting the TLM values (Fig. 1-2) by probit analysis method (Table 1 and 2). The toxicity tolerance values may vary in different size, weight and age groups of the organism in a stable environmental condition. The 96h TLM values for ZnSO<sub>4</sub> (TLM 8.51ppm) than that of CuSO<sub>4</sub> (TLM: 1.81ppm). Metal ions and their complex exhibit widening toxicity to the organism that ranges from sublethal to lethal depending upon the time of exposure and the prevailing conditions in the ambient water.<sup>[14]</sup> LC50 values differ from species to species for the same toxicant due to the mode of action and responses of the animals.<sup>[15]</sup> The toxicity tests have also been influenced by the size, age,<sup>[16]</sup> sex<sup>[17]</sup> and the nutrient supply.<sup>[18,19,20]</sup> The present study reveals that of the two toxicants under investigation the CuSO<sub>4</sub> has caused great harm to the fish. This is evidenced by the fact that a slight higher concentration of CuSO<sub>4</sub> in the medium causes death of a much higher percentage of fish. The lack of proportionality associated with the xenobiotics is more

pronounced in copper. Possibly, Synthesis of the detoxifying proteins (Metallothionein etc) due to zinc induction to combat its poisoning is seemed to be much more prominent than  $\text{CuSO}_4$  (because  $\text{CuSO}_4$  also has a specific detoxification system guided by the proteins like metallothionein or glutathione). On the other hand, the inhibitory effect of  $\text{CuSO}_4$  and  $\text{ZnSO}_4$  prevailing on the enzyme system (controller or bioaccumulation and tissue metabolism of the xenobiotics), probably gets much more prominent than that of zinc induction.

## CONCLUSION

After the above discussion it had been concluded that zinc and copper causes deleterious effects on fishes. In sub lethal concentration it may not be fatal for an individual organism but it does affect the growth rate and reproduction resulting in disturbance to whole community and tropic levels of food chains, ultimately the ecosystem.

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