

Acute Toxicity of Manganese with Respect to LC50 In Freshwater Fish, *Heteropneustes Fossilis*

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Abstract- *Manganese (Mn) is a common contaminant in mine water discharges. Although Mn is an essential element in biological processes, increased concentrations from anthropogenic sources can stress aquatic ecosystems. In the present study, the acute sensitivity of manganese on freshwater catfish, Heteropneustes fossilis was assessed (using MnSO₄). Acute 24h, 48h, 72h and 96h exposures with manganese sulphate generated a mean median lethal (LC50) toxicity estimate of 5.132 mg/l, 4.057 mg/l, 3.494 mg/L and 3.032 mg/L, respectively as determined by Probit graphic method for the ten tests fish with H. fossilis.*

Indexed Terms- *Manganese sulphate, LC50, Probit analysis, Heteropneustes fossilis*

I. INTRODUCTION

Heavy metal pollution is a global environmental issue with serious ecological consequences, particularly for aquatic organisms (Srivastava and Prakash, 2018a). In fish, heavy metals tend to accumulate in various organs such as the skin, gills, intestine, liver, and kidneys, leading to both external and internal damage (Farkas et al., 2002). Although heavy metals are naturally present in the Earth's crust, they are persistent environmental contaminants because they cannot be degraded or destroyed. These metals can enter the body through food, air, and water, gradually accumulating over time (Lenntech et al., 2004). While certain heavy metals are essential for biological functions in fish, they must remain within optimal concentrations. When their levels exceed the required range, these metals start to accumulate in different tissues and organs, causing severe physiological harm and compromising fish health (Azmat et al., 2006).

The introduction of even small amounts of relatively toxic heavy metal cations into aquatic environments can lead to significant physiological changes in aquatic organisms, even at sublethal levels (Srivastava and Prakash, 2018b). The presence and concentration of these metals in fish vary depending on species, age, developmental stage, and other physiological factors. Although certain metals are essential for biological processes, all metals become toxic at higher concentrations. They can induce oxidative stress by generating free radicals or by displacing essential metals in pigments like haemoglobin or haemocyanin, thereby disrupting enzyme functions (Srivastava and Prakash, 2018c). Heavy metals tend to accumulate in various tissues of aquatic organisms, including fish, and when their levels exceed normal limits, they can lead to mortality (Zeeshan et al., 2017). Under specific environmental conditions, these metals may reach toxic concentrations, posing severe ecological threats. Therefore, the presence of heavy metals in aquatic environments represents a significant risk to aquatic organisms, particularly fish.

Toxicity tests are experimental procedures designed to determine the concentration of a toxicant and the duration of exposure required to produce harmful effects in organisms. Toxicity is species-specific, as different organisms may exhibit varying responses to the same dose of a toxic substance (Smith and Stratton, 1986). Toxicity bioassays are commonly employed to detect and evaluate the potential toxic effects of chemicals on living organisms. The data generated from these tests serve as a foundation for assessing the risks posed by toxicants in natural environments. Various methods have been developed to assess the hazards and potential toxicity of chemicals, including acute, sub-acute, and chronic toxicity tests. One widely used parameter is the LC₅₀,

which estimates the concentration or dose required to kill 50% of a test population. This is determined experimentally by exposing groups of organisms to different concentrations of a toxicant and recording the mortality rates at specific time intervals typically 24, 48, 72, and 96 hours. Acute toxicity data are particularly valuable for determining appropriate sublethal concentrations to be used in chronic toxicity studies.

Manganese (Mn) is a naturally abundant element found in the Earth's mantle, present in most rocks and soil types. It plays a crucial role as a component of various enzymes and co-factors, making trace amounts essential for many living organisms. Industrially, manganese is widely used in the production of steel and batteries and is a key ingredient in products such as ceramics, varnishes, fertilizers, and pesticides. Elevated concentrations of manganese are often associated with discharges from mining, smelting, and other industrial activities (Harford et al., 2015). Despite being relatively common, manganese remains a poorly studied element in freshwater ecosystems, where it has the potential to accumulate significantly in aquatic organisms. However, our understanding of the mechanisms underlying manganese toxicity in fish health remains limited.

Probit analysis is a form of regression commonly used to analyze binomial response variables. In toxicology, it is frequently employed to determine the relative toxicity of toxicants or pollutants to living organisms (Singh and Zahra, 2017). This involves exposing organisms to varying concentrations of a toxicant and analysing the concentrations at which specific responses, such as mortality, occur. Among statistical methods, the probit method is widely accepted as one of the most accurate techniques for calculating LC₅₀ values. In this context, the present study was designed to evaluate the acute toxicity of the heavy metal manganese on the freshwater catfish *Heteropneustes fossilis* using probit analysis with the help of SPSS software.

II. MATERIALS AND METHODS

Freshwater catfish *Heteropneustes fossilis* (average length 7.5-8.5 cm and average weight 5.8-6.5gm) was

collected from local fish form and dip in 0.2% of potassium permanganate solution for 2-4 minute. The fishes were acclimatized in laboratory conditions for 10 days. During acclimatization the fishes were fed with commercial diet, egg albumin and small insects. The feeding of Fishes was stopped before experiment. The mortality was recorded after a period of 24, 48, 72 and 96 h and dead fishes were removed when observed.

The test chemical used for the experiment was Manganese sulphate (MnSO₄). A stock solution 1000 mg/L (1000 ppm) of reagent MnSO₄ was prepared by adding 1g of Manganese to 1 liter of distal water and stored in a clean glass bottle. Stock solution of manganese sulphate of various concentrations were prepared and 10 fishes was kept in each rectangular glass aquaria separately to estimate mortality between 0% and 100%. For 96h LC₅₀ test, different concentrations of manganese sulphate taken to find out the narrow range of concentrations. The mortality was recorded after a period of 24, 48, 72 and 96h. Estimation of LC₅₀ values after 24, 48, 72 and 96 h were calculated by SPSS software

III. RESULTS AND DISCUSSION

The percent mortality observed for each dose was calculated (Table1) and converted to probits by means of a SPSS software. Acute toxicity test i.e. LC₅₀ values show susceptibility of fish to particular toxicant or pollutant and reflect their survival potential. In the present study, the LC₅₀ values and the 95% confidence limits of heavy metals, Manganese sulphate for fish, *Heteropneustes fossilis* during 24, 48, 72 and 96 hrs. are presented in Table2.

Table 1. Mortality of Fish, *Heteropneustes fossilis* at different concentrations of Manganese sulphate (mg/L)

Concentration of Detergent (mg/L)	Log Conc. of Detergent	No. of Test Fish	No. of dead fish at different Exposure Period (Hours)			
			24	48	72	96
Control (0.0)	-.301	10	0	0	0	0
0.5	.000	10	0	0	0	0
1.0	.176	10	0	0	0	0
1.5	.301	10	0	0	0	1
2.0	.398	10	0	1	1	1
2.5	.477	10	1	2	2	2
3.0	.544	10	1	2	3	4
3.5	.602	10	2	3	4	6
4.0	.653	10	3	4	6	8
4.5	.699	10	4	5	7	9
5.0	.740	10	5	6	9	10
5.5	.778	10	7	8	10	-
6.0	.813	10	9	10	-	-
6.5	-.301	10	10	-	-	-

Table2. LC50 values with 95% confidence limits for *Heteropneustes fossilis*

Exposure Period (hrs.)	LC ₅₀ Values of Manganese	95% Confidence Limits (mg/L)		Regression Equation	Chi Square Value (P value)	Coefficient of determination (R ² Linear)
		Lower Limit	Upper Limit			
24	5.132	4.671	5.788	$Y = -4.2 + 6.67X$	5.054 (0.929)	0.895
48	4.057	3.581	4.660	$Y = -2.72 + 4.32X$	4.810 (0.903)	0.920
72	3.494	3.106	3.911	$Y = -3.24 + 5.95X$	2.768 (0.973)	0.943
96	3.032	2.666	3.420	$Y = -2.75 + 5.70X$	4.709 (0.788)	0.906

In the present study, the 24h, 48h, 72h and 96 LC₅₀ value of manganese sulphate for the fish, *Heteropneustes fossilis* was 5.132 mg/l, 4.057 mg/l,

3.494 mg/L and 3.032 mg/L, respectively as determined by Probit graphic method (Table2 and Fig.1a-d). The toxicity tolerance of freshwater airbreathing catfish *Heteropneustes fossilis* to MnSO₄ in the present study depends upon concentrations of heavy metals and duration of exposure. It was noticed that percent of mortality increased with an increase in concentration and duration of exposure. LC₅₀ values of the heavy metals showed the susceptibility of catfish to lethal concentrations in acute short-term exposure.

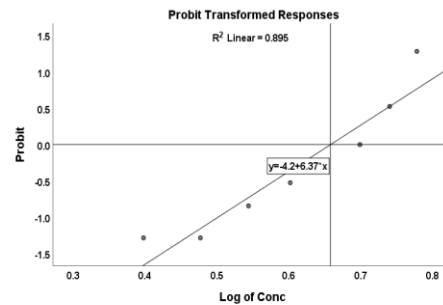


Fig. 1a. Regression Line (Based on Probit analysis) of Log concentration of Manganese sulphate Vs % mortality of *H. fossilis* (at 24hrs)

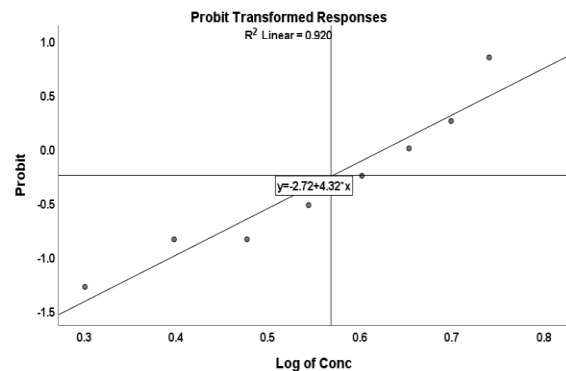


Fig. 1b. Regression Line (Based on Probit analysis) of Log concentration of Manganese sulphate Vs % mortality of *H. fossilis* (at 48hrs)

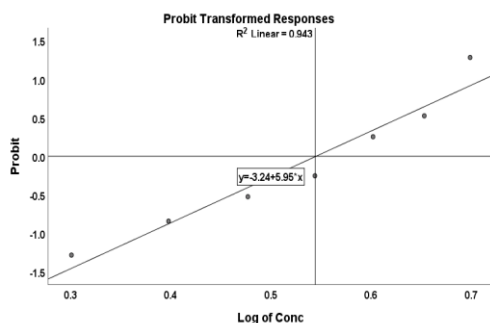


Fig. 1c. Regression Line (Based on Probit analysis) of Log concentration of Manganese sulphate Vs % mortality of *H. fossilis* (at 72hrs)

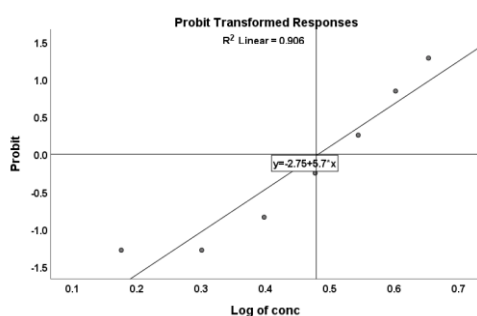


Fig. 1d. Regression Line (Based on Probit analysis) of Log concentration of Manganese sulphate Vs % mortality of *H. fossilis* (at 96hrs)

The death of fish is often attributed to the lethal effects of pollutants, which disrupt physiological and biochemical processes associated with cellular metabolic pathways. The toxicity of any substance to fish varies depending on several factors, including species, sex, age, weight, concentration or dose of the toxicant, duration of exposure, and whether the toxicant is in organic or inorganic form. Even sublethal concentrations of heavy metals can cause significant alterations in the biological organization of fish (Srivastava and Prakash, 2018a). Heavy metals are particularly toxic, even at relatively low concentrations, posing serious threats to the survival of aquatic organisms. According to Stebbing and Fandino (1983), the complex nature of heavy metals largely contributes to their adverse biological effects in aquatic environments. When the concentration of toxicants in water becomes excessively high, it often leads to fish mortality. Historically, the death of an organism has been used as a primary endpoint in toxicological studies (Jones and Reynolds, 1997).

The LC50 values obtained from toxicological studies are crucial for determining the sublethal concentrations of metals. Currently, most research on the effects of heavy metals on aquatic animals focuses on short-term experiments conducted at lethal concentrations. However, such data are insufficient to fully assess the extent of biological damage caused by pollutants. Therefore, it is essential to conduct studies on sublethal toxicity, which are particularly valuable for understanding the sequence of physiological and biochemical responses of test organisms to prolonged exposure at sublethal levels (Gandhewar et al., 2014). In this context, LC50 values play a vital role in deriving appropriate sublethal concentrations and evaluating the biological responses of fish under conditions of increasing aquatic pollution. Consequently, toxicity studies on heavy metals in fish are critical for developing a better understanding of their long-term ecological impacts.

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REFERENCES

- [1] Azmat R, Akhter Y, Talat R, Uddin F. (2006). Persistent of nematode parasite in presence of heavy metals found in edible herbivorous fishes of Arabian Sea. Food and agriculture organization of the United Nation 6, 282-285.
- [2] Farkas A., Salanki J., Specziar A. (2002). Relation between growth and the heavy metal concentration in organs of bream *Abramis brama* L. Populating Lake Balaton. *Arch. Environ. Contam. Toxicol.* 43(2):236-243. doi: 10.1007/s00244-002-1123-5.
- [3] Harford AJ, Mooney TJ, Trenfield MA, van Dam RA (2015) Manganese (Mn) toxicity to tropical freshwater species in low hardness water. *Environmental Toxicology and Chemistry* 34(12) 2856-2863 DOI: /10.1002/etc.3135/
- [4] Gandhewar S S, Zade S B and Sitre S R (2014) Assessment of toxic potential of three different

- heavy metals to *Clarias batrachus* (Linn.) utilizing static acute bioassay. *J. Appl. Nat. Sci.* 6(1), 117-120.
- [5] Jones J C and Reynolds J D (1997) Effects of pollution of reproductive behavior of fishes. *Rev. Fish Biol. Fisheries* 7, 463-491.
- [6] Lenntech (2004). Water Treatment and Air Purification. Water Treatment, Published by Lenntech, Rotterdamseweg, Netherlands.
- [7] Singh A and Zahra K (2017) LC50 assessment of cypermethrin in *Heteropneustes fossilis*: Probit analysis. *Int. J. Fish. Aquatic Stud.* 5(5), 126-130.
- [8] Smith T M and Stratton G W (1986) Effects of synthetic pyrethroid insecticides on non-target organisms. *Res. Rev.* 97, 93-119.
- [9] Srivastava N and Prakash S (2018a) Effect of sublethal concentration of zinc sulphate on the serum biochemical parameters of freshwater cat fish, *Clarias batrachus*. *Indian J. Biol.* 5(2), 113-119.
- [10] Srivastava N and Prakash S (2018b) Morphological, Behavioural and Haematological alterations in catfish, *Clarias batrachus* (Linn.) after acute zinc toxicity. *Int. J. Biol. Sci.* 9(1), 72-78.
- [11] Srivastava N and Prakash S (2018c) Alterations in the organic reserve of zinc exposed fresh water catfish, *Clarias batrachus*. *Flora and Fauna* 24(2), 386-392.
- [12] Stebbing A R D and Fandino V J R S (1983) The combined and separate effects of copper and cadmium on the growth of *Campanularia flexuosa* colonies (Hydrozoa). *Aquatic Toxicol.* 3, 183-193.
- [13] Zeeshan Ali, Ali Muhammad Yousafzai, Ijaz Muhammad, Gul-E-Nayab, Syed Abdul Maajid Aqeel and Syed Toheed Shah (2017). In vitro acute toxicity and bioaccumulation of manganese in common carp fish (*Cyprinus carpio*), *International Journal of Biosciences*, 10(6):160-165.
<http://dx.doi.org/10.12692/ijb/10.6.160-165>