



**RESEARCH ARTICLE**

**Toxic Effects of Tannery Chemical on the Fresh Water Fish, *Catla catla* (Ham.)**

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Received: 18<sup>th</sup> Jan. 2013, Revised: 23<sup>rd</sup> April 2013, Accepted: 30<sup>th</sup> April 2013

**ABSTRACT**

*The toxic effects of tannery chemical on the fresh water fish, Catla catla were studied. The LC<sub>50</sub> values of tannery chemicals at 24, 48, 72 and 96 hrs were determined. The fish exhibited abnormal behavioral movement upon exposure of different concentration of chemical. The study on the rate of mortality shows that higher the concentration greater the rate mortality.*

**Key word:** Toxicity, Tannery chemicals, *Catla catla*, LC<sub>50</sub>.

**INTRODUCTION**

Tannery effluents and discharges of spent water from industries, factories and from domestic waste which are ultimately dumped into sewers are known as "storm sewage" (Ingram 1989). Domestic sewage consist of discharges of spent water from wash basins, bathroom and from other sources which is a complex mixture of mineral and organic matter. Aquatic toxicology have adopted many of the techniques of its classical predecessor as the acute bioassay, whereby the concentration lethal to 50% of a population of fish or invertebrate in a given exposure time is determined (LC<sub>50</sub>). The procedures for carrying out aquatic bioassays have become rather well standardized (APHA 1992). These tannery chemicals are sublethal for fish and their toxicity leads towards the mortality of fish.

**MATERIALS AND METHODS**

The fishes of medium size (150 ± 10 mm in length and 25gm ± 5 in weight) were collected from the river Yamuna, Govt. fish farm Laramada village and from other local market resources of Agra. They were acclimatized for a fortnight under laboratory conditions before conducting the bioassay experiments.

**DETERMINATION OF LC<sub>50</sub> VALUE**

The acute short term static was conducted in 200 litter capacity glass aquaria to determine medium lethal concentration (LC<sub>50</sub>) of the two leather dyes to fishes. The dye solutions of different doses were applied at different time intervals to the fishes and their mortality was noted. The maximum concentration of dyes was selected at concentrations of stock solution from logarithmic scale as suggested by APHA, 1989. Serial dilutions at different doses of the dyes were prepared using dechlorinated tap water. Repetitions of tests were performed for each concentration. Fifty test fishes were exposed ten in each test media at the intervals of 24hrs, 48hrs, 72hrs, 96hrs and one week or more as desired in accordance to the LC<sub>50</sub> values. A control system was also arranged with twenty fishes in dechlorinated tap water with ten fishes for each test dye separately. The cessation of opercular beat was taken as an index of death.

The fishes were divided into five groups (A, B, C, D and E). Each group consisted of ten fishes and was treated with standard solution of experimental test compounds. Five different concentrations were given to each set. The mortality number of fishes was recorded for each concentration after 24hrs, 48hrs, 72hrs and 96hrs. The mortality percentage was noted after 96hrs. The data were analyzed statistically by log-dose/probit regression line method.

Regression line was drawn on the basis of two variables log-doses and empirical probit on a simple graph paper which is used to determine the expected probit necessary for LC<sub>50</sub> determination. The concentration of compound at which 50% of the fishes died was taken as the median lethal concentration. The mortality percentage was calculated and the graph was plotted between the mortality percentage and the concentrations of test compounds. Thus LC<sub>50</sub> values were obtained by the straight line graphical interpolation.

## RESULTS

### FISH BEHAVIOUR

When the fish *Catla catla* was exposed to different concentrations of test chemicals, stress effect was noticed. The movement becomes restless showing spiraling and backward movement. They struggled hard for breathing. The rates of opercular movements become rapid. When exposed to high concentration of test chemicals, the fish showed a grade reduction in the swimming activity and set on the bottom of jars.

The fish died within 96 to 120 hrs of exposure of higher concentration.

### DETERMINATION OF LC<sub>50</sub> VALUE

In order to estimate the LC<sub>50</sub> value, the fishes of different experimental sets have been treated with different concentrations of test compound. Five concentrations each 20, 30, 40, 50 and 60mg/L for Basic chromium sulphate and 60, 70, 80, 90 and 100mg/L for Nigrosine black have been selected and for each concentration, the mortality number of fishes at different time intervals i.e. 24 hrs, 48 hrs, 72 hrs and 96 hrs and percentage mortality for 96 hrs (Table-1,2,4 and 5) have been calculated which was used as final mortality for calculation as per international standards for fishes. The mortality number showed a corresponding increase with the increasing concentrations of the test compounds.

LC<sub>50</sub> values have been calculated by the log dose/probit regression line method (Finney, 1971). The test doses have been converted to their logarithms for ease of calculation (Table-3a and 6a). Empirical probit values corresponding to the percentage mortality have been obtained from standard table (Finney, 1971) and tabulated in the appropriate columns of the respective tables. The empirical probit values have thereafter been plotted against log dose on the graph paper and a provisional line filling the points is drawn (Fig. 1 and 2). From this line, expected probit values 'Y' are noted for the values of log dose 'X' (Table-3a and 6a). The working probit 'y' have been calculated using the following formula:

$$y = y_0 + kp$$

Where  $y_0$  and  $k$  are noted from the table for the expected probit  $Y$  and  $p$  is the percentage mortality.

The weighing coefficient 'n' for each point is also noted from the table (Finney 1971). Each weighing coefficient is multiplied by the number of fishes used and the products have been taken as 'w'. After this, for each row the products of  $wx$ ,  $wy$ ,  $wxy$ ,  $wx^2$ ,  $wy^2$  have been calculated and summed up as  $\sum wx$ ,  $\sum wy$ ,  $\sum wxy$ ,  $\sum wx^2$ ,  $\sum wy^2$  respectively and finally the mean have been calculated by the following formula:

$$\bar{X} = \sum wx / \sum w$$

$$\bar{Y} = \sum wy / \sum w$$

The value of 'b' has been calculated by the following formula:

$$b = (\sum wxy - \bar{X} \sum wy) / (\sum wx^2 - \bar{X} \sum wx)$$

Regression equation-

$$Y = \bar{Y} + b (x - \bar{X})$$

Values of 'Y' corresponding to the original values of 'X' have been calculated and the regression line is drawn.

The variance has been calculated by the following formula:

$$\text{Variance (V)} = \frac{1}{b^2} + \left( \frac{1}{\sum w} \frac{(X - \bar{X})^2}{\sum wx^2 - \frac{(\sum wx)^2}{\sum w}} \right)$$

The fiducial limits with 95% confidence have been obtained by the following formula:

$$m_1 = m + 1.96 V$$

$$m_2 = m - 1.96 V$$

The LC<sub>50</sub> value of Basic chromium sulphate and Nigrosine black for *Catla catla* (Ham.) have been observed to be 32.48mg/L and 79.80mg/L respectively (Table-3b and 6b). Sub-lethal concentrations were 3, 4, 5mg/L and 7, 8, 9mg/L for Basic chromium sulphate and Nigrosine black respectively.

## DISCUSSION

The main criteria for toxicity have been set by using LC<sub>50</sub> value which reveals 50% mortality of test fish for a given dose of toxin. The LC<sub>50</sub> values are useful measures of the acute toxicity of the tested toxicants under certain environmental conditions and it is also important because all the sub-lethal concentrations for experimentations depend on LC<sub>50</sub> value. In the present study, the percent mortality (Table-1 and 4) was converted into probit mortality and was plotted against the different concentrations of both toxicant (Fig. 1 and 2) as a result straight lines were obtained. The probit analysis suggested that the 96hrs LC<sub>50</sub> of Basic chromium sulphate and Nigrosine black on the test fish, *Catla catla* (Ham.) is 32.48mg/L and 79.80mg/L respectively. In both the test chemicals Basic chromium sulphate were more toxic to aquatic environment and its inhabitant organisms in comparison to Nigrosine black. Gupta *et al.* (2006) observed the LC<sub>50</sub> value of chromium sulphates in the *Heteropneustes fossilis* is 250mg/L. It is differ from the present investigation due to different species and mode of action. Raizada and Rana (1998) reported an LC<sub>50</sub> value of 0.86 mg/L to be highly toxic at 96hrs exposure of *Clarias batrachus* (Linn.) to malachite green. Subramanian *et al.* (2007) studied the toxic effect of heavy metal; chromium on *Clarias batrachus* (Linn.) and reported an LC<sub>50</sub> value of 2.3401 mg/L at 96hrs exposure to be highly toxic. Veliek *et al.* (2006) reported 18.40 mg/L LC<sub>50</sub> due to clove oil exposure of *Silurus glanis* (Linn.). Venkatesan and Subramanian (2007) observed an LC<sub>50</sub> value of 0.253 mg/L at 96hrs exposure of *Oreochromis mossambicus* (Peters) to copper sulphate. All these research findings of different LC<sub>50</sub> values are due to different toxic substances and fishes, but are highly toxic to organism, thus supporting the present findings.

**Table- 1:** Mortality of *Catla catla* (Ham.) at different time intervals after treatment with different concentrations of Basic chromium sulphate

S.No.	Concentration (mg/L)	No. of fishes	Mortality number after exposure of			
			24hrs	48hrs	72hrs	96hrs
1	20	10	0	0	0	0
2	30	10	1	1	2	4
3	40	10	1	2	5	7
4	50	10	1	4	6	9
5	60	10	2	6	9	10

**Table- 2:** Survival number and percentage mortality of *Catla catla* (Ham.) after 96 hours of treatment with Basic chromium sulphate

S.No.	Concentration (mg/L)	No. of fishes	Exposure time (hrs)	Mortality number	Percentage mortality	Survival number
1	20	10	96	0	0	10
2	30	10	96	4	40	6
3	40	10	96	7	70	3
4	50	10	96	9	90	1
5	60	10	96	10	100	0

**Table- 3a:** Determination of LC<sub>50</sub> after treatment with different concentrations of Basic chromium sulphate to *Catla catla* (Ham.)

Conc. (mg/L)	No. of Fishes 'n'	% mor.	log conc 'x'	Empirical probit	Expected probit 'y'	Working probit 'y'	Weighting coefficient 'N'	Weight w=n x N	wx	wy	wxy	wx <sup>2</sup>	wy <sup>2</sup>						
20	10	0	1.30	-	-	-	-	-	-	-	-	-	-						
30	10	40	1.47	4.75	4.74	4.750	0.616	6.16	9.05	29.26	43.01	13.31	138.98						
40	10	70	1.60	5.52	5.50	5.524	0.581	5.81	9.29	32.09	51.35	14.87	177.28						
50	10	90	1.69	6.28	6.20	6.278	0.378	3.78	6.38	23.73	40.10	10.79	148.98						
60	10	100	1.77	0.0	6.30	6.805	0.336	3.36	5.94	22.86	40.47	10.52	155.59						
								$\Sigma W =$	19.11	$\Sigma WX =$	30.68	$\Sigma WY =$	107.95	$\Sigma WXY =$	174.93	$\Sigma WX^2 =$	49.50	$\Sigma WY^2 =$	620.85

**Table- 3b:** Toxicity evaluation of Basic chromium sulphate to *Catla catla* (Ham.) specifying fiducial limits

Experimental animal	Compound	Regression equation	LC <sub>50</sub> mg/L	Variance	Fiducial limits
<i>Catla catla</i>	Basic chromium sulphate	$Y = 5.64 + 6.89(X - 1.60)$	32.48	0.001	$m_1 = (+) 1.51196$ $m_2 = (-) 1.50804$

**Table- 4:** Mortality of *Catla catla* (Ham.) at different time intervals after treatment with different concentrations of Nigrosine black

S.No.	Concentration (mg/L)	No. of fishes	Mortality number after exposure time of			
			24hrs	48hrs	72hrs	96hrs
1	60	10	0	0	0	0
2	70	10	0	1	1	3
3	80	10	1	1	3	5
4	90	10	1	2	5	7
5	100	10	1	4	8	10

**Table- 5:** Survival number and percentage mortality of *Catla catla* (Ham.) after 96 hours of Treatment with Nigrosine black

S.No.	Concentration (mg/L)	No. of fishes	Exposure time (hrs)	Mortality number	Percentage mortality	Survival number
1	60	10	96	0	0	10
2	70	10	96	3	30	7
3	80	10	96	5	50	5
4	90	10	96	7	70	3
5	100	10	96	10	100	0

**Table- 6a:** Determination of LC<sub>50</sub> by log-dose/probit regression analysis after treatment of Nigrosine black to *Catla catla* (Ham.)

Conc. (mg/L)	No. of Fishes 'n'	% mor.	Log conc 'x'	Empirical probit	Expected Probit 'y'	Working Probit 'y'	Weighting coefficient 'N'	Weight w=n x N	wx	wy	wxy	wx <sup>2</sup>	wy <sup>2</sup>
60	10	0	1.77	-	-	-	-	-	-	-	-	-	-
70	10	30	1.84	4.48	4.50	4.476	0.581	5.81	10.69	26.00	47.85	19.67	116.40
80	10	50	1.90	5.00	5.00	5.000	0.637	6.37	12.10	31.85	60.51	22.99	159.25
90	10	70	1.95	5.52	5.55	5.523	0.558	5.58	10.88	30.81	60.09	21.21	170.20
100	10	100	2.00	0.0	5.90	5.593	0.471	4.71	9.42	26.34	52.68	18.84	147.33
								$\Sigma W =$ 22.47	$\Sigma W X =$ 43.09	$\Sigma W y =$ 115.01	$\Sigma W X y =$ 221.14	$\Sigma W X^2 =$ 82.72	$\Sigma W y^2 =$ 593.19

**Table- 6b:** Toxicity evaluation of Nigrosine black to *Catla catla* (Ham.) specifying fiducial limits

Experimental animal	Compound	Regression equation	LC <sub>50</sub> mg/L	Variance	Fiducial limits
<i>Catla catla</i> (Ham.)	Nigrosine black	$Y = 5.11 + 7.49(X - 1.91)$	79.80	0.0007	$m_1 = (+) 1.90137$ $m_2 = (-) 1.89863$

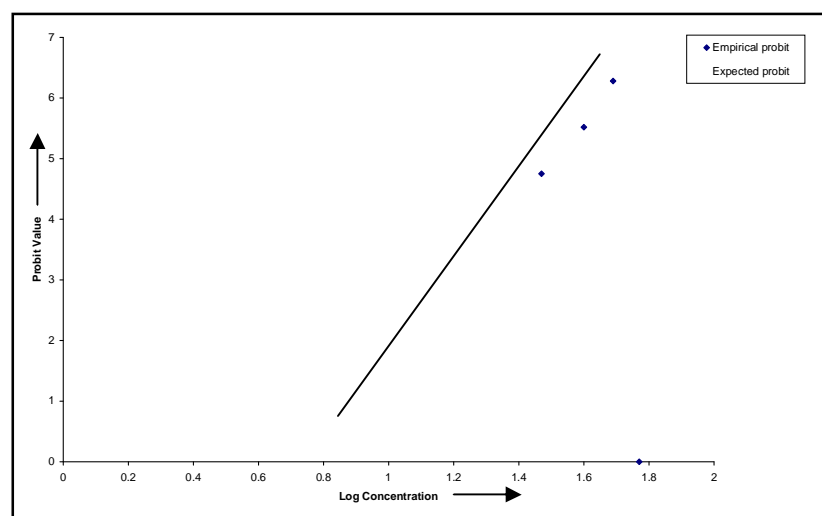
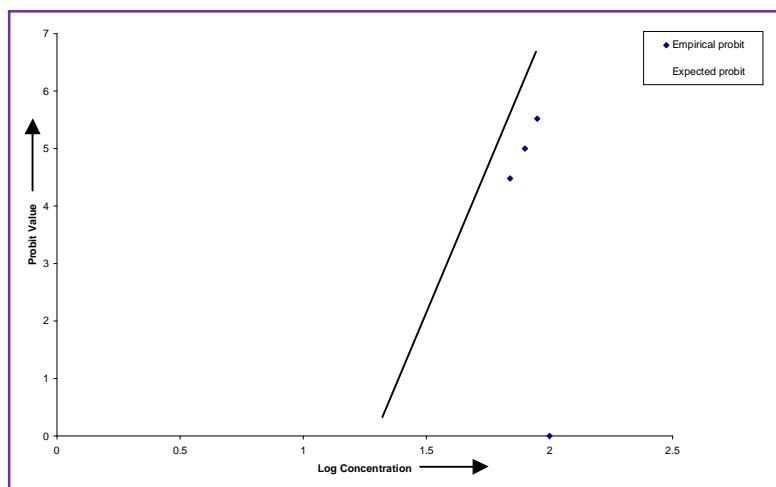
**Fig.- 1:** Graph showing regression line for the determination of LC<sub>50</sub> of Basic chromium sulphate for *Catla catla* (Ham.)

Fig.- 2: Graph showing regression line for the determination of LC<sub>50</sub> of Nigrosine black for *Catla catla* (Ham.)



### ACKNOWLEDGEMENT

The author is highly grateful to Professor K.S. Rana, HOD Deptt. Zoology Agra College, Agra for his constant guidance and also respected thankful for my loving father Sh. R.P. Singh (Principal) support every steps.

### REFERENCES

1. APHA (1989): Standard methods for the examination of water and waste water. 17<sup>th</sup> ed., Washington, D.C.
2. APHA (1992): Standard methods for the examination of water and waste water. 18<sup>th</sup> ed., American Public Health Association, American Water Works Association, Water Environmental Federation, Washington, D.C.
3. Finney D.J. (1971): Probit analysis. 3<sup>rd</sup> Edn. Cambridge university press, Cambridge, 333.
4. Gupta S.S., Kumar A. and Srivastava J.P. (2006): Effect of chromium sulphate on haematological factors of the fish; *Heteropneustes fossilis* (Bloch.). *J. Ecotoxicol. Environ. Monit.*, 16(4): 363-370.
5. Ingram R.G., Bourget E. and Fevire J.L. (1989): Effect of ocean variability on the abundance of the dungeness crab. *Fish Aquat. Sci.*, 50: 1002-4016.
6. Raizada S. and Rana K.S. (1998): Acute toxicity of malachite green to an air breathing teleost; *Clarias batrachus* (Linn.). *J. Environ. Biol.*, 19(3): 237-241.
7. Subramanian J., Nethaji V. and Shasikumar R. (2007): Toxic effects of the heavy metal chromium on the fresh water catfish; *Clarias batrachus* (Linn.). *J.Exp. Zool. India.*, 10(2): 357-362.
8. Veliek J., Wlasow T., Gomulka P., Svobodova Z., Lovotnz L. and Ziomek E. (2006): Effects of clove oil anaesthesia on European cat fish; *Silurus glanis* (Linn.). *Acta. Vet. Brno.*, 75: 99-106.
9. Venkatesan R. and Subramanian N. (2007): Effect of copper sulphate on histopathological changes in the fresh water fish; *Oreochromis mossambicus* (Peters). *J. Ecotoxicol. Environ. Monit.*, 17(4): 353-36.