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# Toxicity evaluation and behavioural responses of *Danio rerio* exposed to raw tannery effluent

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**Abstract**
When exposed to different concentration of a toxicant, the physiology and the whole system of the fish found to be disturbed and affected. In the present investigation, toxicity evaluation and behavioral changes in the fish *Danio rerio* (Zebrafish) were studied. The freshwater fish *Danio rerio* was exposed to tannery effluent in static test for the $LC_{50}$ values for 12 to 96 hrs and the $LC_{50}$ values were 54.314 for 12 hours, 52.940 for 24hrs, 51.967 for 36 hrs, 51.461 for 48 hrs, 50.878 for 60 hrs, 50.171 for 72 hrs, 49.221 for 84 hrs and 48.448 for 96 hrs respectively. The $LC_{50}$ values found to be decreased constantly with increasing period of exposure. This indicated that low concentration of a toxicant is also toxic to the fish *Danio rerio*. Alterations in behavioural patterns were also well marked during the period of the experiment.

**Keywords:** *Danio rerio*, Tannery effluent, toxicity and behavioral alterations.

## 1. Introduction
Environmental pollution is one of the most serious problems that the mankind has been facing in this century [1]. With the rapid growth of industries in the country, pollution of natural water by industrial waste has increased tremendously [2]. The industrial effluent generally contains dissolved and suspended solids, organic and inorganic chemicals, high BOD and COD, oils and grease, that cause deleterious effects on the freshwater fauna when discharged in to water bodies. Tannery effluents when get mixed with natural water bodies cause health risks for human beings and pollute the environment. Effluents from raw hide processing tanneries produce wet blue, crust leather or finished leather that mostly contain compounds of trivalent chromium and sulphides [3]. It is also confirmed that one to ten percent of tannery wastewaters kill fish [4].
The fishes are quite sensitive to the contaminated water since the pollutants significantly damage their physiological and biochemical processes [5]. The tannery wastewaters continue to cause negative effects in aquatic organisms as they also have endocrine disruption effects [6]. Biological changes in fish caused by the contaminants are called biomarkers that can be used for environmental risk assessment [7].
Acute toxicity tests are generally used to determine the concentration of a toxicant that produces specific adverse effect on a specified percentage of test organisms in a short span of time. The most common acute toxicity test is acute lethality test. Experimentally, effect on fifty percent of test organisms is the most reproducible and easily determined measure of toxicity and 48 hrs or 96 hrs is often convenient and useful exposure duration [8]. $LC_{50}$ represents the lethality of a toxicant to a test species in terms of mortality and time. The experimental results often do not contain a single group in which exactly half the animals died. However the concentration that is expected to kill half the number of test species in a single group can be determined by calculating $LC_{50}$.
The present study was thus planned to investigate the toxic effect of raw tannery effluent on the freshwater fish *Danio rerio.*

## 2. Materials and Methods
The present investigation was carried out from March 2014 to May 2014.The fresh water fish *Danio rerio* size 4.0 ± 0.5 cm and weight 290 ± 30 mg were brought from the local fish farm. The fish *Danio rerio* were acclimatized to the laboratory conditions at 28 ± 2 ºC. The fish were fed daily with commercial fish pellets and allowed to acclimate for 30 days. Water was renewed every day to provide oxygen rich freshwater.

The water used for acclimatization and experiments was clear, non-chlorinated and aerated ground water. A batch of 10 fishes was also maintained along with experimental groups as control group. They were kept in a containers of 10 litre capacity each. The raw tannery effluent was collected from Ambur, Vellore District in Tamil Nadu. The experiments were conducted to determine the toxicity in eleven different concentrations of tannery effluent for 12, 24, 36, 48, 60, 72, 84 and 96 hours in static system. The mortality rate was recorded by counting the dead animals. The dead fish were removed immediately. The data of each concentration was pooled up to calculate the $LC_{50}$ values. The un-weighted regression method of probit analysis was used to calculate the $LC_{50}$ values [9]. During this experiment the behavioural changes were also observed. Physico-chemical characteristics of the tannery effluent are given in table 1. Values of different quality parameters were compared with the standards prescribed by Central Pollution Control Board (CPCB).

## 3. Results and Discussion

In the present investigation the test species, *Danio rerio* showed differential toxicity level with varying exposure time. The observed percentage of mortality and $LC_{50}$ values for *Danio rerio* for tannery effluent in static tests conducted for 12, 24, 36, 48, 60, 72, 84 and 96 hours are shown in the Tables 2 and 3. As evidenced by the results the abnormal changes in the fish exposed to lethal concentration of Tannery effluent are time dependent. The highest log concentration of 1.72 showed the highest fish mortality (Table 4 and Figure 1).

It is clear from earlier studies that $LC_{50}$ of effluent for a fresh water fish varies from species to species and in the same species under the influence of number of factors including size and time of exposure. The response is initiated at the threshold dose when increase intensity of dose and exposure time is increased. That is reported on the basic concept of the dose response relation-ship [22, 23].

**Table 1:** Chemical Analysis of Tannery Effluent used for experiments

| Sl. No. | Parameters | Amount present in Tannery effluent | Permissible limit (CPCB) |
|---|---|---|---|
| 1 | **Turbidity NTU** | 465 | 10 |
| 2 | **Electrical conductivity at 25 ºC (ms/cm)** | 27.4 | 2100 |
| 3 | **pH value at 30 $^0$C** | 3.84 | 6.0 to 9.0 |
| 4 | **Nitrate ($NO_3^-$) (mg/l)** | 116 | 10 |
| 5 | **Total solids (mg/l)** | 26470 | 100 |
| 6 | **Lead (Pb) (mg/l)** | 0.07 | No relaxation |
| 7 | **Total Hardness (as CaCO3) (mg/l)** | 1820 | 600 |
| 8 | **Chromium VI (mg/l)** | 0.096 | 0.1 |
| 9 | **Calcium $CaCo_3$ (mg/l)** | 990 | 200 |
| 10 | **Sulphate (as $SO_4)_2$ (mg/l)** | 16300 | 400 |
| 11 | **Chloride (as $Cl)^-$, (mg/l)** | 2950 | 1000 |
| 12 | **Magnesium (mg/l)** | 830 | 100 |
| 13 | **Total Chromium (mg/l)** | 140 | 2.0 |
| 14 | **Dissolved oxygen as $O_2$(ml/l)** | 1.06 | 4 to 6 |
| 15 | **Temperature** | $28 \pm 32$ $^0$C | Shall not exceed 5 $^0$C above the receiving water temperature |
| 16 | **COD (mg/l)** | 11770 | 250 |
| 17 | **BOD (mg/l)** | 3230 | 30-100 |
| 18 | **Nitrite (mg/l)** | 0.235 | 0.02 |

Behavioral study gives direct response of the animals to the pollutant. The behavioral activities of an organism, represent the final integrated results of a diversified biochemical and physiological processes [10]. When *Danio rerio* were introduced to the raw effluent added water, they got excited and were found to swim rapidly with random movements. Similar excitement was also found with light movements and vibrations. There were rapid opercular movements followed by excited swimming and coughing in *Danio rerio* because of improper ventilation or inconvenience in breathing [11, 15, 16, 17]. Such coughing response is a useful tool for evaluating the quality of industrial, distillery and municipal effluents. While in control fish the opercular movements were normal and it was clearly seen when compared with experimental fish, coughing was not observed in control fish [18, 19].

Disruption of schooling behavior of the fish, due to the lethal and sublethal stress at the toxicant, results in increased swimming activity and entails increased expenditure of energy [12], Change in the normal physiological and biochemical aspects in the treated fish in the present study could be attributed to the disruption of the schooling activities [13, 24]. The erratic swimming of the treated fish indicates loss of equilibrium. It is likely that the region in the brain which is associated with the maintenance of equilibrium should have been affected [14]. Increase in fin “flickers” observed in the treated fish is not uncommon. These behavioral changes were seen and confirmed in the present investigation also. When the ventilation rate was increased, repeated opening and closing of the mouth was observed.

The fish were swimming with the belly upwards and in zigzag motion. There were also erratic and parallel movements observed in the fish, indicating the loss of equilibrium while in control the fish were swimming normally without loss of equilibrium. The fish is exposed to cypermethrin showed erratic swimming, being hyper and hypoactive, imbalance in posture, increase in surfacing activity, opercular movement, gradual loss in equilibrium, spreading of excess of mucus all over the surface of the body [20].

The fish regularly came to the surface of water for atmospheric oxygen. Also the gills of fish were affected as indicating the pale red colour when exposed Tilapia to lindane [21]. The abnormal changes in the fish exposed to lethal concentration cypermethrin were reported to be time dependent [12]. Thus it is concluded that the effluent is not safe to aquatic organisms like

fishes. Hence this type of study can be useful to compare the sensitivity of the various species of aquatic animals and to derive safe environmental concentration. Changes in behaviour of fish, *Danio rerio* due to raw tannery effluent stress can be used as a biological indicator of pollution and biological early alarm system of the tannery effluent.

**Table 2:** The $LC_{50}$ Values of Tannery effluent for the fish *Danio rerio* after 12, 24, 36, 48, 60, 72, 84 and 96 of treatment

| Sl. No. | Period | $LC_{50}$ |
|---|---|---|
| 1 | 12 Hours | 54.314 |
| 2 | 24 Hours | 52.940 |
| 3 | 36 Hours | 51.967 |
| 4 | 48 Hours | 51.461 |
| 5 | 60 Hours | 50.878 |
| 6 | 72 Hours | 50.171 |
| 7 | 84 Hours | 49.221 |
| 8 | 96 Hours | 48.448 |

**Table 3:** Tannery effluent Vs *Danio rerio f*or 24 hrs, 48 hrs, 72 hrs and 96 hrs

| 24 Hrs | | | 48 Hrs | | | 72 Hrs | | | 96 Hrs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. of Effluent % | Log conc. | % of mortality | Conc. of Effluent % | Log conc. | % of mortality | Conc. of Effluent % | Log conc. | % of mortality | Conc. of Effluent % | Log conc. | % of mortality |
| 5.1 | 1.71 | 20 | 5.0 | 1.70 | 20 | 4.8 | 1.68 | 10 | 4.6 | 1.66 | 10 |
| 5.2 | 1.72 | 30 | 5.1 | 1.71 | 40 | 4.9 | 1.69 | 30 | 4.7 | 1.67 | 30 |
| 5.3 | 1.72 | 40 | 5.2 | 1.72 | 60 | 5.0 | 1.70 | 40 | 4.8 | 1.68 | 40 |
| 5.4 | 1.73 | 70 | 5.3 | 1.72 | 80 | 5.1 | 1.71 | 70 | 4.9 | 1.69 | 60 |
| 5.5 | 1.74 | 90 | 5.4 | 1.73 | 100 | 5.2 | 1.72 | 80 | 5.0 | 1.70 | 70 |
| 5.6 | 1.75 | 100 | | | | 5.3 | 1.72 | 100 | 5.1 | 1.71 | 90 |
| | | | | | | | | | 5.2 | 1.72 | 100 |

**Table 4:** Tannery effluents vs *Danio rerio* 96 hours after treatment

| No. | Conc. of Effluent % | Log conc. | No. of exposed fish | No of Live fish | No of Died fish | % of mortality | Probit mortality |
|---|---|---|---|---|---|---|---|
| 1 | 4.6 | 1.66 | 10 | 9 | 1 | 10 | 3.63 |
| 2 | 4.7 | 1.67 | 10 | 7 | 3 | 30 | 4.20 |
| 3 | 4.8 | 1.68 | 10 | 6 | 4 | 40 | 4.75 |
| 4 | 4.9 | 1.69 | 10 | 4 | 6 | 60 | 5.30 |
| 5 | 5.0 | 1.70 | 10 | 3 | 7 | 70 | 5.84 |
| 6 | 5.1 | 1.71 | 10 | 1 | 9 | 90 | 6.36 |
| 7 | 5.2 | 1.72 | 10 | 0 | 10 | 100 | 6.88 |

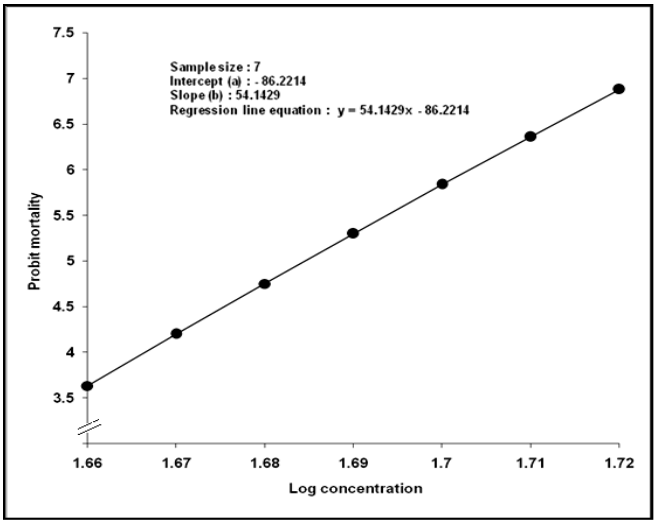


**Fig 1:** The graph showing linear curve between probit mortality of fish against log concentration in *D. rerio* on exposure to tannery effluent.

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## 5. References.

1. Samir AMEZ, Eman GEH, Abd-Rabo TEI, Somaia ZAR. Carbamate toxicity and protective effect of vit-A and vit-E: some bio-chemical aspects of male albino rats. The Egyptian J Hospital Medicine 2000; 1:60–77.
2. Muthuswamy A, Jayabalan N. Effects of factory effluents on physiological and biochemical contents of *Gossypium hirsutum* L. J Environ Biol 2001; 22:237-247.
3. Groganzh GH. Treatment of Tannery Waste Water, Technical Handbook, Tool Foundation, 2002.
4. Srivastava S, Prabhakar P, Srivastava BC. Toxicity and behaviour of the fish *Labeo rohita* and *Channa punctatus* exposed to pulp paper mill effluent. J Ecotoxicol Environ Monit 2007; 17:241-244.
5. Nemesok J, Orban L, Aszatalos B, Vig E. Accumulation of pesticides in the organs of carp *Cyprinus carpio* L. at $4^0$ and 20 $^0$C. Bull Environ Contam Toxicol 1987; 39:370-378.
6. Hewitt LM, Parrott JL, McMaster ME. A decade of research on the environmental impacts of pulp and tannery mill effluents in Canada: sources and characteristics of bioactive substances. J. Toxicol. Environ Health 2006; 9:341-356.
7. Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk

assessment: a review. Environ. Toxicol. Pharmacol 2003; 13:57-149.
8. ASTM Committee E-35 on Pesticides, Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians in Annual book of ASTM standards 1980; 46:1-25.
9. Finney DJ. Probit analysis Edn 3, Cambridge University Press.
10. Warner REKK, Peterson, Burgman L. Behavioral pathology in fish. A quantitative study of sub-lethal pesticide toxication. J Appl Eco 1966; 3:233.
11. Anderson PD, Weber LJ, Toxic response as a quantitative function of body size. Toxicol Appl Pharmacol 1975; 33:471.
12. Rathnamma VV, Vijayakumar M, Philip GH. Acute toxicity and behavioral changes in freshwater fish Labeo rohita exposed to Deltamethrin. J Aqua Biol 2008; 23:165-170.
13. Murthy BN, Ramarao KV. Ethology of fish Tilapia mosambica under lindane toxicity Geobios 1983; 10:230-231.
14. Sandheinrich MB, Atchison GJ. Sublethal toxicant effects on fish. Foraging behaviour: Empirical US. Mechanistic Approach. Environ Toxicol Chem 1990; 9:107-119.
15. Carlson RW. Some characteristic of ventilation and coughing in the blue gill *Lepomis macrochirus* Rafinesque. Environ Pollut 1982; 29-35.
16. Haider G. Studies on the heavy metal poisoning of fishes l. Lead poisoning of rainbow trout (*Salmo gairdneri* Rich.) Z Angew Zool 1964; 51:347-366.
17. Carlson RW, Drummound RA. Fish cough response- A method for evaluating quality of treated complex effluents. W Sater Res 1978; 12, 1.
18. Durve VS, Jain SM. Toxicity of distillery effluent to the cyprinid weed fish *Rasbora Daniconius* (Ham). Acta. Hydrochim 1980; 8: 329-336.
19. Prasanth MS, David M, Mathed SG. Behavioural changes in freshwater fish Ctenopharyng odonidellus(Hamilton) exposed to cypermethrin. J Ecotoxicol Environ Monit. 2005; 26(1):141-144.
20. Konar SK, Ghosh TK. Ethology of fish, Tilapia mosambica under lindane toxicity. Geobios. 1982; 10, 230-231.
21. Murthy BN, Rama Rao KV. Ethology of fish Tilapia mossambica under lindane toxicity. Geobios. 1983, 10: 230-231.
22. Malik GM, Viral RH, Kausar AKH. Toxic effects of effluent on mortality and behaviour changes on fresh water fish. J Environ Res Develop 2012; 7:1036-1039.
23. Pathan TS, Sonawane DI, Khillare YK. Toxicity and behavioral changes in freshwater fish *Rasbora daniconius* exposed to paper mill effluent. Bot Res Inter 2009; 2:263-265.
24. Prashanth MS, Sayeswara HA, Mahesh Anand Goudar. Free Cyanide Induced Physiological Changes in the Freshwater Fish, *Poecilia Reticulata*. Journal of Experimental Sciences 2011; 2:27-31.