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Investigation of acute toxicity and the effect of chrome plating industry effluent on behavior of the guppy (*Poecilia reticulata*)

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Abstract
In this study 96-h LC_{50} value of chromium, is a metal widely used in industry, was determined for the guppy (*Poecilia reticulata*, Peters, 1859). 10 fish were placed in each replicate of each dose. The experiments were performed as three replicates, and behavioural changes in the guppy were determined for each chrome plating effluent concentration. The data obtained were statistically evaluated by the use of EPA computer program based on Finney's Probit Analysis Method and a 96-h LC_{50} value for *P. reticulata* was found to be 1.67 ml/l in a static bioassay test system. Lower and upper confidence limits for the LC_{50} were 1.44 and 1.94 ml/l, respectively. The behavioural changes observed in fish were swimming in imbalanced manner, capsizing, attaching to the surface, difficulty in breathing and gathering around the ventilation filter.

Keywords: Toxicity; LC_{50}; Bioassay; Acute toxicity; Chromium; Guppy; *Poecilia reticulata*

Introduction

Effluents from chrome plating industry containing heavy metals pollute water bodies, causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al.*, 2003). Heavy metal contamination reduces feed utilization in fish resulting in reduced fish metabolic rate and retarded growth (Javed, 2005) [15].

Chromium, a constituent of chrome plating industry effluent is also used in metal finishing, petroleum refining, iron and steel, pulp and paper, electroplating, leather tanning, textile and wood preservation industries (Huaxiao *et al*., 2009; Das and Mishra, 2010 and Poornima *et al*., 2010) [14, 7, 25]. Chromium is found naturally in rocks, plants, soil, volcanic dust, grains, yeast, and animals. Stainless steel consists of 12-15% chromium. Chromium metal is consumed worldwide in amounts of approximately 20,000 tons per year. Wastewaters usually contain about 5 ppm of chromium. Chromium compounds are very persistent in the aquatic environment, mostly bound to sediments and soil.

Chromium is a highly toxic, mutagenic and carcinogenic metal contaminant (Prabakaran *et al.*, 2007) [26], highly mobile and an incorporating metal in food chain (Goldoni *et al.*, 2006) [13]. It occurs in several oxidation states ranging from Cr^{2+}, Cr^{3+} to Cr^{6+} in which Cr^{3+} and Cr^{6+} exist in stable states. The most common forms of chromium that occur in natural waters in the environment are trivalent chromium (chromium-3) and hexavalent chromium (chromium-6). Chromium-3 is an essential human dietary element and is found in many vegetables, fruits, meats, grains and yeast. Chromium (III) compounds are water insoluble because these are largely bound to floating particles in water (Bahijri and Mufti, 2002) [5]. Hexavalent chromium (Cr (VI)) is the most toxic form. It is a well known carcinogen, and mutagen (Kotas and Stasicka, 2000; Ramakrishna and Ligy, 2005 and Ashwini and Seeta, 2009) [19, 27, 4] due to its high solubility, ability to penetrate the cell membrane and strong oxidizing ability (Shanker and Pathmanabhan, 2004).

Several authors have already evaluated the toxicity of heavy metals on aquatic organism. Cr (VI) has an adverse effect on the haematological and biochemical parameters of fish (Oner *et al.*, 2009 and Zaki *et al.*, 2009) [23, 36]. Blood cell changes associated with Cr (VI) include decreased erythrocyte count and haematological indices (Firat and Kargin, 2009) [11]. The objective of this study is to estimate the toxic effect of Chrome plating industrial effluent on the freshwater fish, *Poecilia reticulata* Peters 1859.

2. Material and methods

2.1 Analysis of Physico - Chemical characteristics of effluent

Chrome plating industry effluent was collected from a small scale industry in a private working area, Ozhuhinasery (8.184’’ and 77.433’’), Tamilnadu, India. Physico- chemical analysis studies were carried out to characterize the effluent. pH, Total Dissolved Solids, Total Suspended Solids, Electrical Conductivity, Turbidity, Dissolved Oxygen, Biochemical Oxygen Demand, Chemical Oxygen Demand (COD), Total Hardness, Oil and Grease, Potassium, Sodium, Calcium, Chlorides, Sulphates, Magnesium, total Chromium, Copper, Zinc, Cadmium, Nickel, Cobalt, Iron, Lead were estimated following methods suggested by APHA (Standard Methods, 1985).

2.2 Collection and acclimatization of the test fish

The freshwater fish, *P. reticulata* belonging to the same size group were collected from the college pond and maintained in the wet laboratory of Scott Christian College (Autonomous) Nagercoil. The fish were acclimatized in large FRP tanks containing tap water. Care was taken to avoid any sudden changes in temperature, salinity and pH. The fish were acclimatized for about 15 days before the commencement of the experiments.

2.3 Static Bioassay

Static bioassay studies (Sprague, 1973) [34] were carried out to assess the toxicity of chrome plating industry effluent. The stock toxicant solution was prepared by mixing 5.0 ml of the effluent in 1*l* of deionized water. The stock toxicant solution was diluted to obtain 9 different concentrations of the effluent 1.0ml/l, 1.5ml/l, 2.0ml/l, 2.5ml/l, 3.0ml/l, 3.5ml/l, 4.0ml/l, 4.5ml/l and 5.0ml/l. For each set, three replicates were maintained. The mortality of the fish was observed after 12, 24, 36, 48, 60, 72, 84 and 96 hours of exposure and further analysed using probit analysis (Finney, 1971) [10]. Log dose and probability of mortality values were calculated. LC_{50} values were derived through probit analysis and the upper and lower confidence intervals were calculated.

Table 1: Physico-chemical characteristics of chrome plating industry effluent

Sl. No.	Parameter	Quantity	Permissible Limits (CPCB) 2013-2014
1	pH	1.75	5.5-9.0
2	Total Dissolved Solids (mg/l)	39860	2100
3	Total Suspended Solids, (mg/l)	2100	600
4	Electrical Conductivity (μScm^{-1})	78	2100
5	Turbidity (NTU)	150	30
6	Dissolved Oxygen (mg/l)	6.49	4-6
7	Biochemical Oxygen Demand (mg/l)	ND5	-
8	Chemical Oxygen Demand (mg/l)	2228	-
9	Total Hardness (mg/l)	2000	-
10	Oil and Grease, (mg/l)	59	20
11	Potassium (mg/l)	129	-
12	Sodium (mg/l)	750	60
13	Calcium (mg/l)	500	-
14	Chlorides (mg/l)	1600	1000
15	Sulphates (mg/l)	1765	1000
16	Magnesium (mg/l)	1500	-
17	Total Chromium (mg/l)	15147.50	2.0
18	Copper (mg/l)	287.6	3
19	Zinc (mg/l)	84.5	15
20	Cadmium (mg/l)	ND1	-
21	Nickel (mg/l)	798.6	3
22	Cobalt (mg/l)	ND1	-
23	Iron (mg/l)	232	-
24	Lead (mg/l)	ND2	-

CPCB – Central Pollution Control Board

ND1- Not Detected upto 20ppm, ND2 - 2ppm, ND5- 5 ppm

Table 2: Mortality of *P. reticulata* at different concentrations of the effluent

Sl. No	Exposure in hours	LC_{50} in % concentration	No. of fish exposed	% of mortality	Regression equation Y= a+bx
1	12	4.1	10	50	Y=6.57+(-5.66)x
2	24	3.4	10	50	Y=10.74+(-11.47)x
3	36	3.2	10	50	Y=8.56+(-7.88)x
4	48	2.8	10	50	Y=8.77+(-7.72)x
5	60	2.7	10	50	Y=7.51+(-5.74)x
6	72	2.3	10	50	Y=7.70+(-5.44)x
7	84	2.0	10	50	Y=8.50+(-6.12)x
8	96	1.6	10	50	Y=7.41+(-3.97)x

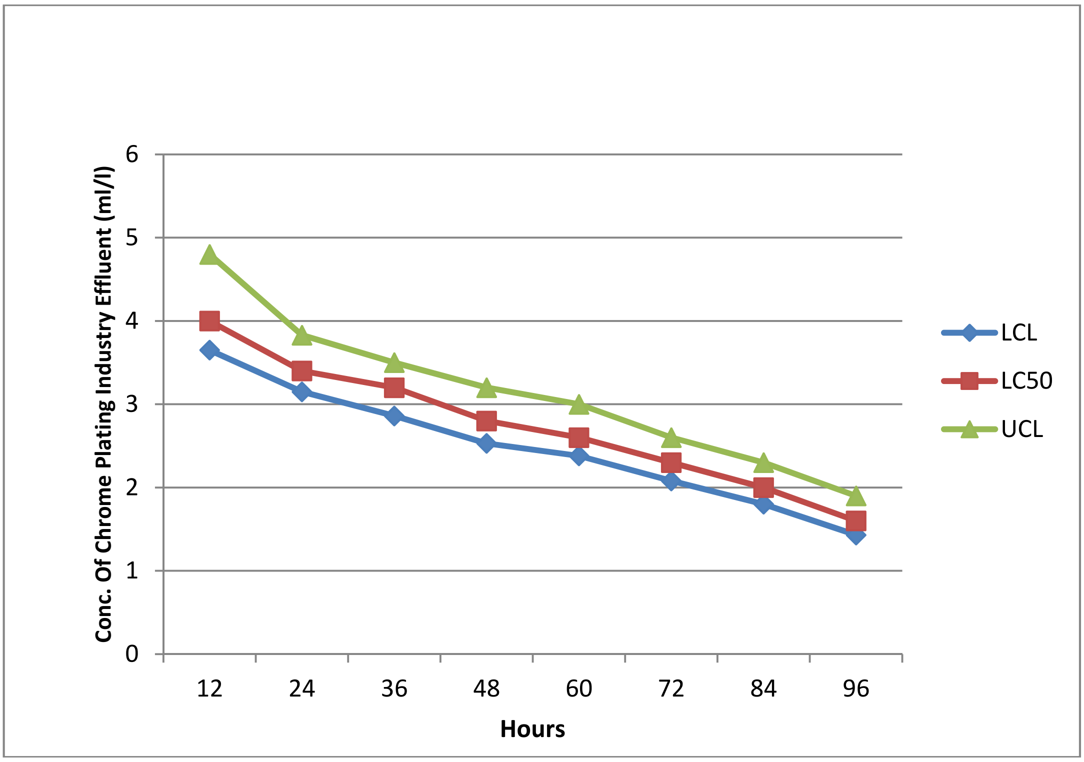

Fig. 1: LC_{50} values and their fiducial limits of the effluent to *P. reticulate*

3. Results and discussion

The various physico-chemical characteristics of chrome plating industrial effluent were above the CPCB permissible limits (Table1). pH of the effluent was 1.75. This value was lower than the observations made by other authors who reported the pH range of chrome electroplating effluent as 3.0 (Kaur and Kaur, 2006) [18], and higher than 1.17 to 1.55 (Nirgude *et al*., 2013) [22]. TSS of the effluent sample was 2100 mg/l. This value was lower than the findings of (Singh *et al*., 2014) [33], who reported COD value of 2848 mg/l in tannery effluent. High COD level indicates the high degree of pollution (Joshi and Santani, 2012) [16]. In the present study, chromium content recorded for the chrome plating industrial effluent was 1514.50 mg/l. This level was too high when compared to the observation of Kaur and Kaur (2006) [18] who reported 34 mg/l of chromium content in the nickel-chrome plating effluent which is completely deviated when compared to this present study.

The mortality response of the fish exposed to different concentration of chrome plating industrial effluent was presented in table 2. After 12h of exposure 20 percent mortality was recorded in the concentration of 3ml/lit and all the exposed fish died at 5ml/lit. At 1 ml/ liter 10 percent mortality was recorded after 96h exposure and all the exposed animals were died in 3 ml/lit after 36h exposure.

The results of the mortality analysis of chrome plating industry effluent against *P. reticulata* recorded the 96h LC_{50} value of 1.67 ml/l and it was supported by the observations made by many authors Shuhaimi-Othman *et al.* (2010) [32] reported a LC_{50} value of 37.9 µg/l at 96 hours when the fish was exposed to copper sulphate treated water. Ezeonyejiaku *et al*. (2011) observed a LC_{50} value of 58.84 mg/l for *Oreochromis niloticus* at 96h of exposure treated with copper sulphate solution.

It was also observed that the mortality rate of the fish increased with the increased time of exposure to chrome planting industry effluent. The lethal concentration (LC_{50}) was inversely proportional to duration of exposure (Roopadevi and Somashekar 2012) [30]. The mortality rate of *Cyprinus carpio* remained directly proportional to duration of exposure and concentration of the effluent. Similar observations were made by Gabriel and Okey (2009) [12] in catfish hybrid exposed to textile industrial effluent. This study also demonstrated the necessity to regulate the discharge of effluent containing Cr from domestic and industrial sources into the aquatic systems.

The probit analysis of the mortality response of fish to 24h the $\overline{X}$ value was 1.54 and $\overline{Y}$ was 5.08, the b value was 10.62 µl/lit. The corresponding value recorded for the 48h exposure was 1.47, 5.17 and 8.69 µl/lit. The probit analysis of the mortality response of the fish to 72h exposure the $\overline{X}$ value was 1.39 and $\overline{Y}$ was 5.17 the b value was 8.06 µl/lit. The corresponding value recorded for the 96h exposure was 1.24, 5.15 and 7.44 µl/lit. The 12, 24, 36, 48, 60, 72, 84 and 96h LC_{50} value of CPIE to *P. reticulata* are 4.19, 3.48, 3.20, 2.87, 2.70, 2.36, 2.07 and 1.67% respectively.

Morphological behaviour like erratic swimming increased activity inconsistent jumping were observed in fish exposed to the industrial effluent. These behavioural responses of fish was in response to toxicants present in the sample at different duration of exposure and the prevailing specific environmental conditions as opined by Bobmanuel *et al*., (2006) [6]. Endocrine regulation of testis revealed that, the leydig cells are markedly injured and showed cytoplasmic vacuolization and shrinking of the cells after exposure to industrial effluent against *P. reticulata* (Rokade, 2012) [29].

The freshwater environment being polluted by various pollutants have adverse effects on aquatic organisms. The freshwater organism particularly fishes are more susceptible to these pollutants. The effects of pollutants generally produce and impact on the survival, reproduction or growth due to physiological alternation in the animal. The physical chemical and biological components of the environment play an important role in manifestation of biological response to pollutants. The toxicity of particular pollutant, depend upon many factor such as animal weight (Pickering, 1968) [24] developmental stage (Kamaldeep and Joor, 1975) [17], period of exposure and physico-chemical characters of the water medium (Mcleese, 1974) [20].

4. Conclusions

The chrome plating industry effluent affects the aquatic flora and fauna. If the effluent could be bio-remediated, the damage caused could be minimized.

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