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Acute toxicity of heavy metal, nickel and chromium on fresh water edible fishes

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Abstract
Industrial effluent is the major sources of heavy metal pollution and it is released into fresh waterbodies. *Catla catla* and *Clarias batrachus* were exposed to Nickel and Chromium for 24, 48, 72 and 96 hrs. The median lethal concentration (LC_{50}) of nickel to *Catla catla* and *Clarias batrachus* for 96 h of exposure were 5.77 mg/l and 7.595mg/l, respectively. The median lethal concentration (LC_{50}) of chromium to *Catla catla* and *Clarias batrachus* for 96 h of exposure were 16.468 mg/l and 34.476 mg/l, respectively. The result also revealed that mortality rate depends upon concentrations of heavy metals and duration of exposure. The acute toxicity levels were derived from LC_{50} concentrations of the heavy metals. Both the heavy metals produced lethality at smaller doses. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. All these observations can be considered to monitor the quality of the aquatic ecosystem and the severity of pollution.

Keywords: Probit analysis, LC_{50}, chromium, nickel, *Catla catla, Clarias batrachus*

Introduction

Pollution of aquatic ecosystem with heavy metals has become a serious health concern in recent years. The discharges from the industries constitute biohazard to man and other living organisms in the environment because they contain toxic substances detrimental to health. These metals are introduced into the aquatic ecosystem through various routes such as industrial effluents and wastes, agricultural runoff, domestic garbage dumps and mining activities (Srivastava and Prakash, 2018a) [21]. The introduction of many relatively toxic heavy metal cations in small amounts into an aquatic environment causes various changes in the internal dynamic of aquatic organism, even at sublethal levels (Srivastava and Prakash, 2018b; Verma and Prakash, 2020) [22, 28]. Under certain environmental conditions like the metals can accumulate to toxic concentrations and cause ecological damage (Srivastava and Prakash, 2018c) [23]. Heavy metal contamination severely interfere with ecological balances of an ecosystem and produces devastating effects on environment quality anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation (Prakash and Verma, 2019a; Verma and Prakash, 2019a) [12, 26]. Thus the heavy metal in the aquatic environments has been as a potential threat to the aquatic organisms including fishes. Metals are known to inhibit the several biochemical and physiological mechanism vital for fish metabolism. Among the heavy metals, cadmium, lead, mercury, copper, zinc, chromium and nickel are comparatively notorious toxicants and most of their compounds are water soluble and non-degradable (Bose *et al.*, 2013) [4]. Increased discharge of heavy these metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high concentrations of metals. These excess amounts in addition to naturally occurring levels gradually build up to toxic levels causing damage to the biota of the aquatic ecosystem. The presence and concentration of any metals varies between fish species; depending on age, developmental stage and other physiological factors.

Toxicity tests are experiments designed to predict the concentrations of toxicant and its duration of exposure required to produce an effect. Toxicity is species-specific because individuals have different levels of response to the same dose of a toxic substance (Smith and Stratton, 1986). The toxicity bioassays are used to detect and to calculate the potential toxicological effects of chemicals on organisms. These tests provide a data base that can be used to assess the risk associated with a situation in which the organisms live. A variety of methods have been developed to evaluate the hazard and potential toxicity of chemicals to organisms, such as acute toxicity test, sub-acute toxicity test or chronic toxicity test.

LC_{50} is the estimation of the dose or concentration necessary to kill 50% of a large population of the test species. Experimentally, this is done by administrating a toxicant at different concentration to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48 72 and 96 hr. The acute toxicity data are important and beneficial in the fixation of sub lethal concentrations for chronic toxicity tests. A lot of works have been done regarding effects of cadmium, zinc and arsenic on fishes (Srivatava and Prakash, 2019; Kumar *et al.*, 2019; Prakash and Verma, 2019b, 2020a, 2020b, 2020c; Verma and Prakash, 2019b) [24, 10, 13, 14, 15, 16, 27].

Probit analysis is a type of regression used to analyze binomial response variables. Probit analysis is commonly used in toxicology to determine the relative toxicity of toxicant or pollutant to living organism (Singh and Zahra, 2017) [19]. This is done by testing the response of an animal at different concentrations of toxicant and then comparing the concentrations at which a response occurs. Probit method is widely accepted and most accurate method for calculating LC_{50}. Therefore the present investigation aimed to evaluate the acute toxicity bioassay of heavy metals, Nickel and Chromium against water fishes, *Catla catla* and *Clarias batrachus*.

Materials and Methods

Indian major carp, *Catla catla* fingerling (average length 7.0-8.0 cm and average weight 5.5-6.2 g) and fresh water catfish, *Clarias batrachus* (average length 6-8 cm and average weight 8-10 g) were collected from local fish form and dip in 0.1% of potassium permanganate solution for 2 minute. The fishes were acclimatized in laboratory conditions for 15 days in a glass aquarium filled with dechlorinated water. During acclimatization the fishes were fed with commercial diet, egg albumin and small insects. The feeding of Fishes were stopped before experiment. The mortality was recorded after a period of 24, 48, 72 & 96 h and dead fishes were removed immediately after death during observation. Stock solution of both heavy metals 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 LC_{50} test, separate 10-12 concentrations both heavy metals were taken to find out the narrow range of concentrations.

Stock solutions of Nickel sulphate and Potassium dichromate of various concentrations were prepared by dissolving analytical grade nickel sulphate ($NiSO_4 6H_2O$ from E. Merck) and Potassium dichromate ($K_2Cr_2O_7 \cdot 7H_2O$ from E. Merck) respectively in double distilled water and 10-10 individuals of both experimental fishes were kept separately in each rectangular glass aquaria to estimate mortality between 0% and 100%. For 96h LC_{50} test, separate 10-12 concentrations both heavy metals were taken to find out the narrow range of concentrations.

Acute Toxicity test was conducted in accordance with standard methods (APHA, 2005) [2]. Probit analysis was carried out as suggested by Finney (1971) [6]. Regression lines of probit logarithmic transformations of concentrations were made and confidential limits (Upper and Lower) of the regression line with Chi-Square test were calculated by SPSS software.

Result and Discussion

The percent mortality observed for each dose was calculated and converted to probits by means of a SPSS software. Static bioassay tests, being an important tool to assess the toxic potential of various toxicants and sensitivity of various organisms at different trophic level in ecosystem has been widely used for evaluation of toxicity (APHA 2005) [2]. Acute toxicity test *i.e.* LC_{50} values show susceptibility of fish to particular toxicant and reflect their survival potential.

In the present study, the percent mortality, and probit mortality of heavy metal, Nickel for fishes, *Catla catla* and *Clarias batrachus* at and 96 hrs are presented in table 1 and for Chromium in table 2. However, fishes exposed to dechlorinated tap-water were observed to be healthy and normal. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional.

Results show that the median lethal concentration (LC_{50}) of nickel to *Catla catla* for 24, 48, 72 and 96 h of exposure are 10.113 mg/l, 9.065 mg/l, 7.386 mg/l and 5.77 mg/l, respectively and to *Clarias batrachus* for 24, 48, 72 and 96 h of exposure are 11.662 mg/l, 10.572 mg/l, 8.944 mg/l and 7.595mg/l, respectively (Table 1). Abdulla and Javad (2006) reported the 96h LC_{50} of Nickel for 30 days *Catla catla* fingerling was 11.83 mg/l.

Table 1: LC_{50} (With 95% confidence limit) of *Catla catla* and *Clarias batrachus* for Nickel

Exposure Period (Hours)	LC_{50} mg/l	Confidence Limit		Regression Equation	Chi Square value	Coefficient of determination (R^2 Linear)
		Lower Limit	Upper Limit			
Catla catla						
24	10.113	8.358	12.145	Y= -3.48+3.40X	3.514	0.950
48	9.065	7.531	10.753	Y= -3.84+3.95X	2.846	0.956
72	7.386	5.669	9.281	Y= -2.40+2.75X	5.077	0.876
96	5.777	4.307	7.232	Y= -2.40+3.16X	2.570	0.944
Clarias batrachus						
24	11.662	10.131	13.221	Y= -5.58+5.23X	2.957	0.938
48	10.572	8.831	12.684	Y= -3.59+3.42X	5.396	0.876
72	8.944	6.790	12.287	Y= -2.32+2.49X	4.379	0.817
96	7.595	5.833	9.606	Y= -2.37+2.66X	5.844	0.849

Results show that the median lethal concentration (LC_{50}) of chromium to *Catla catla* for 24, 48, 72 and 96 h of exposure are 22.173 mg/l, 20.323 mg/l, 18.111 mg/l and 16.468 mg/l, respectively and to *Clarias batrachus* for 24, 48, 72 and 96 h of exposure are 42.089 mg/l, 38.757 mg/l, 36.034 mg/l and 34.476 mg/l, respectively (Table 2). Johnson and Radhakrishnan (2015) reported the 24, 48, 72 and 96 hours LC_{50} of Chromium for *Clarias batrachus* were 40.56 ppm, 38.15 ppm, 36.65 ppm and 35.50 ppm, respectively.

Table 2: LC_{50} (With 95% confidence limit) of *Catla catla* and *Clarias batrachus* for Chromium

Exposure Period (Hours)	LC_{50} mg/l	Confidence Limit		Regression Equation	Chi Square value	Coefficient of determination (R^2 Linear)
		Lower Limit	Upper Limit			
Catla catla						
24	22.173	20.839	23.478	Y= -17.6+13.08X	0.917	0.994
48	20.323	18.973	21.662	Y= -15.28+11.68X	1.120	0.990
72	18.111	16.767	19.418	Y= -13.51+10.73X	0.853	0.996
96	16.468	15.149	17.766	Y= -12.34+10.14X	0.942	0.988
Clarias batrachus						
24	42.089	40.187	44.043	Y= -27.71+17.04X	1.356	0.990
48	38.757	36.938	40.587	Y= -29.11+18.32X	0.829	0.993
72	36.034	34.251	37.835	Y= -27.58+17.71X	0.861	0.986
96	34.476	32.645	36.483	Y= -22.09+14.32X	2.748	0.923

Observations on the upper and lower confidence limits revealed a decreasing trend from 24h to 96h. From the fitted regression equation, it is evident that an increase in exposure period influences an increase in mortality (Table 1 & 2).

The toxicity tolerance of freshwater fishes, *Catla catla* and *Clarias batrachus* to Nickel and Chromium in the present study are 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. The result of the present study revealed that fish *Catla catla* was more sensitive than *Clarias batrachus* for both heavy metals. The calculated Chi-Square value indicated a good fit on the regression line. Therefore the acute toxicity test clearly confirmed the potential hazardous effects of chromium and nickel to the experimental fishes, *Catla catla* and *Clarias batrachus.*

Acute toxicity of fingerlings ranged in between 1.133 to 1.220 µg Hg/l for *Catla catla*; 1.051 to 1.06 µg Hg/l for *Labeo rohita* and 1.091 to 1.183µg Hg/l for *Cirrhinus mrigala* (Ashwani and Ashok, 2006) [3]. The 96h LC_{50} of $CdCl_2$ for fish, *Scorpaena guttata* which is 25 mg/l (Brown *et al*., 1984) [5], 30.4 mg/l for fish, *Poecili reticulate* (Mehnet Yilmaz *et al*., 2004) [11] and 8.13 ppm (Selvanathan *et al*., 2011) [18]. As well as 35.0 mg/l (Gandhewar *et al*., 2014) [7] for fish, *Clarias batrachus* as well as 96h LC_{50} of $CuSO_4$ for fish, *Clarias batrachus* which is 15 mg/l (Gandhewar *et al*., 2014) [7]. The median lethal concentration (LC_{50}) of chromium to *C. batrachus* for 24, 48, 72 and 96 h of exposure were 120 ppm, 115 ppm, 110 ppm and 102 ppm respectively (Rani *et al*., 2011) [17].

In the present study acute toxicity of nickel was significantly higher when compared to chromium after 96 h of exposure of *Catla catla* and *Clarias batrachus*. It was also observed that the fish, *Catla catla* was more sensitive than *Clarias batrachus* for both heavy metals. It is therefore biologically reactive and gives rise to acute poisoning. This difference could be due to the biological diversity and functional variability of cells and tissues to chemical pollutants. Toxicity of metals may vary depending upon their permeability and detoxification mechanisms (Rani *et al*., 2011) [17].

Heavy metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem and this is attributed to bioaccumulation, whereby metal ions are taken up from the environment by the organism and accumulated in various organs and tissues (Rani *et al*., 2011) [17]. Metals also become increasingly concentrated at higher trophic levels, possibly due to food-chain magnification (Wyn *et al*., 2007) [29]. However, sublethal concentrations of heavy metals also induce substantial changes in the biological organization of fish (Srivastava and Prakash, 2018a) [21]. Stebbing and Fandino (1983) [25] reported that, the adverse biological effects of heavy metals in the aquatic environment are mainly due to their complex nature. When the toxicant concentration in the water body is very high, it results in the death of fish. So, the death of an organism was taken as the end point of toxicological studies previously (Jones and Reynolds 1997) [9]. The behaviour of both fish remarkably changed due to the treatment of nickel and chromium when compared to the control. The behavioural responses showed by both fish due to sublethal concentrations of nickel and chromium during initial stage of exposure included restlessness, erratic and fast swimming, abrupt change in position and direction, jumping and overall hyperactivity were noticed. The fishes showed surfacing tendency throughout the experimental period. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. Neurological symptoms like jerking movements, frightening and loss of balance were not observed in nickel and chromium treated *Catla catla* and *Clarias batrachus*.

Conclusion

In the present investigation *Catla catla* and *Clarias batrachus* were highly sensitive to nickel and chromium toxicity along with behavioral changes these heavy metals are capable of causing all forms physiological changes. Thus it can be concluded that heavy metals are relatively toxic, even at fairly low concentrations and affect the survival of aquatic organisms. The death of fish could be due to the lethal action of toxicants that causes alterations in physiological and biochemical process related to cellular metabolic pathway. So acute toxicity test and behavioural responses can be considered to monitor the quality of the aquatic ecosystem and the severity of pollution.

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