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ORIGINAL ARTICLE

Acute Toxicity Bioassay and Determination of LC_{50} of Cadmium Chloride in *Trichogaster (Colisa) fasciata*

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Abstract
Cadmium chloride is a metal salt used in industries in a large scale. *Trichogaster (Colisa) fasciata* (common name banded gourami) is an air-breathing fresh water fish with both nutritional and ornamental values. In this present study, as per EPA guidelines, acute toxicity bioassay was performed in a 96-h static test method in four replicates, each replicate having one control and five concentrations (25, 50, 75, 100 and 125 mg/L, respectively) of the metal salt. A total of 192 fish was used in the study in four series with each replicate containing 8 fish per concentration. The water temperature was maintained at 20–22°C during the study. The data observed were analysed statistically on the basis of Finney's Probit Analysis method using SPSS software. The estimated 96-h LC_{50} value of cadmium chloride for the fish was 49.5 mg/L.

Keywords Acute toxicity · Static bioassay · LC_{50} · Cadmium chloride · *Trichogaster fasciata*

Introduction

Industrial revolution in India along with its multiple beneficial aspects has one detrimental effect on the environment, as heavy metals, which are toxic and used in industries in large scale, are increasingly being discharged into environment, especially into aquatic ecosystems. A diverse array of heavy metals of toxic category like cadmium, copper, lead, zinc and mercury from the industrial waste waters are reported to be directly discharged into the aquatic bodies and found to be harmful for the aquatic biota like fish and others living there [1–3]. Of all the heavy metals used in industries and discharged into environment, cadmium (Cd) is found in maximum concentrations and has emerged as a major concern worldwide because of its deleterious effects on animal health [4].

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Environmental Protection Agency has listed cadmium as one of those 129 toxicants of priority [5]. Silver-cadmium and nickel–cadmium are the prime sources of this heavy metal used predominantly in battery industries, sewage-sludge and mining units [3]. As cadmium is not bio-degradable, therefore, after being released into water, it bio-accumulates in the tissues of different aquatic animals, like mussels, oysters and fish as globally reported from various researches on ecotoxicology [6, 7]. Researchers from all across the world have unanimously argued for the necessity of a constant monitoring of this bioaccumulation of cadmium in aquatic biota, most importantly in fish, as fish is a major component of human diet globally [8].

Cadmium as an element is not essential to organisms, as it has no significant biological role [9, 10]. Natural concentration of cadmium in water is very low [11]. Its accumulation in water is mainly due to anthropogenic activities and even smallest concentrations of cadmium accumulated in water exert adverse effects on aquatic animals [12]. Accumulation of cadmium (Cd) was reported in various fish tissues, predominantly in kidney, liver, and gills of fresh water fish [13], with reports of their accumulation even in heart [14] and other tissues [15]. Cadmium was found to effect severe pathological alterations in above mentioned tissues [16] and thus was reported as cytotoxic, hemotoxic, genotoxic, carcinogenic, nephrotoxic and immunotoxic pollutant [17]. Cadmium-exposed fishes had shown damaged renal tubules and occurrence of kidney stone, decremented phagocytic activity by macrophages and cytotoxicity by natural killer cells, reduced haemoglobin concentration and erythropoiesis [18, 19].

Trichogaster (Colisa) fasciata (Bloch and Schneider, 1801) [20] (common name banded gourami) is a fresh water fish with accessory respiratory organs in the form of paired supra-branchial chambers. It has an elongated, compressed body with small and protrusible mouth [21]. Body is green in colour having prominent oblique blue or orange bars across the body [22]. It prefers bentho-pelagic weedy waters like ponds, lakes, rivers and even paddy fields [23]. This species has good market demand both as a table fish for its delicious taste [24, 25] and as an ornamental fish for its prominent colour [26, 27]. It is a hardy species due to having accessory respiratory organs and can be bred easily in captivity also [25]. For its least concern status according to IUCN red list [28], easy availability, shorter generation time and ability to breed in captive conditions, it is considered an effective model organism in toxicological researches [29–31].

In ecotoxicology, acute toxicity tests are commonly performed to evaluate the deleterious effects of a toxicant on an aquatic organism [32, 33]. Such tests allow the ecotoxicologists to estimate a dose response relationship between concentrations of a particular toxicant and the extent of their respective harmful effects on the aquatic organism chosen in the tests. Median lethal concentration or LC_{50} is the concentration of a toxicant, given at once, that causes mortality of 50% of a population of test organisms. LC_{50} is a standard approach to evaluate the short-term (acute) harmful toxicity of the toxicant. Until date, information on LC_{50} evaluation of cadmium chloride in *Trichogaster (Colisa) fasciata* is not available as such. The aim of this study was to estimate and evaluate the LC_{50} of cadmium chloride in this fish species.

Materials and Methods

This present study was performed in the laboratory of the PG Department of Zoology, Vidyasagar College, University of Calcutta. Healthy specimens of fresh water fish *Trichogaster (Colisa) fasciata* (Bloch and Schneider, 1801) (common name banded gourami), collected from Subhas Sarobar, Beleghata, Kolkata, of almost the same size (length 65 ± 12 mm, weight

7.4 ± 3.3 g), were used for the experiment. In the laboratory, the fish were bathed twice in 0.05% solution of potassium permanganate for 2 min as a prophylactic measure to check dermal infections. After permanganate bath, they were released immediately in a cement tank of 500-L capacity containing dechlorinated tap water and kept there for a duration of 15 days for acclimation following the method of Singh and Manjeet [34]. Fish were fed on Organic Valueman Aqua Fish Feed Floating Pellets produced by Valueman Organic Agritech Private Limited twice daily with complete renewal of water at 24-h intervals. An air compressor with air stones was used for oxygenation of water. Periodic recordings of parameters of quality of water were done. After 15 days' acclimation in the cement tank, healthy fish were selected for the experiment [34]. The lengths and weights of the selected fish were recorded. Periodic determination of several parameters of water quality like dissolved O_2 (DO_2), temperature, pH and salinity was done both before and during the experiments using standard protocols [35]. Dissolved O_2 was on average 8.8 mg/L, and proper aeration of the experimental media was done so that the oxygen level never drops below 4 mg/L. Temperature of test water was maintained between 20 and 22℃ with an average ~ 21℃. The pH of test water was 8.2 on average. On the first day of experiment, the average salinity value was 0.162%, and it increased up to 0.275% on final day of experiment.

Estimation of LC_{50}

Cadmium chloride ($CdCl_2$, H_2O, Merck) was mixed in experimental tap water to be used as toxicant in this static bioassay. The selected healthy fish as test organisms were distributed randomly in 50-L aquaria at the rate of 8 fish per aquarium. On the basis of various literatures studied on similar experiments of cadmium chloride in other fish species [5, 36], a range-finding pilot study was conducted using ten different concentrations of cadmium chloride (viz. 6.25, 12.5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/L, respectively). On the basis of the mortality of fish observed in the pilot study, five different concentrations of cadmium chloride, viz. 25, 50, 75, 100 and 125 mg/L, were chosen for the final trial and were added in five separate aquaria containing 8 test organisms in each. The mortality of fish in each aquarium was recorded after 24, 48, 72 and 96 h. The entire experiment was conducted in four replicates. Simultaneously an aquarium of control group of eight fish, which contained only the experimental water but without the toxicant, was used along with the exposure groups with all other parameters kept same. The duration of the experiment was 96 h, during which the numbers of dead fish were recorded at an interval of 24 h, and dead fish were taken out immediately from the aquaria, if any. The obtained data were statistically analysed to observe whether there is any influence of different treatments (concentrations) on the mortality of fish. Statistical software SPSS version 20 was used at $P < 0.05$ to analyse the data. The lethal concentration (LC) values and their 95% confidence limits (both lower and upper) for different exposure times were calculated using Finney's Probit Analysis method [37]. The same software was used to obtain probit regression line against log of concentration of the toxicant.

Results

The relationships between the various concentrations of cadmium chloride ($CdCl_2$) and the consequent rates of mortality of *Trichogaster (Colisa) fasciata* at various time intervals are shown in Table 1. Control group in each replicate showed zero mortality of fish, which

Table 1 Data of mortality rates of *Trichogaster (Colisa) fasciata* at various concentrations of cadmium chloride

Concentration (mg/L)	Number of fish exposed	Number of mortality				Percentage mortality at 96 h
		24 h	**48 h**	**72 h**	**96 h**	
Control (0 mg/L)	8	0	0	0	0	0
25 mg/L	8	0	0	1	1	12.5
50 mg/L	8	0	1	3	4	50
75 mg/L	8	1	4	5	5	62.5
100 mg/L	8	4	5	7	8	100
125 mg/L	8	8	8	8	8	100

Table 2 Probit analysis of mortality of *Trichogaster (Colisa) fasciata* at various concentrations of cadmium chloride

	Number	Concentration (mg/L)	Number of subjects	Observed responses	Expected responses	Residual	Probability
Probit	1	25	8	1	0.679	0.321	0.085
	2	50	8	4	4.060	– 0.060	0.508
	3	75	8	5	6.381	– 1.381	0.798
	4	100	8	8	7.367	0.633	0.921
	5	125	8	8	7.748	0.252	0.969

was suitable for upper and lower confidence limits of LC_{50} and was found fit for regression equation.

The probit analysis of the mortality of *Trichogaster (Colisa) fasciata* at various concentrations of cadmium chloride is shown in Table 2. It is evident from the table that observed and expected responses in probit analysis do not deviate much.

LC values of cadmium chloride estimated by statistical software SPSS version 20 and their respective 95% upper and lower confidence limits at 96 h are shown in Table 3. It is observed from the table that the LC_{50} value of cadmium chloride at 96 h for *Trichogaster (Colisa) fasciata* is 49.5 mg/L at 95% confidence limit, and its lower and upper limits are 34.1 mg/L and 63.8 mg/L, respectively.

The software generated regression line between the probit mortality of *Trichogaster (Colisa) fasciata* and the log values of the concentrations of cadmium chloride is shown in Fig. 1. R^2 linear = 0.973 implies almost perfect fitting of data with the predicted regression.

Discussion

The results of the present study show a clear positive correlation between the mortality of *Trichogaster (Colisa) fasciata* and the duration of exposure as well as concentrations of cadmium chloride, as increase in both exposure time and concentration of the toxicant showed increased deaths of the fish species as evident from Table 1 and Table 2.

From Table 3, it becomes clear that 95% confidence limits of estimated lethal concentration values of cadmium chloride at 96 h also show a trend of gradual increase, from LC_{10}

Table 3 Data of estimated lethal concentration values of cadmium chloride at 96 h for *Trichogaster (Colisa) fasciata* with lower and upper (95%) confidence limits

LC values	95% confidence limits for concentration at 96 h		
	Estimate	Lower limit	Upper limit
LC_{10}	26.168	10.329	36.908
LC_{20}	32.576	15.903	43.592
LC_{30}	38.149	21.501	49.621
LC_{40}	43.662	27.508	56.062
LC_{50}	49.532	34.102	63.811
LC_{60}	56.191	41.370	74.225
LC_{70}	64.310	49.407	89.835
LC_{80}	75.313	58.705	116.361

values of 26.1 mg/L, to LC_{50} values of 49.5 mg/L, to LC_{80} values of 75.3 mg/L, indicating that more concentrations of a toxicant are required to make more individuals of a test population susceptible.

Finally, the linear regression line in the scatter plot of Fig. 1 supports the positive linear correlation observed in probit values of fish mortality and log concentration of toxicant. These observations are in conformation with various workers who performed bioassays of cadmium in other fish species, like the works of Muley et al. (2000) in common carp, *Cyprinus carpio* [38], Garcia et al. (2006) in Nile tilapia, *Oreochromis niloticus* [39], and Dutta and Kaviraj (2001) in Rohu, *Labeo rohita* [40].

A summary of median lethal concentration (LC_{50}) values of cadmium and some other toxic heavy metals in various fish species as observed in acute toxicity bioassays performed by other workers is presented in Table 4.

In a study by Yilmaz et al. in 2004, LC_{50} value of cadmium chloride in guppy, *Poecilia reticulata*, was 30.4 mg/L [41]. LC_{50} values of mercury, cadmium and lead in tench, *Tinca tinca* were 1 mg/L, 6.5 mg/L and 300 mg/L, respectively, as reported by Shah and Altindag in 2005 [42]. 4.53 mg/L was the LC_{50} value of cadmium chloride in catla fish, *Catla catla* in the study of Sobha et al. in 2007 [43]. In a similar acute toxicity study by Singh et al. in 2010, LC_{50} value of cadmium chloride in singhi, *Heteropneustes fossilis*, was 50.4 mg/L [5]. Parvin et al. in 2010 reported 191.49 mg/L as LC_{50} value of cadmium chloride and 1.015 mg/L as LC_{50} value of lead chloride in *Anabas testudineus* [44]. In a comparative study by Abedi et al. in 2012, LC_{50} values of cadmium chloride in scaled common carp, *Cyprinus carpio* and scaleless sutchi catfish, *Pangasius hypophthalmus*, were 84.8.4 mg/L and 64.89 mg/L, respectively [36]. The differences in the LC_{50} values of cadmium chloride in these two separate species were attributed by the authors to the presence or absence of scales over the skin. Scales covering the epidermis of common carp or any scaled fish in general serve as protective shield against excess permeability of toxicants resulting in higher LC_{50} values and thereby greater tolerance to the toxicants compared to scaleless sutchi catfish [36]. In *Cyprinus carpio*, the estimated LC_{50} values of mercuric chloride, lead chloride and zinc sulphate were 0.93 mg/L, 58 mg/L and 41.1 mg/L, respectively, in the study of Hedayati et al. in 2013 [45]. Sadeghi and Imanpoor in 2015 reported LC_{50} values of mercuric chloride, lead chloride and zinc sulphate in silver dollar fish, *Metynnis fasciatus* as 0.94 mg/L, 86.84 mg/L and 32.24 mg/L, respectively [46]. Singh and Manjeet in 2015 estimated the LC_{50} value of lead nitrate in *Labeo rohita* as 34.2 mg/L [34]. In *Percocypris pingi*, Yuan et al. in 2017 estimated lower LC_{50} values of 0.081 mg/L and

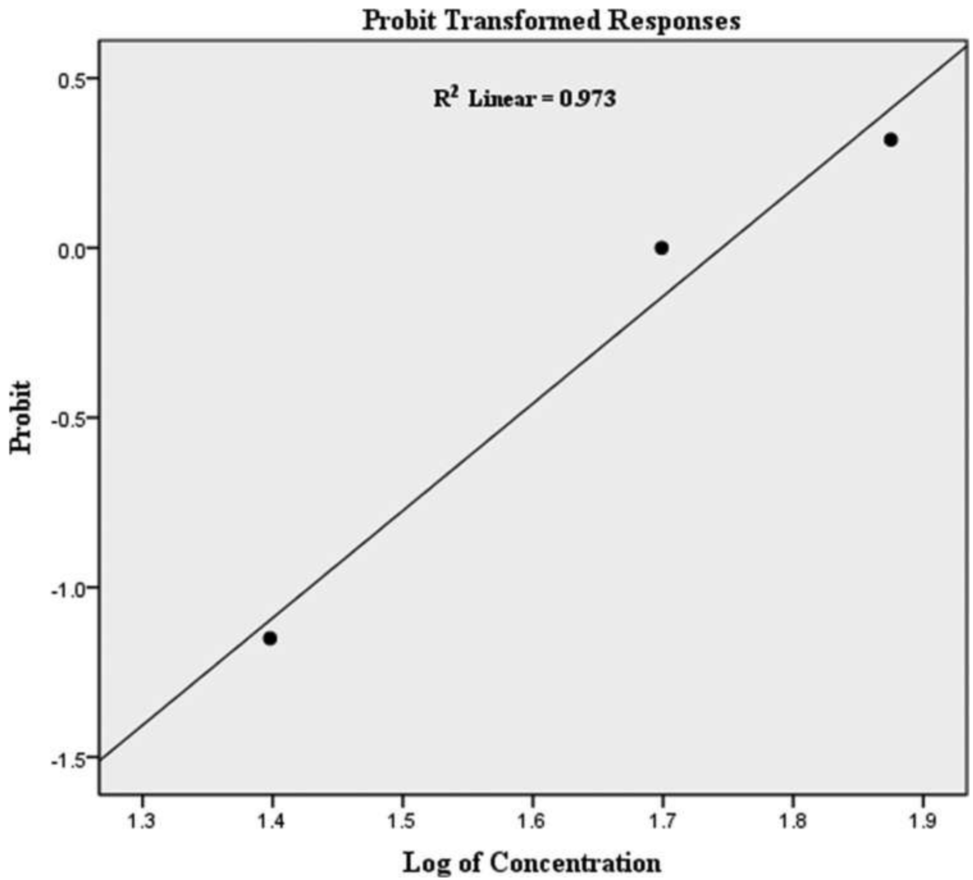

Fig. 1 Regression line between the probit mortality of *Trichogaster (Colisa) fasciata* and the log concentrations of cadmium chloride [R^2 = coefficient of determination]

Table 4 Summary of estimated median lethal concentration (LC_{50}) values of some heavy metals in acute toxicity bioassays of various fish species

Common name	Scientific name	Heavy metal	Duration (hours)	LC_{50} values (mg/L)	References
Common carp	***Cyprinus carpio***	**Cadmium chloride**	96	121.80	**Muley et** al. [38]
Nile tilapia	***Oreochromis niloticus***	**Cadmium chloride**	96	24.66	**Garcia et al.** [39]
Rohu	***Labeo rohita***	**Cadmium chloride**	96	89.50	**Dutta and Kaviraj** [40]
Guppy	***Poecilia reticulata***	**Cadmium chloride**	96	30.40	**Yilmaz et al.** [41]
Tench	***Tinca tinca***	**Cadmium**	96	6.50	**Shah and Altindag** [42]
Tench	***Tinca tinca***	**Mercury**	96	1.00	**Shah and Altindag** [42]
Tench	***Tinca tinca***	**Lead**	96	300.00	**Shah and Altindag** [42]
Catla	***Catla catla***	**Cadmium chloride**	96	4.53	**Sobha et al.** [43]
Singhi	***Heteropneustes fossilis***	**Cadmium chloride**	96	50.40	**Singh et al.** [5]
Climbing perch	***Anabas testudineus***	**Cadmium chloride**	96	191.49	**Parvin et al.** [44]
Climbing perch	***Anabas testudineus***	**Lead chloride**	96	1.02	**Parvin et al.** [44]
Common carp	***Cyprinus carpio***	**Cadmium chloride**	96	84.80	**Abedi et al.** [36]
Sutchi catfish	***Pangasius hypophthalmus***	**Cadmium chloride**	96	64.89	**Abedi et al.** [36]
Common carp	***Cyprinus carpio***	**Mercuric chloride**	96	0.93	**Hedayati et al.** [45]
Common carp	***Cyprinus carpio***	**Lead chloride**	96	58.00	**Hedayati et al.** [45]
Common carp	***Cyprinus carpio***	**Zinc sulphate**	96	41.10	**Hedayati et al.** [45]
Silver dollar	***Metynnis fasciatus***	**Mercuric chloride**	96	0.94	**Sadeghi and Imanpoor** [46]
Silver dollar	***Metynnis fasciatus***	**Lead chloride**	96	86.84	**Sadeghi and Imanpoor** [46]
Silver dollar	***Metynnis fasciatus***	**Zinc sulphate**	96	32.24	**Sadeghi and Imanpoor** [46]
Rohu	***Labeo rohita***	**Lead nitrate**	96	34.20	**Singh and Manjeet** [34]
Perch carp	***Percocypris pingi***	**Cadmium chloride**	96	0.08	**Yuan et al.** [47]
Perch carp	***Percocypris pingi***	**Mercuric chloride**	96	0.33	**Yuan et al.** [47]
Banded gourami	***Trichogaster (Colisa) fasciata***	**Cadmium chloride**	96	49.5	**This study**

0.327 mg/L for cadmium chloride and mercuric chloride, respectively [47]. Variations in the results of LC_{50} values of different heavy metals in all these studies support the fact that lethality of fish under toxic stress depends mostly on the species concerned and the heavy metal involved along with both concentration of the toxicant as well as exposure time [48], as evident in the present study also. Moreover, various other factors like salinity, pH and temperature of water, size, age and feeding habits of fish also influence LC_{50} values [43, 49]. Shah and Altindag [42] studied the effect of heavy metal on 96-h LC_{50} values in tench, *Tinca tinca*, and reported that temperature of water plays a significant role in the valuation of LC_{50}, as increase in water temperature decreases the dissolved oxygen and consequently decreases the LC_{50} value in fish [50]. Hence, maintenance of fairly constant water quality parameters like temperature, pH, salinity and selection of fish of almost uniform size and age is crucial in such acute toxicity bioassays as done in this present study. Tripathi et al. [51] studied the toxic effects of cadmium sulphate on the biochemical parameters of *Colisa fasciatus* and reported cadmium-induced significant alterations in total protein, glycogen, DNA and RNA and body weights in the reproductive cycles of the fish. More investigations of cadmium toxicity bioassays can further highlight the impact of toxic stress imposed by cadmium in the physiology of this species. As summarised in Table 4, median lethal concentration (LC_{50}) values revealed from acute toxicity bioassays in various fish species can range from 0.08 [47] to 191.49 [44] for cadmium chloride, which is in good agreement with the result of this present study. Furthermore, as evident in Table 4, the LC_{50} values of lead chloride in various fish species can range from 1.02 [44] to 300.00 [42]. Comparing these observations, cadmium can be preliminarily considered more toxic than lead in fish. More comparative investigations of acute toxicity in fish involving the two metals are required to affirm this view. However, development and use of such acute toxicity bioassays are very much essential to generate data of toxic responses of different fish species to a common heavy metal as well as a common fish species to different heavy metals, which could be analysed and used further in predictive toxicology and risk assessment [36].

Finally, comparison of the above observations with the present study further supports the report of Canadian Environmental Protect Act, 1994, suggesting species-specific variations in cadmium toxicity in fish.

Conclusion

From the present study, it can be concluded that aquatic organisms like fish can become susceptible to increasing concentrations of toxic heavy metals like cadmium in water. Accumulation of toxic heavy metals in the tissues not only affects their physiological activities, but also poses the threat of biomagnification through food chain in organisms of higher trophic levels. This study further allows us to validate a permissible level of the toxicant in aquatic ecosystem for the chosen fish species. Similar tests of acute toxicity of other toxic metals can give the measure of their tolerable limits in water for the aquatic organisms like fish to survive in it. Finally, such studies can help us to analyse the extent of damage to aquatic ecosystem due to heavy metal contamination and also help in implementation of proper policies to protect the ecosystem from it.

Author Contribution The authors Saikat Roy, Debasish Karmakar and Sarmila Pal designed the experimental works of the study, performed the statistical analysis and wrote the draft manuscript in collaboration. All authors read and approved the final manuscript.

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Data Availability The fish samples were collected from Subhas Sarobar, Beleghata, Kolkata. The data were collected from experiments performed by the authors in the laboratory.

Declarations

Ethical Approval Test organism for the study is fresh water fish *Trichogaster (Colisa) fasciata* (Bloch and Schneider, 1801) (common name banded gourami). It is included in the risk group – Aquatic Organism Biosafety Level 1 (AqBSL-1), i.e., uninfected and does not pose health threat to humans. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Consent to Participate Not applicable.

Consent for Publication The authors hereby give full consent for publication of the data.

Conflict of Interest The authors declare no competing interests.

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