E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2024; 12(1): 63-66

www.fisheriesjournal.com
Received: 25-12-2023
Accepted: 30-01-2024

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Sub-lethal toxic effect of cadmium chloride ($CdCl_2$) on freshwater murrel *Channa punctata* (BLOCH)

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DOI: https://doi.org/10.22271/fish.2024.v12.i1a.2893

Abstract
The purpose of this study was to look into the effects of cadmium chloride ($CdCl_2$) on freshwater murrel *Channa punctata* (Bloch). The impacts in fishes include changes in morphological behaviour i.e., changes in opercular activity, hypoxic condition and colour changes in gills, profuse mucus secretion, alteration of protein levels in gill and liver tissues when exposed to sub-lethal doses of $CdCl_2$ (at 5 ppm and 10 ppm) after the estimation of protein content of these tissues. When exposed to 5 ppm dose, the protein level in gill was found to be 70.3±0.25 mg protein/gram of tissue and at 10 ppm it was 65.3±0.30 mg protein/gram of tissue. Similarly the protein content of liver tissue at 5 ppm and 10 ppm doses was found to be 79.3±0.20 and 71.3±0.25 mg protein/gram of tissue respectively. These values showed deviation from the Control which were at 82.5±0.20 89.7±0.15 mg protein/gram of tissue respectively for the gill and liver tissues.

Keywords: Heavy metal toxicity, Cadmium Chloride ($CdCl_2$), *Channa punctata* (Bloch)

Introduction
Toxicity is defined as the extent to which any substance can harm an organism. The effect can be referred to a whole organism, which would be any animal, plants or bacterium even the effect on a substance of the organism ranging from cytotoxity to hepatotoxicity. The ever increasing population and the rapid industrialization has led to tremendous rise in pollution level of water bodies which are linked with disposal from different agricultural and industrial sources. The toxicants which are water soluble that originates from municipal and industrial wastes reaches soil and gets transferred to water bodies. Aquatic toxicity is a very serious and fatal environmental problem. Aquatic toxicity is caused by variety of materials such as industrial pollutants, heavy metal pollution etc., which are very much toxic to aquatic organisms. Fishes serve as an essential bio-indicator in the aquatic eco-system for determining the presence of heavy metals. They offer various special advantages in characterizing the natural properties of an aquatic environment and gaining access to changes in habitat. Fishes are vulnerable to accumulation of heavy metals in them as they are located at the highest trophic level in the aquatic ecosystem. Research conducted in both laboratory and field settings has demonstrated that the concentration of metals in water and the length of exposure are the primary determinants of the accumulation of heavy metals in a tissue. However, other environmental factors, including pH, TDS, oxygen concentration, temperature, and other biochemical parameters, are also important in the bioaccumulation of heavy metals in fish. Pollutants are accumulated more readily by fish in their fatty tissues, such as the liver, and the consequences become noticeable when the concentration in these tissues reaches a certain point. Fish are among the aquatic animal species that are unable to flee the harmful consequences of pollution. Research conducted on several kinds of fish has shown that heavy metals can change the biochemical characteristics in blood and tissues. Acute toxicity in freshwater fishes resulting from various toxicants can be assessed using quantitative metrics such as test specimen survival and mortality as well as sensitivity to metal toxicity. Species with usually varying susceptibilities or metabolic activities, particularly those that are readily available and common in the area where the toxicant may occur, should be used to gain information on the pollutant toxicity range.

In order to determine which species yield data appropriate for assessing the eco-toxicity of the pollutant under investigation, comparative toxicity studies ought to be created.

The Heavy metal cadmium (Cd) is extremely hazardous and non-essential and therefore considered as one of the major environmental pollutants and produces toxic effects in living organisms. Aquatic environments have been contaminated as a result of the significant growth in cadmium production, use, and emissions to the environment over the 20th century due to its industrial application in batteries, electro plating, plastic stabilizers, and pigment. It has been demonstrated that this heavy metal primarily builds up in the kidney, liver, and gills of freshwater fish, but it can also deposit in other tissues, including the heart, and induce clinical and anatomical changes of differing degrees of severity.

Effects of cadmium in fish

Oxidative stress, which is caused by an increase in Reactive Oxygen Species (ROS) levels like Super oxide, hydrogen peroxide, and hydroxyl radicals, is one of the main effects of Cd toxicity. This damages proteins, lipids and DNA. There is evidence of morphological and histological changes in the livers of fish exposed to cadmium. Cadmium and other heavy metal pollution in freshwater reservoirs has received attention for a long time.

Experimental Organism

Taxonomic position of *Channa punctata* (Bloch)

Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Channidae
Genus: *Channa*
Species: *punctata*

Fig 1: Channa punctata

Materials and Methods

Collection and maintenance of test fish

Channa punctata were collected from culture pond near Serispore T.E., Hailakandi, Assam, India and brought to the laboratory in aerated containers. In the laboratory, the fishes were maintained in an aquarium with constant aeration. Fishes were fed with commercially available fish meal twice daily and were acclimatized in the aquarium for a week before the experiment.

Experimental design of fish bio-assay

For test setup, *Channa punctata* were kept in a tank (Group-I) with heavy metal ($CdCl_2$) for seven days. A control with no treatment was also run simultaneously in another tank. After seventh day, the protein content of liver was examined.

Experimental Design

The fishes were randomly divided into three groups *viz.*, control, low dose, sub-lethal-1 (SL-I, 5ppm) and high dose sub-lethal 2(SL-II, 10ppm). They were kept in three different buckets containing 5 litres of water obtained from a pond and were labelled as control, SL-1(5ppm) and SL-2 (10ppm). The experiment was run for seven days. Fishes were taken out from all the tanks after seven days and were anesthetized and the total length and body weight were recorded. Test fishes were sacrificed and tissues like gills and liver were dissected and stored at refrigerator until further analysis.

Procedure for estimation of total protein content

Estimation of protein content

Total protein content was estimated by the protocol developed by Lowry *et al.* (1951).

Reagents used

A. Alkaline copper Reagent.

Reagent A: 2 % Na_2CO_3 in 0.1 N NaOH.
Reagent B: 0.5% $CuSO_4.5H_2O$ in sodium potassium tartarate
The alkaline copper reagent was made by mixing 50 ml of reagent A and 1 ml of reagent B.

B. Folin Ciocalteu phenol reagent (It is commercially available which is diluted with distilled water in the ratio of 1:2
C. 0.1N NaOH
D. 70% alcohol
E. Protein (stock) Standard solution: 100mg % Bovine Serum Albumin in 0.1 N NaOH.

Working standard

10 ml of the stock was diluted to 100ml with distilled water.

The protocol

1 ml of 70% alcohol was added to the test tube after 0.2 ml of tissue homogenate was pipetted out. For ten minutes, the tubes were centrifuged at 5,000 rpm. After discarding the supernatant, 1 ml of 0.1N NaOH was used to dissolve the precipitate. 5 ml of Alkaline Copper Reagent was added, and it was left at room temperature for ten minutes. The Folin-Ciocalteu phenol reagent was added after ten minutes, and it was left in the dark for thirty minutes. The UV-Vis Spectrophotometer was used to measure the absorbance at 620 nm against a blank for the reagent. Simultaneously, a series of graded protein standard volumes were run. The data are given as milligrams of protein per gram of tissue weight that is moist.

Table 1: Morphological and behavioural changes in fishes when exposed to doses (5 ppm and 10 ppm) of $CdCl_2$ at different exposure time

Exposure Time	Dose	Colour Changes	Opercular activity	Bottom to surface movement	Resting at Bottom	Mucous Secretion	Loss of Equilibrium
Day 1	Control						
	SL-I	=	*	+	=	+	+
	SL-II	*	*	+	=	+	*
Day 2	Control						
	SL-I	+	=	=	*	+	=
	SL-II	=	=	*	*	+	*
Day 3	Control						
	SL-I	+	*	=	=	+	=

	SL-II	=	*	*	=	+	*
Day 4	Control						
	SL-I	+	*	=	*	+	*
	SL-II	=	*	=	=	+	*
Day 5	Control						
	SL-I	+	=	=	*	*	=
	SL-II	=	+	*	+	=	*
Day 6	Control						
	SL-I	+	*	=	+	=	*
	SL-II	+	=	+	=	=	+
Day 7	Control						
	SL-I	*	=	+	+	=	=
	SL-II	+	=	*	+	*	+

SL-I = 5 ppm; SL-II = 10 ppm
"=" indicates no change or no increase/decrease
"+" indicates occurrence of changes or increase in change
"*" indicates occurrence of changes or decrease in change

Table 2: Protein Content of the tissues (gill and liver) of *C. punctata* at different concentrations of $CdCl_2$ after 7 days of exposure

Treatments	Duration	Gill	Liver
5 ppm $CdCl_2$ (mg/L)	7 Days	70.3±0.25	79.3±0.20
10 ppm $CdCl_2$ (mg/L)	7 Days	65.3±0.30	71.3±0.25
Control	7 Days	82.5±0.20	89.7±0.15

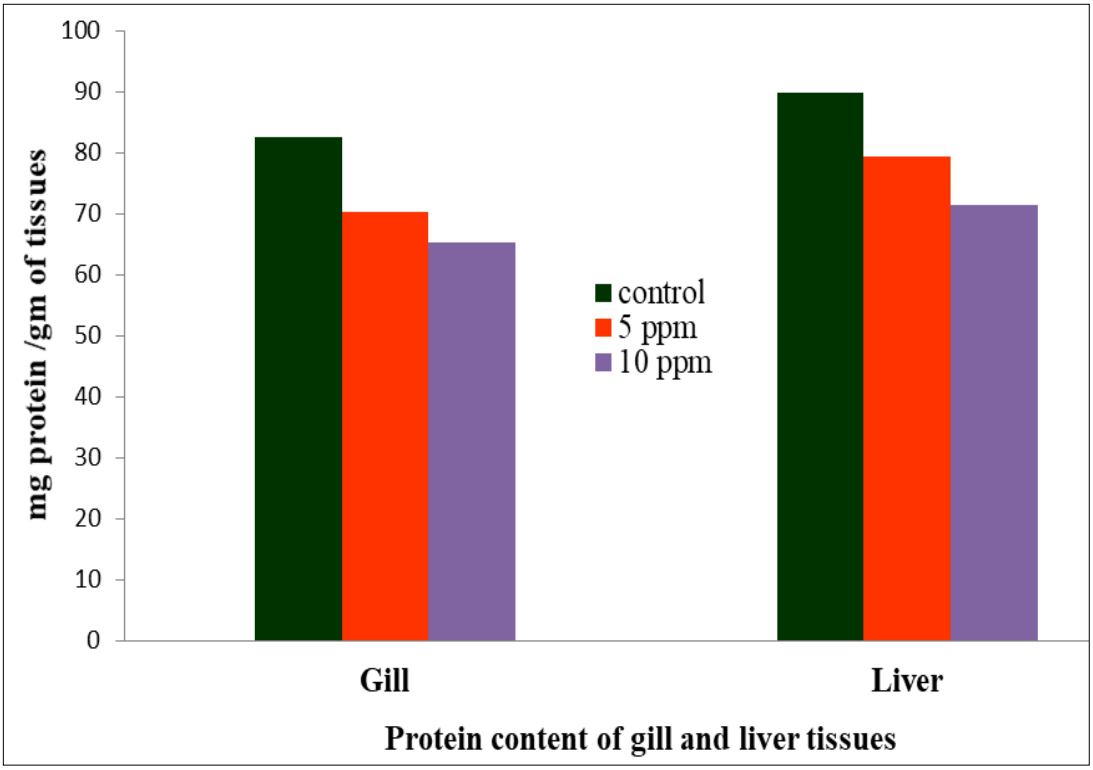

Fig 1: Protein Content of the tissues (gill and liver) of *C. punctata* at 5 ppm and 10 ppm of $CdCl_2$ after 7 days of exposure

Results and Discussion

The present study indicated that the heavy metal Cadmium Chloride ($CdCl_2$) has a dreadful effect on the freshwater fish *Channa punctata* (Bloch) (Qureshi et al, 2011) [3]. From the behavioural responses of fish exposed to sub-lethal concentration of the heavy metal showed that they were under stress. The increase in opercular movement and the bottom to upward movement of the fishes to overcome hypoxic condition was seen in the 5th and 7th day of exposure. Mucus secretion, starting of depletion of scales, skin lesions on dorsal and lateral side of the body, copious mucus, clumping of gills increased with the increasing concentration of the toxicant (Rastogi, *et al*., 2018) [5]. Colour changes of the body from blackish to pale yellowish and restlessness again confirmed the anxiety level of the fishes in the experiment (Table 1). Also many visible lesions were observed near operculum, mouth, pectoral and pelvic fins and also near the caudal fins.

Also from the total protein content assay, it was observed that the gills and liver tissues when exposed to sub lethal heavy metal concentration of $CdCl_2$ (5ppm and 10ppm) showed gradual decline in the protein content with the increase in exposure time, which further confirms the toxicity of the heavy metal (Table 2). This result indicates that the vital organs like gills and liver if affected proves lethal in survival of the fishes (Agrahari *et al*., 2007) [1].

Conclusion

From the experiment, it can be concluded that the presence of Cadmium Chloride in aquatic bodies greatly affects the

aquatic organisms which is confirmed from the present experiment by its (heavy metal) exposure to freshwater fish *Channa punctata*. The heavy metals proved to be very much fatal to the aquatic organisms and its heavy exposure may also prove to be detrimental to the aquatic life. Therefore, the use of heavy metals should be restricted in every aspect.

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