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Research Article

Toxic effects of cadmium chloride on behavior and histopathology of butter catfish *Ompok pabda* (Hamilton, 1822)

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ABSTRACT

Cadmium is very harmful to aquatic organisms and the environment as a toxicant. The present study investigated cadmium's morphological, behavioral, and histopathological effects on *Ompokpabda*. Initially, the fish were divided into 6 groups and exposed to different concentrations (20 mg/L, 40 mg/L, 80 mg/L, 160 mg/l, 320 mg/L, 400 mg/L) of $CdCl_2$. After 96 hours of exposure, the LC_{50} value of $CdCl_2$ for *O. pabda*was calculated as 190.9 mg/L. Finally, based on LC_{50}, three concentrations of $CdCl_2$ viz., 100 mg/L, 190.9 mg/L, and 300 mg/L, respectively, with three replica and control groups were used to observe morphological, behavioral, and histopathological changes in fish. There were no abnormalities in behavior or deaths in the control group at any point in the experiment. At the same time, the behavioral abnormalities of the $CdCl_2$-treated fish were increased as the dose increased. The behavioral abnormalities observed were loss of balance, breathing difficulty, hyperactivity, frequent surfacing, excessive mucus secretion, and gasping. After 96 hours of exposure to the final treatment, tissue samples (gill and intestine) were collected. Histopathological results revealed clear and significant alterations in the gill and intestine tissues. The alteration in the gill was characterized by hyperplasia of primary and secondary lamellae, epithelial edema, lamellar aneurism, and necrosis. In contrast, the intestine was characterized by intact serosa, less organized mucosa, a consequent fusion of mucosa, and edema between the intestinal submucosa and lamina propria. Compared with the control group, *Ompokpabda* of treatment groups showed severe intestinal and gill tissue injury. The result revealed that acute cadmium toxicity has a detrimental effect on the exposed fish's normal behavior and essential organs.

Introduction

Pollutants in aquatic systems, particularly heavy metals, are a severe global issue (Soltan et al., 2018). Aquatic systems are a major source of water pollution, as they are subjected to a variety of pollutants produced mainly through wastewater discharged from industries, wastewater treatment facilities, and drainage systems from urban and agricultural regions that contain suspended particles, fertilizers, organic and inorganic compounds, and other hazardous metal compounds (Silva and Martinez, 2014). Uncontrolled agricultural chemical discharge into water bodies has caused damage to aquatic ecosystems, affecting all kinds of aquatic organisms. Heavy metals, which are not degraded by biological breakdown, are the most common contaminants in aquatic bodies worldwide. Heavy

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metals cause environmental problems by interfering with various physiological, biochemical, and cellular processes (Ferro et al., 2019). Fish growth rates, physiological functions, mortality, and reproduction may be hampered by the harmful effects of different heavy metals (Ebrahimi and Taherianfard, 2011). Several studies have found that fish exposed to metals have a weak immune system and a higher mortality risk. Heavy metals can increase genotoxicity by causing toxicity in other chemical agents, either directly or indirectly (Pretto et al., 2011). Heavy metals can also increase genotoxicity by generating toxicity in other chemical agents (Pretto et al., 2011). Heavy metals seriously threaten many aquatic organisms by degrading water quality (Garcia et al., 2006).

Cadmium is a heavy metal linked to a detrimental effect on aquatic species (Nordberg et al., 2007). It enters the aquatic environment as a result of both natural and manmade activities (Heath, 1987). With the increase in pollution in aquatic bodies and pisciculture, studies on heavy metal toxicity in fish are receiving more and more attention worldwide. Cadmium chloride, a heavy metal that poses a serious threat to organisms due to its high toxicity and tissue accumulation, contaminates aquatic environments from air and land (Kumar et al., 2006). Cadmium pollution is caused by organic and inorganic materials in household and industrial effluents (McCarty et al., 1978). In Bangladesh's economy, the aquatic ecosystem is extremely important. In the aquatic ecology, fish serve as bioindicators. Pollutants from the water and food chain accumulate directly and indirectly in aquatic species. As a result, discharging contaminants in water has a negative impact on the health of fish and other aquatic species. As a result, fish production is often hampered, and fishermen suffer significant financial losses. It is vital to monitor and understand the pathophysiology of pollutants to reduce negative effects and protect public health (Pandey et al., 2008).

Ompok pabda (Hamilton Bouchanan, 1822) is a valuable commercial species in Bangladesh and other Southeast Asian countries (Talwar and Jhingran, 1991). *O. pabda* is a small freshwater catfish native to Bangladesh, colloquially known as pabda. This species is also a well-established model for toxicological study because of its ease of handling, culture, and maintenance in the laboratory and its rapid response to environmental changes. The objectives of the experiment were to determine the LC_{50} value of Cadmium Chloride ($CdCl_2$) for *O. pabda* along with observing morphological and behavioral changes of fish due to cadmium chloride exposure and to identify histological changes of gill and intestine of *O. pabda* due to cadmium chloride exposure.

Materials and Methods

Collection of samples

The fingerlings of *O. pabda* were collected from Babul Motsho Hatchery and Nursery at Ishwargonj, Mymensingh. Almost identical-sized fish were used for the experiment. The fish were carried to the laboratory of the Department of Fisheries, University of Dhaka. The average weight of the sample was 12.05±0.12 gm, and the average length was 10.27±0.23 cm.

Acute toxicity

Short-term acute toxicity tests were conducted using the renewal bioassay method (Reish and Oshida 1975; USEPA, 2002) for 96 h and different concentrations of toxicants. To perform this experiment, 98% of Cadmium chloride hemi (pentahydrate) was used as a toxicant, and this chemical was collected from Shanghai Titan Scientific Co. Ltd. in its original package form.

Pre-exposure acclimation of the test animals

After arrival in the laboratory, the fishes were immediately released into three big aquariums containing filter water and maintained there for about 7 days. Fish were fed on artificial feed once daily. Any debris or unwanted particles were removed from the tank after feeding. The water was changed at 24-hour intervals to remove the metabolic waste products. Aerators were used for the continuous oxygenation of water. The water quality parameters of the acclimation tank were studied daily. However, only healthy fishes were transferred to the experimental system after acclimation.

Experimental system and dose preparation

The static bioassay LC_{50} was conducted using the recommendation by Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). For the LC_{50} assay, seven independent glass aquaria were used. Each aquarium was 30 cm x 20 cm x 25 cm, containing 10 liters of water. The water was aerated for one day before starting the experiment. Stone aerators connected to a compressed air supply were used to maintain an adequate dissolved oxygen level in each aquarium. Cadmium chloride was measured, and serial dilutions were made with deionized water. Six concentrations (0, 20, 40, 80, 160, 320 and 400 mg/L of $CdCl_2$) were used as stock solution. Fresh solutions were prepared and used for each test on the same day. The solution was directly mixed with test water.

Samples are in Treatment

An organism loading of approximately 1.0 g/L of water was maintained in all the tests as recommended by APHA (1985). Eight randomly selected fish were transferred to each aquarium. In all cases, control groups of fish were maintained. Each experimental trial was carried out for 96 hours, as Sprague (1969, 1970) and APHA (1985) recommended. According to Sprague (1969), 96 hours of LC_{50} is the most reproducible. The mortality of the fish was recorded at logarithmic time intervals (Sprague, 1970), that is, after 6, 12, 24, 48, 72, and 96 hours of exposure.

Table 1. Concentration of $CdCl_2$ for final treatment.

Serial No.	Treatment	Concentrations of $CdCl_2$ (mg/L)
1	Control	0.00
2	T1	100.00
3	T2	190.90
4	T3	300.00

Each treatment: 3 replication.

Measurement and analysis of water quality

The physicochemical characteristics of the water, such as temperature, pH, salinity, and oxygen concentration, were frequently conducted following the standard procedures described in APHA (1985). Temperature, Salinity, DO, and P^H were measured with a thermometer, Salinometer, DO meter, and P^H meter, respectively.

Evaluation of Median Lethal Concentration (LC_{50})

The concentration of $CdCl_2$ at which 50 percent of the test organisms die during a particular period, or the concentration lethal to 50 percent of the test population, is called the median lethal concentration (LC_{50}). The LC_{50} values of $CdCl_2$ were estimated using Finney's probit method and ExcellProbit analysis.

Final Experiment

Pre-exposure acclimation of the test fish

The fish were immediately released into twelve tanks containing filter water and maintained there in a static condition for about one day. They were fed artificial feed once daily, and aerators were used to oxygenate the water.

Dose Preparation

The next day, Cadmium chloride was measured, and serial dilutions were made with deionized water. Three different concentrations were used as stock solutions. Different test doses were prepared by diluting the stock concentration (Table 1). Fresh solutions were prepared and used for each test on the same day.

Samples are in the final treatment

The final treatment was conducted using the recommended method (Ahmed et al., 2014). An organism loading of approximately 1.0 g/L of water was maintained in all the tests as recommended by APHA (1985). Eight randomly selected fish were transferred to each aquarium. In all cases, control groups of fish were maintained. Each experiment was carried out for 96 hours, as Sprague (1969, 1970) and APHA (1985) recommended. Initial length and weight were recorded on the 1st day of the exposed chemical. The physicochemical characteristics test of the water, such as temperature, pH, salinity, and oxygen concentration, were conducted frequently following the standard procedures described in APHA (1985). Temperature, Salinity, DO, and PH were measured with a thermometer, Salinometer, DO meter, and PH meter. Aerators were used to oxygenate the water. Fish were fed on artificial feed once daily. The mortality of the fish was recorded at logarithmic time intervals (Sprague, 1970), that is, after 24, 48, 72, and 96 hours of exposure. External behavior was observed according to (Test Guideline No. 203, Fish, Acute Toxicity Testing) after 24, 48, 72 and 96 hours of exposure.

Behavioral changes

Behavioral changes were observed every 24 hours. Loss of schooling, abnormal vertical orientation, hyperactivity, etc., were observed every day. Those behavioral activities were observed because they showed differences between the control and different treatments.

Histopathology

After 4 days, tissue samples of the gill and intestine were isolated from the treated fish of all treatments. The gill and intestine were aseptically removed by sacrificing from the study fish and transferred to vials with 10% formalin, which was immediately processed for further analysis.

Results

Acute Toxicity Estimation for LC_{50}

The water quality parameters were measured during the experimental period (Table 2). Here, the range of temperature, dissolved oxygen, ^{pH,} and salinity did not fluctuate much. All values are in the optimal range for treatment. However, there was a linear relationship between increased $CdCl_2$ and P^H. With the increased $CdCl_2$ concentration, the P^H also increased. However, this is also in the optimal range for every treatment.

In the initial experiment, no behavioral changes were observed in the control group, but various abnormalities gradually increased with increasing $CdCl_2$ concentration and number of days of exposure (Table 3). No behavioral changes or deaths occurred in the control group during the trial. All control fish were active and swimming normally. Behavioral abnormalities include loss of schooling, vertical orientation, hyperactivity, spiral swimming, gulping, gasping, surface activity, and increased mucus secretion. These toxic effects increase as the dose is increased.

The first visible behaviors were observed at the highest concentrations of 400 mg/L and 320 mg/L on day one. On the first day, at 400 mg/L and 320 mg/L, loss of schooling, abnormal vertical orientation, hyperactivity, gulping, gasping, surface escape, secretion of mucus, and dark skin color were observed. On the second day at 160 mg/L and 80 mg/L, loss of schooling, abnormal vertical orientation, hyperactivity, gulping, gasping, surface escape, mucus secretion and darkening of skin color were observed. On day four of 40 mg/L, loss of schooling, hyperactivity, abnormal vertical orientation, gulping, gasping, and mucous secretions were observed. However, at a concentration of 20 mg/L, no significant behavior change was observed during the four days of treatment. No behavioral changes were observed in the control treatment.

Table 2. Water quality parameters during LC_{50} determination of different experimental aquariums.

Concentration of $CdCl_2$ (mg/L)	Physical-chemical properties			
	Temperature (ºC) (January)	Dissolved oxygen (mg/L)	pH	Salinity
0.0 (Control)	20.3± 0.17	8.21 ± 0.07	7.35±0.05	0
20	20.2± 0.12	6.79 ± 0.50	7.42± 0.08	0
40	20.1± 0.19	8.75±0.11	7.49± 0.10	0
80	20.3± 0.90	8.66± 0.09	7.50± 0.11	0.1
160	20.3±0.18	8.99± 0.16	7.68±0.16	0.1
320	20.0±0.21	8.02± 0.13	7.77± 0.15	0.2

Table 3. Fish behaviors observation on LC_{50} treatments.

Clinical Sign	Doses for LC_{50} (mg/L)						
	0.0 (Control)	20	40	80	160	320	400
Loss of Schooling	×	×	×	×	√	√	√
Vertical orientation	×	×	×	×	√	√	√
Hyper activity	×	×	×	×	√	√	√
Spiral swimming	×	×	×	×	√	√	√
Gulping	×	×	×	×	√	√	√
Gasping	×	×	×	×	√	√	√
Cannabalism	×	×	×	×	√	√	√
Skin color darkness	×	×	×	√	√	√	√
Mucous Secretion	×	×	×	√	√	√	√

x- no response, √- showed response

Table 4. Cumulative mortality (%) of 96 hours exposure time for LC_{50}.

Treatment	Concentration of $CdCl_2$(mg/L)	Exposure time (hours) Cumulative mortality (%) of 8 fishes						
		6	12	18	24	48	72	96
Control	0.0	0	0	0	0	0	0	0
1	20	0	0	0	0	0	0	0
2	40	0	0	0	0	0	0	0
3	80	0	0	0	0	0	0	0
4	160	0	0	0	0	12.5	25	37.5
5	320	0	25	100	100	100	100	100
6	400	12.5	100	100	100	100	100	100

Observation of fish mortality for LC_{50} treatment

Table 4 show the cumulative mortality rate (percentage) for $CdCl_2$ treatment in the preliminary trials for LC_{50} determination. No mortality occurred at control, 20 mg/L, 40 mg/L, and 80 mg/L of $CdCl_2$ after 96-h exposure. Mortality increased steadily as the concentration of $CdCl_2$ increased. The cumulative mortality (%) of the initial treatment for $CdCl_2$ is presented in Table 4. After 96 hours of exposure to 20 mg/L, 40 mg/L, and 80 mg/L of $CdCl_2$, no mortality was recorded. At 160 mg/L of $CdCl_2$, 12.5%, 25%, and 37.5% mortalities were recorded after 48 hours, 72 hours, and 96 hours of exposure, respectively. At 400 mg/L $CdCl_2$, however, complete death (100%) occurred within 12 hours.

Table 5. Relation between the concentration of $CdCl_2$ and the percentage mortality of the fish.

Concentration (mg/L)	Log10 transfer	Mortality (%)	Probit
20	1.301029996	0	0
40	1.602059991	0	0
80	1.903089987	0	0
160	2.204119983	37.5	4.82
320	2.505149978	100	7.37
400	2.602059991	100	7.37

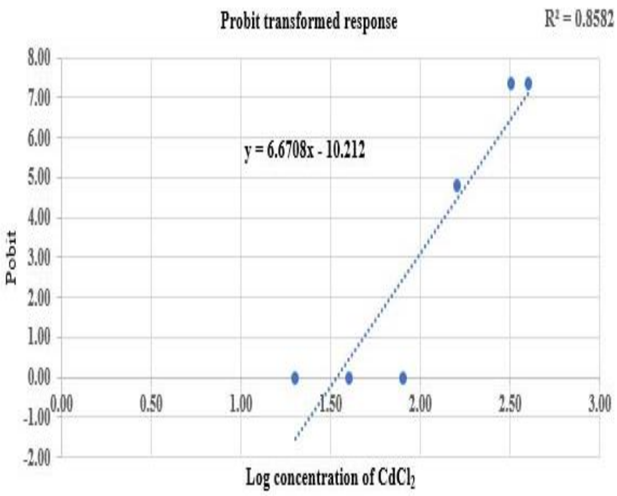


Fig. 1. Regression line between the probit of *O. pabda* and log concentration of $CdCl_2$.

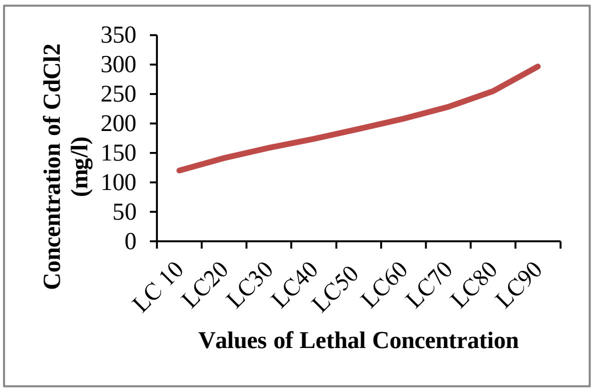


Fig. 2. Different values of lethal concentration of $CdCl_2$ on *O. pabda.*

Prediction of lethal concentration

After the treatment of $CdCl_2$, fish mortalities data were analyzed using ExcellProbit Analysis software. LC_{50}, LC_{60}, LC_{70}, LC_{80}, and LC_{90} values were calculated after analyzing the mortalities data.

According to probit analysis the LC_{50}, LC_{60}, LC_{70}, LC_{80}, LC_{90} values were 190.90 mg/L, 207.89 mg/L, 228.20 mg/L, 254.86 mg/L, 296.66 mg/L, respectively (Table 5; Fig. 1 & 2).

Acute toxicity estimation for final treatment

Potential hazardous consequences can be seen through behavioral changes, the most sensitive signs. Behavioral abnormalities were observed in *O. pabda* when treated with various doses of $CdCl_2$ while conducting the initial treatment for LC_{50} determination. Behavioral alterations became more pronounced and lasted longer as the concentration increased. The following is a list of the behavioral changes seen in *O. pabda* (Table 6).

Control group: During the experiment, no behavioral changes or deaths were observed in the control group. All fish were active and swimming normally.

Table 6. Fish behaviors observation on final treatments.

Clinical Sign	Different concentrations for final treatment (mg/L)											
	C_1	T_1R_1	T_1R_2	T_1R_3	C_2	T_2R_1	T_2R_2	T_2R_3	C_3	T_3R_1	T_3R_2	T_3R_3
	0.0	100	100	100	0.0	190.9	190.9	190.9	0.0	300	300	300
Loss of schooling	×	√	√	√	×	√	√	√	×	√	√	√
Vertical orientation	×	√	√	√	×	√	√	√	×	√	√	√
Hyper activity	×	×	×	×	×	√	√	√	×	√	√	√
Spiral swimming	×	√	√	√	×	√	√	√	×	√	√	√
Gulping	×	√	√	√	×	√	√	√	×	√	√	√
Gasping	×	×	×	×	×	√	√	√	×	√	√	√
Surface escape	×	×	×	×	×	√	√	√	×	√	√	√
Cannibalism	×	×	×	×	×	×	×	×	×	×	×	×
Skin color darkness	×	×	×	×	×	√	√	√	×	√	√	√
Mucus secretion	×	×	×	×	×	√	√	√	×	√	√	√

C = Control, T = Treatment, R = Replica

Treatment 1 group (100 mg/L): After 72 hours, behavioral abnormalities, such as a spiral swimming pattern, floating motionless on the water's surface, and gulping, were observed, and fish tended to gather on the surface.

Treatment 2 group (190.9 mg/L): After 12 hours, the fish's movement became very slow, and they developed behavioral abnormalities such as overturn in the water, breathing problems, loss of coordination, swimming disorders, gulping, and gasping. Increased mucus was also seen on the body's surface.

Treatment 3 (300 mg/L): The fish lost coordination immediately after the CdCl2 was added. Swimming difficulties were seen, and fish had trouble breathing and congregating. The fish initially sank to the bottom of the aquarium and remained immobile. The first fish died after 6 hours, while the rest died within 48 hours.

Observation on fish mortality for final treatment

The cumulative mortality (%) data for the final treatment is shown in Table 7. No mortality was observed in the control, which $CdCl_2$ did not treat. No mortalities were observed in three replicas of treatment 1, which were treated with 100 mg/L of $CdCl_2$. 53.5% average mortality wasobserved at a concentration of 190.9 mg/L of $CdCl_2$ of treatment 2 with three replica, but 100% mortality was noticed in concentration of 300 mg/L (Table 7; Fig. 3).

Histopathology in gill and intestine of *O. pabda*

Histopathological changes in the gill and intestine of *O. pabda* were observed after 96 hours of treatment.

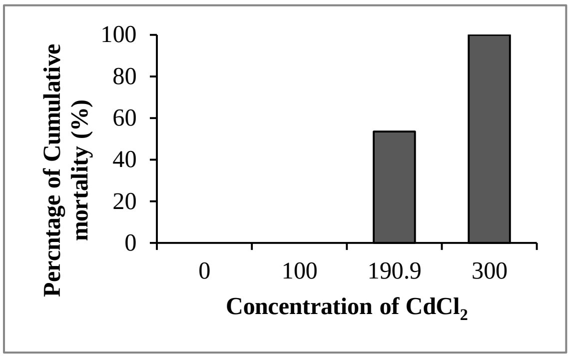
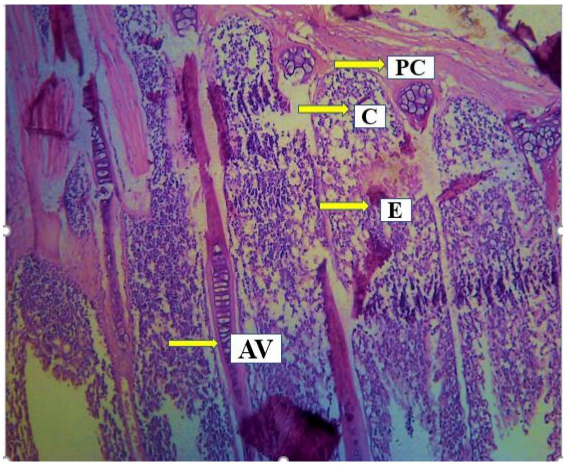


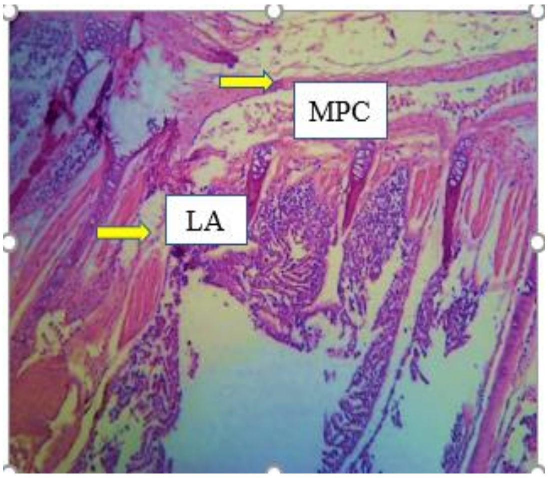
Fig. 3. Cumulative mortality (%) at different concentrations of $CdCl_2$ after 96 hours of exposure time for LC_{50} determination.

Table 7. Cumulative mortality (%) of 96 hours exposure time for final treatment.

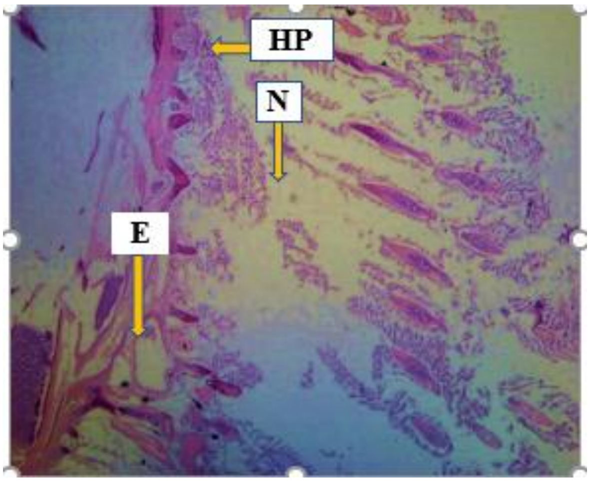
Treatment	**Concentration of $CdCl_2$ (mg/L)**	**Exposure time (hours)**			
		Cumulative mortality (%) of 8 fishes			
		24	**48**	**72**	**96**
Control 1	0.0	0	0	0	0
$T_1 R_1$	100	0	0	0	0
$T_1 R_2$	100	0	0	0	0
$T_1 R_3$	100	0	0	0	0
Control 2	0.0	0	0	0	0
T_2R_1`	190.9	0	25	37.5	60.5
T_2R_2	190.9	0	12.5	25	50
T_2R_3	190.9	0	12.5	25	50
Control 3	0.0	0	0	0	0
T_3R_1	300	37.5	75	100	100
T_3R_2	300	37.5	87.5	100	100
T_3R_3	300	37.5	87.5	100	100



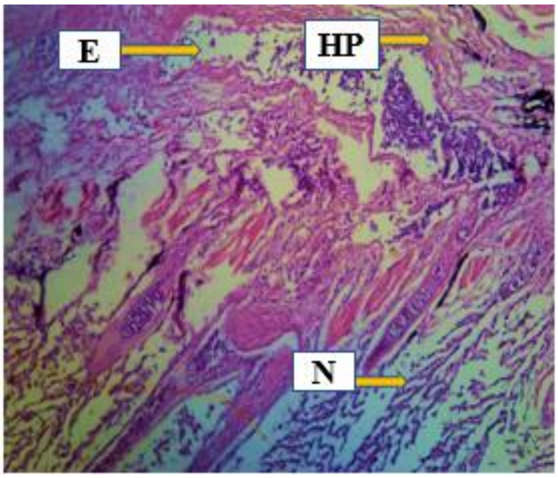
a. Gill tissue of control fish which was not treated by $CdCl_2$.



b. Gill tissue of treatment 1 (treated by 100 mg/L of $CdCl_2$).



c. Gill tissue of treatment 2 (treated by 190.9 mg/L of $CdCl_2$).



d.Gill tissue of treatment 3 (treated by 300 mg/L of $CdCl_2$).

Fig. 4 (a-d): Pathological changes in gill tissues. AV: afferent vessel; C: chloride cell; E: erythrocyte; N: neutrophil; LA: lamenar aneurism; E: edema; MPC: moderately disorganized primary cell; HP: hyperplasia; PC: pillar cell;

Observation of gill tissue

There were no histological alterations in the gill tissue of the fish in the control group. A consistent arrangement of gill lamellae and filaments with inter lamellar space (ILS) was seen throughout the experiment, and no modifications were noticed. At the same time, pillar cells, epithelial cells, primary gill lamellae, and secondary gill lamellae were observed well structured, as shown in Fig. 4a. Chloride cells and mucous cells were present and well structured. Histopathological examination of the gill section after 96 hours of 100 mg/L $CdCl_2$ of treatment 1 exposure showed moderate fusion of secondary lamellae and lamellar aneurism that led to eluding of the tips of the secondary lamellae (Fig. 4b). Pillar cell systems were found to be moderately disorganized (Fig. 4b).

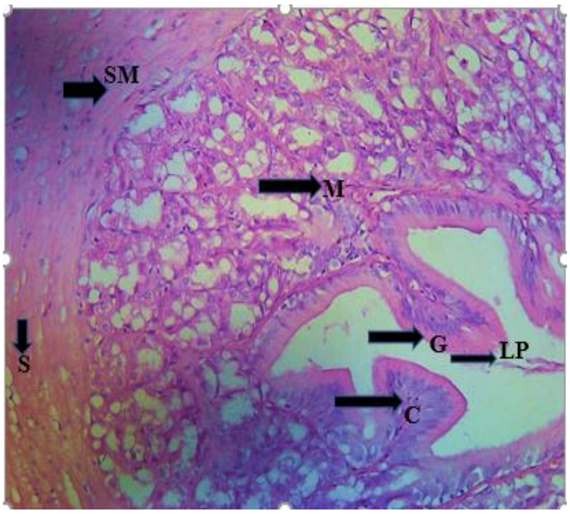
After 96 hours of exposure, the gill of treatment 2, treated by LC_{50} doses, 190.9 mg/L of $CdCl_2$, showed severe histopathological changes. Primary lamellae and secondary lamellae were severely damaged. Gill hyperplasia and interlamellar spacing between secondary lamellae were observed in fish gills. Edema was detected in the resulting interlamellar gaps. Secondary lamellae were found to be shorter in length, and the length of secondary lamellae was shown to besignificantly shorter (about 50 percent or less than normal as shown as Fig. 4c. The gill of fish after 96 hours of exposure at 300 mg/L of $CdCl_2$ showed severe hyperplasia of primary lamellar and secondary lamellar epithelium, and edema was observed in the resulting interlamellar spaces (Fig. 4d). The number of chloride cells in the primary lamellar epithelium increased significantly (Fig. 4d). Histopathological results indicated that the gill was the primary target tissue affected by $CdCl_2$. The most common changes at all doses of $CdCl_2$ were hyperplasia of secondary lamellae (Table 8).

Table 8. Summary of histopathological effects in the gills of *O. pabda* treated with different concentrations of $CdCl_2$ and control fish.

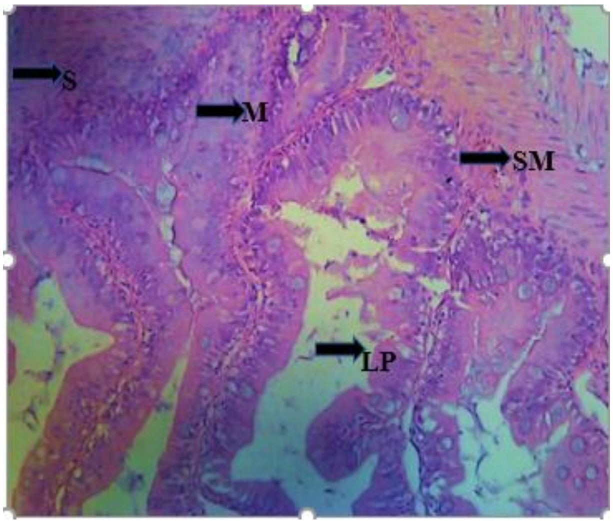
Treatment	Concentration of $CdCl_2$ (mg/L)	Edema	Hyperplasia of Primary lamellae	Hyperplasia of secondary lamellae
Control	0.0	-	-	
1	100	-	-	+
2	190.9	++	++	+
3	300	+++	+++	+++

None (−), mild (+), moderate (++), and severe (+++)

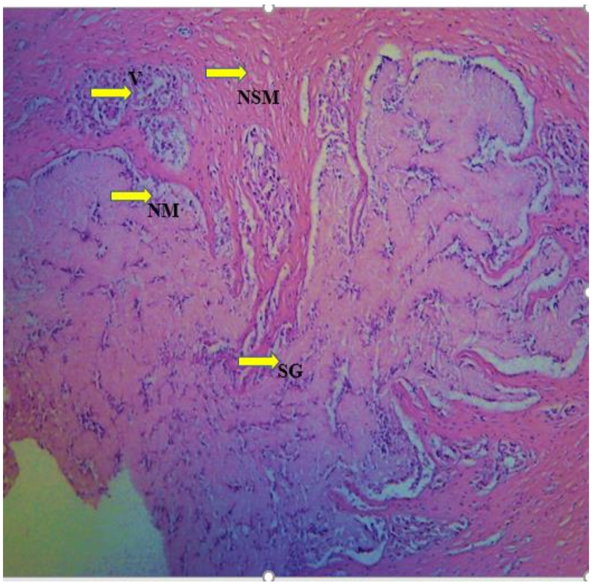
Observation of intestinal tissue



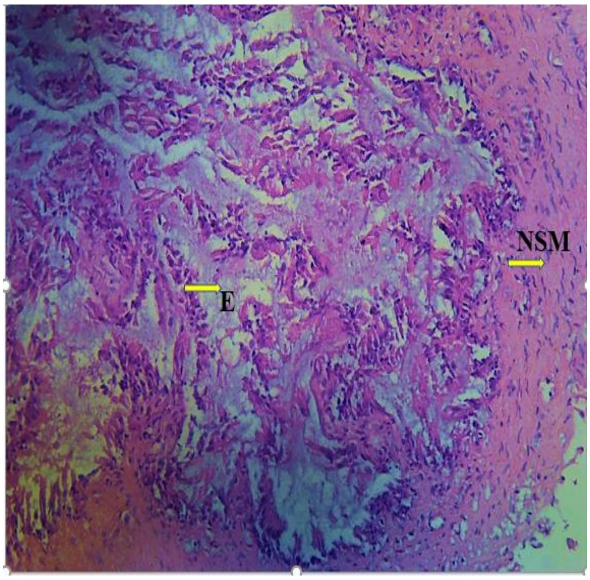
a. Intestine of control fish, which was not treated by $CdCl_2$.



b. Intestine of treatment 1 (treated by 100 mg/L of $CdCl_2$).



c. Intestine of treatment 2 (treated by 190.9 mg/L of $CdCl_2$).



b. Intestine of treatment 1 (treated by 100 d. The intestine of treatment 3 (treated with 300 mg/L of $CdCl_2$).

Fig. 5 (a-d): Pathological changes in intestine tissues. C: columner cells; E: erosion; G: goblet cells; LCT: loose connective tissue; LP: lamina propia; M: mucosa; NM: necrotic mucosal layer; NSM: necrotic submucosal layer; S: serosa; SG: swelling of goblet cell; V: vacuoles; SM: Submucosal Layer.

In the control group, the photomicrograph of the intestine depicts normal columnar cells, and goblet cells and normal intestinal submucosa with blood capillaries. The photomicrograph of the intestine in the control group shows ordinary columnar and goblet cells, as well as normal intestinal submucosa and blood capillaries, in contrast to the experimental group. Mucosa, intestinal submucosa and lamina propria were well organized (Fig. 5a). The intestine of O. pabda exposed to $CdCl_2$ underwent progressively harmful changes that increased in severity as the concentration of $CdCl_2$ increased. The intactness of serosa, less organized intestinal mucosa, submucosa, and lamina propria was observed in treatment 1 treated with 100 mg/L $CdCl_2$ (Fig. 5b). Photomicrograph of intestine of treatment 2 treated by 190.9 mg/L of $CdCl_2$ shows degenerative changes in tips of villi, loss of structural integrity of mucosal layers, degenerative changes of mucosal epithelium, swelling of goblet cell and necrosis (Fig. 5c). The intestine tissue of treatment 3, treated by 300 mg/L, was eroded. Mucosa, serosa, intestinal submucosa, and lamina propria were totally damaged (Fig. 5d).

Discussion

Bioassays are the best tool for evaluating the effects and destiny of toxins in the aquatic ecosystem. Heavy metals of anthropogenic origin, such as Cd, are recognized as significant pollutants in the aquatic environment. In this study, we elucidated the effects of the acute toxicity of cadmium on the behavior and histopathology of the freshwater fish *Ompokpabda*. As can be seen from the results of this study, the water quality parameters were within the optimal range for different treatments. According to APHA (1985), temperature fluctuations should not exceed 4°C and the dissolved oxygen of warm water fish should not be below 4 mg/L. In this experiment, the temperature fluctuations were small (20.35 and 21.15°C), and the dissolved oxygen was in the optimal range of 7.86 and 8.33 mg/L. Evidence available indicates that trace amounts of toxicants can cause abnormal behavioral changes in fish through responsive impairment (Kabir and Begum, 1978).

In aquatic organisms, behavioral changes have been identified as sensitive indicators of chemically induced stress (Sharma and Agarwall, 2005). Stress-related changes in behavior are susceptible indicators of possible toxic effects. Fish treated to various toxicant doses displayed distinct behavioral changes. In the current study, fishes were observed darting around and attempting to escape toxic water. Fishes appeared to trigger mucus secretion over the gills, forcing the opercula to move quickly and exert significant effort. Loss of schooling, vertical orientation, hyperactivity, spiral swimming, gulping, gasping, surfacing activity, and increased mucus secretion were all noted in fish. As the dose was increased, the toxic effects became more pronounced. Our findings are consistent with the findings of the following researchers: According to Woodal et al. (1988), cadmium LC_{50} values on *Salmo gairdneri* and *Xenopus larvae* were between 80 and 100 mg/L; however, Muley et al. (2000) observed that the 96-hour LC_{50} value of cadmium on the fish *Cyprinuscarpio* was 121.8 mg/L and Sehgal and Pandey (1984) determined that the 96 hours LC_{50}values of Cadmium for *Heteropneustesfossilis* are 360.50 mg/L (Rai et al., 2008).

Cadmium's LC_{50} value for the goldfish *Carassiusauratus* was found to be 46.8 mg/L (McCarty et al., 1978). The cadmium LC_{50} value for the fish *Labeorohita* was reported to be 89.5 mg/L after 96 hours (Dutta and Kaviraj, 2001). It should be noted that the toxicities of a single toxicant to different types of fish species are hard to compare since they are impacted by parameters such as the test water's temperature, dissolved oxygen content, pH, hardness, and (Schoettger, 1970).The concentration of $CdCl_2$ for acute exposure was calculated based on the results of LC_{50} values from the experiment. The LC_{50} value of $CdCl_2$ for *O.pabda*was found to be 190.9 mg/L, according to the findings of this experiment. Compared to other fishes' 96-hour LC_{50} values, this study shows that *O. pabda*is more resistant to cadmium and might be called the least sensitive fish. Abnormal behavior was observed every 24 hours. $CdCl_2$ in aquariums causes irregular movement, fast operculum movement, jumping out of the experimental media, lateral swimming, and loss of balance. Abnormal reactions in the fish body occur due to the neurotoxic effects. Toxicants are responsible for changing the behavior of jumping out of water. An irritation of the skin is responsible for secretion of mucus from fish body. Damage to the nervous system impaired lateral swimming and caused a loss of equilibrium (Sinha and Kumar, 1992), as found in the current study. In addition to serving as indicators of numerous anthropogenic contaminants, histopathological biomarkers also resemble the general health of the whole population in an ecosystem. Many studies employ alterations in cells and tissues in fish as biomarkers. After 96 hours of exposure, the harmful effect of Cd on fish gills and gut tissues was observed in the present study. On the basis of histological changes from our study, it is speculated that a significant disequilibrium in physiological processes may have resulted in Cd-exposed fishes.

Gills are extremely sensitive organs in the fish body that regulate respiratory, osmoregulatory, and excretory activities. Poisoning of heavy metals is responsible for occurring respiratory distress (Matos et al., 2017). High levels of cadmium taken through gills are very harmful to fish health. In this present study, hyperplasia of primary and secondary lamellae, epithelial edema, lamellar aneurism, and necrosis were the most prominent histopathological abnormalities detected in the gills of *O. pabda* exposed to $CdCl_2$. These toxic effects increased with the dose increased.

Conclusions

As aquatic organisms, fish are constantly exposed to water contaminated with heavy metals like cadmium. This research provides information on the LC_{50} of $CdCl_2$ for *O. pabda* and the histopathological changes in the gills and intestine of *Ompokpabda*exposed to different $CdCl_2$ concentrations. The present study revealed that the LC_{50} value of $CdCl_2$ for *O. pabda* was 190.9 mg/L. About 53.5% of fish mortality was observed in this dose, and all fish showed behavioral abnormalities. Significant histopathological changes were observed in gill and intestine of fish after exposing this toxicant. From the findings of this experiment, it can be concluded that acute cadmium chloride toxicity has a detrimental effect on the exposed fish's normal behavior and essential organs. The findings clearly show that the usage of a heavy metal such as cadmium must be strictly regulated.

Author contributions

Atakiya Galiba: Sample Collection and preparation, Data interpretation and Visualization and Writing Original Draft. **Md. Mostavi Enan Eshik**: Concept Development, Study Design, Supervision, Data interpretation, reviewing, and Writing. **Mohammad Shamsur Rahman:** Concept Development, Study Design, Data interpretation, Supervision, Writing-Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahmed MK, Parvin E, Islam MM, Akter MS, Khan S and Al-Mamun MH. Lead-and cadmium-induced histopathological changes in gill, kidney and liver tissue of freshwater climbing perch *Anabas testudineus* (Bloch, 1792). *Chem. Ecol.* 2014; 30(6): 532-540.

APHA, AWWA and WPCF. Standard methods for the examination of water and wastewater. 15th ed. APHA, Washington: DC, 1985. p.7.

Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for acute toxicity tests with fish, macroinvertebrates. and amphibians. U.S. Environmental Protection Agency, *Ecol. Res. Serv. EPA*, 1975; 660/3-75-009. 61 pp.

Dutta TK and Kaviraj A. Acute toxicity of cadmium to fish *Labeorohita* and copepod *Diaptomus forbesi* preexposed to CaO and $KMnO_4$. *Chemosphere*, 2001; 42: 955-958.

Ebrahimi M and Taherianfard M. Effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor river. *Iran. J. Fish. Sci.* 2011; 10: 13-24.

Ferro JP, Campos LB, Ossana N, Ferrari L and Eissa BL. Effects of cadmium on the behaviour of *Cnesterodon decemmaculatus*. *Int. J. Environ. Health,* 2019; 4(9): 372-379.

Garcia S, Fontaínhas FA and Wilson JM. Cadmium tolerance in the Nile tilapia (*Oreochromis niloticus*) following acute exposure: assessment of some ion regulatory parameters. *Environ. Toxicol.* 2006; 21: 33-46.

Heath AG. Water Pollution and Fish Physiology. CRC Press:Florida; 1987. p245.

Kabir SMH and Begum R. Toxicity of three organophosphorus insecticides to Singhi fish, *Heteropneustesfossilis* (Bloch). Dhaka University Studies. Part B. 1978; 26: 115-122.

Kumar P, Prasad Y, Sharma R and Patil RD. Histopathological changes in liver and kidney of *Clariasbatrachus* due to cadmium toxicity and its neutralization by Vitamin C, Taurine and Garlic. Proceedings of a national conference held in Chenai, Aquatic Toxicology. 2006; 10: 42.

Matos LA, Cunha ACS, Sousa AA, Maranhão JPR, Santos NRS, de MC Gonçalves M, de M. Dantas SMM, de C. e Sousa JM, Peron AP, da Silva FCC, de Alencar MVOB, Islam MT, de Aguiar

RPS, de C. Melo-Cavalcante AA, Bonecker CC and Junior HFJ. The influence of heavy metals on toxicogenetic damage in a Brazilian tropical river. *Chemosphere*, 2017; 185: 852-859.

McCarty LS, Henry JAC and Houston AH. Toxicity of cadmium to goldfish, *Carassius auratus,* in hard and soft water. *J. Fish. Res. Board Can.* 1978; 35: 35-42.

Muley DV, Kamble GB and Bhilave MP. Effect of heavy metals on nucleic acids in *Cyprinuscarpio. J. Environ. Biol.* 2000; 21: 367-370.

Nordberg GF, Fowler BA, Nordberg M and Friberg L. Handbook on the toxicology of metals. Academic press, Amsterdam: 2007. p.1024.

Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F and Raisuddin S. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channapunctata* Bloch. *Chem. Biol. Interact*. 2008;174(3):183-192.

Pretto A, Loro VL, Baldisserotto B, Pavanato MA, Moraes BS, Menezes C, Cattaneo R, Clasen B, Finamor IA and Dressler V. Effects of water cadmium concentrations on bioaccumulation and various oxidative stress parameters in Rhamdiaquelen. *Arch. Environ. Contam. Toxicol.* 2011; 60: 309-318.

Rai R, Mishra D, Srivastav S and Srivastav AK. Acute toxicity of cadmium against catfish, *Heteropneustes fossilis* (siluriformes heteropneustidae) in static renewal bioassays. *Ethiop. J. Biol. Sci.* 2008; 7(2): 185-191.

Reish DL and Oshida PS. Manual of methods in aquatic environment research. Vol. 10. Food & Agriculture Org., 1975.

Schoettger RA.Toxicology of thiodan in several fish and aquatic invertebrates: Investigations in Fish Control. Series No. 35, U.S. Fish and Wildlife Service, 1970.

Sehgal R and Pandey AK. Effect of cadmium chloride on testicular activities in guppy, *Lebistesreticulatus*. *Comp. Physiol. Ecol.* 1984; 9: 225-230.

Sharma RK and Agrawal M. Biological effects of heavy metals: an overview. *J. Environ. Biol.* 2005; 26(2): 301-313.

Silva AO and Martinez CB. Acute effects of cadmium on Osmoregulation of the freshwater teleost *Prochiloduslineatus*: enzymes activity and plasma ions. *Aquat. Toxicol.* 2014; 156: 16-168.

Sinha TKP and Kumar K. Acute toxicity of mercuric chloride to *Anabas testudineus* (Bloch). *Environ. Ecol.* 1992; 10(3): 720-722.

Soltan HAH, Ata AM and El-Mamlouk EAK. Chemical components and cytotoxic effects of four different types of wastewater. *Egypt. J. Agric. Res.* 2018; 96(2): 547-560.

Sprague JB. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. *Water Res.* 1969; 3(11): 793-821.

Sprague JB. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. *Water Res.* 1970; 4(1): 3-32.

Talwar PK and Jhingran AG. Inland fishes of India and adjacent countries. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi, 1991. p.1158.

USEPA, Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 4th edn, U.S. Environmental Protection Agency, Washington, DC, 2002.

Woodal C, Maclean N and Crossley F. Responses of trout fry (*Salmo gairdneri*) and *Xenopuslaevis* tadpoles to cadmium and zinc. *Comp. Biochem. Physiol.* 1988; 89: 93-99.