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Full Length Article

Acute Toxicity of Cadmium and its Bio-accumulation in the Carnivorous Fish Species *Channa marulius*, *Mystus seenghala* and *Wallago attu*

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Abstract

Acute toxicity of Cadmium (Cd) using 96 h LC_{50} and lethal concentrations was determined for the three fish species viz. *Channa marulius, Mystus seenghala* and *Wallago attu.* These toxicity tests were performed with three length groups (50, 100, 150 mm) of each fish species at constant water temperature (28ºC), pH (8) and total hardness (250 mg L^{-1}). Fish mortality data were analyzed using Probit analysis method at 95% confidence interval to calculate 96 h LC_{50} and lethal concentrations of Cd. At the end of acute experiments, the organs (gills, kidney, liver, muscles) of dead fish were analyzed for Cd accumulation. Among the three carnivorous fish species, *M. seenghala* showed significantly higher sensitivity towards Cd toxicity followed by *W. attu* and *C. marulius*. However, 50 mm length group of all the three fish species exhibited significantly higher sensitivity towards Cd, followed by 100 and 150 mm length groups with significant differences. The organs of all fish species showed significant variations in their ability to amass Cd. However, *C. marulius* exhibited significantly ($p<0.05$) higher ability to amass Cd in its tissues followed by that of *W. attu* and *M. seenghala.* Among the fish organs, liver and kidney showed significantly higher ability to amass Cd during acute exposure of metal. This study will help in sustainable conservation of carnivorous fish species in the natural aquatic bodies of Pakistan.

Keywords: Cadmium; Acute toxicity; Carnivorous fish; Bioaccumulation

Introduction

The natural aquatic habitats in Pakistan have become contaminated with wide-ranging pollutants, including heavy metals and their mixtures, due to indiscriminate discharge of untreated effluents originating from diversified industries and domestic sewage. This situation is not only creating health hazards to the aquatic organisms, inhabiting these water bodies, but also affecting the human health by consuming contaminated food (Ghaffar *et al*., 2015). Heavy metals are regarded as the most toxic due to their ability to bio-accumulate and intoxicate in the natural aquatic food chains (Kousar and Javed, 2015). Therefore, toxicity of heavy metals in the natural water bodies of Pakistan have become a growing threat to the native fish fauna, especially to the carnivorous fish species like *Channa marulius*, *Mystus seenghala* and *Wallago attu* that are on the verge of extinction in the natural water bodies. These carnivorous fish species are residing on the top of aquatic food chain and, therefore, exhibit greater propensity to bio-accumulate metals from the aquatic environment.

Cadmium (Cd) is a non-essential, noncorrosive in nature and highly toxic metal which is discharged into the aquatic environment by industrial sources such as plating processes, mining and refining of ores, pigments, Ni-Cd batteries, gasoline containing lead by fishery boats and the use of phosphate fertilizers (ICdA, 2000). Biological techniques are implied for the appraisal of metallic ions toxicity and its consequences towards fish and other animal behavior, physiological sensitivity and morphological indices (Yang *et al.*, 2014; Kousar and Javed, 2015). Acute toxicity tests i.e., 96 h LC_{50} and lethal concentration indices allow us to gauge rapidly the impacts of toxicants on the organisms, like fish. In acute toxicity, fish mortality is used as a criterion to its final response for a particular toxicant (Kazlauskiene *et al*., 1999). Therefore, on the basis of acute toxicity tests, the sensitivity of a particular organism, belonging to variable ranks of phylogeny and at several growing phases, to diverse toxicants are frequently equated (Kazlauskiene *et al*., 1996; Mahboob *et al*., 2014).

In Pakistan, *C. marulius*, *M. seenghala* and *W. attu* are the fast growing and high priced carnivorous fish species in Pakistan. However, over the last few decades these fish species have suffered substantial decline in population due to higher rate of heavy metal contamination in their natural habitats. The conservation of these food fish species will help in the economic growth of the country. The previous studies have assessed the toxic effects of metals on primary consumer fish (Athikesavan *et al*., 2006; Ameer *et al*., 2013; Shaukat and Javed, 2013) but no studies have been

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conducted on the effects of water-borne metals on secondary and tertiary consumers i.e. carnivorous fish species in Pakistan. Thus, the objectives of this study were to determine the acute toxicity of water-borne Cd, in terms of 96 h LC_{50} and lethal concentrations, for the three carnivorous fish species, *C. marulius*, *M. seenghala* and *W. attu*, belonging to three length groups, to metal toxicity and its accumulation in the fish.

Materials and Methods

Acute Toxicity Assay

Three fish species viz. *C. marulius*, *M. seenghala* and *W. attu* were procured from the Shanawan fish hatchery, Head Qadirabad, and kept in cemented tanks supplied with flow through aerated water and acclimated for 6 days before conducting acute toxicity tests. Keeping in view their carnivorous feeding habits, fish were provided with ample feed with 45% protein and gross energy of 3.50 kcal g^{-1}. The fish were fed daily @4% of their body weight at 11 and 15 h daily. Each fish species was divided into three length groups viz. 50, 100 and 150 mm having weights given in Table 1.

Toxicity tests (96 h LC_{50} and lethal concentrations) for the experimental fish species were conducted at constant water temperature (28ºC), pH (8) and total hardness (250 mg L^{-1}) with three replications for each test concentration. The glass aquaria of 50 L water capacity were washed thoroughly and filled with 35 L de-chlorinated tap water. A group of ten fish were selected and tested against each test concentration of Cd, separately, under controlled laboratory conditions. Fresh air was supplied to each aquarium through a pump to maintain sufficient oxygen for fish respiration. Fish were not fed during acute toxicity trials. Stock solution (1000 ppm) of Cd was prepared in de-ionized water by using analytical grade cadmium chloride ($CdCl_2.H_2O$). The test concentration of metal was started from zero with an increment of 0.01 and 0.1 mg L^{-1} for low and high concentrations, respectively, up to the concentration at which 50 and 100% fish mortality occurred during 96 h. In order to minimize stress on the fish, the concentration of Cd in each aquarium was increased gradually as 50% test concentration maintained within 3 h and full toxicant concentration in 6 h. Fish mortality data were collected and analyzed through Probit analysis method (Hamilton *et al*., 1977).

Bioaccumulation Assay

At each 96 h LC_{50} and lethal concentration of Cd, dead fish were isolated and lightly blotted dry at the time of mortality. No mortality was observed among control fish groups. The dead fish were dissected and their organs (gills, kidney, liver, muscles) separated, rinsed with distilled water and analyzed for Cd concentration by following the methods of S.M.E.W.W. (1989) using Atomic Absorption Spectrophotometer (AAnalyst 400 Perkin Elmer, USA).

Statistical Analyses

Three replicates for treatment were used to calculate mean values of 96 h LC_{50} and lethal concentrations for each fish species with 95% confidence interval by using the Probit analyses (Hamilton *et al*., 1977) with the help of MINITAB computer package. Means were computed by using Tukey's Student Newman-Keul tests and a value of $p<0.05$ was accepted as statistically significant (Steel *et al*., 1996).

Results

Acute Toxicity of Cd to the fish

Among the three fish species *C. marulius* exhibited significantly higher average 96 h LC_{50} value of 101.25 mg L^{-1} for all the three length groups. However, *M. seenghala* showed significantly higher sensitivity with least value (53.28 mg L^{-1}) of lethal concentration of Cd for all length groups. At both 96 h LC_{50} and lethal concentrations, 50 mm length groups of all the three fish species were significantly more sensitive to Cd followed by that of 100 mm and 150 mm length groups (Table 2).

Bioaccumulation of Cd in Fish

Three length groups of all fish species showed significantly variable ability to bio-accumulate Cd in their bodies during 96 h LC_{50} exposure (Table 3). Among the three fish species, *C. marulius* showed significantly greater ability to amass Cd in its body followed by that of *W. attu* and *M. seenghala*. However, the fish with 150 mm length showed significantly higher ability to accumulate Cd with a mean value of 193.21 µg g^{-1} followed by 100 and 50 mm length groups. The liver of all length groups of three fish species exhibited significantly higher ability to amass Cd followed by kidney, gills, blood and muscles. However, the tendency of three fish species to amass Cd followed the order: *C. marulius* > *W. attu* > *M. seenghala.* At lethal concentration exposures, Cd accumulation in the fish body was significantly variable in three length groups and that varied among various fish organs also. *C. marulius* exhibited significantly higher ability to amass Cd followed by *W. attu* and *M. seenghala*. The liver of all the three fish species accumulated significantly higher Cd (812.24 µg g^{-1}) while muscles showed significantly least tendency to amass this metal with a mean value of 50.30 µg g^{-1} (Table 3). The tendency of fish to accumulate this metal followed the order: liver > kidney > gills > blood > muscle.

Discussion

In Pakistan metallic ion pollution of aquatic habitats has become serious concern to the sustainability of aquatic animals, including fish (Javed, 2015). The prevailing met`allic ion pollution is causing adverse effects on the diversity and condition factor of indigenous fish species in the natural water bodies as they are in direct contact with

Table 1: Average wet weights (g) of three fish species

Length Groups	Fish Species	Average weight (g)
	Channa marulius	3.22±0.56
50 mm	*Mystus seenghala*	3.28±0.47
	Wallago attu	4.37±0.64
	Channa marulius	5.32±0.65
100 mm	*Mystus seenghala*	4.06±0.45
	Wallago attu	8.23±1.10
	Channa marulius	7.49±0.94
150 mm	*Mystus seenghala*	6.48±0.53
	Wallago attu	15.65±2.57

Table 2: Acute toxicity of cadmium (96 h) for three fish species

Treatments	Length groups	Fish species			
		Channa marulius	*Mystus seenghala*	*Wallago attu*	Overall Means*
96 h LC_{50}	50 mm	86.74±3.88a	23.86±1.72c	29.45±1.93 b	46.67±34.81c
	100 mm	97.00±3.76 a	27.95±2.08 c	39.04±2.10 b	54.66±37.08 b
	150 mm	120.01±3.89a	39.04±2.10 c	49.65±2.19 b	69.57± 44.01 a
Overall Means		101.25±17.04 a	30.27±7.87 c	39.38±10.10 b	
Lethal concentrations	50 mm	150.23±7.56 a	41.83±3.23 c	50.97±3.70 b	81.01±60.12 c
	100 mm	158.19±7.84 a	52.83±4.26 c	65.17±4.30 b	92.06±57.60 b
	150 mm	180.23±7.51 a	65.17±4.30 c	78.23±4.58 b	107.88±63.00 a
Overall Means		162.88±15.54 a	53.28±11.68 c	64.79±13.63 b	

Means with similar letters in a single row and *column are statistically similar at p<0.05

Table 3: Accumulation of cadmium (μgg^{-1}) in the fish body organs during 96 h acute toxicity exposures

Tissues	Gills	Kidney	Liver	Muscles	Blood	Overall Means*
At 96 h LC_{50}						
Length groups						
50 mm	134.85±79.92 c	140.56±59.30 b	160.06±72.99 a	7.90±3.03 e	89.66±25.23 d	106.61±60.91 c
100 mm	154.67±89.42 c	188.51±118.02 b	222.20±105.49a	29.28±11.76 e	117.66±37.66 d	142.46±74.25 b
150 mm	164.73±96.55 c	285.58±129.79 b	307.24±79.75 a	47.20±22.86 e	161.31±40.18 d	193.21±105.69 a
Overall Means	151.42±15.20 c	204.88±73.88 b	229.83±73.89 a	28.13±19.68 e	122.88±36.11 d	
Fish species						
Channa marulius	243.01±28.18 c	309.71±113.07 a	307.10±68.84 b	39.08±26.52 e	151.63±40.09 d	210.11±119.67 a
Mystus seenghala	6 6.68±12.43 d	107.10±45.60 b	141.67±67.28 a	15.07±8.48 e	85.00±27.75 c	83.11±54.73 c
Wallago attu	144.56±144.56 c	197.83±70.04 b	240.73±95.57 a	30.22±24.78 e	132.00±41.07 d	149.07±87.99 b
Overall Means	151.42±88.36 c	204.88±101.49 b	229.83±83.25 a	28.13±12.14 e	122.88±34.24 d	
At 96 h Lethal Concentrations						
Length groups						
50 mm	548.41±50.93 c	594.55±40.34 b	613.32±45.72a	31.88±11.43 e	201.48±47.31 d	397.93±264.71 c
100 mm	599.31±87.57 c	770.49±52.43 b	822.61±156.23 a	46.63±15.25 e	303.17±87.07 d	508.44±328.27 b
150 mm	695.45±102.37 c	990.80±79.92 b	1000.80±66.74 a	72.40±20.13 e	399.45±96.51 d	631.78±398.75 a
Overall Means	614.39±74.67 c	785.28±198.54 b	812.24±193.95 a	50.30±20.51 e	301.37±99.00 d	
Fish species						
Channa marulius	685.53±105.31 c	835.93±213.05 b	895.26±210.26 a	63.40±22.50 e	367.02±120.89 d	569.43±347.93 a
Mystus seenghala	528.59±52.76 c	725.84±171.60 a	721.58±195.22 b	33.54±14.52 e	217.13±73.16 d	445.34±305.02 c
Wallago attu	629.06±68.11 c	794.07±211.93 a	819.89±196.77 a	53.97±24.80 e	319.94±104.60 d	523.39±327.54 b
Overall Means	614.39±79.49 c	785.28±55.57 b	812.24±87.09 a	50.30±15.26 e	301.37±76.65 d	

Means with similar letters in a single row and *column are statistically similar at p<0.05

pollutants and cannot avoid the harmful effects of a wide variety of toxicants exerting profound effects on their physiology (Vutukuru, 2005). *M. seenghala* showed significantly higher sensitivity towards Cd toxicity followed by *W. attu* and *C. marulius*. Significant variations in the sensitivity of three fish species towards Cd were attributed to significant changes that accrued in the physiology of different fish species during both acute and chronic exposure stress (Kousar and Javed, 2015). Bull trout has been reported as the most tolerant species against toxicity of both Cd and Zn than rainbow trout. This tolerance ability appeared species specific and characterized by the less ability to lose Ca^{2+} (Kamunde and MacPhail, 2011). All length groups of three fish species showed significant variations for their sensitivity to tolerate Cd toxicity. The 50 mm length group of fish showed significantly higher sensitivity followed by 100 and 150 mm groups with statistically significant differences. This revealed metallic ion toxicity on the fish species that decreased with fish size (Ansari *et al*., 2006) relating to their age (Kousar and Javed, 2014). This might be due to significant changes in fish metabolism (James *et al*., 2003). Younger fish has been reported significantly more sensitive to the toxicity of Co and Cd by Yaqub and Javed (2012).

The knowledge on the distribution of metals in fish tissues is important to forecast their sensitivity against various metallic ions and to see the patterns of metals bio-accumulation and the rate of amassing in different organs of fish (Gbem *et al*., 2001). The metals would be accumulated in the fish body when the absorption rate exceeds the elimination rate of the body (Zhou *et al*., 2008). During both 96 h LC_{50} and lethal concentration, the accumulation of metals in the organs of all the three fish species followed the order: liver > kidney > gills > blood > muscle. Physiological functions of various fish organs are responsible for the accumulation of metals (Karuppasamy, 2004). Liver, being a regulatory organ, is a main site, where bioaccumulation of metals takes place because of its detoxifying nature through the production of metallothionein (Canli and Atli, 2003). The cysteine rich metallothioneins are synthesized by the liver to regulate metal ions in the fish body (Demirak *et al*., 2006). However, the amount of various metals in the fish organs depends upon their affinity to uptake specific metals (Erdogrul and Ates, 2006; Kousar and Javed, 2015) along with ability of various metals to react with sulfur, oxygen carboxylate, nitrogen of mercapto group or amino groups of liver metallothionein (Al-Yousuf *et al*., 2000). Fish liver showed significantly higher accumulation of Cd followed by kidney and gills. This shows metallic ion movement from the tissues and blood towards liver and kidney to detoxify the tissues (Vinodhini and Narayanan, 2008) and ultimately resulting in significant lowering of metallic ions in the fish muscles.

Among the three fish species, *C. marulius* showed significantly higher ability to accumulate Cd in its body while these accumulations followed the order: *C. marulius* > *W. attu* > *M. seenghala*. A less sensitive fish species exhibited significantly higher ability to amass metals in its body during acute exposures. The accumulation of Cd in three length groups of fish followed the order: 150 mm > 100 mm > 50 mm. These findings indicate size/growth related accumulation of Cd that was correlated positively with the extent of their sensitivity towards metallic ions that changed with fish length/age (Kotze *et al*., 1999). Kousar and Javed (2015) has also reported species and metallic ions specific accumulations of metals in *Cyprinus carpio* and *Ctenopharyngodon idella*. The nature of metallic ion species and the level of metallothioneins, ability of gills to transport various metals across their lamellae and fish metabolic rate are the other factors affecting bio-accumulation of various metals in the fish body (Chen and Folt, 2000).

Conclusion

Present investigation revealed variable toxicity of Cd to the three carnivorous fish species. Among the three fish species, *M. seenghala* showed significantly higher sensitivity towards Cd toxicity followed by *W. attu* and *C. marulius*. However, 50 mm length group of all the three fish species exhibited significantly higher sensitivity towards Cd, followed by 100 and 150 mm length groups with significant differences. The tendency of three fish species to accumulate Cd in their body followed the order: liver > kidney > gills > blood > muscle.

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