INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0794/2017/19–6–1566–1570
DOI: 10.17957/IJAB/15.0472
http://www.fspublishers.org

Full Length Article

Bioaccumulation of Metals in Fish, *Channa marulius*, *Mystus seenghala* and *Wallago attu* during Acute Toxicity Exposures

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Abstract

Laboratory experiments were conducted to ascertain the acute toxicity of chromium (Cr), cobalt (Co), copper (Cu) and nickel (Ni) for three carnivorous fish species viz. *Channa marulius*, *Mystus seenghala* and *Wallago attu*. During acute exposure, the tendency of these fish species to accumulate metals has also been determined. Mean sensitivity of three fish species, determined in terms of 96 h LC_{50}, towards metals was Cu>Cr>Co>Ni. During both 96 h LC_{50} and lethal concentrations, the accumulation pattern of metals in the organs of all the three fish species was liver>kidney>gills>blood>muscle. However, bioaccumulation tendency of all metals in the fish organs showed positive dependence on their uptake affinity. Among the three fish species, *C. marulius* exhibited significantly higher ability to bio-accumulate all metals in its body followed by *W. attu* and *M. seenghala*. The variable accumulation patterns of all the four metals in three fish species were correlated positively ($p<0.05$) to their sensitivity towards toxicity of metals. © 2017 Friends Science Publishers

Keywords: Carnivorous fish; 96 h LC_{50}; Lethal concentration; Accumulation

Introduction

Heavy metals like Cu, Pb, Cr, Mn, Fe, As, Hg, Cd, Zn, Ni and Co are the common river pollutants in the Punjab province. These metals cause adverse health hazards on the indigenous fish fauna (Rauf *et al*., 2009; Javed, 2015). The exposure of metals may modify the fish behaviour, metabolism, physiology, growth and reproduction (Gohil and Mankodi, 2013). Metals contaminated waters are adversely affecting the ecological balance and biodiversity of the recipient environment (Joshi, 2014). Fish are among the major components of aquatic habitats therefore, they may act as bio-indicators of metal pollution in the aquatic ecosystems. Conservation of fish in their natural habitat make it essential to determine their growth potentials and ability to bio-accumulate metals during acute exposure of waterborne metals. Some metals are essential for normal metabolic processes of fish like Zn, Cu and Fe, while others viz. Hg, Pb and Cd have no known function in the organisms. The essential metals are taken up from the surrounding water or food by the fish but their excessive intake may cause lethal effects on the fish (Maret, 2016).

The curiosity in toxicological studies has been steered towards development of various laboratory tests for the determination of water-borne acute toxicity of metals (Anandhan and Hemalatha, 2009). Acute toxicity testing is an imperative tool to measure the conceivable consequences of specific toxicants, like metals, that are frequently persistent in the natural aquatic habitats (Shuhaimi-Othman *et al*., 2010). The 96 h LC_{50} and lethal concentrations of a particular metal are used to determine the quantifiable factors like survival and mortality of the test organisms and to compare the sensitivity of various fish species toward toxicity of metals and other compounds (Azmat *et al*., 2012; Ilyas and Javed, 2013). In fish, the determination of 96 h LC_{50} and lethal concentration values are used as a standard tool for the assessment of sensitivity of a fish towards a specific metal (Reda *et al*., 2010; Kousar and Javed, 2015). However, the vulnerability of fish against different metals varied significantly among fish species. Any metal that is non-toxic at higher concentration to a particular fish species may be less or more toxic at the same concentration to the other organisms (Kaushal and Mishra, 2013).

Acute toxicity tests play a significant role in sustainable management and conservation of fish natural aquatic habitats. *C. marulius*, *M. seenghala* and *W. attu* are fast growing high priced carnivorous fish species in Pakistan. In order to develop plans for their sustainable conservation in the aquatic bodies, it is necessary to define their tolerance limits against persistent metallic ion pollutants like Cr, Co, Cu and Ni that may impose genetic injury to the natural populations of these species. These metals can also adversely affect the fish growth, well-beings and may become genotoxic due to higher ability of carnivorous fish to bio-magnify metals in the food chain. The conservation of these food fish species will help in the

To cite this paper: Javed, M., S. Abbas and F. Latif, 2017. Bioaccumulation of metals in fish, *Channa marulius*, *Mystus seenghala* and *Wallago attu* during acute toxicity exposures. *Int. J. Agric. Biol*., 19: 1566–1570

economic development of the country. Therefore, present research work was conducted to ascertain the acute toxicity of Cr, Co, Cu and Ni for the three carnivorous fish species viz. *C. marulius*, *M. seenghala* and *W. attu* and their tendencies to bioaccumulate these metals.

Materials and Methods

Three fish species viz. *C. marulius*, *M. seenghala* and *W. attu* of 150 mm length groups were collected from Shanawan Fish Hatchery, Head Qadarabad and kept separately in cemented tanks for 10 days for acclimatization to laboratory conditions. To reduce predation among individuals, fish were fed with high protein feed (45% DP; 3.50 kcal g^{-1} DE), to satiation, thrice a day. Exposure media were renewed after every 24 h to remove feeding debris and fish fecal matter.

Preparation of Metal's Stock Solutions

Stock solutions of $CrCl_3.6H_2O$, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$ and $NiCl_2.6H_2O$ were prepared, separately, by using analytical grade compounds of Sigma Aldrich in de-ionized water by following standard procedure.

Acute Toxicity Assay

The acute toxicity of metals was determined in terms of 96 h LC_{50} and lethal concentrations for each fish species viz. *C. marulius* (7.49±0.94 g), *M. seenghala* (6.48±0.53 g) and *W. attu* (15.65±2.57 g), separately, each metal. Ten number of three fish species were taken separately for the collection of their mortality data during 96 h exposure of various concentrations of each metal. The fish were starved two days prior to the experiment and did not feed throughout the acute toxicity trials. However, in order to avoid cannibalism, each fish was kept in a separate aquarium with three replications for each test dose of metals. All acute toxicity trials, with three fish species, were conducted at constant water temperature, pH and total hardness of 28ºC, 8 and 250 mg L^{-1}, respectively. The stock solutions of metals were diluted to obtain the desired concentrations for the determination of 96 h LC_{50} and lethal concentrations for three fish species. The control fish were kept in metal free water for comparison. All the glass aquaria were filled with 35 L water and concentration of each metal was increased gradually to avoid any stress on the fish. The 50% test concentration of metal was reached in 3 h, while full concentration in 6 h. For the estimation of acute toxicity, the metal concentration was started from zero with an increment of 0.01 and 0.1 mg L^{-1} for low and high doses, respectively. Fresh air was continuously supplied to all the aquaria water to maintain sufficient oxygen for fish respiration. Fish mortality data were collected during 96 h and analyzed through Probit analysis method (Hamilton *et al.*, 1977).

Bio-accumulation of Metals during Acute Toxicity Exposures

At the end of each 96 h LC_{50} and lethal concentration exposure of each metal, the dead fish were isolated and lightly blotted dry at the time of mortality. No mortality was recorded in the control fish groups. Dead fish were removed from the test media, dissected and their liver, kidney, gills and muscle isolated, while blood samples were taken from the fish near to death. Samples of selected fish organs (wet) were digested in nitric acid and perchloric acid (3:1V/V) by following SMEWW (1989) to determine Cr, Co, Cu and Ni concentrations through Atomic Absorption Spectrophotometer (AAnalyst-400 Perkin Elmer, USA). The data were statistically analysed by using Factorial design (RCBD), while means were compared through Analysis of Variance and Tukey/student Newman-Keul tests.

Results

Acute Toxicity of Metals for the Fish

96 h LC_{50}: Table 1 shows significant ($p<0.01$) variations among *C. marulius*, *M. seenghala* and *W. attu* for their sensitivity to all the metals. Among three fish species, *C. marulius* were less sensitive to the toxicity of tested metals while *W. attu* showed significantly higher sensitivity. *C. marulius*, *M. seenghala* and *W. attu* were significantly ($p<0.05$) more sensitive to Cu with the average 96 h LC_{50} values of 72.65, 20.55 and 28.16 mg L^{-1}, while significantly least sensitive to Ni with LC_{50} values of 170.47 and 101.82 mg L^{-1}, respectively. *M. seenghala* exhibited significantly least sensitivity towards Co with the mean 96 h LC_{50} value of 78.67 mg L^{-1}. The average sensitivity of three fish species towards metals followed the order: Cu>Cr>Co>Ni.

Lethal Concentration of Metals for the Fish

The mean lethal concentrations of four metals for three fish species varied significantly (Table 1). There existed non-significant differences among replications for each fish species and treatment. Among the metals, Cu caused significantly higher toxicity to all three fish species. *C. marulius* and *W. attu* showed significantly least sensitive to Ni with the average lethal concentrations of 211.33 and 132.58 mg L^{-1}, respectively. However, *M. seenghala* showed significantly least sensitivity towards Co (118.15 mg L^{-1}). *M. seenghala* were significantly more sensitive, while *C. marulius* showed least sensitivity towards toxicity of all metals. However, the mean sensitivity of three fish species towards all metals followed the order Cu>Cr>Ni>Co with statistically significant differences at $p<0.05$.

Table 1: Acute toxicity of different metals to three fish species

Exposure time	Species	Metal treatments				*Means±SD
		Chromium	Cobalt	Copper	Nickel	
96 h LC_{50}	*Channa marulius*	156.39±2.80 c	175.57±3.56 b	90.97±2.76 d	192.89±2.95 a	153.96±44.56 a
	Mystus seenghala	59.37±2.56 c	96.32±2.89 a	26.25±1.32 d	85.32±2.49 b	66.82±31.17 c
	Wallago attu	62.64±2.67 c	98.11±2.72 b	36.87±1.24 d	112.78±2.73 a	77.60±34.36 b
	Means±SD	92.80±55.09 c	123.33±45.25 b	51.36±34.71 d	130.33±55.89 a	
96 h lethal concentration	*Channa marulius*	194.40±6.06 c	233.90±7.73 b	126.61±5.52 d	234.28±6.23 a	197.30±50.70 a
	Mystus seenghala	91.08±5.21 c	135.90±6.51 a	49.46±2.65 d	117.19±5.26 b	98.41±37.45 c
	Wallago attu	97.28±5.53 c	133.84±5.12 b	58.46±2.41 d	147.72±6.75 a	109.33±40.03 b
	Means±SD	127.59±57.95 c	167.88±57.18 a	78.18±42.19 d	166.40±60.74 b	

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

Bio-accumulation of Metals in Fish During Acute Exposures

Accumulation of metals in fish at 96 h LC_{50} exposures: At 96 h LC_{50} exposure of each metal the dead fish were isolated from the media and their respective exposure metal was determined in their gills, kidney, liver, muscle and blood. Three fish species showed significant differences for the ability to amass metals in their body organs (Table 2). Fish gills, kidney, liver, muscle and blood showed significant variations for the accumulation of Cr during 96 h LC_{50}. However, these accumulations followed the order: liver > kidney > gills > blood > muscle. Cobalt concentrations in all the organs of three fish species showed significant variability. The abilities of three fish species to amass Co varied significantly as *C. marulius* >*W. attu* > *M. seenghala*. Liver in all of three fish species showed significantly higher ability to bio-accumulate Co. The ability of different fish organs to amass Co followed the order: liver > kidney > gills > blood > muscle. The 96 h LC_{50} exposure of Cu to the three fish species caused significant bio-accumulation of Cu that varied among organs. The liver of three fish species showed significantly higher quantity of Cu, while fish muscle had significantly lower content of Cu. *C. marulius* accumulated significantly higher Ni than the other two species of fish. Significantly higher quantity of Ni was accumulated in the fish liver, followed by kidney, gills, muscle and blood.

Accumulation of metals in fish at lethal concentration exposures: Among three fish species, *C. marulius* showed significantly higher Cr, followed by *W. attu* and *M. seenghala*. However, the concentration of Cr varied significantly among fish organs as liver > kidney > gills > blood > muscle. All the three fish species accumulated significantly ($p < 0.05$) variable quantity of Co in their body organs during lethal concentration exposures. Fish liver exhibited greatest ability to amass Co, followed by kidney, gills, blood and muscle with significant differences. Copper accumulation in all the three fish species varied significantly with a higher mean accumulation of 527.59 $\mu g g^{-1}$ in *C. marulius*, followed by *W. attu* and *M. seenghala*. Considering the overall responses of three fish species towards Ni accumulation, *M. seenghala* showed significantly least tendency to bio-accumulate this metal while the same was maximum in the body organs of *C. marulius*. Liver showed significantly higher while that of muscle exhibited significantly lower ability to amass all the metals during 96 h lethal concentration exposures (Table 3).

Discussion

During present investigation, the fish mortality criterion was used as metal's toxicity index. The sensitivity of three fish species, in terms of acute toxicity (96 h LC_{50} and lethal concentrations) of water-borne Cr, Co, Cu and Ni varied significantly at $p < 0.05$. *M. seenghala* showed more sensitivity towards all metals followed by *W. attu* and *C. marulius*. Significant variations in the sensitivity of three fish species to metals are attributed to significant changes that occurred in the physiology of different fish species during acute exposure stress (Shaukat, 2015). The heavy metals toxicity and their bioaccumulation in fish have been reviewed (Adami *et al*., 2002; Al-Weher, 2008; Dimari and Hati, 2009). Remarkable changes in the tolerance limits of *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* for Cr toxicity have also been reported (Azmat and Javed, 2011).

Among the metals, Cu was significantly more toxic to the three fish species determined in terms of both 96 h LC_{50} and lethal concentration. Copper is a Fenton metal with an ability to participate in the redox cycling to form reactive oxygen species "the hydroxyl (-OH) radicals". The formation of reactive oxygen species (ROS) in the animals can induce DNA damage, while DNA repair process is brought about due to binding of copper (Cu^{2+}) to the critical sites of specific enzymes (Guecheva *et al*., 2001). As compared to the other tested heavy metals, Cu caused significant mortality in the fish, hence proved to be the most toxic (Aldoghachi *et al*., 2016). Grosell *et al*. (2002) described an acute toxicity of Cu to the rainbow trout due to inhibition of gills bronchial Na^+ and Cl^- uptake that ultimately lead to mortality of fish.

The knowledge on the metals distribution in the fish organs is important to forecast their sensitivity against various metallic ions and to see the patterns of metals bioaccumulation and the rate of amassing in different organs of fish (Gbem *et al*., 2001). During acute toxicity exposure of Cr, Co, Cu and Ni, the accumulation of these metals in the organs of all the three fish species followed the order:

Table 2: Accumulation of metals (µg g^{-1}) in the body organs of fish during 96 h LC_{50} exposures

Metal	Fish species	Organs					*Overall means
		Gills	Kidney	Liver	Muscle	Blood	
Chromium	*Channa marulius*	255.37±49.85 c	310.11±79.86 b	347.72±113.36 a	50.85±37.01 e	187.66±47.60 d	230.34±123.96 a
	Mystus seenghala	73.35±8.26 d	130.99±50.50 b	159.65±103.47 a	20.42±12.82 e	113.01±39.29 c	99.49±68.27 c
	Wallago attu	165.49±13.46 d	232.29±125.68 b	234.57±55.79 a	40.59±30.89 e	169.71±45.19 c	168.53±92.04 b
	Overall means	164.74±91.01 c	224.47±89.81 b	247.31±94.68 a	37.29±15.48 e	156.79±38.97 d	
Cobalt	*Channa marulius*	278.14±36.53 b	347.69±130.58 a	347.97±75.33 a	58.16±40.02 d	214.50±54.43 c	249.29±128.52 a
	Mystus seenghala	126.18±52.04 d	180.75±109.24 b	216.76±89.21 a	32.27±22.14 e	164.97±37.82 c	144.18±88.11 c
	Wallago attu	177.90±21.32 d	228.21±73.80 b	274.17±96.62 a	46.96±32.74 e	190.10±49.62 c	183.47±94.25 b
	Overall means	194.07±77.26 c	252.22±86.02 b	279.63±65.78 a	45.80±12.99 e	189.85±24.76 d	
Copper	*Channa marulius*	217.70±36.53 c	275.39±59.33 b	298.02±95.87 a	35.23±25.32 e	118.65±42.26 d	188.99±113.15 a
	Mystus seenghala	64.05±18.87 c	77.66±35.55 b	113.07±47.53 a	10.92±6.99 e	62.40±27.52 d	65.62±42.74 c
	Wallago attu	106.89±9.73 c	169.31±52.31 b	201.50±76.11 a	22.61±20.48 e	94.74±31.77 d	119.01±74.74 b
	Overall means	129.55±79.29 c	174.12±98.95 b	204.20±92.50 a	22.92±12.16 e	91.93±28.23 d	
Nickel	*Channa marulius*	284.16±53.47 c	352.14±97.76 b	378.65±95.70 a	62.85±40.28 e	238.18±54.12 d	263.19±130.82 a
	Mystus seenghala	113.76±48.80 d	164.51±90.70 b	201.37±88.07 a	28.60±19.98 e	139.73±39.59 c	129.59±80.82 c
	Wallago attu	191.62±19.61 d	285.42±100.36 b	287.85±123.14 a	52.69±37.94 e	211.46±50.42 c	205.81±110.01 b
	Overall means	196.51±85.30 c	267.35±95.11 b	289.29±88.65 a	48.05±17.59 d	196.46±50.91 c	

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

Table 3: Accumulation of metals (µg g^{-1}) in the body organs of fish during 96 h lethal concentration exposures

Metal	Fish species	Organs					*Overall means
		Gills	Kidney	Liver	Muscle	Blood	
Chromium	*Channa marulius*	799.48±151.48 c	898.60±247.16 b	926.77±258.04 a	68.08±23.71 e	408.10±115.45 d	620.21±376.96 a
	Mystus seenghala	614.77±168.81 c	636.40±56.12 b	807.41±202.18 a	41.65±16.36 e	247.84±73.72 d	469.61±309.66 c
	Wallago attu	708.92±178.68 c	782.55±208.27 b	853.09±241.60 a	58.90±23.72 e	373.39±132.49d	555.37±341.62 b
	Overall means	707.73±92.36 c	772.52±131.39 b	862.42±60.23 a	56.21±13.42 e	343.11±84.31 d	
Cobalt	*Channa marulius*	722.52±112.82 c	924.36±274.26 b	943.56±220.99 a	73.21±24.16 e	441.15±131.94 d	620.96±370.90 a
	Mystus seenghala	634.41±72.81 c	792.30±207.07 b	874.02±219.11 a	53.08±19.19 e	300.68±88.18 d	530.90±342.57 c
	Wallago attu	686.42±90.39 c	856.26±258.04 b	918.94±207.06 a	68.36±21.55 e	408.03±128.60 d	587.60±353.56 b
	Overall means	681.11±44.29 c	857.64±66.04 b	912.17±35.26 a	64.88±10.51 e	383.29±73.43 d	
Copper	*Channa marulius*	627.46±88.62 c	805.69±233.31 b	839.92±185.50 a	54.84±18.74 e	310.02±107.28 d	527.59±336.39 a
	Mystus seenghala	496.12±34.69 c	646.91±165.69 b	724.22±193.46 a	25.09±12.47 e	192.18±62.16 d	416.90±294.37 c
	Wallago attu	600.36±72.91 c	685.28±174.97 b	782.93±177.00 a	46.97±22.49 e	275.58±101.82 d	478.23±303.47 b
	Overall means	574.65±69.34 c	712.63±82.85 b	782.36±57.86 a	42.30±15.42 e	259.26±60.59 d	
Nickel	*Channa marulius*	769.61±120.52 c	919.29±233.43 b	989.54±267.64 a	79.95±25.50 e	457.88±136.24 d	643.25±379.00 a
	Mystus seenghala	608.49±73.10 c	770.71±207.39 b	844.87±203.27 a	48.86±17.67 e	277.09±74.86 d	510.01±333.95 c
	Wallago attu	728.57±96.37 c	819.40±238.18 b	938.25±218.96 a	71.51±21.46 e	427.64±129.93 d	597.07±351.20 b
	Overall means	702.22±83.73 c	836.47±75.75 b	924.22±73.35 a	66.77±16.07 e	387.54±96.83 d	

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

liver > kidney > gills > blood > muscle. The accumulation pattern of four metals in the fish organs followed the order Ni > Co > Cr > Cu as amassing of metals is dependent upon the physiological functions of various fish organs (Karuppasamy, 2004). Fish liver showed significantly higher ability to accumulate all metals, followed by kidney and gills. This shows metallic ion movement from the tissues and blood towards liver and kidney for the purpose of detoxification process in the liver (Vinodhini and Narayanan, 2008; Javed *et al.*, 2016) and ultimately resulted into significant lowering of metallic ions in the fish muscle. Liver was the main site for bioaccumulation of metals due to its detoxifying nature through production of metallothioneins (Ghedira *et al*., 2010). Increased exposure of metals to the fish induces the production of metals binding proteins i.e., metallothioneine (MT) in the body organs (M'kandawire *et al*., 2017). MT helps in detoxification of metals through their accumulation in the liver and regulation in the body (Oliveira *et al*., 2010). Among the three fish species, *C. marulius* showed significantly higher ability to bio-accumulate all the metals in its body. However, the accumulation pattern of these metals in three fish species followed the order *C. marulius* > *W. attu* > *M. seenghala*. These significantly variable accumulation patterns of metals in three fish species correlated to their metallic ions sensitivity. Therefore, the

less sensitive fish showed significantly higher ability to accumulate metals in its body organs during acute exposures.

Conclusion

Mean sensitivity of three fish species, determined in terms of 96 h LC_{50} followed the order: Cu > Cr > Co > Ni while lethal concentrations of metals for three fish were Cu >Cr > Ni > Co with statistically significant differences. At both 96 h LC_{50} and lethal concentration exposures, the accumulation of metals in the organs of three fish species followed the order: liver > kidney > gills > blood > muscle. Among the three fish species, *C. marulius* showed significantly higher ability to bio-accumulate all metals in its body, followed by *W. attu* and *M. seenghala*.

Acknowledgements

This research work was conducted under the project No. PSF/NSLP/P-AU (285) granted by the Pakistan Science Foundation, Islamabad (Pakistan) to the principal author.

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(Received 19 July 2017; Accepted 13 September 2017)