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Full Length Article

Toxicity and Bioaccumulation of Metals (Al and Co) in Three Economically Important Carnivorous Fish Species of Pakistan

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Abstract

The acute toxicity of aluminum (Al) and cobalt (Co), in terms of both 96 h LC_{50} and lethal concentrations, was determined for three fish species viz. *Channa marulius*, *Mystus seenghala* and *Wallago attu* under controlled laboratory conditions. The experiments were performed at constant water temperature (28ºC), pH (8) and total hardness (250 mg L^{-1}). At the end of toxicity trials, the dead fish were dissected, digested and then analyzed for respective exposure metal's concentrations in various body organs viz. gills, liver, kidney, heart, gut, intestine, muscles, bones and skin. There existed statistically significant variations at $p<0.05$ among the three fish species toward Al and Co acute toxicities (96 h LC_{50} and lethal concentrations) that followed the order: *C. marulius*>*W. attu*>*M. seenghala*. Statistically all the three species of fish were found more sensitive to Al than Co toxicity. During acute concentrations exposure, *C. marulius* exhibited significantly higher potential to accumulate Al and Co in its body followed by *W. attu* and *M. seenghala*. At both 96 h LC_{50} and lethal concentration exposures, all the three carnivorous fish species had significantly ($p<0.05$) higher tendency to concentrate both metals in their liver, followed by the kidney, gills, heart, gut, intestine, bones and skin. However, they exhibited significantly lower Al and Co concentrations in their muscles. The overall accumulation pattern of metals in the bodies of all three carnivorous fish species followed the order: Co>Al.

Keywords: Carnivorous fish; Metal toxicity; 96 h LC_{50}; Lethal concentration; Bioaccumulation

Introduction

Industrially developing countries, including Pakistan, are facing a severe threat to the stability of their freshwater ecosystems due to the metallic ions pollution (Chen *et al*., 2011). Owing to the non-biodegradability and ability to bio-concentrate in various organs, metals produce toxic effects in the aquatic organisms (Mendil *et al*., 2005). The main toxic effects result from metallic ions exposure are the production of reactive oxygen species and damage to the DNA (Zeeshan *et al*., 2017). The elevated concentrations of metals may cause various metabolic, physiological and behavioral alterations in the aquatic animals (Li *et al*., 2010). Among the aquatic organisms, carnivorous fish species are more susceptible to the contamination as they reside at the higher trophic level of aquatic food chain (Subotic *et al*., 2013). Hence, they could better indicate the pollution status of their respective ecosystem (Stergiou and Karpouzi, 2002). Aluminum (Al) does not have any biological function and is readily transferred through the food chains (Fallah *et al*., 2011). However, it enters into the aquatic environs as a pollutant through mining activities, water treatment plants as they employ alum (aluminum sulphate) for removal of suspended solid particles from water and through industrial effluents discharge. In rivers, acid precipitation has led to enhanced concentrations of trace element (i.e., Al), which may eventually result into huge fish mortality (Selvam *et al*., 2014). Cobalt (Co) serves as an integral component of cobalamin or vitamin B_{12} and hence considered as a vital element for the organisms (Blust, 2012). The combustion of fossil fuel, mining, industrial and agricultural wastes are predominant sources of Co discharge into the aquatic environments (Kim *et al*., 2006). Exposure of metals such as Al and Co may adversely affects development, behavior and viability of fish (Howe *et al*., 2014).

The characteristic property of an organism's response toward a chemical at a specific dosage or concentration for a specific period of time is the toxicity (Johnson and Radhakrishnan, 2015). In order to assess the potential toxic impacts of chemicals on the biology of aquatic animals and to evaluate their effects for a short time interval usually exposure for 96 h, the acute toxicity assays are designed (Haloi *et al*., 2014). The tolerance limits of different fish species toward metal toxicity could be evaluated by quantitative parameters such as survival or mortality of the test organism (Reda *et al*., 2010). The study related to accumulation of xenobiotics in various fish tissues provide

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insight about the pollution level of any aquatic ecosystem along with the possible harms of contaminated fish consumption (Shukla *et al*., 2007).

The population of three economically important carnivorous fish species viz. *Channa marulius*, *Mystus seenghala* and *Wallago attu* in riverine systems of Pakistan is declining (Rafique and Khan, 2012) because of uncontrolled and increasing metals contamination from the last several decades. This necessitates the determination of toxic effects of commonly found contaminants such as Al and Co to the three carnivorous fish species so that water quality guidelines could be devised in order to conserve the nutritionally significant fish species of Pakistan. Thus, the objectives of present study were to ascertain acute toxicity of Al and Co, in terms of 96 h LC_{50} and lethal concentrations, for the three carnivorous fish species viz. *C. marulius*, *M. seenghala* and *W. attu* and their accumulation in the body organs of fish.

Materials and Methods

The research work was conducted in the laboratories of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. The tolerance limits of three fish species viz. *C. marulius*, *M. seenghala* and *W. attu* were determined for Al and Co, separately, in terms of both 96 h LC_{50} and lethal concentrations. Three fish species were obtained from Head Qadirabad and brought to the wet laboratory for acclimatization in cemented tanks for two weeks. The fish were fed pelleted feed having 40% digestible protein and 3.50 kcal g^{-1} energy, thrice a day. However, fish were not fed during acute toxicity experiments. After acclimatization, the healthy individuals of 100 mm total lengths, for each fish species, were selected for acute toxicity trials. The average wet weights for each of the three fish species exposed separately to Al and Co are shown in Table 1.

Chemically pure compounds of Al ($AlCl_3.6H_2O$) and Co ($CoCl_2.6H_2O$) were dissolved, separately, in 1000 mL deionized water and stock solutions prepared for each metal. The toxicity tests were conducted in glass aquaria of 50 L water capacity at constant water temperature, pH and total hardness of 28°C, 8 and 250 mg L^{-1}, respectively.

Determination of 96 h LC_{50} and Lethal Concentrations

Ten individuals of each fish species were placed in each aquarium, with three replications for each test dose. The concentration of exposed metals was started from zero with an increment of 0.01 and 0.1 mg L^{-1} for low and high concentrations, respectively. The concentration of metals in each aquarium was increased gradually and 50% test concentration maintained within 3 h and full toxicant concentration in 6 h. Each exposure concentration was tested with three replications, separately for each fish species. To prevent any lowering of the exposed metals concentration, the media were renewed and the metallic ions concentration maintained after every 24 h. Constant aeration of the aquarium water was done with an air pump fitted with capillary system. During acute toxicity trials, the observations on fish mortality were made after every 2 h. The metals exposure concentrations were started from zero up to that concentration at which 50% (LC_{50}) and 100% mortality of fish (lethal concentration) occurred during 96 h. The dead fish were immediately removed from the test media and mortality data were recorded. During the whole experimental period, the physico-chemical variables of the test media viz. dissolved oxygen, carbondioxide, total ammonia, electrical conductivity, calcium, magnesium, sodium and potassium were analyzed on 12 h basis following the methods of APHA (2012).

Determination of Metals in Fish Body Organs

At the end of each acute toxicity trial of metals, dead fish were dissected and their gills, liver, kidney, heart, gut, intestine, muscles, bones and skin isolated. Fish organs were digested in HNO_3 and $HClO_4$ (3:1 V/V) by following the methods of SMEWW (1989) for the determination of respective exposure metal (µg g^{-1}) using Atomic Absorption Spectrophotometer (Perkin Elmer, AAnalyst-400).

Statistical Analyses

The acute toxicities of metals (Al and Co) were computed by using Probit analysis method (Hamilton *et al*., 1977). Mean values of 96 h LC_{50} and lethal concentrations of metals for each fish species were obtained at 95% confidence intervals. Data were statistically analyzed by using Factorial experiments (RCBD), while means were compared for statistical differences by employing Tukey's/Student Newman-Keul test (Steel *et al*., 1996).

Results

Toxicity

96 h LC_{50}: There existed statistically significant differences among three fish species towards Al toxicity. *M. seenghala* was significantly more sensitive to Al than *W. attu* and *C. marulius*, in terms of mean 96 h LC_{50} values. Statistically significant ($p<0.05$) variations were found for Co toxicity, among all the fish species. The tolerance limits of three fish species towards Co, in terms of mean 96 h LC_{50} values, followed the order: *C. marulius*>*W. attu*>*M. seenghala*. Al was found significantly more toxic to all the three carnivorous fish species than Co (Table 2).

96 h lethal concentrations: The mean lethal concentrations of Al for the fish species varied significantly between 75.56 and 164.85 mg L^{-1} as determined for *M. seenghala* and *C. marulius*, respectively. Among the fish species, *C. marulius* was significantly more tolerant to Co toxicity having mean

lethal concentration value of 201.04 mg L^{-1} (confidence interval of 192.37–215.11 mg L^{-1}), followed by *W. attu* and *M. seenghala*. Regarding overall toxicity of metals, Co was relatively less toxic to the fish than Al (Table 2).

Bioaccumulation

During 96 h LC_{50} exposure: All the three fish species exhibited ($p<0.05$) variable ability to concentrate Al in their bodies. Among the three carnivorous fish species, *C. marulius* had significantly greater potential to accumulate Al, followed by *W. attu* and *M. seenghala*. The overall pattern of Al accumulation in the selected body organs of three fish species differed significantly with the mean higher concentration in the liver, followed by kidney, gills, heart, gut, intestine, bones, skin and muscles. However, *C. marulius* had significantly higher mean metal concentration in its kidney, followed by liver, gills and heart. The order of Al accumulation in *M. seenghala* was liver > kidney > heart > gills ≥ gut > intestine. All the three species of fish exhibited significantly least Al amassing in their muscles (Table 3).

M. seenghala had significantly lesser Co concentration in its body organs than other two fish species. All the three fish species had significantly higher Co concentration in their liver while had lower concentration in skin and muscles. *W. attu* exhibited significantly higher concentration of Co in its liver, followed by that of kidney, heart, gills, gut, intestine and bones.

During 96 h lethal concentration exposure: Among the three species of fish, *C. marulius* exhibited significantly higher mean Al concentration in its body organs, followed by *W. attu* and *M. seenghala*. All the three carnivorous fish species had significantly ($p<0.05$) higher tendency to concentrate Al in their liver, followed by kidney, gills, heart, gut, intestine, bones, skin and muscles. However, *M. seenghala* exhibited greater potential to accumulate Al in its kidney, followed by liver, gills and heart (Table 3).

During 96 h lethal concentration exposure of Co, statistically significant variations were observed toward metal accumulation, among all the three carnivorous fish species. The order of Co accumulation in bodies of three fish species was *C. maruilus*>*W. attu*>*M. seenghala*. All the three fish species exhibited significantly greater potential to bioconcentrate Co in their liver, followed by kidney, gills, heart, gut, intestine, bones and skin while had significantly lesser ability to accumulate the same in their muscles.

Table 4 shows the overall accumulation pattern of metals (Al and Co) in the bodies of three carnivorous fish species, at 96 h acute (LC_{50} and lethal concentration) exposures. During 96 h LC_{50} exposure, all the three fish species exhibited significantly higher ability to amass Co (146.52 µg g^{-1}) in their bodies than Al (124.03 µg g^{-1}). During 96 h lethal concentrations exposure, the overall accumulation of metals in the bodies of all the three fish species followed the order: Co>Al.

Table 1: Average wet weights of 100mm fish used for Al and Co acute toxicity trials

Treatment/Metal	Species	Average wet weight (g)
Al	*C. marulius*	5.32±0.15
	M. seenghala	4.76±0.11
	W. attu	9.28±0.24
Co	*C. marulius*	4.66±0.17
	M. seenghala	4.29±0.09
	W. attu	9.53±0.21

Table 2: Calculated mean 96 h LC_{50} and lethal concentrations of metals for three fish species

Species	Treatments		*Overall Means
	Al	Co	
96 h LC_{50}			
C. marulius	114.97±3.28 a	158.90±2.96 a	136.94±31.06 a
M. seenghala	45.52±2.44 c	80.01±3.07 c	62.77±24.39 c
W. attu	74.24±2.66 b	92.36±2.62 b	83.30±12.81 b
Overall Means	78.24±34.90 b	110.42±42.43 a	
96 h lethal concentrations			
C. marulius	164.85±7.54 a	201.04±5.42 a	182.95±25.59 a
M. seenghala	75.56±4.94 c	120.62±5.84 c	98.09±31.86 c
W. attu	109.13±5.06 b	127.51±5.05 b	118.32±13.00 b
Overall Means	116.51±45.10 b	149.72±44.57 a	

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

Discussion

The discharge of metallic ions into the natural water bodies of Pakistan has caused serious issues such as; (i) mortality of carnivorous fish resulting into a substantial decline in their density and diversity, and (ii) accumulation of metals in the various body organs of carnivorous fish hence, making them unfit for human consumption. The results of present investigation showed that there existed significant ($p<0.05$) variations among the three carnivorous fish species regarding their abilities to tolerate the metals. The mean 96 h LC_{50} and lethal concentration values showed that *M. seenghala* was more sensitive to both Al and Co, followed by *W. attu* and *C. marulius* (Table 2). This may be due to the fact that inherent potential to tolerate the metallic ions stress varied from one species to another. The variations in toxicity of even the same metal to different fish species is linked to the differential metabolic and physiological rates of each fish species (Kaushal and Mishra, 2013). The metals after being taken up by the individual affect the activity of certain enzymes either by displacing or completely blocking the enzymes required for normal metabolic activities and also alter the normal immune system functioning (Torres *et al*., 2008; Authman *et al*., 2015). During present acute toxicity trials, all the three carnivorous fish species were found significantly more sensitive to Al than Co toxicity.

The individual effects of both these metals (Al and Co) were variable since each metal has a specific mode of action to cause toxicity to the exposed organism. However, both of these are the potential genotoxicants (Figgitt *et al*., 2010; Garcia-Medina *et al*., 2011).

Table 3: Accumulation of metals (µg g^{-1}±SD) in the body organs of fish during acute concentrations exposure

Species	Organs									
	Gills	Liver	Kidney	Heart	Gut	Intestine	Muscles	Bones	Skin	*Means±SD
96 h LC_{50} exposure										
Al										
C. marulius	242.34±0.32 c	309.54±0.35b	327.29±0.39 a	222.36±0.28 d	197.62±0.25 e	121.18±0.22 g	44.95±0.09 i	132.84±0.23 f	68.19±0.18 h	185.15±100.41 a
M. seenghala	75.61±0.21 d	138.77±0.27a	106.44±0.26 b	80.99±0.19 c	74.53±0.17 d	51.79±0.15 e	22.82±0.06 h	29.56±0.10 g	36.30±0.13 f	68.53±37.98 c
W. attu	155.29±0.28 c	225.36±0.36a	182.37±0.33 b	136.45±0.26 d	115.28±0.22 e	94.65±0.19 f	30.16±0.05 i	75.40±0.21 g	50.82±0.08 h	118.42±63.24b
Means±SD	157.75±83.39 c	224.56±85.39a	205.37±112.21b	146.60±71.23 d	129.14±62.71e	89.21±35.01 f	32.64±11.27 i	79.27±51.75 g	51.77±15.97 h	
Co										
C. marulius	282.18±0.27 c	375.25±0.24a	345.82±0.20 b	247.52±0.38 d	208.15±0.33 e	177.35±0.27 f	57.16±0.08 i	135.70±0.23 g	79.15±0.17 h	212.03±111.47 a
M. seenghala	103.29±0.19 c	177.06±0.15a	123.31±0.23 b	96.18±0.27 d	77.60±0.14 f	82.22±0.18 e	32.05±0.08 i	52.42±0.06 g	38.39±0.02 h	86.95±45.37 c
W. attu	166.10±0.24 d	244.10±0.13a	198.25±0.12 b	175.23±0.29 c	153.52±0.29 e	140.69±0.26 f	43.92±0.07 i	86.13±0.18 g	57.40±0.11 h	140.59±66.42b
Means±SD	183.86±90.76 c	265.47±100.81a	222.46±113.21b	172.98±75.70 d	146.42±65.56e	133.42±47.98f	44.38±12.56 i	91.42±41.89 g	58.31±20.40 h	
96 h lethal concentration exposure										
Al										
C. marulius	651.65±0.45 c	754.80±0.53 a	706.33±0.51 b	629.89±0.44 d	510.60±0.38 e	427.11±0.33 f	56.78±0.07 i	314.07±0.29 g	162.68±0.24 h	468.21±247.11 a
M. seenghala	517.89±0.38 c	582.12±0.42 b	621.05±0.40 a	463.14±0.33 d	387.42±0.27 e	288.39±0.21 f	42.03±0.08 i	199.59±0.16 g	101.05±0.12 h	355.85±209.96c
W. attu	552.20±0.43 c	704.36±0.56 a	668.92±0.52 b	507.05±0.46 d	468.09±0.41 e	395.65±0.38 f	49.15±0.11 i	220.36±0.32 g	139.14±0.26 h	411.66±230.87b
Means±SD	573.91±69.47 c	680.43±88.79 a	665.43±42.75 b	533.36±86.43 d	455.37±62.57e	370.38±72.73f	49.32±7.38 i	244.67±60.99 g	134.29±31.10h	
Co										
C. marulius	702.19±0.34 c	986.91±0.29 a	867.27±0.27 b	683.30±0.63 d	570.08±0.52e	492.88±0.44 f	69.25±0.10 i	375.61±0.42 g	254.22±0.37 h	555.75±292.59a
M. seenghala	645.54±0.21 c	904.25±0.19 a	735.38±0.31 b	527.12±0.56 d	402.46±0.43 e	339.11±0.31 f	48.31±0.09 i	132.59±0.18 h	159.30±0.20 g	432.67±293.78 c
W. attu	672.63±0.30 c	954.82±0.16 a	769.41±0.15 b	604.59±0.42 d	539.13±0.36 e	436.57±0.29 f	65.82±0.10 i	308.47±0.22 g	198.47±0.16 h	505.55±283.19b
Means±SD	673.45±28.33 c	948.66±41.67 a	790.69±68.47 b	605.00±78.09 d	503.89±89.19e	422.85±77.80 f	61.13±11.23 i	272.22±125.50g	204.00±47.70 h	

Means with different letters within a single row and *column are statistically significant at $p<0.05$

Table 4: Overall accumulation (µg g^{-1}±SD) of metals in the fish during 96 h exposure to acute concentrations of metals

Species	Treatments(µg g^{-1} of tissue)		
	Al	Co	*Overall means
96 h LC_{50} exposure			
C. marulius	185.15±100.41 b	212.03±111.47 a	198.59±19.01 a
M. seenghala	68.53±37.98 b	86.95±45.37 a	77.74±13.02 c
W. attu	118.42±63.24 b	140.59±66.42 a	129.51±15.68 b
Overall Means	124.03±58.51 b	146.52±62.75 a	
96 h lethal concentration exposure			
C. marulius	468.21±247.11 b	555.75±292.59 a	511.98±61.90 a
M. seenghala	355.85±209.96 b	432.67±293.78 a	394.26±54.32 c
W. attu	411.66±230.87 b	505.55±283.19 a	458.61±66.39 b
Overall Means	411.91±56.18 b	497.99±61.89 a	

Means with different letters in a single row and *column are statistically

Al toxicity involves oxidative damage to the tissues, chromosomal aberrations, DNA strand breaks, production of micronuclei and cell apoptosis, ultimately lead towards fish mortality (Ternjej *et al*., 2010). The exposure of elevated Co concentrations results in excessive generation of reactive oxygen species causing damage to the DNA hence, may affects the survival of fish (Alarifi *et al*., 2013).

The accumulation of metals in the bodies of three carnivorous fish species differed significantly ($p<0.05$) and followed the order: *C. marulius>W. attu>M. seenghala*. In the present investigation, we found that all the three fish species accumulated a higher amount of Co than Al (Table 4). The accumulation of metallic ions in the body of fish depends on ecological needs, feeding habitats, species, metabolism of the fish, metal's concentration and the route by which they get absorb i.e. either water or food (Mziray and Kimirei, 2016). Each fish species has differential ability to absorb, regulate and detoxify the metals within the body (Qadir and Malik, 2011). The metals interact with Ca^{2+}ATPases of the plasma membrane and hence enter into the cell (Ballatori, 2002). After absorption in the fish body, the metals are transferred by the blood stream to the various body organs where either they are detoxified, accumulated or excreted (Weber *et al*., 2013). During 96 h LC_{50} and lethal concentration exposures, it was found that overall accumulation pattern of metals in the body organs of fish varied significantly and followed the order: liver > kidney > gills > heart > gut > intestine > bones > skin > muscles. The inconstant amassing of metals in different organs of fish is related to the variable metabolic and physiological rates of each organ.

The metals accumulation in fish induces various enzymatic and histopathological alterations (Lionetto *et al*., 2000; Giari *et al*., 2007). As a result of elevated metallic ions concentration, the production of metallothioneins increases in the liver and kidney that ultimately bind the metals with them in order to avoid the possible harms to the living system. This situation leads to enhanced metal concentrations in the liver as this organ is principally involved in detoxification processes (Amiard *et al*., 2006; Zhao *et al*., 2012). Kidney is also considered as one of the important organs for metal's accumulation as it is involved

in reabsorption and excretion processes (Barbier *et al*., 2005). The gills are associated with continuous gas exchange mechanisms due to which the water-borne toxicants remain in direct contact with them, and hence amass higher amounts of metallic ions (Oliveira-Filho *et al*., 2010). The variable amassing of metals in the heart, gut, intestine, bones and skin of the fish is attributed to the differential exposure, absorption, regulation, retention and excretion rates of these organs (Storelli *et al*., 2006). During present investigation, the accumulation of Al and Co in muscles of all the carnivorous fish species was found minimum (Table 3) as they are metabolically less active than all the other studied organs (Elnabris *et al*., 2013).

Conclusion

Significant differences were observed among the three carnivorous fish species for the acute toxicity of metals, in terms of 96 h LC_{50} and lethal concentration values. Al was found more toxic to all the fish species than Co. During 96 h LC_{50} and lethal concentrations exposure, *C. marulius* exhibited higher propensity to accumulate both the metals in its body than *W. attu* and *M. seenghala*. All these carnivorous fish species showed a greater tendency to concentrate the metals in liver followed by the kidney, gills, heart, gut, intestine, bones and skin and the lowest in their muscles.

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