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RESEARCH ARTICLE

Heavy Metals Toxicity and Bioaccumulation Patterns in the Body Organs of Four Fresh Water Fish Species

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ABSTRACT

Various environmental pollutants, including metals can cause toxicological effects on aquatic animals especially fish species. Laboratory experiments were conducted to determine acute toxicity and bioaccumulation patterns of arsenic (As), nickel (Ni) and zinc (Zn) in 150-day old fish species (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*), separately, in glass aquaria under constant water temperature (30°C), total hardness (300 mg L^{-1}) and pH (7.5). *Catla catla* showed significantly ($P<0.05$) highest sensitivity to metals while *L. rohita* was least sensitive. Significantly variable accumulation of metals was observed in fish that followed the order: Zn>Ni>As. Among exposed fish species, *Cirrhina mrigala* exhibited significantly higher ability to amass Ni (146.8±149.1 µg g^{-1}) and Zn (243.0±190.5 µg g^{-1}), followed by *Ctenopharyngodon idella*, *Labeo rohita* and *Catla catla* at 96-h LC_{50}. Liver showed higher tendency to accumulate Ni, followed by gills and kidney with significant differences while kidney showed higher tendency to accumulate As, followed by liver. Fins and scales exhibited significantly ($P<0.05$) least tendency to accumulate all the three metals. Accumulation of metals in different fish species is the function of their membrane permeability, which is highly species specific. Due to this reason different fish species showed different amount of metal accumulated in their bodies. This study also reveals that the metals, being conservative in nature have higher ability of biomagnifications.

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INTRODUCTION

The contamination of natural aquatic resources, with heavy metals released from industrial, domestic and other anthropogenic activities has become a matter of concern over the past few decades (Waqar *et al*., 2013). Harmful effects of heavy metals on aquatic organisms can be detected by performing toxicity tests that allow establishing a dose-response relationship (Akter *et al*., 2008; Javed, 2013) which help us in predicting acute damage to aquatic fauna as well as in regulating toxic chemical discharges into the water bodies (APHA, 2005). Due to deleterious effects of metals on aquatic organisms, it is important to monitor their bioaccumulation patterns. This will give an indication of temporal and spatial extant of metal accumulation, as well as assessment of potential impact on human health (Ladipo *et al*., 2012). Fish being the top consumer in the aquatic food chain accumulate large amounts of heavy metals in their body (Chezhian *et al*., 2010). The chemicals once absorbed are transported by the blood to either a storage point (bones, liver), or further transported to other organs (kidney, gills, fat) (Dural, 2007).

Among metallic pollutants, As is one of the most dangerous single substance toxicants in the environment which is known to be carcinogenic and genotoxic (Chen *et al*., 2005) causing chromosomal abnormalities (Wen-Chien *et al*., 2001) to humans through the consumption of drinking water. As, in the form of trioxide, is commonly used in pharmaceuticals, pesticides, veterinary products and decolorizing agents making human beings more prone to its exposure (Bohrer *et al*., 2005). Ni is usually considered as immunotoxic, genotoxic and carcinogenic to living organisms (Kasprzak *et al*., 2003). Uptake and accumulation of Ni in different organs may result in disturbed tissue metal content ratio, alterations of organism's metabolism (Funakoshi *et al*., 1996) and lipid peroxidation (Chakrabarti and Bai, 1999). In spite of the well-known Ni toxicity in mammals, its mechanism of action in aquatic organisms, particularly in fish is still unclear (Pane *et al*., 2005). Zn, required as micronutrient by

the fish, can be obtained from water and diet (Wood, 2001). However, higher intake of Zn could cause harmful effects on fish health (Hayat *et al*., 2007).

In Pakistan, the fresh water reservoirs have been polluted due to several contaminants including heavy metals. The trace metals including Ni and Pb reflects low while Fe, Micronuclei, Zn, Cu, Cr and Co reflects their higher concentration in the system in the system under present scenario. The main reason of water pollution in Pakistan is the discharge of untreated industrial effluents that result in high level of pollution in the surface water as well as ground water. Despite elevated metal levels reported for many industrial receiving waters, these metal contaminants get little research interest for their toxicity to freshwater fish species. In order to conserve the indigenous fauna in their natural habitats (water bodies), it is important to determine their sensitivity and inherent potentials for the uptake and accumulation of metals.

MATERIALS AND METHODS

This experiment was conducted in wet laboratory at Fisheries Research Farms, University of Agriculture, Faisalabad.

Fish collection and acclimatization: 150-day old fish (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*) were collected from Fish Seed Hatchery, Faisalabad. Fish were transported to the laboratory in polythene bags with appropriate care and handling. Fish were acclimatized to laboratory conditions using dechlorinated tap water in cemented tanks for 10 days. During acclimatization, fish were fed to satiation on feed (34% DP and 3.00Kcal/g DE) twice daily however, the fish were not fed during acute toxicity tests. Water medium was replenished at 24-h intervals in order to remove feeding debris and the fecal matter.

Stock solution preparation: Stock solutions (10,000 mg L^{-1}) of As_2O_3 (Merck), $NiCl_2.2H_2O$ (Merck) and $ZnCl_2.2H_2O$ (Merck) were prepared by mixing their appropriate amount in 1 L deionized water, separately. The acute toxicity bioassay was conducted to determine 96-h LC_{50} and lethal concentrations of As, Ni and Zn for fish. Prior to start experiment, all the aquaria and glassware were washed thoroughly. After acclimatization fish with following weights and lengths were shifted from stock tanks to 50 L experimental glass aquaria.

Fish Species	Wet Weights (g)	Total Lengths (mm)
Labeo rohita	14.5±0.4	110.3±2.9
Cirrhina mrigala	11.3±0.7	101.5±1.2
Catla catla	19.7±0.2	121.4±2.4
Ctenopharyngodon idella	10.6±0.3	99.8±1.6

Appropriate volume of stock solutions of the each metal were poured slowly in the test media (aquaria), separately, to obtain desired exposure concentrations. To avoid sudden stress to experimental fish, metal concentration in aquarium water was increased gradually and 50% test concentration was maintained within 3.5-h and total toxicant concentration in 7-h. Dissolved oxygen of the test media was maintained with the help of aerator. Water temperature (30°C), total hardness (300 mg L^{-1}) and pH (7.5) were kept constant during experiment. Water temperature was maintained through electric heaters. The chemical i.e. $CaSO_4$ and EDTA were used to increase and decrease the water hardness, respectively. For acute toxicity trails, test concentrations of each metal for fish were started from zero with an increment of 0.05 mg L^{-1} and 5 mg L^{-1} for low and high concentrations, respectively. Each exposure concentration was tested in triplicate. Fish mortality during 96-h exposure period was obtained against each test concentration at 12-h intervals. Dead fish were taken out from the test media and slightly blotted dry. Mean values for 96-h LC_{50} and lethal concentrations were calculated for each test dose/treatment with 95% confidence intervals using probit analyses method (Ezeonyejiaku and Obiakora, 2011).

Metals bioaccumulation assay: Dead fish obtained from acute toxicity trial were dissected and bones, fins, gills, gut, kidney, liver, muscles, scales and skin were separated, rinsed with distilled water, blotted with blotting paper. Samples of fish organs (wet) were digested in HNO_3 and $HClO_4$ (3:1V/V) by following S.M.E.W.W. (1989) to determine metals viz. As, Ni and Zn concentrations through Atomic Absorption Spectrophotometer (Analyst-400 Perkin Elmer, USA). The data on various parameters were analyzed statistically by using Factorial design (RCBD), Analysis of Variance and Tukey/student Newnan-Keul tests.

RESULTS

Acute toxicity of metals: All the four fish species were tested for acute toxicity (96-h LC_{50} and lethal concentrations) of As, Ni and Zn. The data presented in Table 1 shows that 96-h LC_{50} and lethal concentrations of metals for the four fish species varied significantly.

The 96-h LC_{50} values of As were recorded as 30.0±0.0, 24.5±0.1, 10.2±0.2 and 22.2±0.0 mg L^{-1} for *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*, respectively. While the highest value of lethal concentration of As was recorded as 40.2±0.0 mg L^{-1} for *Labeo rohita* followed by 32.1±0.0, 29.7±0.0 and 14.1±0.2 mg L^{-1} for *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Catla catla*, respectively (Table 1).

Mean 96-h LC_{50} values of Ni varied significantly between *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*. Whereas, *Cirrhina mrigala* was least sensitive species having significantly higher mean LC_{50} value (100.69±0.16 mg L^{-1}) than *Labeo rohita, Catla catla* and *Ctenopharyngodon idella*. However, non-significant difference was observed between *Catla catla* and *Ctenopharyngodon idella* for their 96-h LC_{50} values. Furthermore, mean lethal concentration of Ni varied significantly between 131.8±0.3 mg L^{-1} (*Cirrhina mrigala*) and 58.1±0.3 mg L^{-1} (*Catla catla*) (Table 1).

Labeo rohita showed significantly higher tolerance limits, in- terms of 96-h LC_{50} and lethal concentrations, for Zn followed by *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Catla catla* (Table 1).

Metal Bioaccumulation: Table 2 shows the accumulation patterns of As, Ni and Zn in fish body organs at 96-h LC_{50} and lethal concentrations exposure. There were significant

Table 1: Mean 96 h LC_{50} and lethal concentrations (mg L^{-1}±SD) of metals for 150-day fish species

Hours/Metals	Fish Species			
	L. rohita	*C. mrigala*	*C. catla*	*C. idella*
96-h LC_{50}				
Arsenic	30.0±0.0a	24.5±0.1b	10.2±0.2d	22.17±0.0c
Nickel	92.3±0.3b	100.7±0.2a	43.4±0.2c	43.78±0.2c
Zinc	165.3±0.4a	108.8±0.1b	41.8±0.3d	99.57±0.1c
96-h Lethal Concentration				
Arsenic	40.2±0.0a	32.1±0.0b	14.1±0.2d	29.67±0.0c
Nickel	120.7±0.1b	131.8±0.3a	58.1±0.3d	64.24±0.3c
Zinc	212.9±0.9a	140.7±0.1b	57.3±0.6d	129.22±0.1c

Means with similar letters in single row are non-significant P<0.05.

Table 2: Metals accumulation (µgg^{-1}±SD) in fish species at 96-h LC_{50} and lethal concentration exposure

Exposure/ Metals	*L. rohita*	*C. mrigala*	*C. catla*	*C. idella*
LC_{50} Exposure				
Arsenic	13.7±17.0c	18.2±21.4b	13.3±11.3c	20.2±23.4a
Nickel	106.0±141.2c	146.8±149.1a	92.7±83.1d	129.0±124.9b
Zinc	190.5±145.5c	243.0±190.5a	176.2±167.7d	200.5±131.9b
Lethal Concentration Exposure				
Arsenic	19.6±26.9c	26.8±32.0a	16.4±14.2d	26.2±27.0b
Nickel	155.4±209.8c	244.9±234.3a	133.1±123.7d	181.1±170.3b
Zinc	328.8±279.0c	401.5±335.0a	229.9±178.4d	349.2±216.2b

Means with similar letter in a single row are non-significant (P>0.05).

(P<0.05) differences for accumulation of these metals in different organs of all the four fish species. Regarding ability of fish species to accumulate Ni and Zn at 96-h LC_{50} and lethal concentrations *Cirrhina mrigala* showed highest tendency, followed by *Ctenopharyngodon idella*, *Labeo rohita* and *Catla catla*. Among fish species highest As accumulation was observed in *Ctenopharyngodon idella*, followed by *Cirrhina mrigala* while the same was statistically non-significant between *Labeo rohita* and *Catla catla* at 96-hr LC_{50}. Acute exposure of Ni and Zn showed significantly higher amassing of metals in fish liver while As showed highest accumulation in fish kidney followed by liver, gills, gut, bones, muscles, skin, fins and scales (Table 3). Minimum amassing of As and Zn was observed in scales of fish while minimum Ni accumulation was observed in the fish fins. Metals burden in different organs of *Labeo rohita*, *Cirrhina mrigala, Catla catla* and *Ctenopharyngodon idella* were in the order of liver > gills > kidney > gut > bones > skin > muscles > scales > fins; liver > kidney > gills > gut > skin > scales > bones > muscles > fins; gills > liver > kidney > gut > bones > skin > muscles > scales > fins and liver > kidney > gills > gut > skin > bones > muscles > fins > scales, respectively (Table 3). During 96-hr LC_{50} and lethal concentration exposure *Cirrhina mrigala* showed significantly higher ability to amass metals while *Catla catla* showed significantly least tendency.

DISCUSSION

Acute toxicity (96-h LC_{50} and lethal concentrations) of metals varied significantly among fish species. The fish that is highly susceptible to a metal may show higher resistance to another metal at the same concentration (Biuki *et al*., 2010). During present investigation, it observed that As was highly toxic metal as compared to Ni and Zn. *Catla catla* showed highest sensitivity to all metals while *Cirrhina mrigala* showed the least. For both 96-hr LC_{50} and lethal concentrations, *Catla catla* were reported significantly more susceptible to Al toxicity, followed by that of *Labeo rohita* and *Cirrhina mrigala* (Azmat *et al*. 2012). Leblond and Hontela (1999) reported that rainbow trout was more susceptible to acute toxicity of mercury followed by Zn and Cd. Waterborne heavy metals exposure caused marked hypersensitivity in fish (Javed, 2012). Metals may enter the fish through contaminated water and food intakes, start accumulating in liver, kidney, gills, skin, fins, muscles and bones (Rauf *et al*., 2009). However, patterns of metals bioaccumulation in different organs of fish varied significantly (P<0.05). These variations among ability of different fish species to accumulate metals in their bodies appeared to be species specific (Giguere *et al*., 2004). Furthermore, position of each tissue in the fish body and the physiological differences can also influence the accumulation of a particular metal (Kotze, 1997). Liver plays a central role in detoxification and accumulation of heavy metals (Yousafzai, 2004). Synthesis of metallothioneine, a metal binding protein, is induced in fish due to elevated levels of heavy metals. This protein helps in detoxification and accumulation of metal ions in liver (Hogstrand and Haux, 1991).

Table 3: Metals accumulation (µgg^{-1}±SD) in fish body organs at 96-h LC_{50} and lethal concentration exposure

Metals/ Species	Organs								
	Liver	Kidney	Gills	Gut	Muscles	Skin	Bones	Fins	Scales
LC_{50} Exposure									
Arsenic	41.9±12.0b	53.3±13.8a	12.8±3.6c	10.6±2.6d	6.2±1.7f	6.3±2.3fg	7.4±2.9e	4.5±1.7h	4.2±3.3hi
Nickel	295.4±74.9a	226.3±132.4c	288.6±48.6b	97.9±48.9d	31.7±14.4g	32.6±12.4h	41.2±19.1e	16.4±5.2i	37.7±45.9f
Zinc	423.3±71.1a	330.6±68.5b	422.1±87.9a	238.9±80.8c	74.4±21.3f	119.5±41.0d	103.8±21.8e	58.4±20.2g	52.0±13.6h
Species x Organs									
L. rohita	256.9±193.2a	156.8±161.6c	244.5±207.8b	102.1±120.5d	32.6±31.8f	46.9±65.1e	47.6±41.2e	20.4±19.0h	23.1±30.6g
C. mrigala	295.2±133.5a	287.1±194.7b	260.5±232.7c	160.8±176.0d	39.5±39.7h	55.3±62.7e	43.1±53.4g	30.3±38.8i	52.3±51.1f
C. catla	205.3±173.9b	151.5±106.0c	243.6±246.7a	83.2 ± 89.1d	35.2±27.1g	37.1±28.2f	45.8±42.1e	21.4±19.2i	23.2±13.4h
C. idella	256.8±196.8a	218.2±136.9b	215.5±172.2c	117.1± 90.2d	42.5±48.3g	72.1±82.6e	66.5±63.3f	33.5±37.9h	26.3±35.3i
Lethal Concentration Exposure									
Arsenic	55.6±15.1b	73.3±21.02a	19.9±7.02 c	15.6±5.25 d	8.5±2.36f	8.1±2.81 fg	9.5±3.83 e	5.2±3.2h	4.6±4.02 i
Nickel	453.2±114.4a	323.3±216.12c	427.7±69.18b	149.3±71.64d	49.2±15.07g	47.3±17.66h	57.4±23.90f	29.0±7.3i	71.4±97.85e
Zinc	696.4±202.4a	562.9±133.86c	613.9±160.62b	405.0±131.80d	114.6±43.68g	211.8±99.95e	179.6±70.85f	85.6±37.7h	76.5±12.25i
Species x Organs									
L. rohita	411.4±314.5a	271.1±314.0c	404.1±353.0b	193.5±255.4d	38.6±35.4f	68.4±96.1e	68.6±60.4e	28.4±25.7g	27.4±35.5h
C. mrigala	515.0±433.6a	462.5±320.3b	424.7±383.7c	250.9±261.7d	59.8±53.2g	102.0±121.9e	60.3±68.8f	42.2±49.5h	102.4±107.2e
C. catla	266.4±212.2b	218.3±166.7c	289.5±256.7a	114.6±112.2d	55.2±43.5f	51.2±42.9g	76.7±87.2e	29.6±27.1i	36.9±27.2h
C. idella	414.2±347.8a	327.6±242.2b	297.1±234.8c	200.9±172.6d	76.1±87.4g	134.6±173.5e	123.0±140.3f	59.5±65.3h	36.6±46.3i

Means with similar letter in a single row are non-significant (P>0.05).

Fish liver showed significantly ($P<0.05$) higher tendency to accumulate heavy metals than other tissues (Javed, 2012) due to alterations in biochemical parameters (Nayaranan and Vinodhini, 2008). Overall higher Zn amassing in all tissues of fish may probably be due to increased metal concentration in ambient water or may be due to slow rate of excretion from fish body (Yousafzai and Shakoori 2008a). Fish exposed to As showed 3-fold greater As accumulation in liver as compared to non-exposed fish (Bears *et al*., 2006). Ahmed and Bibi (2010) reported higher lead accumulation in the liver and intestine of *Catla catla*. Fish exposed to water borne heavy metals generally showed higher metal load in gills than digestive tract because of their direct contact to the water-borne metals. Yousafzai and Shakoori (2008b) reported the order of metal accumulation in the gills of *Tor putitora* as: Zn > Pb > Cu > Ni > Cr. Monitoring of metal accumulation in fish muscle tissue is much important because it is consumed by people. Yousafzai *et al*. (2010) reported the order of metals accumulation in the muscle of *L. dyocheilus* as Zn > Cr > Cu > Pb > Ni > Cd. As skin is in direct contact with metallic ions in the surrounding water, therefore, the probability of metal's amassing in it is equally high. Fish skin is mostly consumed along with muscle, therefore, this organ is important for metal accumulation point of view (Yousafzai and Shakoori, 2006).

Conclusion: Present investigation showed variable toxicity of As, Ni and Zn for fish. Regarding metals toxicity, zinc was found as significantly ($P<0.05$) least toxic while As was reported as highly toxic metal. Among the four fish species C*atla catla* showed highest sensitivity, followed by *Ctenopharyngodon idella*, *Cirrhina mrigala* and *Labeo rohita*. Dose dependent metal accumulation in fish was also observed. Highest metal accumulation was recorded in fish liver, followed by that of kidney and gills.

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