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RESEARCH ARTICLE

Chronic Dual Exposure (Waterborne+Dietary) Effects of Cadmium, Zinc and Copper on Growth and their Bioaccumulation in *Cirrhina mrigala*

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ABSTRACT

During this research endeavor, the chronic effects of dual exposure (waterborne+dietary) of cadmium (Cd), zinc (Zn) and copper (Cu) on the growth performance and tissue-specific bioaccumulation of metals in three age groups (60-, 90- and 120-day) of *Cirrhina mrigala* were determined. The exposure of all metals caused significant effects on the weight and total length gains in all three age groups of fish. Fish growth was significantly affected due to Cu, followed by Cd and Zn exposures. However, growth of treated fish was significantly lesser than control (un-stressed). Fish condition factor did not change significantly due to treatments. Zn exposure induced significantly better feed intake in fish than Cd and Cu treatments. Feed conversion efficiency (FCE) of fish varied significantly due to treatments that changed with age also. Exposure of fish to metals caused significant alterations in fish feeding patterns that caused profound effects on fish growth and FCE. Therefore, fish growth has been found reliable end point in chronic dual exposure of Cd, Zn and Cu to predict various processes associated with fish bioenergetics. The chronic dual exposure of metals resulted into significantly inconstant buildup of Cd, Zn and Cu in fish body organs also. In general, all metals exhibited their bioaccumulations in fish that followed the order: liver > kidney > gills > muscles > fins > skins > bones. The 60-dayfish displayed significantly higher propensity to accrue all metals while Zn accumulation was significantly higher than both Cd and Cu in the fish body.

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INTRODUCTION

Cirrhina mrigala is one of the most important commercial fish species in Pakistan that is grown in earthen ponds under poly- and composite culture systems. Cd, Zn and Cu are the major contaminants of aquatic habitats affecting the growth and longevity of fish (Bu-Olayan and Thomas, 2008; Yaqub and Javed, 2012). Most of metals are essential micronutrients for fish but they become toxic at higher concentrations, commonly found in aquatic habitats of Pakistan (Jabeen *et al*., 2012). Acute and sub-lethal effects of various metals on fish have been studied by Shaukat and Javed (2013) and Ameer *et al.* (2013) while kousar and Javed (2014) reported significant amassing of various metals in fish. All such toxicological studies are commonly dealing with the effects of either waterborne or dietary metals on fish growth. However, in natural habitats animals are exposed simultaneously to both waterborne and dietary metals. Furthermore, the metal's toxicity can exhibit pronounced effects on animals when found in mixture form (Naz and Javed, 2013; Naz *et al*., 2013; Ilyas and Javed, 2013). Toxic effects of various metals, either waterborne or dietary, on fish could be studied by examining their growth and condition factor as these parameters provide significant information regarding toxicity relating to fish recruitment and longevity (Kerambrun *et al*., 2012). The waterborne metals can also affect the fish sensory system and making them difficult to locate food and ultimately reducing the fish feeding activity (McGeer *et al*., 2000). Heavy metals cause toxicity in fish through oxidative stress created due to generation/production of reactive oxygen species (Valko *et al*., 2005). Metals like cadmium, nickel and chromium may also reduce the affinity of hemoglobin towards binding of oxygen (Witeska and Kosciuk, 2003). Metals occur naturally in aquatic ecosystems both individually or in a mixture form and animals are simultaneously exposed to both waterborne and dietary metals. When metals coexist in a water body, the speciation properties and bioavailability of individual metals are affected (Jabeen *et al*., 2012). Various metals compete for their uptake routes, target sites, mechanism of transport and excretion within the fish. Zn and Cu acts as a part of Cu/Zn superoxide dismutase, cytochrome oxidase and tyrosinase (Stern *et al*., 2007). Zn acts as cofactor for various metalloenzymes to take part in gene regulation and its expression in fish (Watanabe *et al*., 1997). Most of studies have been conducted to evaluate the toxic effects of either waterborne or dietary Cd, Zn and Cu separately on growth and their bioaccumulation in fish during chronic exposures. However, in natural conditions fish are exposed simultaneously to both waterborne and dietary sources of metals. Therefore, the aim of this study was to determine the effects of dual exposure of waterborne+dietary Cd, Zn and Cu on the growth and their bioaccumulation in the body organs of *Cirrhina mrigala* under controlled laboratory conditions.

MATERIALS AND METHODS

During this investigation, 60-, 90-, and 120-day age groups of *Cirrhina marigala* were tested for their growth and metal's bioaccumulation under dual exposure of waterborne+dietary Cd, Zn and Cu in glass aquaria under controlled laboratory conditions. Each metal was tested for three age groups of fish separately for 12 weeks growth trials. After acclimatization to laboratory conditions, each age group of fish (n=10) was exposed simultaneously to waterborne and dietary each metal (Cd, Zn and Cu) separately at 1/3 of their LC_{50} and LD_{50} in glass aquaria at constant pH, hardness and temperature of 7, 200mg $L^{-1}$and 30°C, respectively. The average initial wet weights of each age group and their metallic ion exposure concentrations are presented in Table 1.Ten fish of each age group, with three replications for each treatment, were grown under dual exposure of waterborne+dietary metals, separately. The fish were simultaneously exposed to waterborne concentrations (1/3 of LC_{50}) and fed the diets (30% digestible protein and 290 Kcal g^{-1} digestible energy) containing sub-lethal (1/3 of LD_{50}) dietary concentration of each metal twice a day to satiation. The control fish groups were kept in metal free water and fed a diet deprived of any added metal for comparison. Fish growth was monitored interms of increase in weight, total length, feed intake and FCE on weekly basis. After each growth trial, the fish body organs viz. kidney, liver, muscle, fins, gills, skin and bones were isolated and analyzed for respective metal exposure treatment by following the standard procedures of APHA (2005) through Perkin Elmer, Analyst-400 Atomic Absorption Spectrophotometer. After verification of normality of distribution and variance homogeneity, the data were analyzed by following two-way analysis of variance. Comparisons of mean values for each parameter were performed using statistical program.

RESULTS

The data on fish growth i.e. increase in weight, length, condition factor, feed intake and FCE under three treatments viz. Cd, Zn and Cu exposures and control are presented in Table 2. Significant differences were observed among three age groups of fish for their increase in weights and lengths due to treatments. However, mean increments in weight and length were significantly higher in 120-day, followed by 90- and 60-day age groups of fish under all treatments. Cu and Cd stress caused lower weight gains to the fish while control fish attained significantly higher weights. Increase in total length of fish varied significantly among treatments also with the mean lower increment due to Cu exposure. However, there was non-significant difference between Cd and Zn treatments to cause effects on total length gains in all age groups of fish. The condition factor of fish did not change significantly among three age groups of treated fish. The stress of all metals caused significant reduction in fish feed intake while Cu exposure caused significant lowering of feed intake, followed by Cd and Zn treatments. The 120-day fish exhibited significantly higher feed intake than 90- and 60-day age groups. The FCE of fish varied significantly among three age groups and treatments. Cd stress caused significant lowering of fish FCE, followed by Zn and Cu treatments. However, FCE values of all treated fish were significantly lower than control. Among three age groups, 120-day fish groups exhibited significantly better FCE than 90- and 60-day fish but the difference between 60- and 90- day age groups was non-significant.

Tissue specific bioaccumulation platters of Cd, Zn and Cu in the body organs of fish are presented in Table 3.

Table 1: Average initial wet weights and exposure concentrations of metals for the fish

Metal	Fish age (Days)	Average fish weight (g)	Waterborne concentration (mg L^{-1})	Dietary concentration (µg g^{-1})
Cd	60	4.31±1.58	51.47	56.25
	90	9.05±2.87	52.33	58.83
	120	12.27±2.59	55.01	60.03
Zn	60	4.27±1.56	25.84	65.92
	90	8.25±2.58	27.99	67.77
	120	12.49±3.89	29.10	69.19
Cu	60	4.37±2.89	20.07	58.56
	90	7.72±2.58	20.95	58.99
	120	13.04±2.98	21.11	60.02

Table 2: Growth performance of fish under chronic dual exposure of metals

		Fish age (days) groups		
		60	90	120
Increase in weight (g) ±SD	Cd	3.31±0.08c	5.48±0.14b	7.24±0.11a
	Zn	5.19±0.11b	5.12±0.22b	7.59±0.09a
	Cu	4.41±0.04c	5.24±0.18b	6.08±0.12a
	Control	8.60±0.13b	8.79±0.05b	15.83±0.08a
Increase in total length (mm)	Cd	17.97±0.91c	19.32±1.15b	25.21±1.51a
	Zn	17.66±1.47c	20.44±1.36b	25.59±0.45a
	Cu	16.99±0.62c	19.01±0.50b	24.20±1.42a
	Control	22.27±1.93b	23.02±0.88b	33.41±1.03a
Condition factor	Cd	1.33±0.26a	1.41±0.04a	1.43±0.07a
	Zn	1.27±0.10a	1.38±0.05a	1.38±0.04a
	Cu	1.37±0.22a	1.47±0.18a	1.44±0.07a
	Control	1.66±0.19a	1.46±0.12a	1.46±0.06a
Feed intake (g)	Cd	6.77±0.10b	12.39±0.40a	12.78±0.22a
	Zn	9.95±0.21b	10.58±0.22b	13.35±0.14a
	Cu	7.75±0.34c	10.17±0.14b	11.19±0.09a
	Control	12.31±0.05c	14.25±0.25b	23.58±0.11a
FCE (%)	Cd	48.89±1.66b	44.23±2.38c	56.65±3.35a
	Zn	52.16±2.35b	48.39±3.00c	56.85±2.56a
	Cu	56.90±2.42a	51.52±2.54b	54.33±3.48a
	Control	48.74±2.55c	61.68±3.04b	67.10±2.96a

Mean±SD with different letters in a row for each parameter differ statistically ($P<0.05$).

Table 3: Accumulation of metals (mgL^{-1}) in different organs of fish

Fish organs	Cadmium		Zinc		Copper	
	Treated	Control	Treated	Control	Treated	Control
Kidney	43.12±6.95b	2.21±1.20a	47.40±9.07b	2.77±1.53a	45.65±6.92b	2.06±1.18ab
Liver	49.35±4.72a	2.48±0.98a	50.35±7.47a	1.98±1.02b	49.36±7.38a	2.21±1.04ab
Muscle	13.44±4.26d	0.95±0.85c	14.22±5.56d	0.97±0.99d	12.75±3.93d	0.86±0.70c
Fins	11.81±3.47e	1.06±1.02c	11.47±6.63e	1.26±1.28c	11.02±2.64e	0.80±0.85c
Gills	29.80±5.57c	1.70±0.91b	30.90±6.07c	1.95±0.99b	29.51±4.20c	1.77±0.82b
Skin	10.40±3.06f	0.98±0.96c	10.81±4.84f	1.28±0.93c	9.79±3.07f	1.03±0.96c
Bone	8.67±2.48g	0.91±1.85c	9.27±2.45g	1.22±1.10c	8.81±2.39f	1.18±1.14c

Mean±SD with same letters in a single column are statistically similar

All organs of treated fish showed significantly variable abilities to accumulate Cd, Zn and Cu. The amassing of Cd, Zn and Cu in treated fish body organs varied significantly as Zn > Cu ≥ Cd. Fish liver acted as an organ that accumulated significantly higher quantity of metals while bones showed least tendency to amass them. In general, all metals were accumulated in fish body as liver > kidney > gill > muscle > fins > skin > bones. The fish body showed significantly higher aptitude to accrue Zn, followed by Cu and Cd but the difference between Cu and Cd accumulations was non-significant. Cd, Zn and Cu accumulation in the body organs of three age groups of fish varied significantly also. The younger fish (60-day) exhibited significantly higher ability to amass metals than 120- and 90-day age groups. In control fish, all organs had significantly lower quantity of all metals than the fish exposed to Cd, Zn or Cu treatments (Table 3).

DISCUSSION

Fish growth was significantly affected by Cd, Zn and Cu treatments, based on fish tolerance limits (1/3 of LC_{50} and LD_{50}). Fish growth was significantly affected by Cu, followed by Cd and Zn treatments. However, treatments did not cause any significant effect on the condition factor of three age groups of fish. Control fish exhibited significantly better growth and condition factor than treated fish. Russell *et al.* (2010) reported non-significant effects of diet-borne Cu, Cd, Pb and As on rainbow trout, fathead minnow and channel catfish. However, rainbow trout showed reduced growth under arsenic toxicity which was dose-dependent and attributed by slow rate of feeding that resulted in to significantly lower FCE. Among treatments, Cu caused significantly lesser feed intake by the fish, followed by Cd and Zn treatments. Zn acts as an essential mineral but may become toxic to the fish at higher concentrations (Javed, 2012) as significantly better intake of feed under Zn treatment did not result into better fish growth and FCE. Various compounds of zinc can damage the gills and enhance the discharge of mucus by the fish. Studies on the effects of chronic sub-lethal Zn exposure demonstrated growth inhibition in Murrel (Shukla and Panday, 2006) and *Cirrhinus mrigala* (Mohanty *et al*., 2009). Feed intake by the fish was affected significantly due to treatments that caused significantly lower feed intake and consequently resulted into lower weight and length gains in all age groups of *Cirrhina mrigala*. FCE of fish varied significantly with age also. In toxicology, Cd seeks distinctive consideration due to its impending threats to aquatic animals (Barber and Sharma, 1998). Cd is a common pollutant of natural waters and is highly toxic to the aquatic animals even at very low concentration (Yaqub and Javed, 2012) that can result into significantly lower FCE of fish as observed during present investigation. Various metals have been reported to change the fish feeding patterns under both laboratory and natural aquatic environments (James *et al*., 2003). Hanan (2007) reported significant distortion in the gills and liver of Nile Tilapia due to chronic exposure of Cu, Zn and Cd. Cu exposure caused dilated primary gill lamellae while Zn produced epithelial cells sloughing and hypertrophy of chloride cells, epithelial hyperplasia, mucus secretion and severe congestion of blood vessels of primary lamellae. Cu and Zn caused hyperplasia of pyknotic nuclei focal necrosis and hepatic degeneration while Cd exposure caused severe injury to the cell membranes, disorganization of hepatic cords and nuclei hyperplasia. The higher concentrations of Cd, Zn and Cu in fish liver was due to the presence of metalothionin protein that has high affinity for these metals and being a transportation source had direct correlation with fish growth (Fernandes *et al*., 2008). Therefore, fish growth can be used as a respective and reliable consequence in chromic metal's exposure experiments that could predict changes in fish physiology to reveal and assess the impacts of metals on several processes relating to fish bioenergetics. Condition factor is considered one of the growth parameters to predict degree of fish well beings under a certain set of environmental conditions. The impact of treatments on fish condition factor was significant. Significant effects of waterborne Cu on feed intake, growth, FCE and condition factor of tilapia has been reported by Ali *et al.* (2003). The toxic effects of individual metals (Vosyliene *et al.,* 2003) and metal mixtures on fish feed intake, as a result of increase in fish metabolism during chronic exposure stress, has been reported by Naz *et al*. (2013). Significant impact of different concentrations of waterborne Cu on tilapia growth was reported by Hansen *et al*. (2002). The present results show that fish growth can provide a reflective measure to study the chronic effects of dual (waterborne+dietary) exposure of Cd, Zn and Cu to the fish, *Cirrhina mrigala*.

The dual exposure of Cd, Zn and Cu caused significant amassing of all metals in fish body organs. Fish liver showed significantly higher tendency to accumulate all metals, followed by that of kidney. However, all three metals were found significantly low in fish bones. Keeping in view the role of liver as storage, regulating and detoxification organ, it amassed significantly higher quantities of all metals during 12-week growth trials. In general, the accumulation of all metals in fish body organs followed the ordered: liver >kidney>gills>muscle>fins> skin>bones. Murugan *et al.* (2008) reported accumulation pattern of Zn in *Channa punctatus* organs as: liver>kidney>intestine>gill > muscle after chronic exposure. Zn accumulation in fish body organs was significantly higher than both Cd and Cu. The effects of dietary metals, unlike waterborne, on fish health and vitality are still indistinct. However, various studies revealed Cu accumulation in fish (Kamunde *et al*., 2002) and its impacts on growth (Kousar and Javed, 2012). The results of these studies are variable due to nature of fish species used, the profiles of salinities experienced and exposure situations. Fish bones exhibited significantly least ability to concentrate all metals during stress of metals while both liver and kidney showed significantly higher tendency to accumulate all metals, indicating tissue specific amassing of these metals that can act as key indicator of chronic dual exposure of waterborne+dietary uptake of metals in *Cirrhina mrigala*.

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