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Chronic Effects of Nickel and Cobalt on Fish Growth

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Abstract

Chronic exposure impacts of waterborne and dietary nickel (Ni) and cobalt (Co) on the growth performance of juvenile major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* were studied under static water bioassay. Fish growth was monitored in terms of wet weight and fork length increments, condition factor, feed intake and feed conversion efficiency (FCE). All the three treated fish species showed exploratory behavior during first few hours of both waterborne and dietary exposure of metals. However, these responses were more pronounced in the fish exposed to waterborne than that of dietary metals. The exposure of both Ni and Co caused significant effects on weight and fork length increments, feed intake and FCE of all the three fish species. Exposure of waterborne metals caused significantly lower weight and fork length gains to all the three fish species, while feed intake, FCE and condition factor were significantly better due to dietary treatments. All the three control fish species exhibited significantly higher feed intakes that resulted into significantly better FCE than the fish either exposed to waterborne or dietary metals. Dietary exposure of both Ni and Co increased the feed intake by the fish that showed positive relationship with its growth, while waterborne exposure of both metals resulted into significant loss of apatite to cause significant reduction in growth of all the three fish species. *L. rohita* showed significantly higher sensitivity to metallic toxicity as evident from its lower weight increment than *C. mrigala*, while the difference between *C. catla* and *L. rohita* was non-significant. The condition factor and feed intake of all the three fish species did not change significantly due to the toxicity of both waterborne and dietary exposure of metals. The response of three fish species towards FCE fluctuated significantly that followed the order: *C. mrigala* > *L. rohita* > *C. catla*. © 2013 Friends Science Publishers

Keywords: Fish; Growth; Chronic exposure; Nickel; Cobalt

Introduction

Metals are found naturally in the aquatic habitats or as a result of anthropogenic activities, representing an imperative factor of exposure to the aquatic animals, including fish (Jabeen *et al*., 2012). Metals can affect the fish growth, population dynamics and reproduction (Saeed, 2000). Most metals are essential, in trace amounts, for the physiological processes in fish (Watanabe *et al*., 1997), while above the permissible limits and under variable environmental conditions, metals may affect fish growth energetics and hence resulting into behavioral and biochemical changes leading to fish mortality (Azmat *et al.,* 2012). Ni is present in living organisms in trace amounts, while its elevated levels in water would become perilous to the environment and animals living in it (Rauf *et al*., 2009). An extensive use of Ni in different industries i.e., electroplating, steel and ceramic processing is producing Ni by-products that are discharged, untreated, into the natural habitats of Punjab province (Jabeen and Javed, 2011). Co in trace amounts is beneficial for animals as a part of vitamin B_{12} that is essential for fish health (Ahilan and Jeyaseelan, 2001). However, its too high concentration can harm the fish health. Therefore, Ni and Co contamination is a real threat to the aquatic organisms due to their persistence nature and ability to bio-magnify in the aquatic food chain (Ubaid-ullah *et al*., 2004).

Unlike water-borne exposure, the consequences of dietary metals on fish are undecided. Much less is known about Ni and Co regulation and metabolism in fish, although in contaminated environments fish may take up metals through both gut and gills (Dallinger *et al*., 1987). Despite substantial literature pertaining to metal's uptake via gills or gut (McDonald and Wood, 1993; Handy, 1996), the relationship between these two routes of uptake are yet to be clearly understood. The requirement of metals for fish is much more composite than that of mammals due to their potential uptake through gills and skin. Furthermore, metals are ubiquitously present in the aquatic environments due to natural and anthropogenic activities that could harm the aquatic animals, including fish. Toxic responses of various fish species exposed to elevated waterborne concentration of metals have been studied (Ali *et al*., 2003; Ololade and Oginni, 2010; Naz *et al*., 2012), while little research has been conducted on the effects of dietary source of metals on fish despite diet being a significant route of contamination in a wild fish. Significant data are required to set suitable limits of Ni and Co in fish feeds to assure health of fish for consumer safety. The responses of various fish species at different stages of their development to various toxicants are generally compared on the basis of fish tolerance limits (Azmat *et al*., 2012). However, in natural habitats, fish are influenced by long-term exposure of toxicants at low concentrations. Therefore, laboratory tests at sub-lethal concentrations are imperative to anticipate probable consequences regarding fish adaptability to survive under contaminated environments (KaiSun *et al*., 1995; Jabeen *et al*., 2012). The interaction of dietary and waterborne metal's uptake has yet to be explicitly demonstrated, a finding that could permit the fortitude of relative contribution of waterborne and dietary metals in fish nutrition and toxicity. Ni and Co are the common pollutants of rivers in the Punjab province entering them with untreated industrial and municipal waste waters. Therefore, higher metallic ion toxicity in the rivers of Pakistan are severely affecting the indigenous fish fauna, including major carps viz. *C. catla, L. rohita* and *C. mrigala*. This demands evaluation of fish growth potentials under chronic exposures of Ni and Co to devise strategies for sustainable conservation of these cyprinids in their natural habitats.

Materials and Methods

Static water bioassay tests were performed with three fish species viz. *C. catla, L. rohita* and *C. mrigala* to monitor their growth under chronic sub-lethal concentrations (1/3 of LC_{50}) of waterborne and dietary Ni and Co, separately. Before the experiments, fish were acclimatized to laboratory conditions for two weeks. Stock solutions of each metal were prepared, separately, by using $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$ (Aldrich, USA) and diluted to specific concentrations (Table 1) for each species of fish (Javed and Yaqub, 2008).

Fish Growth under Waterborne Metals

Ten fish (120-days old) of each species were grown, separately, in glass aquaria containing 50 L water with sub-lethal concentrations (Table 1) of Ni and Co, seperately. Each treatment was tested with three replications against control (metal free water). All the growth trials were conducted at constant water hardness, temperature and pH of 200±1.00 mg L^{-1}, 30^{o}C±0.05 and 7±0.05, respectively. The water media were replaced partially on regular basis to maintain the desired metal concentrations in water by determining them through APHA (1998) by using Atomic Absorption Spectrophotometer (Analyst 400 Perkin Elmer, USA). All the three fish species were fed on metal-free extruded feed (35% DP and 2.90 Kcalg^{-1} DE), to-satiation, once daily for 12 weeks.

Fish Growth under Dietary Metals

Another set of experiments was conducted by placing groups (n=10) of 120-day fish in glass aquaria containing clean metal free water. Fish were with diets (35% DP and 2.90 Kcal g^{-1} DE), already mixed with sub-lethal concentrations (1/3rd of LD_{50}) of each metal, separately (Table 1). Fish were fed, to-satiation, on daily basis for 12 weeks, while control fish were grown in metal deprived water and fed on metal- free diet. Each treatment was tested on three fish species, separately, with three replications.

All the three fish species were tested for waterborne and dietary treatments of Ni and Co, separately, for their growth and monitored on weekly basis for increase in their average wet weights and fork lengths, condition factor, feed intake and FCE. Data on each variable were collected and articulated as means ±SD and analyzed through two-way analysis of variance and means were compared for statistical differences (Steel *et al*., 1996). The correlations among defined variables were also computed to find out possible relationships.

Results

During first 2-3 days of both waterborne and dietary exposure of metals, all the three fish species showed variable behavioral responses, while these responses were more pronounced due to waterborne than dietary treatments. All the three fish species gave significantly lesser weights due to waterborne Co (Table 2). Within treatments, fish exposed to dietary Ni and Co exhibited significantly better growth in terms of increase in their average weights and fork lengths, and condition factor, while these increments were significantly lower than that of control fish. Feed intakes in all the three fish species were significantly lesser due to waterborne Ni while waterborne Co and dietary Ni exposure resulted into significantly higher feed intake by the fish. However, feed intakes were significantly better among control fish groups that caused significantly higher FCE than those exposed to waterborne or dietary metals. Within treatments, significantly better feed intakes by the fish were observed due to waterborne Co that did not product into significantly better FCE (Table 3). Non-significant difference between Ni and Co treatments was observed for their influence to escalate fish weights, condition factor and FCE. Weight increments and feed intakes of fish were significantly higher due to exposure of Co than Ni treatment. Control fish species exhibited significantly higher increments in their weight, fork length, condition factor, feed intake and FCE, followed by Ni and Co treatments (Table 3). Among three fish species, *C. mrigala* attained significantly better weight, fork length and FCE. The differences among three fish species for their condition factor and feed intake were statistically non-significant. Dietary exposure of both Ni and Co caused significantly better gain in weight, fork length, condition factor, feed intake and FCE of fish than that observed due to waterborne treatments. However, all the control fish species performed significantly better than that of both waterborne and dietary treatments (Table 3).

Feed intake of fish under waterborne Ni and Co exposure showed positive but non-significant correlation with weight increments of all the three fish species, while it

Table 1: Sub-lethal exposure concentrations of metals for the fish

Metal	Fish species	Average Weight (g)	Average Fork Length (mm)	Exposure Concentration	
				Waterborne (1/3rd of LC_{50} in mg L^{-1})	Dietary (1/3rd of LD_{50} in µg g^{-1})
Control	*Catla catla*	80.70±9.10	80.70±9.10	-	-
	Labeo rohita	74.40±4.19	74.40±4.19	-	-
	Cirrhina mrigala	80.10±4.16	80.10±4.16	-	-
Ni	*Catla catla*	83.33±2.12	87.90±2.12	24.63	70.40
	Labeo rohita	84.20±3.80	74.20±2.10	25.79	71.99
	Cirrhina mrigala	97.00±4.16	75.10±2.16	21.93	79.11
Co	*Catla catla*	87.94±6.32	83.30±6.19	30.43	74.34
	Labeo rohita	86.49±7.20	84.10±6.02	38.34	80.87
	Cirrhina mrigala	81.33±5.25	74.12±6.13	39.67	67.77

Table 2: Growth performance of metal stressed fish

Parameters	Waterborne Exposure		Dietary Exposure		Control
	Nickel	Cobalt	Nickel	Cobalt	
Increase in Weight (g)					
Catla catla	1.50±0.04 d	1.25±0.12 d	14.22±0.79 c	16.54±2.19 b	34.75±1.72 a
Labeo rohita	4.67±0.03 c	3.38±0.09 d	13.98±1.11 b	13.01±2.11 b	31.77±2.35 a
Cirrhina mrigala	5.44±0.02 c	3.98±0.11 d	13.16±1.16 b	13.98±1.65 b	40.75±2.96 a
Increase in Fork Length (mm)					
Catla catla	4.70±0.07 e	8.35±0.18 d	16.37±0.97 c	22.87±1.75 b	31.43±1.21 a
Labeo rohita	6.17±0.09 d	6.76±0.46 d	16.16±1.08 b	13.84±1.02 c	32.47±1.37 s
Cirrhina mrigala	6.43±0.17 d	6.99±0.39 d	14.42±1.22 c	20.50±1.07 b	38.57±1.12 a
Condition Factor (K)					
Catla catla	1.22±0.15 c	1.02±0.13 c	3.21±0.03 a	1.96±0.25 b	2.92±0.02 a
Labeo rohita	1.61±0.04 d	1.40±0.11 d	2.79±0.14 c	2.02±0.13 b	3.19±0.04 a
Cirrhina mrigala	1.23±0.13 e	1.72±0.05 d	2.69±0.09 b	2.28±0.11 c	2.99±0.12 a
Feed Intake (g)					
Catla catla	3.89±0.22 d	8.51±1.06 b	9.39±1.61 b	7.86±1.19 c	15.40±1.12 a
Labeo rohita	4.76±0.81 d	8.53±1.29 b	8.74±0.96 b	7.65±0.93 c	15.78±1.73 a
Cirrhina mrigala	5.60±0.05 c	8.29±0.66 b	8.17±1.17 b	8.42±1.11 b	15.57±1.29 a
Feed Conversion Efficiency (%)					
Catla catla	38.56±2.25 d	14.68±1.97 e	151.43±3.12 c	211.07±4.22 b	225.65±4.57 a
Labeo rohita	98.11±4.29 d	39.62±2.16 e	159.95±2.90 c	170.07±4.23 b	201.33±4.65 a
Cirrhina mrigala	97.14±3.46 c	48.01±2.12 d	161.08±3.27 b	166.03±4.24 b	261.72±5.26 a

FCE= gain in weight (g) /feed intake (g) x 100; Condition factor = $W \times 10^5 \div L^3$, where W = fish weight (g); L = fork length (mm); Means with same letters in a single row are statistically similar at $p < 0.05$.reshearch

Table 3: Responses of treatments, species and metal exposure sources towards fish growth

Characteristics	Increase in weight (g)	Increase in Fork Length (mm)	Condition Factor (K)	Feed Intake (g)	FCE (%)
Treatments					
Nickel	8.83±2.01 b	10.71±2.99 c	1.35±0.09 b	6.71±1.78 c	117.85±16.46 b
Cobalt	8.69±3.23 b	13.21±3.23 b	1.38±0.50 b	8.20±0.33 b	108.24±10.86 b
Control	35.76±4.57 a	34.16±3.86 a	3.03±0.14 a	15.58±0.19 a	229.57±20.38 a
Species					
Catla catla	13.65±1.74 b	16.74±3.82 ab	2.06±0.98 a	9.01±2.15 a	128.28±17.24 c
Labeo rohita	13.36±1.34 b	15.08±2.65 b	2.20±0.76 a	9.04±1.07 a	133.81±34.62 b
Cirrhina mrigala	15.46±1.82 a	17.38±3.18 a	2.18±0.71 a	9.21±2.74 a	146.97±10.67 a
Exposure Sources					
Water-borne	3.37±0.70 c	6.56±0.12 c	1.36±0.02 c	6.50±0.73 c	56.01±10.99 c
Diet-borne	14.14±0.51 b	17.36±2.86 b	2.48±0.57 b	8.33±0.49 b	170.08±7.40 b
Control	35.76±2.57 a	34.16±3.86 a	3.03±0.14 a	15.58±0.19 a	229.57±11.38 a

Means with same letters in a single column are statistically similar at $p < 0.05$

was negative but non-significant with FCE. However, FCE showed positive but non-significant correlation with fish weights while it was non-significantly negative with feed intake. Condition factor of fish had non-significantly direct relationship with weight escalation, feed intake and FCE of all the three fish species. Dietary exposure of both Ni and Co increased the feed intake by the fish that resulted into positive correlation with weight increments of all the three fish species. However, correlation coefficient was significant for *C. mrigala* only. FCE of fish had significantly positive correlation with feed intake of both *C. catla* and *L. rohita*. Condition factor of three fish species exhibited inverse but non-significant correlation with feed intake and FCE due to dietary Ni, while Co exposure caused negative relationship with feed intake of both *C. catla* and *L. rohita*. Both feed intake and FCE of control fish were significantly and positively correlated with fish weight increments. Condition factor showed positively significant correlation with feed intake and FCE of all the three fish species grown under metal free environment (Table 4).

Table 4: Correlation coefficients among growth parameters of fish

Treatments	Application mode	*Catla catla*			*Labeo rohita*			*Cirrhina mrigala*		
		Increase in weight	Feed intake	FCE	Increase in weight	Feed intake	FCE	Increase in weight	Feed intake	FCE
Control	Feed Intake	0.8840	-	-	0.5840	-	-	0.6210	-	-
	FCE	0.8204	0.6632	-	0.6255	0.7180	-	0.5950	0.9254	-
	Condition Factor	0.7847	0.5824	0.5882	0.8837	0.5810	0.5985	0.6019	0.6555	0.6126
Waterborne Metals										
Nickel	Feed intake	0.2870	-	-	0.2038	-	-	0.2510	-	-
	FCE	0.3775	0.0948	-	0.1663	- 0.2886	-	0.0675	0.3683	-
	Condition Factor	0.4388	0.0363	0.3695	0.4769	0.2988	0.5056	- 0.1454	0.3940	0.1413
Cobalt	Feed intake	0.1525	-	-	0.3458	-	-	0.0292	-	-
	FCE	0.2653	0.1049	-	0.3708	- 0.1669	-	0.2256	0.3429	-
	Condition Factor	0.0994	0.0982	- 0.2075	0.3048	0.40322	0.1079	0.4800	0.0361	0.5000
Dietary Metals										
Nickel	Feed Intake	0.4667	-	-	0.3240	-	-	0.6580	-	-
	FCE	0.4900	0.6066	-	0.5999	0.6408	-	0.4426	0.5662	-
	Condition Factor	0.3925	- 0.3166	- 0.2438	0.4395	- 0.0366	0.3995	0.4546	- 0.1313	- 0.4965
Cobalt	Feed intake	0.4903	-	-	0.2785	-	-	0.4754	-	-
	FCE	0.2811	0.6828	-	0.5546	0.5915	-	0.2883	0.6692	-
	Condition Factor	0.4481	- 0.5039	0.2997	0.3189	- 0.0967	0.5176	0.4429	0.2719	0.4524

Critical value (2-tail, 0.05 = + or – 0.5540)

Discussion

Besides hypersensitivity and pronounced behavioral changes, significant reduction in the feeding activity was monitored in all the three fish species during first few hours of chronic exposure of both waterborne and dietary exposure of metals. Fish apatite recovered slowly but did not match with the control fish. Metals are known to agitate the fish through physical irritation to their external tissues and muddling of metabolism (Azmat *et al*., 2012). All the three fish species reacted instantly under water-borne than dietary exposure of metals, presenting aversion and reduced exploratory behavior. The exposure of waterborne Ni and Co caused significantly lower feed intake by the fish than that of dietary exposures. Fish weights and fork lengths were affected significantly ($p<0.05$) due to exposure of both waterborne and dietary metals also. Feed intake and FCE of fish were significantly affected due to waterborne than dietary exposure of both Ni and Co. Significant effect of dietary Co in reducing growth performance of ells, due to reduction in feed intake, has been reported by Heinsbroek *et al*. (2007). Waterborne metals have also been reported to cause disturbances in the growth and development of aquatic flora and fauna (Dojlido and Best, 1993) that could adversely affect the human health (Baralkiewicz and Siepak, 1999). Significant reduction in feed intake due to dietary exposure of Cu to Salmon (Buckley *et al*., 1982) and deleterious effects of Ni on the hematological parameters of *Clarias gariepinus* fingerlings have been reported by Ololade and Oginni (2010). Dietary exposure of both Ni and Co caused significantly better growth, feed intake and condition factor to all three fish species than the fish exposed to waterborne metals. Dietary Co (at 25 mg L^{-1}) has been reported to cause significant effects on the growth performance of *Carassius auratus* (Ahilan and Jeyaseelan, 2001). Significant loss of apatite in fish, due to exposure of waterborne Ni and Co, was resulted into reduced fish growth. However, the effect of waterborne Ni was significantly more pronounced on weight and fork length gains in all the three fish species, while that of dietary Co appeared significantly least. Some studies have reported fish tissue specific accumulation of metals during chronic exposure to cause adverse effects on their growth due to significant drop in their feed intake and FCE (Kamunde *et al*., 2002). Very little is known about Ni and Co metabolism in fish although fish can uptake these metals from the contaminated water and feed (Dallinger *et al*., 1987; Jabeen *et al*., 2012).

L. rohita exhibited significantly higher sensitivity to metallic toxicity for its response to gain weight than *C. mrigala*. However, the difference between *C. catla* and *L. rohita* was statistically non-significant. Condition factor and feed intake of all the three fish species did not change significantly due to waterborne or dietary exposure of metals, while the response of three fish species towards FCE fluctuated significantly that followed the order: *C. mrigala* > *L. rohita* > *C. catla*. Exposure of waterborne metals caused significantly lesser gain in weights of all the three fish species (Hayat *et al*., 2007) than those exposed to dietary ones. Weight increments of fish, exposed to waterborne Ni and Co, showed positive but non-significant relationships with feed intake, condition factor and FCE while feed intake of fish under dietary exposure was positively correlated with FCE. Feed intake, FCE and condition factor of control fish exhibited significantly positive correlation with their weight gains. Metals have the ability to modify the feeding behavior of fish (James *et al*., 2003) that would ultimately affects their growth (Hayat *et al*., 2007). Therefore, fish growth is employed as an accessible and reliable consequence of chronic stress of a wide range of pollutants to forecast physiological course of action in evaluating the impacts of pollutants associated with feed ingestion, its metabolism and excretion (Bhavan and Geraldine, 2000). Fish growth is a culmination of several biochemical phenomenon and pollutant intoxication that would aggravate biochemical modifications before initiating reduction in fish growth. Significant variations in feed intake, feeding behavior and growth performance of *C. mrigala* due to chronic exposure of sludge containing substantial quantities of copper, zinc, lead, nickel, chromium and cadmium was reported by Pereira *et al.* (2001). Vincent *et al.* (1996) reported decrease in FCR of *C. catla*, while increasing exposure concentration of water-borne chromium. Low levels of feed ingestion in the fish exposed to waterborne metals was suggestive of reduction in assimilated energy that ultimately caused reduction in fish weight increments (Ali *et al.*,2003).

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