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ACUTE TOXICITY OF METALS MIXTURES FOR FISH, *Catla catla, Labeo rohita* AND *Cirrhina mrigala*

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Acute toxicity (96-hr LC_{50} and lethal concentrations) of water-borne metal mixtures viz. iron, zinc, lead, nickel and manganese was investigated in three fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) under laboratory conditions at constant pH (7), total hardness (200 mgL^{-1}) and water temperature (30°C). All the three fish species showed significantly variable tolerance limits in terms of 96-hr LC_{50} and lethal concentrations against 19 mixtures of five metals. Amongst 19 mixtures/treatments, the mixture of five metals (Fe+Zn+Pb+Ni+Mn) caused significantly higher toxicity to the fish in terms of 96-hr LC_{50} and lethal concentrations of 49.18±11.10 and 79.20±14.73 mgL^{-1}, respectively. However, Pb+Mn and Zn+Pb mixture was significantly least toxic to the fish with the mean LC_{50} and lethal concentrations of 85.42±10.50 and 134.86±30.46 mgL^{-1}, respectively. Regarding overall sensitivity of three fish species, *Labeo rohita* showed significantly least sensitivity to metal mixtures with a mean LC_{50} and lethal concentrations of 81.73±12.73 and 128.80±19.95 mgL^{-1}, respectively.

Keywords: LC_{50}, lethal concentration, metal toxicity, *Catla catla, Labeo rohita, Cirrhina mrigala*

INTRODUCTION

Environmental pollution is one of the most devastating issues being faced by human life over the last few decades. This includes pollution of land, air and water. However, aquatic pollution is more difficult to be measured than land and air pollution (Barton, 2003). Metals can affect the organisms directly by contaminating their bodies or indirectly by transferring them to the next trophic level in the food chain (Shah and Altindag, 2005). Therefore, aquatic pollution, with heavy metals, has become a burning issue for the developing countries. Fish can be used as bio-indicator of metal's pollution in aquatic bodies (Javed, 2005). Most of the heavy metals when present in trace amounts are important for physiological functions in fish to regulate biochemical processes. However, histological and biochemical changes may occur in fish due to persistence of metals in the form of mixtures above the tolerance limits of fish (Bu-Olayan and Thomas, 2004). Heavy metals are considered to be mutagenic and carcinogenic and also affecting the immune system of fish resulting in decreased population, pathogenocity and its mortality (More *et al.*, 2003). Acute methods are employed to determine the toxicity of metals to the fish because this can quickly assess the effects of contaminants on aquatic organisms (Kai Sun *et al.*, 1995). Iron is an essential micronutrient for fish but can become harmful when exceeds 10 mgL^{-1} in water that starts damaging the fish gills (Bury and Grosell, 2003). Manganese and nickel are naturally occurring micro-nutrients in the aquatic environments (Holmes, 2010). Nickel is less injurious when present in low concentration in freshwater and cause morphological transformation and chromosomal aberration in the cells (Clark and Keasling, 2002). Zinc is essential micro-nutrient while its uptake beyond permissible limits would become toxic to the fish due to disturbance in acid-base balance, ion regulation, disruption of gill tissues and hypoxia (Hogstrand *et al.,* 1994).

Metals are usually existed in the form of mixtures in the aquatic environment due to diversified sources of their discharge. The eco-toxicological studies are mostly concentrated on the effect of single metal exposure rather than their binary exposures (Lange *et al*., 2002). The impact of metal mixtures is much different than that of individual metals (Vosyliene *et al*., 2003). The existence of metal-metal interaction occurring at low environmental relevant concentrations has been reported to cause impact on the growth of different fish species also (Birceanu *et al*., 2008). Therefore, in order to conserve freshwater fisheries in the Punjab province, it is pertinent to reveal their tolerance limits against mixtures of iron, lead, nickel, manganese and zinc under acute exposures in the laboratory.

MATERIALS AND METHODS

Acute toxicity (96-hr LC_{50} and lethal concentrations) of water-borne metal mixture of five metals viz. iron, zinc, lead, nickel and manganese, was studied in three fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) under laboratory conditions at constant pH (7), total hardness (200 mgL^{-1}) and water temperature (30°C) by using static water system in glass aquaria. Fresh air was supplied to each aquarium through a pump fitted with capillary system. Stock solution of iron, lead, manganese, nickel and zinc were prepared. By dissolving required quantities of different stock solutions of metals, the following 19 mixtures were prepared on metallic ion equivalence basis.

Mixture#	Metal Mixture	Mixture#	Metal Mixture
1	Fe+Pb	11	Fe+Zn+Pb
2	Fe+Ni	12	Fe+Zn+Ni
3	Fe+Mn	13	Fe+Zn+Mn
4	Fe+Zn	14	Zn+Pb+Ni
5	Zn+Pb	15	Zn+Pb+Mn
6	Zn+Ni	16	Pb+Ni+Mn
7	Zn+Mn	17	Fe+Zn+Pb+Ni
8	Pb+Ni	18	Fe+Zn+Pb+Mn
9	Pb+Mn	19	Fe+Zn+Pb+Ni+Mn
10	Ni+Mn		

Thoroughly rinsed metal free aquaria were filled with 50 liter de-chlorinated tap water of desired hardness (200 mgL^{-1}) and pH (7) while water temperature was maintained at 30^0C by using automatic heaters. Each fish species was tested, separately, for acute toxicity tests (96-hr LC_{50} and lethal concentrations) against each metal mixture at constant hardness, pH and water temperature. Each test dose was evaluated with three replications. Fish mortality observations were made twice a day. In order to avoid immediate stress to the fish, the concentration of metal mixture within the experimental aquaria was gradually increased from 0 to 50% in 6 hours to finally 100% in 8 hours. The test concentrations for each metal mixture and fish species were started from zero with an increment of 0.05 and 5 mgL^{-1} (as total concentration on metallic ion basis) for low and high concentrations, respectively, for both LC_{50} and lethal concentration tests with each species of fish. The fish were not fed during 96 hours of each acute trial. A group of 10 fish of each species were tested against metal mixture for the determination of their acute responses during 96 hour duration. The dead fish were separated immediately at the time of mortality. No mortality was observed among the control fish kept under metal free water environment.

The analyzed data coincided the desired metallic ion concentrations in the test media quite satisfactorily. The 96-hr LC_{50} and lethal concentrations were estimated at 95% confidence intervals through the Probit analyses method (Hamilton *et al.*, 1977). The mean weight and length of three fish species used for these toxicity tests are presented in Table 1. Analysis of variance and Tukey's/Student Newnan-Keul tests (Steel *et al.*, 1996) were performed to find out statistical differences among the variables defined for this study.

RESULTS

Three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, were exposed 90 days, separately to 19 mixtures and recorded the following findings.

***Catla catla*:** The fish, *Catla catla* exhibited significantly variable sensitivity for all the 19 mixtures of five metals except between mixture # 2 and 5, and 11 and 12. However, the sensitivity of fish increased significantly against HMM # 19 (Fe+Zn+Pb+Ni+Mn) with a mean 96-hr LC_{50} value of 42.26±0.58 mgL^{-1} while this fish was significantly least sensitive to a mixture of Pb+Mn (HMM # 9) with a mean LC_{50} of 85.96±0.45 mgL^{-1}. The sensitivity of fish against various metal mixtures, determined as mean lethal concentrations, followed almost similar trend as that observed for 96-hr LC_{50} values. A mixture of five metals (HMM # 19) was significantly more toxic (with a mean lethal concentration of 66.86±0.79 mgL^{-1}) to the fish than rest of the mixtures. This fish showed significantly least sensitivity to a mixture of Fe+Ni with a mean lethal concentration of 128.53±0.69 mgL^{-1} (Table 2).

***Labeo rohita*:** The data on sensitivity of *Labeo rohita* to short term exposure of 19 mixtures of metals determined as their 96-hr LC_{50} and lethal concentrations are also presented in Table 2. This fish was significantly more sensitive to a mixture of five metals (Fe+Zn+Pb+Ni+Mn) with the mean LC_{50} of 61.94±0.42 mgL^{-1} while fish was significantly least sensitive to metal mixture # 4 (Fe+Zn). Non-significant differences existed among the mixture # 5, 6 and 9; between 1 and 16; 10 and 14; 17 and 18. Mean lethal concentrations for *Labeo rohita* ranged between 170.00±0.60 (mixture # 5) and 95.51±0.62 mgL^{-1} (Fe+Zn+Pb+Ni+Mn). The differences between these two mean values were statistically significant (Table 2).

***Cirrhina mrigala*:** Mean 96-hr LC_{50} values for *Cirrhina mrigala* varied significantly with a lowest mean value of 43.32±0.57 mgL^{-1} for mixture # 18 (Fe+Zn+Pb+Mn) closely followed by that of 43.35±0.78 mgL^{-1} determined for mixture #19. However, the difference between these two mean values was statistically non-significant (Table 2). *Cirrhina mrigala* exhibited significantly least sensitivity against a mixture of iron and zinc (Mixture # 4) followed by that of mixture # 9 (Pb+Mn). Mean lethal concentrations for fish varied significantly within 19 mixtures. Fish was significantly more sensitive to mixture # 19 with a mean concentration of 75.22±0.45 mgL^{-1}, followed by that of HMM # 18 (80.46±0.63 mgL^{-1}). This fish showed significantly least sensitivity against the mixture of iron and zinc (HMM # 4) followed by that of Pb+Mn mixture (Table 2).

Regarding overall response of fish towards 19 mixtures, mean sensitivity of fish to the mixture of five metals (Fe+Zn+Pb+Ni+Mn) was significantly high followed by that of four metal mixtures (Fe+Zn+Pb+Mn) with statistically

Table 1. Average weight (g), fork and total length (mm) of three fish species used for acute toxicity trials

Fish species	Average weight (g)	Average fork length (mm)	Average total length (mm)
Catla catla	3.28±1.87	57.04±11.89	65.86±12.07
Labeo rohita	5.59±2.08	75.05±11.85	84.12±11.29
Cirrhina mrigala	4.46±2.02	72.88±11.80	81.52±11.35

Table 2. Tolerance limits of three fish species, as 96-hr LC_{50} and lethal concentrations (mgL^{-1}), against various metal mixtures

Mixture #	Mean 96-hr LC_{50} (mgL^{-1})				Mean lethal concentrations (mgL^{-1})			
	Catla catla	***Labeo rohita***	***Cirrhina mrigala***	**Overall species means**	***Catla catla***	***Labeo rohita***	***Cirrhina mrigala***	**Overall species means**
Mixture 1 (Fe+Pb)	59.26±0.61 k	74.12±0.68 h	68.93±0.64 e	67.44±7.54 j	104.66±0.53 g	121.67±0.52 j	109.29±0.59 e	111.87±8.80 k
Mixture 2 (Fe+Ni)	78.75±0.56 b	96.99±0.72 b	64.44±0.70 h	80.06±16.30 c	128.53±0.69 a	140.98±0.46 d	100.35±0.46 h	123.29±20.28 d
Mixture 3 (Fe+Mn)	74.13±0.57 e	82.64±0.47 g	72.38±0.47 c	76.38±5.49 f	117.69±0.55 c	131.27±0.64 h	114.52±0.66 d	121.16±8.90 f
Mixture 4 (Fe+Zn)	61.17±0.38 j	107.17±0.60 a	76.16±0.60 a	81.50±23.50 b	100.08±0.56 i	155.70±0.90 b	118.56±0.55 a	124.78±28.33 c
Mixture 5 (Zn+Pb)	78.24±0.39 b	95.65±0.66 c	71.55±0.36 cd	81.81±12.40 b	118.63±0.74 c	170.00±0.60 a	115.96±0.55 c	134.86±30.46 a
Mixture 6 (Zn+Ni)	69.89±0.94 f	94.84±0.58 c	72.38±0.54 c	79.04±13.70 d	109.78±0.87 e	141.90±0.41 d	114.52±0.59 d	122.07±17.34 e
Mixture 7 (Zn+Mn)	65.24±0.24 h	85.58±0.54 e	65.23±0.52 gh	72.02±11.70 g	113.38±0.20 d	132.38±0.41 g	106.84±0.72 f	117.53±13.27 i
Mixture 8 (Pb+Ni)	50.38±0.36 n	72.63±0.46 i	64.75±0.48 h	62.59±11.30 l	81.41±0.42 j	115.89±0.57 m	114.05±0.41 d	103.78±19.40 n
Mixture 9 (Pb+Mn)	85.96±0.45 a	95.64±0.72 c	74.67±0.41 b	85.42±10.50 a	122.49±0.60 b	144.41±0.61 c	117.35±0.36 b	128.08±14.37 b
Mixture 10 (Ni+Mn)	75.95±0.45 d	87.45±0.63 d	67.99±0.46 e	77.13±9.78 e	108.94±0.36 f	145.06±0.63 c	106.95±0.64 f	120.32±21.45 g
Mixture 11 (Fe+Zn+Pb)	63.17±0.33 i	82.91±0.72 fg	56.04±0.35 j	67.37±13.90 j	99.44±0.41 i	139.28±0.46 e	106.77±0.49 f	115.16±21.20 j
Mixture 12 (Fe+Zn+Ni)	62.91±0.74 i	70.17±0.56 j	56.00±0.72 j	63.03±7.09 k	102.45±0.64 h	120.26±0.59 k	95.64±0.29 i	106.12±12.17 m
Mixture 13 (Fe+Zn+Mn)	61.17±0.34 j	83.94±0.49 f	70.86±0.59 d	71.99±11.40 h	100.08±0.43 i	136.24±0.46 f	118.42±0.41 a	118.25±18.80 h
Mixture 14 (Zn+Pb+Ni)	77.20±0.39 c	86.80±0.43 d	66.15±0.48 f	76.72±11.30 f	118.41±0.50 c	130.05±0.60 i	107.40±1.03 f	118.62±11.33 h
Mixture 15 (Zn+Pb+Mn)	55.78±0.55 i	63.08±0.40 l	54.64±0.57 k	57.83±4.58 n	80.17±0.40 j	102.07±0.50 p	95.20±0.34 i	92.48±11.20 p
Mixture 16 (Pb+Ni+Mn)	68.62±0.62 g	72.20±0.75 h	66.15±0.39 fg	68.99±3.04 i	107.84±0.63 f	116.99±0.59 l	107.40±0.41 f	110.74±5.41 l
Mixture 17 (Fe+Zn+Pb+Ni)	52.37±0.34 m	68.71±0.41 k	60.50±0.61 i	60.53±8.17 m	81.39±0.34 j	108.77±0.38 n	103.62±0.55 g	97.93±14.55 o
Mixture 18 (Fe+Zn+Pb+Mn)	46.60±0.59 o	68.70±0.43 k	43.32±0.57 l	52.87±13.80 o	79.48±0.59 k	106.97±0.47 o	80.46±0.63 j	88.97±15.60 q
Mixture 19 (Fe+ Zn+Pb+ Ni+Mn)	42.26±0.58 p	61.94±0.42 m	43.35±0.78 l	49.18±11.10 p	66.86±0.79 l	95.51±0.62 q	75.22±0.45 k	79.20±14.73 r
*Overall means (Treatments)	64.69±11.96 b	81.73±12.73 a	63.99±9.54 b		101.70±16.72 c	128.80±19.95 a	105.80±12.08 b	

Means with similar letters in a single column and row are statistically non-significant at $p< 0.05$.

significant difference. Amongst three fish species, *Cirrhina mrigala* and *Catla catla* showed significantly higher sensitivity toward metal mixtures with LC_{50} and lethal concentrations of 63.99±9.54 and 101.70±16.72 mgL^{-1}**,** respectively (Table 2).

DISCUSSION

The toxicity of various metals to the fish fluctuates extensively as a function of their diverse physicochemical characteristics (Azmat *et al*., 2012). Majority of research work has been conducted on the toxic effects of single metal species. However, in natural waters organisms are typically exposed to the mixtures of metals. Therefore, in order to provide data supporting the usefulness of freshwater fish as indicator of heavy metal's pollution, the acute toxicity of mixture of five metals viz. iron, zinc, lead, nickel and manganese has been determined for three commercially important fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*.

Static bioassay tests were performed to determine the acute toxicity of mixture of iron, zinc, lead, nickel and manganese, separately, on 90-day old *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. The present investigation reveals that all the three fish species showed significantly variable tolerance limits, in terms of 96-hr LC_{50}, against mixture of five metals. Amongst five fish species, significant differences were observed for their sensitivity to mixture of metals (Handy, 1992; Azmat *et al*., 2012). Amongst 19 treatments, a mixture of five metals (Fe+Zn+Pb+Ni+Mn) caused significantly higher toxicity to the fish, in terms of 96-hr LC_{50} and lethal concentrations. Regarding overall sensitivity of five fish species, *Labeo rohita* were significantly least sensitive. The present results are confirmatory with the results of Hua and Qixing (2009). They conducted an experiment to predict the isolated and combined effects of metals (Cd and Zn) on grass carp (*Ctenopharyngodon idella*). The calculated LC_{50} (96-hr) values indicated that acute toxicity of cadmium was significantly higher than zinc. The 96-hr LC_{50} of zinc and cadmium were 33.14 and 26.86 mgL^{-1}, respectively. Metals mixture exposure for 48 hours was antagonistic while it was synergistic due to 96-hour exposure period. Acute methods allow us to evaluate quickly the effects of toxicants on the organisms. The criterion of lethal toxicity is mortality, the final response of an organism to a toxic effect. On the basis of these methods, the sensitivity of organisms of different phylogenetic ranks and various developmental stages to toxicants has been mostly compared (Kazlauskiene *et al*., 1999; Abdullah and Javed, 2006; Javed and Abdullah, 2006; Azmat, 2011). The acute toxicity of heavy metal mixture (Zn+Pb) to *Callianassa Kraussi* was evaluated in hard water by Jackson *et al*. (2005). The mixture of zinc and lead appeared more toxic than the individual metals with significantly additive effects on fish tolerance limits. The tolerance limits of three fish species, as determined by their lethal concentrations against metals mixture varied significantly also (Azmat and Javed, 2011). The overall sensitivity of three fish species varied significantly for 19 mixtures with the mean higher and lower lethal concentrations of 134.86±30.46 and 79.22±14.73 mgL^{-1} for Zn+Pb and Fe+Zn+Pb+Ni+Mn mixtures, respectively. Paired bioassay was used to determine the relative sensitivity of two fish species viz. *Salvelinus confluentus* and *Oncorhynchus mykiss* to cadmium and zinc in single and binary form. *Salvelinus confluentus* was about twice as tolerant of cadmium and about 50% more tolerant to zinc than *Oncorhynchus mykiss*. The toxicity of cadmium alone was equivalent to the toxicity of Cd+Zn mixture to the fish (*Oncorhynchus mykiss*). However, the toxic effect of Cd+Zn mixture to *Salvelinus confluentus* was higher than Cd alone (Hansen *et al*., 2002). Acute toxicity of heavy metals (cadmium, zinc and copper) was determined individually or in mixture form for Chinese minnow (*Gobiocyprius rarus*). The sensitivity of this fish to heavy metals was different that depends on exposure period and developmental stages. Mixtures of Cu+Cd and Cu+Zn showed synergistic lethal effects. The enhanced toxicity of fish suggested inclusion of metal mixture considerations in risk assessment of heavy metals (Zhou *et al*., 2011).

In conclusion, fish were significantly more sensitive to mixture # 19 (Fe+Zn+Pb+Ni+Mn), followed by that of 18 (Fe+Zn+Pb+Mn) with statistically significant difference. Amongst three fish species, *Labeo rohita* were significantly least sensitive towards 19 metal mixtures both in terms of LC_{50} and lethal concentrations.

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