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Investigation of acute toxicity of lead-manganese mixture to fish under laboratory conditions

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Abstract

The aim of this study was to investigate acute toxicity (96-hr LC_{50} and lethal concentrations) of waterborne metal mixture (Pb+Mn) for the fish, *Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. The extent of metals bio- accumulation in fish body organs viz. gills, liver, kidney, fins, bones, muscle and skin at both 96-hr LC_{50} and lethal concentrations were also determined. The criteria of toxicity used during these experiments were mortality upon the 90-day old fish species. The tests were performed, separately, at constant pH (7), temperature (30°C) and hardness (200 mg L^{-1}) of water with three replications for each test dose. The overall sensitivity of five fish species, determined in terms of LC_{50} and lethal concentrations, against metals mixture (Pb+Mn) varied significantly. Among five fish species, *H. molitrix* were significantly more sensitive to metals mixture with mean 96 hr-LC_{50} and lethal concentrations of 69.36±0.03 and 114.51±0.02, respectively. Regarding overall responses of five fish species for their ability to accumulate metals, kidney appeared as an organ to amass significantly higher amounts of metals, followed by that of liver while it was significantly least in fish muscles. Accumulation of the metals in fish body followed the general order: kidney>liver>fins>gills >skin>bones>muscle.

Keywords: Acute toxicity, Bioaccumulation, Metal mixture, Fish, Pb, Mn.

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Introduction

The aquatic environment is the eventual recipient of the pollutants originated from natural and anthropogenic sources. Many contaminants can persist in the aquatic environment for a longer period of time can threaten the survival and physiology of the organisms by inducing genetic alterations which may lead to mutations and cancer (Russo *et al*., 2004). Heavy metals are ubiquitous in the biosphere where they occur as a part of the natural back ground of chemicals. Anthropogenic activities have also been introduced huge quantities of metals into the environment (Cheista *et al.*, 2006; Yaqub and Javed, 2012). The awareness regarding potential hazards caused by various heavy metals in the aquatic environments has stimulated a lot of interest in the use of fish as indicator to monitor the environmental carcinogens and mutagens (VanDer-Oost et al., 2003). The occurrence of pollutants in the aquatic environment influences the immune system, health and survival of the fish (Sweet and Zelikoff, 2001). The fish can accumulate heavy metals from their diet and water (Evans, 1987). Heavy metals mostly enter in the fish body through the gills, skin and food (Ni *et al*., 2005).

Lead is distributed into the environment by natural and anthropogenic sources. The concentration of lead in fish tissues corresponds to the environmental pollution levels and varies significantly with geographical area and demographic factors (Georgiou and Alouminas, 2000). Manganese in water can be significantly bio-concentrated by aquatic biota at lower trophic levels. Uptake of manganese by aquatic invertebrates and fish significantly increases with temperature and decreases with pH, whereas it was not significantly affected by dissolved oxygen. Uptake of manganese has been found to increase with decreasing salinity (Geneva, 2004).

Mixtures are characterized by antagonistic effects, while others by synergetic ones. Natural waters are frequently contaminated with trace metals as a result of human activities. In such conditions aquatic organisms are often exposed to a mixture of metals rather than a single element (Rainbow, 2002). Essential and non-essential metals may interact with each other affecting uptake, bioaccumulation and toxicity. The results of such interactions are highly variable ranging from antagonism to synergism depending on the species of metal, its concentration and exposure scenario and length of exposure (Norwood *et al*., 2003). The unsafe concentrations of heavy metals in our riverine systems and their negative influence on fish necessitate to plan and conduct this research project to see the toxic effects of lead and manganese mixtures on fishes viz. *Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*.

Materials and methods

Five fish species viz. *C. catla, L. rohita, C. mrigala, C. idella* and *H. molitrix* were brought to the wet laboratory of fisheries research farms and acclimated to laboratory conditions in cemented tanks for two weeks. During this acclimation period, fish were fed to satiation on feed crumbles with 32 % digestible protein and 3.00 Kcal g^{-1} digestible energy, but they were not fed during the last 24 hours of adaptation and throughout the test

duration. Acute metal mixture (Pb+Mn) toxicity tests were conducted in glass aquaria (60 liter) that had already been washed with HNO_3 and thoroughly rinsed with water prior to use. The pure chloride compounds of Pb ($PbCl_2$) and Mn ($MnCl_2.H_2O$) were dissolved in distilled water to prepare the desired stock solutions and further diluted as per required metal mixture concentrations.

Waterborne acute toxicity tests

Thoroughly rinsed metal free aquaria were filled with 50 liter de-chlorinated tap water of desired hardness (200 mg L^{-1}), pH (7) and water temperature (30^{o}C). 90-day fish species of the following average weight, fork and total lengths were tested for their tolerances against metal mixture (Table 1).

Table 1: Average weight, fork length and total length of test fish species.

Species	Average Weight (g)	Average fork length (mm)	Average total length (mm)
Catla catla	3.30±1.89	59.04±11.90	67.86±12.09
Labeo rohita	5.60±2.09	75.07±11.87	84.13±11.30
Cirrhina mrigala	4.48±2.01	72.89±11.81	81.52±11.35
Ctenopharyngodon idella	4.01±1.66	70.31±12.28	80.01±12.01
Hypophthalmichthys molitrix	3.84±11.78	65.07±11.56	74.92±11.73

Acute toxicity tests viz. 96-hr LC_{50} and lethal dose were conducted, separately, for each fish species against metal mixture (Pb+Mn). Tests for each toxicity dose were performed with three replications. Each metal mixture test dose in the aquaria started from zero and was gradually increased to avoid any stress to the fish with 50 % test concentration being used in 6-hr and full toxicant concentrations in 8-hr. The metal mixture test concentrations started from zero with an increment of 0.05 and 5 mg L^{-1} (as total concentration on metallic ion basis) for low and high concentrations, respectively, for both LC_{50} and lethal acute toxicity trials with each species of fish.

Ten fish of each species were placed in separate aquaria (3 replicates for each metal mixture) for the determination of 96-hr LC_{50} and lethal concentrations. The dead fish were separated and weighed individually after being lightly blotted dry at the time of mortality. No mortality was observed among the control (placed in metal free water) fish. After LC_{50} and lethal exposures of metal mixtures the dead fish were analyzed for corresponding metals in their body organs viz. kidney, liver, skin, muscle, fins, gills and bones by following the methods as described in SMEWW (1989) by using atomic absorption spectrophotometer (Analyst 400, Perkin Elmer, USA). The analyzed data obtained confirmed the desired metallic ions concentrations for each metal mixture in the test dose throughout the experimental period.

Statistics

Both 96-hr LC_{50} and lethal concentrations of each waterborne metal mixture were determined along with 95 % confidence interval and were observed by using Probit

Static Bioassay test. The 96-hr LC_{50} values and their 95% confidence interval were determined by Trimmed Spearman- Karbar method (Hamilton *et al.*, 1977). The data obtained from acute toxicity and accumulation of metals in the fish body during 96-hr LC_{50} and lethal tests were subjected to statistical analysis by using factorial experiment (RCBD) to statistically analyze the differences among various parameters (Steel *et al*., 1996). Relationships between acute toxicity of metal mixtures to the fish were determined by Pearson correlation coefficients.

Results

The overall tolerance limits of five fish species determined in terms of 96-hr LC_{50} against binary mixture of lead and manganese are presented in Table 2. The differences among all the five fish species, for their ability to tolerate metal mixture, varied significantly. Regarding overall sensitivity of five fish species, *H. molitrix* were significantly more sensitive to the metal mixture, followed by that of *C. mrigala*, *C. catla*, *L. rohita* and *C. idella*.

Table 2: Responses of five fish species for their 96-hr LC_{50} and lethal concentrations (mg L^{-1}) of metals mixture (Pb+Mn).

96-hr	Fish Species				
	Catla catla	***Labeo rohita***	***Cirrhina mrigala***	***Ctenopharyngodon idella***	***Hypophthalmychthys molitrix***
LC_{50}	85.08±0.02^{c}	96.56±0.04^{b}	76.45± 0.09^{d}	99.39± 0.07^{a}	69.36±0.03^{e}
Lethal concentrations	123.41±0.07^{c}	143.28±0.34^{b}	117.42±0.18^{d}	141.20± 0.05^{a}	114.51±0.02^{e}

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

The data regarding accumulation patterns of lead and manganese in the body organs of five fish species due to acute exposure to Pb+Mn mixture are presented in Table 3. *C. idella* and *C. catla* exhibited significantly higher accumulation of lead and manganese, respectively during 96-hr LC_{50} exposure of Pb+Mn mixture. *H. molitrix* and *L. rohita* had significantly lowest mean levels of lead and manganese, respectively after chronic exposures. Lead and manganese accumulations were significantly highest in fish kidney and liver, respectively. Lethal exposure of mixture resulted in significant accumulation of lead and manganese in the bodies of *H. molitrix* and *C. catla* while the same were significantly lowest in *C. catla* and *L. rohita*, respectively. Both lead and manganese accumulations were significantly highest in fish liver while they were significantly lowest in muscle tissue. Fish showed significantly higher tendency for the uptake and accumulation of manganese than that for lead.

Table 3: Accumulation patterns of metals (µg g^{-1}) in fish organs during 96-hr LC_{50} and lethal concentrations exposure of metal mixture (Pb+Mn).

Metals	Fish Species	Organs							
		Kidney	Liver	Skin	Muscle	Fins	Gills	Bones	*Means
LC_{50}									
Pb	*C. catla*	48.53±0.59^{d}	52.43±1.34^{e}	2.36±1.02^{e}	14.32±2.25^{b}	25.77±4.61^{c}	7.66±3.22^{e}	9.90±1.73^{d}	**23..00±20.14^{d}**
	L. rohita	65.93±0.63^{b}	6.66±0.80^{g}	3.19±1.66^{e}	6.83±1.02^{c}	77.86±0.61^{a}	23.43±0.37^{a}	7.82±2.46^{d}	**27.39±31.28^{c}**
	C. mrigala	59.38±0.66^{c}	271.33±1.15^{a}	35.34±3.44^{a}	24.13±2.40^{a}	5.35±1.32^{e}	8.63±3.54^{c}	17.85±3.69^{a}	**60.28±94.83^{b}**
	C. idella	491.90±0.41^{a}	148.33±2.88^{b}	16.89±2.71^{b}	0.93±0.56^{d}	49.87±3.23^{b}	18.58±2.93^{b}	10.27±0.17^{cd}	**105.25±177.78^{a}**
	H. molitrix	25.00±2.75^{e}	9.85±0.83^{de}	19.57±0.37^{b}	23.58±0.35^{a}	13.57±1.75^{d}	3.71±0.14^{d}	14.89±0.78^{b}	**15.74±7.60^{e}**
	Overall Means	**138.15±198.36^{a}**	**97.72±112.66^{b}**	**15.47±13.57^{d}**	**13.95±10.21^{d}**	**34.48±29.50^{c}**	**12.40±8.25^{e}**	**12.15±4.10^{e}**	
Mn	*C. catla*	282.08±2.60^{c}	670.25±2.38^{a}	116.93±2.14^{a}	24.54±1.47^{a}	116.41±4.21^{c}	38.08±0.31^{c}	28.02±1.26^{b}	**182.33±233.15^{a}**
	L. rohita	115.37±2.50^{e}	103.72±1.17^{g}	19.67±0.50^{d}	7.36±0.68^{d}	135.71±5.61^{b}	56.77±3.63^{a}	19.33±0.19^{cd}	**65.42±52.54^{c}**
	C. mrigala	301.67±1.66^{b}	466.33±4.04^{b}	12.01±0.58^{e}	6.45±0.18^{d}	50.03±0.32^{e}	25.37±1.82^{d}	13.69±0.38^{c}	**125.08±183.70^{c}**
	C. idella	307.92±1.90^{a}	365.00±5.00^{c}	64.90±1.61^{b}	9.69±0.12^{cd}	145.17±2.51^{a}	21.27±0.97^{e}	34.42±1.00^{a}	**135.48±145.24^{b}**
	H. molitrix	240.83±2.60^{d}	217.00±1.00^{d}	25.96±1.98^{c}	15.45±0.59^{b}	58.10±1.68^{d}	42.08±1.45^{b}	15.90±1.17^{d}	**87.90±97.73^{d}**
	Overall Means	**249.58±79.47^{b}**	**364.46±220.01^{a}**	**47.89±43.65^{d}**	**12.70±7.49^{e}**	**101.08±44.25^{c}**	**36.71±14.14^{e}**	**22.27±8.71^{f}**	
Lethal concentrations									
Pb	*C. catla*	107.97±0.74^{e}	127.16±1.66^{e}	43.60±1.65^{c}	38.18±0.26^{a}	193.14±2.91^{a}	53.03±1.64^{d}	101.43±0.43^{d}	**94.93±55.54^{e}**
	L. rohita	427.84±0.72^{c}	528.59±1.77^{c}	71.28±0.24^{a}	10.47±1.53^{d}	120.82±2.08^{c}	204.78±1.42^{a}	144.54±0.68^{b}	**215.47±191.51^{c}**
	C. mrigala	283.67±2.51^{d}	298.51±1.69^{d}	55.28±2.99^{b}	24.42±2.44^{b}	159.25±1.08^{b}	166.71±4.23^{b}	105.49±0.48^{c}	**156.19±105.47^{d}**
	C. idella	808.33±1.67^{a}	677.50±2.50^{b}	38.19±2.55^{b}	19.96±1.61^{c}	190.90±0.51^{a}	123.35±1.88^{c}	68.88±2.79^{e}	**275.30±325.63^{b}**
	H. molitrix	777.08±1.90^{b}	896.67±1.44^{a}	52.57±0.44^{b}	26.68±1.63^{b}	110.80±0.39^{b}	36.85±2.65^{e}	246.97±2.95^{a}	**306.80±371.28^{a}**
	Overall Means	**480.98±306.48^{b}**	**505.69±303.77^{a}**	**52.18±12.69^{f}**	**23.94±10.10^{g}**	**154.98±38.35^{c}**	**116.94±71.99^{e}**	**133.46±68.90^{d}**	
Mn	*C. catla*	577.78±12.06^{a}	993.33±3.81^{a}	130.52±1.09^{a}	31.69±0.58^{a}	243.70±1.12^{b}	40.20±2.06^{e}	72.39±1.31^{e}	**298.52±360.80^{a}**
	L. rohita	265.71±3.26^{e}	294.17±2.50^{e}	92.24±1.88^{b}	12.72±2.00^{e}	151.11±1.88^{c}	173.02±2.21^{a}	89.85±2.50^{b}	**154.11±100.32 e**
	C. mrigala	433.34±1.65^{c}	542.78±2.54^{b}	38.52±1.78^{e}	21.77±0.34^{b}	131.17±2.63^{d}	113.00±0.44^{d}	117.42±1.43^{a}	**199.71±203.68^{c}**
	C. idella	538.89±2.54^{b}	517.50±1.25^{d}	80.41±0.67^{c}	15.15±0.45^{e}	490.25±2.68^{a}	163.34±2.34^{b}	45.50±1.56^{d}	**264.43±239.62^{b}**
	H. molitrix	398.75±1.25^{d}	534.99±1.66^{c}	54.78±3.21^{d}	16.75±0.21^{e}	72.41±1.21^{e}	143.05±3.69^{c}	25.58±0.78^{e}	**178.05±205.33^{d}**
	Overall Means	**442.89±123.33 b**	**576.56±254.85^{a}**	**79.30±35.55^{c}**	**19.62±7.52^{g}**	**217.72±164.31^{a}**	**126.52±53.44^{d}**	**70.15±36.13^{f}**	

Comparison of means

Metals	Mean Accumulation in fish body (µg g^{-1})	
	*LC_{50}	*Lethal concentrations
Pb	46.33 ± 91.60^{b}	209.74 ± 239.06^{b}
Mn	119.24 ± 150.60^{a}	218.97 ± 227.27^{a}

Means with the same letters in a single row and * column are statistically similar at $p<0.05$.
C. catla= Catla catla; *L. rohita= Labeo rohita*; *C. mrigala= Cirrhina mrigala*; *C. idella= Ctenopharyngodon idella*; *H.molitrix=Hypophthalmichthys molitx*

Discussion

The susceptibility of species of different phylogenetic positions and various developmental stages to toxicants has often been compared by using acute methods (Kai Sun *et al*., 1995; Kazlauskiene *et al*., 2003; Abdullah and Javed, 2006). However, in nature many species have a direct impact of long-term exposure of lower concentration of toxicants or their mixtures. Significant differences have been reported for the toxicity of single metals against their mixture to the fish, *Synodontis clarias* and *Tilapia nilotica* (Obiakor *et al*., 2010). Heavy metals mixtures showed more toxicity than single metals (Kazlauskiene and Vosyliene, 2008).

The overall sensitivity of all the five fish species against various metal mixtures varied significantly. *H. molitrix* were significantly more sensitive to the mixture of metals, followed by the tolerance limits exhibited by *C. mrigala*, *C. catla*, *L. rohita* and *C. idella*. Among the three fish species *L. rohita* were less sensitive to Cr followed by those of *C. mrigala* and *C. catla* (Azmat and Javed, 2011). Acute (LC_{50}) toxicity of copper and cadmium (alone and in combined form) to the fish (*Oreochromis mossambicus*) has been evaluated by Munshi *et al*. (2005) who reported significant difference in the toxicity of various mixtures of metals and their accumulation in the fish body from that of individual metal exposures. The acute toxicity of copper and zinc (singly and in mixture form) can affect the developmental stages of rainbow trout, *Oncorhynchus mykiss* (Kazlauskiene and

Vosyliene, 2008). Marked changes were also observed in aeration rate of gills of fish. The exposure of mixture altered fish (*Clarias gariepius*) behavior such as loss of equilibrium, agitated swimming and air gulping activities (Vosyliene and Jankaite, 2006).

The acute exposure of metal mixture to fish caused significantly higher accumulation of metals in their kidney>liver>fins>gills>skin>bones>muscle. Therefore, pattern of metals bio-accumulation in various body tissues/organs of fish may be considered as a valuable indicator of environmental contamination (Jabeen *et al*., 2012; Javed, 2012). Fish kidney appeared as an organ, next to liver, that accumulated significantly higher metals in the fish body. This shows the importance of the kidney to act as the second most important site to store metals in all the five fish species. This corresponds to the study conducted by Azmat *et al*. (2012) that pointed out the kidney as a suitable indicator of metal contamination in major carps. Water-borne metals can be transported to various organs to induce histopathological and cellular alterations leading to genetic modification in animals (Tkatcheva *et al*., 2000). The uptake and accumulation of both essential and non-essential metals by the aquatic organisms, including fish, are similar. However, their bioaccumulation and toxicity effects vary significantly in different fish species (Luoma and Rainbow, 2005; Abdullah *et al*., 2011). Therefore, metabolic behavior of organisms is reflected in terms of growth that could potentially be affected by the interaction among the toxicity of various metals in a mixture form (Adhikari *et al*., 2009). Bio-accumulation of metals is dependent upon their concentration, route of uptake, availability, storage and excretion mechanisms of animals (Vijver *et al*., 2004). Apart from other functions and processes, metal-metal interactions have been found dependent upon the targeted organ/tissue, duration of exposure and exposure concentration of metals in mixtures.

Amongst five fish species, *C. idella* showed significantly lower sensitivity while *H. molitrix* exhibited significantly higher sensitivity to Pb+Mn for both 96-hr LC_{50} and lethal concentrations. Liver, kidney and fins were the three prime sites of metal bio-accumulation and their loads in the fish muscle were significantly low.

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