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Full Length Article

Manganese-Induced Acute Toxicity in *Labeo rohita*, *Cyprinus carpio* and *Oreochromis niloticus*: A Comparative Study

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Abstract

This study was undertaken to determine the acute toxicity of manganese in terms of 96-h LC_{50} and lethal concentrations for 120-days old three freshwater fish species viz. *Labeo rohita*, *Cyprinus carpio* and *Oreochromis niloticus* at constant water temperature (26°C), pH (7.60) and total hardness (240 mg L^{-1}). Pre-acclimatized fish were exposed to different test concentrations of manganese and dead fish were collected during 96-h exposure period. Data regarding fish mortality were analyzed by using Probit analysis method in order to calculate the mean 96-h LC_{50} and lethal concentrations of manganese for three fish species with 95% confidence intervals. The results revealed that the mean 96-h LC_{50} values of manganese for the three fish species ranged from 74.07±2.23 to 92.50±4.66 mg L^{-1}. The sensitivity of three fish species to manganese toxicity varied significantly that followed the order: *L. rohita* > *C. carpio* > *O. niloticus*. The obtained data could prove helpful in conservation of these valuable fish species. © 2017 Friends Science Publishers

Keywords: Aquatic pollution; LC_{50}; Manganese; Freshwater fish; Acute toxiciy

Introduction

Heavy metals are potentially harmful for the aquatic environment and human beings because they can cause high toxicity, bioaccumulation and biomagnification in the food chain (Yang *et al*., 2014; Javed, 2015). Although essential metals are required by the living organisms for various physiological functions but beyond specific concentrations they may become toxic to the organisms due to generation of free radicals leading to oxidative stress (Merciai *et al*., 2014). The key factors influencing the differential toxicity of metallic ions are metals specific, its solubility and mode of action along with physicochemical properties of the test medium (Naz and Javed, 2012). Manganese (Mn) exists naturally as a constituent of about hundred different mineral compounds (ATSDR, 2012). It plays vital roles in various metabolic activities, mineralization of bone and skeleton, reproductive process and cellular protection (Santamaria, 2008). Higher levels of manganese in the aquatic ecosystems are mainly attributed to various industrial and mining operations (Morillo and Usero, 2008). Literature is available on Mn toxicity in fish (Vieira *et al*., 2012) but the concentrations at which Mn become toxic is dependent upon its chemical compound and composition of aquatic medium, life stage and species of fish (Fish, 2009; Javed *et al*., 2016).

Survival and mortality of fish serve as valuable quantitative parameters for the assessment of acute toxicity of various pollutants (Azmat *et al*., 2012). *Labeo rohita*, *Cyprinus carpio* and *Oreochromis niloticus* are three freshwater and worldwide culturable fish species that show sensitivity to toxic compounds during toxicity bioassays conducted in the laboratories and therefore, serve as suitable tools in bio-monitoring studies (Osman *et al*., 2012). Due to lack of effective strategies for discharge of wastewater, the heavy metal load in aquatic reservoirs of Pakistan have exceeded their permissible limits (Azmat *et al*., 2012) that has seriously affected the freshwater fish fauna (Jabeen *et al*., 2012; Kousar and Javed, 2015). Keeping in view the current issues relating to freshwater fisheries in Pakistan the present research work was carried out to determine the acute toxicity of Mn to three freshwater fish species viz. *L. rohita*, *C. carpio* and *O. niloticus* that will help in the development of proper strategies for sustainable conservation of these valuable fish species.

Materials and Methods

Acute toxicity tests were conducted in the wet laboratories of Fisheries, Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The fingerlings of three freshwater fish species viz. *L. rohita*, *C. carpio* and *O. niloticus* were procured from the Fish Seed Hatchery, Faisalabad and transported to the wet laboratory in oxygenated polyethylene bags. Fish fingerlings were placed in large cemented tanks containing

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well aerated, dechlorinated tap water and acclimated under fluorescent light consisting of 12 h photoperiod in order to adapt them to laboratory conditions for two weeks prior to commencement of acute toxicity tests. During the entire acclimatization period, fish were fed with pelleted feed (30% DP and 3.00 kcal g^{-1} DE) twice a day to satiation. However, feeding was withheld three days before and throughout the 96 h time period of each acute toxicity trials. The 120-day old healthy fish belonging to each species, with almost similar average wet weights and total lengths were selected for acute toxicity tests (Table 1). Stock solution of manganese ($MnCl_2.4H_2O$: Mn) was prepared in distilled water that was further diluted for the preparation of desired test concentrations of the metal ranging from 0 to 150 mg L^{-1}. Before carrying out acute toxicity experiments, glass aquaria of 50 L water capacity were washed with clean water and then filled with thirty five liters of un-chlorinated tap water.

Fingerling of fish were divided into different groups, each containing 10 individuals that were transferred to experimental glass aquaria equipped with aerators to supply dissolved oxygen. Fish were exposed to different test concentrations of manganese separately, in each aquarium that were maintained throughout the course of experiment at constant level by periodic renewal of metallic test media. During 96 h LC_{50} and lethal toxicity trials concentration of Mn causing mortality of 50% fish population was considered as LC_{50}, while the concentration that results in hundred percent fish death was regarded as the lethal value. Following initial test concentration of 0 mg L^{-1}, the exposure concentrations of metals were continuously increased up to 0.05 mg L^{-1} (as low concentration) and 5 mg L^{-1} (as high concentration) until the onset of median (LC_{50}) and lethal concentration (LC_{100}). Because sudden exposure of each test concentration can cause stress to the fish, therefore, 50% test dose was gradually administered into glass aquaria in 3.5 h, while it reached the full concentration in 7 h.

Throughout the 96 h duration of acute toxicity tests, three physico-chemical parameters of the test media viz. water temperature, pH and total hardness were maintained at constant levels of 26°C, 7.60 and 240 mg L^{-1}, respectively. All test aquaria were carefully examined after every 4 h time interval during the 96 h period of acute toxicity trial in order to record fish mortality. Dead fish were removed from the water media, slightly blotted dry and their lengths and weights were recorded. Fish death was only observed in metal treated groups, while in batches of control fish no mortality was found.

Data regarding fish mortality were analyzed by using Probit analysis method (through MINITAB computer package) in order to calculate the mean 96 h LC_{50} and lethal concentrations of Mn for three fish species with 95% confidence intervals (Hamilton *et al*., 1977). Comparison of means was performed to find out statistical differences among them (Steel *et al*., 1996).

Results

The data on percentage mortality of *L. rohita*, *C. carpio* and *O. niloticus* at different exposure concentrations of Mn showed that fish mortality increased continuously with gradual increase in Mn concentrations of the test media (Fig. 1). Mn exposure at concentrations of 70, 80 and 90 mgL^{-1} resulted in 50% mortality of *L. rohita*, *C. carpio* and *O. niloticus*, while it caused 100% mortality of three fish species at concentrations of 120, 150 and 160 mg L^{-1}, respectively. Acute toxicity of Mn to the three fish species determined in terms of mean values of 96 h LC_{50} and lethal concentrations with 95% confidence intervals varied significantly at $P<0.05$. Among the three fish species, *L. rohita* appeared more sensitive to Mn exposure with mean 96 h LC_{50} value of 74.07±2.23 mg L^{-1} followed by that of *C. carpio* (84.80±5.08 mg L^{-1}) and *O. niloticus* (92.50±4.66 mg L^{-1}), respectively. The mean lethal concentrations of manganese for the three fish species ranged from 124.19±2.36 to 145.77±5.64 mg L^{-1} (Table 2).

The mean values of various physico-chemical parameters of the Mn test media are presented in Table 3. At different exposure concentrations of Mn, the mean dissolved oxygen contents of the *O. niloticus* test media were significantly lower as compared to *C. carpio* and *L. rohita* test media while total ammonia and CO2 concentrations of the *O. niloticus* test media were significantly higher than other two fish species.

Discussion

Acute toxicity in fish mainly involves immediate and delayed toxic effects. Dizziness and shortened breath are the initial symptoms of sudden metal stress, while delayed effects include changes in skin coloration and ultimately fish death occurs (Abdullah and Javed, 2006). During the present investigation, the percentage fish mortality increased gradually with increase in Mn concentrations (Fig. 1). Morcillo *et al*. (2015) reported that arsenic, cadmium, mercury and lead caused dose and time dependent acute toxic effects in *Dicentrarchus labrax.* Pandey *et al*. (2005) reported dose and time dependent 96 h acute toxicity of mercuric chloride and malathion to *Channa puctatus.* An important factor for the determination of 96 h LC_{50} is species-specific sensitivity of fish towards particular metals. The findings of the present study revealed significantly variable 96 h LC_{50} and lethal concentrations of Mn for the three fish species viz. *L. rohita*, *C. carpio* and *O. niloticus*. The lowest mean 96 h LC_{50} value was observed for *L. rohita* while *O. niloticus* showed significantly highest 96 h LC_{50} which indicated that *L. rohita* was considerably more sensitive to Mn toxicity as compared to other two species which exhibited greater tolerance against Mn toxicity (Table 2). Such variations in 96 h LC_{50} values of Mn for three fish species can be attributed to differences in their physiological conditions.

Table 1: Length-Weight measurements of the three fish species taken during acute toxicity tests of manganese

Fish Species	Average wet weights (g)	Average wet fork lengths (mm)	Average wet total lengths (mm)
L. rohita	10.67±0.39	84.69±3.23	92.86±2.75
C. carpio	9.99±0.28	81.45±4.63	89.19±4.87
O. niloticus	11.00±0.34	90.38±4.078	98.21±4.10

Table 2: Acute toxicity (96-h LC_{50} and lethal concentrations) of manganese for three fish species

Fish Species	Mean 96-h LC_{50} (mgL^{-1})	95% C.I. (mgL^{-1})	Mean lethal concentrations (mgL^{-1})	95% C.I. (mgL^{-1})
L.rohita	74.07±2.23c	63.87-82.19	124.19±2.36c	111.73-146.52
C.carpio	84.80±5.08b	72.69-93.61	141.06±8.82b	128.23-163.74
O.niloticus	92.50±4.66a	80.69-101.03	145.77±5.64a	133.07-169.05

Mean± standard deviation, C.I. = Confidence Interval; Means with similar letters in a single column (for each fish species) are statistically non-significant at P>0.05

Table 3: Mean water quality parameters of the manganese test media

Characteristics	Fish Species		
	L. rohita	*C. carpio*	*O. niloticus*
Temperature (°C)	26.01±0.00a	26.01±0.01a	26.02±0.01a
pH	7.60±0.00a	7.61±0.00a	7.60±0.00a
Total Hardness (mgL^{-1})	240.01±0.01a	240.01±0.00a	240.01±0.00a
Dissolved Oxygen (mgL^{-1})	5.43±0.25a	5.42±0.23a	5.29±0.22b
Total Ammonia (mgL^{-1})	0.65±0.11c	0.72±0.16b	0.78±0.20a
Carbon dioxide (mgL^{-1})	0.33±0.08b	0.35±0.11b	0.46±0.10a
Calcium (mgL^{-1})	26.74±1.12a	26.84±1.32a	26.74±1.17a
Magnesium (mgL^{-1})	44.12±2.64a	44.04±2.49a	44.11±2.40a
Sodium (mgL^{-1})	308.11±12.95a	308.15±13.40a	307.82±13.71a
Potassium (mgL^{-1})	8.87±1.49a	8.78±1.43a	8.87±1.49a

Means with similar letters in a single row (for each fish species) are statistically non-significant at P<0.05

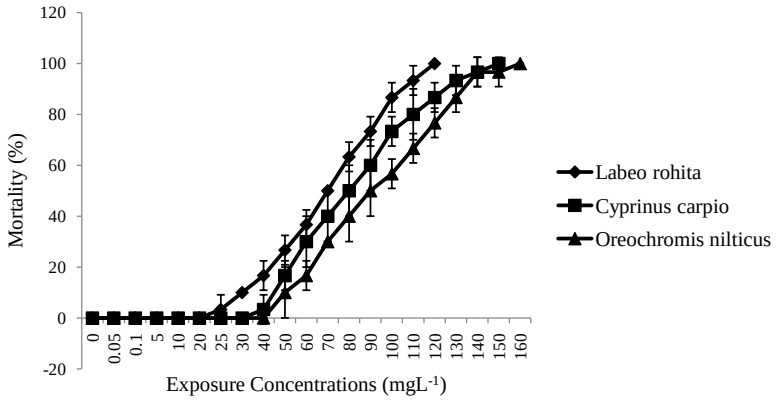

Fig. 1: The percentage mortality of three fish speciesat different concentrations of manganese during 96-h acute toxicity tests

Javid *et al*. (2007) also reported significant variability in mean 96 h LC_{50} of lead for *Catla catla*, *L. rohita*, *Cirrhina mrigala* that were found as 20, 30 and 45 mg L^{-1}, respectively. These data also corroborate the finding of Svecevicius (2010) regarding the sensitivity of five fish species viz. roach (*Rutilus rutilus*), dace (*Levciscus levciscus*), perch (*Perca fluviatilis*), rainbow trout (*Onchorhynchus mykiss*) and three spined stickleback (*Gasterosteus aculeatus*) to waterborne nickel. The 96 h LC_{50} value of Mn for embryonic *Danio rerio* has been reported to range from 3.34 Mm to 80 Mm (Hernandez *et al*., 2015). A laboratory study by Hoseini *et al*. (2014) revealed that 96-h median lethal concentration (LC_{50}) of manganese for *R. rutilus caspicus* was 300 mg L^{-1}. Philippe *et al*. (2017) determined the acute toxicity (24 h LC_{50}) of copper for *Nothobranchius furzeri* that was found to be 53.93 µg L^{-1}. Filho *et al.* (2017) reported that sensitivity of *Phalloceros caudimaculatus* to 4 metals in terms of LC_{50} (96 h) was in the following order: Lead (9.70±0.29 mg L^{-1}) > Cadmium (13.99±0.57 mg L^{-1}) > Zinc (14.23±0.63 mg L^{-1}) > Chromium (57.49±6.63 mg L^{-1}).

The toxicity of metals in fish is likely to be influenced by environmental variables such as water temperature, pH, dissolved oxygen, hardness, salinity and concentration of other metallic ions etc. (Witeska and Jezierska, 2003; Abdullah and Javed, 2006). A greater reduction in dissolved oxygen content of the *O. niloticus* test media as compared to other fish species in the present study can be explained by the fact that due to gradual increase in manganese concentrations the excretion of ammonia by *O. niloticus* increased to a greater extent and test media becomes more toxic. Thus, under such stressful condition the fish consumed large quantities of dissolved oxygen (Table 3). These results are supported by the findings of Abdullah and Javed (2006) who reported greater oxygen consumption and ammonia excretion by *C. catla* under acute stress of nickel, zinc, lead, manganese and iron.

Conclusion

The Mn becomes toxic to fish beyond a specific concentration because in natural aquatic ecosystems different anthropogenic sources are continuously increasing the concentration of dissolved manganese. Moreover, among the three fish species *L. rohita* appeared significantly most sensitive to manganese toxicity followed by that of *C. carpio* and *O. niloticus*. The physico-chemical characteristics of water also has greater impact on the toxicity of manganese to the fish.

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