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Research Article

Comparative Assessment of the Acute Toxicity, Behavior and Catalase Activity in *Cirrhina mrigala* Exposed to Fe+Ni+Pb+Zn Mixture

Huma Naz[1,*], Sajid Abdullah[2], Sidra Naz[3], Sidra Abbas[2], Wardah Hassan[2], Shakeela Perveen[2], Moazama Batool[1], Laiba Shafique[2]

[1]*Department of Zoology, Government College Women University, Sialkot, Pakistan*
[2]*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Physics, University of Agriculture, Faisalabad, Pakistan*

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Authors' Contributions
HM executed the research. SA supervised and guided in planning the research work. SN did statistical analysis. SA assisted in compiling the data. MB and WH helped in lab work. SP and LS helped in writing the manuscript.

Keywords
Acute toxicity, Metals mixture, Fish, Behavior, CAT.

Abstract | The 96 h LC_{50} and lethal concentration of Fe+Ni+Pb+Zn mixture for the fish, *Cirrhina mrigala* was determined in this research work. The fish were exposed to each of the 19 different concentrations (5-90 mg/L) of Fe+Zn+Pb+Ni mixture to determine 96 h LC_{50} and lethal concentration. During acute toxicity test, behavioral changes and catalase activity in liver, kidney, gills, brain and muscle of the fish were also studied. The mean 96 h LC_{50} and lethal value for *C. mrigala* was determined as 47.56±0.04 and 93.89±0.07 mgL^{-1}, respectively. The selected concentration of Fe+Ni+Pb+Zn concentrations showed strong and direct relationship with, fin movement followed by mucous secretion, hyperactivity and swimming rate for *C. mrigala* with R^2 value of 0.858, 0.853, 0.216 and 0.005, respectively. The Fe+Ni+Pb+Zn mixture exposure at 96 h LC_{50} concentration caused significant decrease of catalase activity in liver, kidney, gills, muscle and brain of *C. mrigala* as compared to control. This study suggests that the behavioral and biochemical parameters can be used as a valuable bio-marker to identify the toxic effects of pollutants in aquatic systems.

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Introduction

Aquatic pollution is a hot issue in different areas of Pakistan which is rising rapidly due to industrial revolution. Heavy metals are the major source of environmental contaminants, on entering aquatic ecosystem, cause serious problems to aquatic fauna due to their persistent nature (Farombi *et al.*, 2007). Aquatic ecosystems contained these metals in combined form due to diversified sources of their discharge (Lange *et al.*, 2002). Metals in combined form showed much varied effects than in single form (Vosyliene et al., 2003). The complex mixtures of toxicants in water bodies may cause injurious effects on the water living animals (Lopez-Lopez *et al.*, 2011). Water pollution mainly affects the aquatic animals in various ways such as biochemical or physiological disturbance in response to toxicity caused by those pollutants (Begam, 2004).

Among aquatic animals, fishes are most susceptible to changes in their surrounding environment. Fish can be used as a best indicator for assessing the condition of particular water body (Mokhtar *et al.*, 2009; Gupta *et al.*, 2009). The toxicants present in aquatic medium may affect the water quality as well as swimming, feeding, delayed hatching and maturation period of fish (Kumar *et

*Corresponding author: Huma Naz
humanaz98@yahoo.com

al., 2007; Laovitthayanggoon, 2006; Atif *et al*., 2005). At individual level response, behaviour is the one that is result of physiological and molecular modifications as well as ecological processes in organisms (Scott and Sloman, 2004; Weis, 2005). Toxicants have ability to amass in various tissues of fish (Rao and Padmaja, 2000). Therefore, in ecological, toxicological and pathological studies, the most commonly used fish organs are liver, gills, kidney and muscles (Sauer and Watabe, 1989; Velcheva, 2002; Heier *et al*., 2009) because all these organs are have higher ability to amass the toxicant due to their metabolic activity (Andres *et al*., 2000; Karadedeh, 2000; Marcovecchio, 2004).

Oxidative stress due to metals toxicity cause the production of reactive oxygen species (ROS), such as H_2O_2, O^{-2} and OH^- that cause oxidation of lipids, proteins, RNA and DNA, which disrupts the structure of cell finally cause the cell death (Cao *et al*., 2010). Fish tissues are gifted with this system to save them from oxidative stress induced by metals (Atli and Canli, 2008). Antioxidant defense system consists of enzymes like superoxide dismutase, glutathione peroxidase, catalase, glutathione-reductase and glutathione S-transferase (Pinto *et al*., 2003; Tripathi *et al*., 2006). Catalase is a key component of defensive system scavenges hydrogen peroxide which has ability to interact with biological membranes and also inhibit some enzyme activities. Evaluation of oxidative stress biomarker such as catalase activity is considered to be a useful measure before any injurious effects appeared in fish (Sanchez *et al*., 2005; Gul *et al*., 2004). The heavy metals in the riverine systems are exist in the form of mixtures and their unsafe concentrations exert their negative impacts on the fish, necessitated this research work, that was performed to estimate the acute toxicity of waterborne Fe+Ni+Pb+Zn mixture and its effect on catalase activity in different organs of freshwater fish, *Cirrhina mrigala*.

Materials and Methods

96 h LC_{50} and lethal concentration

Cirrhina mrigala (Mori) was selected as an experimental fish. The experimental fish was purchased from Fish Seed Hatchery, Faisalabad. The acute toxicity test (96 h LC_{50} and lethal concentration) was conducted in the wet laboratory at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. The fish were placed in cemented tank for acclimatization to laboratory conditions for 15 days. Fish were fed with a commercial fish feed twice a day at 3% body weight during the period of acclimation but were starved for 24 h prior to the experiment and throughout the experiment. No mortality was observed during the acclimatization. During acclimatization, 12 h light and 12 h dark photoperiod was maintained. Chlorides Salts of iron ($FeCl_2.4H_2O$), zinc ($ZnCl_2$), lead ($PbCl_2$) and nickel ($NiCl_2.6H_2O$) were used to make stock solutions of metal. Metals mixture of required concentration was ready by dissolving a suitable volume of stock solution in deionized water. The experimental fish *C. mrigala* was exposed to each of the 19 different concentrations (5-90 mg/L) of heavy metals mixture (Fe+Zn+Pb+Ni) to determine 96 h LC_{50} and lethal concentration. The trial was conduct with three replications in 100 L glass aquaria each having ten *C. mrigala* (90-day old).

Physico-chemistry of test media

The temperature, hardness and pH of water were maintained as 30°C, 225 mgL^{-1}, and 7.5, respectively. The other water quality parameters *viz.* carbon dioxide, ammonia, electric conductivity, dissolved oxygen, potassium, magnesium, sodium, and calcium, were also measured (APHA, 2005).

Behavioral study

During the acute test, the behavioral abnormalities such as hyperactivity, swimming rate, mucous secretion and fin movement of the healthy (control) and metals mixture exposed fish were regularly observed.

Biochemical study

After acute exposure of metals mixture, fish were sacrificed and organs *viz.* gills, liver, kidney, muscle and brain of fish was separated to evaluate the catalase activity.

Preparation of homogenate

The dissected organs (gills, liver, kidney, muscle and brain) were, separately, homogenized in cold phosphate buffer in ration of 1:4 (w/v) by using a blender. Organ homogenates were centrifuged for 15 min at 10,000 rpm and 4°C. The clear supernatants were used for catalase assay.

Catalase assay

Catalase activity was noted according to the method of Chance and Mehaly (1977). Buffer substrate solution of H_2O_2 (10 mM) was prepared in 60 mM phosphate buffer (pH 7.0). Reaction mixture contained 2ml of buffered substrate solution and 0.05 ml of enzyme extract. The absorbance was recorded at 240nm.

Statistical analyses

The whole experiment was performed with three replicates. Probit Analysis was applied to calculate the LC_{50} and lethal values of metals mixture for fish *C. mrigala* (Hamilton *et al*., 1977) at 95% confidence interval. Correlation analysis was performed to find out relationship among physico-chemical parameters with Fe+Zn+Pb+Ni mixture concentrations. Statistical differences among different variables were found by performing ANOVA. A regression analysis was applied to check the relationship between various mixture concentration and behavioral parameters under study (Steel *et al*., 1997).

Results and Discussion

96 h LC_{50} and lethal concentration

The obtained data on fish mortality with mean 96 h

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LC_{50} and lethal values, concentration of metals in mixture, exposure duration, 95% lower and upper confidence interval limits with their calculated chi-square values are given in Table I. The LC_{50} and lethal value (96 h) for *C. mrigala* was computed as 47.56±0.04 and 93.89±0.07 mgL^{-1}, respectively. The Deviance Chi-Square and p-value for *C. mrigala* was determined as 3.72 and 0.994, respectively. The mortality of the fish, *C. mrigala*, increased with increasing concentrations of Fe+Ni+Pb+Zn mixture and duration of exposure. Figure 1 shows the relationship between the concentrations of Fe+Ni+Pb+Zn mixture and mortality of *C. mrigala*. Mean LC_{50} and lethal concentrations of Pb+Ni for *Ctenopharyngodon idella* (56.42±2.51 and 120.98±7.18 mgL^{-1}) and *Hypophthalmichthys molitrix* (55.85±2.84 and 128.44±9.25 mgL^{-1}) was reported by Naz and Javed (2013). Abdullah *et al*. (2011) calculated the 96 h LC_{50} value of Pb, Zn, Ni and Mn for *C. mrigala* (90-day old) as 32.68, 40.58, 23.96 and 71.24 mgL^{-1}, respectively. Naz and Javed (2012) find the 96 h LC_{50} and lethal concentrations of Fe+Zn+Pb+Ni+Mn mixture for *Cirrhina mrigala* as 43.35±0.78 and 75.22±0.45 mgL^{-1}, respectively. The 96 h LC_{50} and lethal value of Fe+Ni mixture for the fish *C. mrigala* was estimated as 64.44±0.70 and 100.35±0.46 mgL-1, respectively (Naz and Javed, 2013).

Behavioral study

Behavioral abnormalities in the any examined individual are the most responsive signs of toxicant exposure. During acute toxicity test period (4 day) fish behavior was observed. Fish, *C. mrigala* exposed to various concentration of Fe+Ni+Pb+Zn mixture for 96 h showed a marked change in behavior. The alter behavior of fish such as hyper-activity, swimming rate, mucous secretion and fin movement were increased with increasing the concentration of metals mixture and finally fish became settled at the bottom and all activities were stopped before death. Figure 2 represents the relationship between fish behavior and Fe+Ni+Pb+Zn concentrations used during this experiment. The exposed Fe+Ni+Pb+Zn concentrations showed strong and direct relationship with, fin movement followed by mucous secretion, hyperactivity and swimming rate for *C. mrigala* with R^2 value of 0.858; 0.853; 0.216 and 0.005, respectively. In control fish, no behavioral changes were recorded. The response and survival of aquatic organism mainly depends on the biological conditions of organisms and physico-chemical parameter of water. In addition, it also depends upon toxicity, type and nature of toxicants (Batool *et al*., 2014). According to Remyla *et al.* (2008) modifications in behavioral patterns of organisms is the major responsive sign of anxiety caused by contact to chemicals.

Batool *et al.* (2014) checked the acute impacts of cadmium and copper on behavioral responses of *Channa marulius and Wallago attu*. During the acute exposure of both metals, the fish showed increased surface behavior, hyperactivity and erratic swimming. These results are also similar to the findings of Biuki *et al*. (2010). Exposure of high concentration of cadmium chloride to fish (*Chanos chanos*) showed behavioural changes such as swimming disorder and fin movements. According to Susan and Sobha (2010) exposure of fenvalerate change the behavior (such as swimming at the water surface, increased mucus production and hyperexcitation) of *C. catla, L. rohita and C. mrigala*. Hesni *et al*. (2010) reported that the milkfish (*Chanos chanos*) exposed to lead nitrate showed behaviour changes such as downward and vertical swimming patterns, increased mucus secretion, hyperactivity and loss of balance. The abnormal behavior like hyperactivity was dose and duration dependent (Tiwari *et al*., 2011).

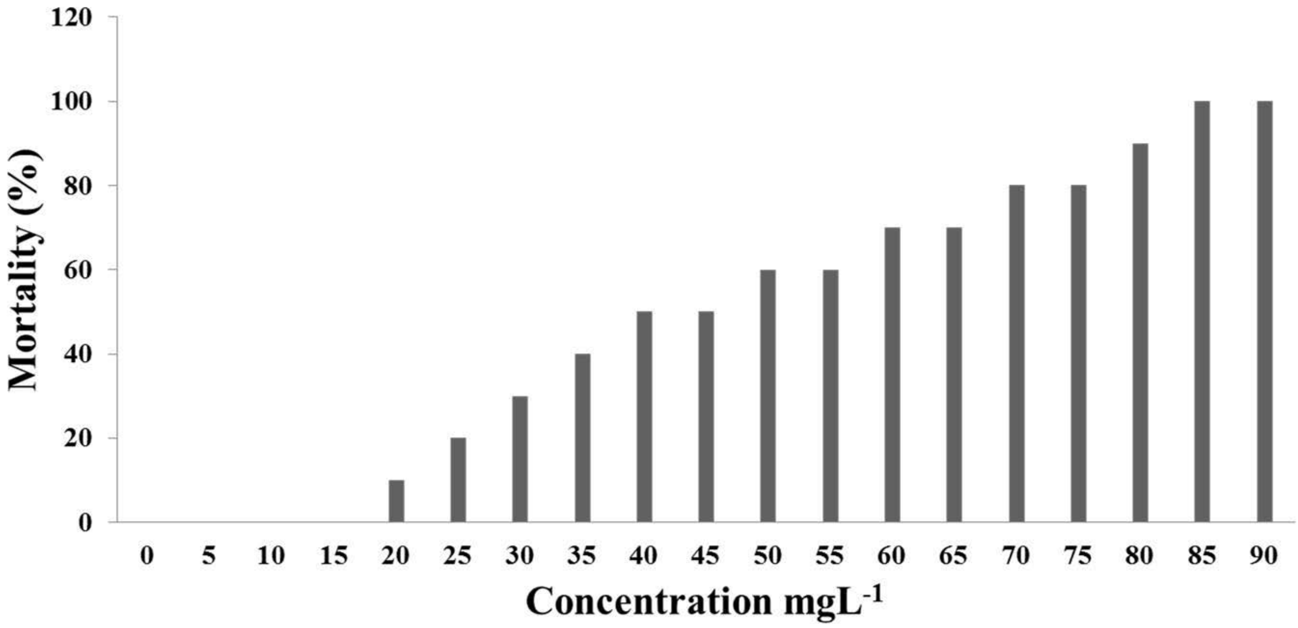

Figure 1: Relationship between *C. mrigala* mortality (%) and exposed concentrations of Fe+Ni+Pb+Zn mixture (mgL^{-1}) during 96 h acute toxicity test.

Table I: 96 h acute toxicity of heavy metals mixtures (mgL^{-1}) for *Cirrhina mrigala*.

Fish species	Metals mixture	Mixture ratio	LC_{50}	95% CI (LCL-UCL)	Lethal conc.	95% CI (LCL-UCL)	Pearson goodness of fit tests		
							Chi-Square	DF	p-value
Cirrhina mrigala	Fe+Ni+ Pb+Zn	1:1:1:1	47.56	38.93-53.62	93.89	84.07-111.44	3.72	13	0.994

CI, confidence interval (mgL^{-1}); LCL, lower confidence limit (mgL^{-1}); UCL, upper confidence limit (mgL^{-1}); Lethal Conc., lethal concentrations (mgL^{-1}); DF, degree of freedom.

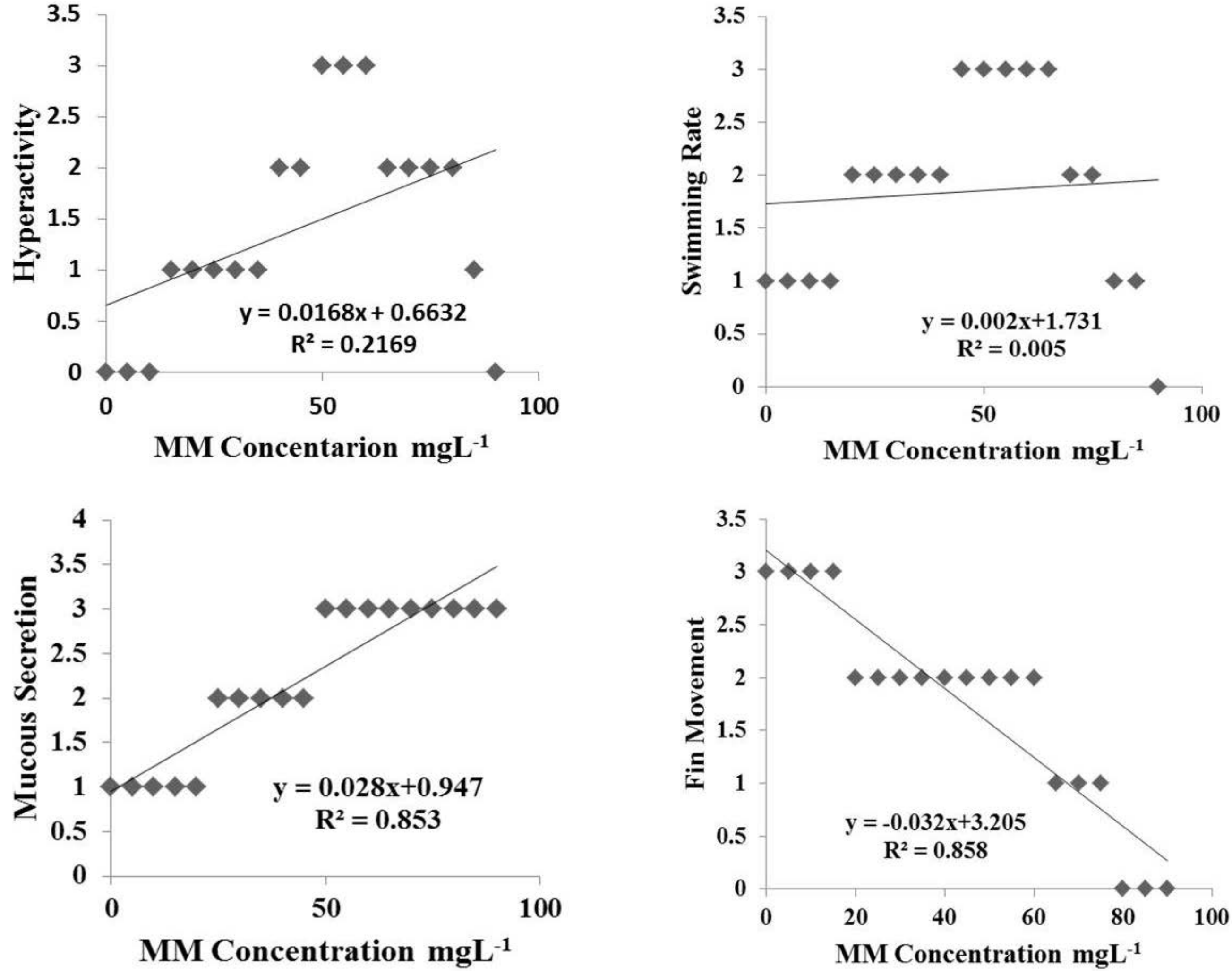

Figure 2: Behavioral response of *C. mrigala* exposed to various concentrations of Fe+Ni+Pb+Zn mixture.

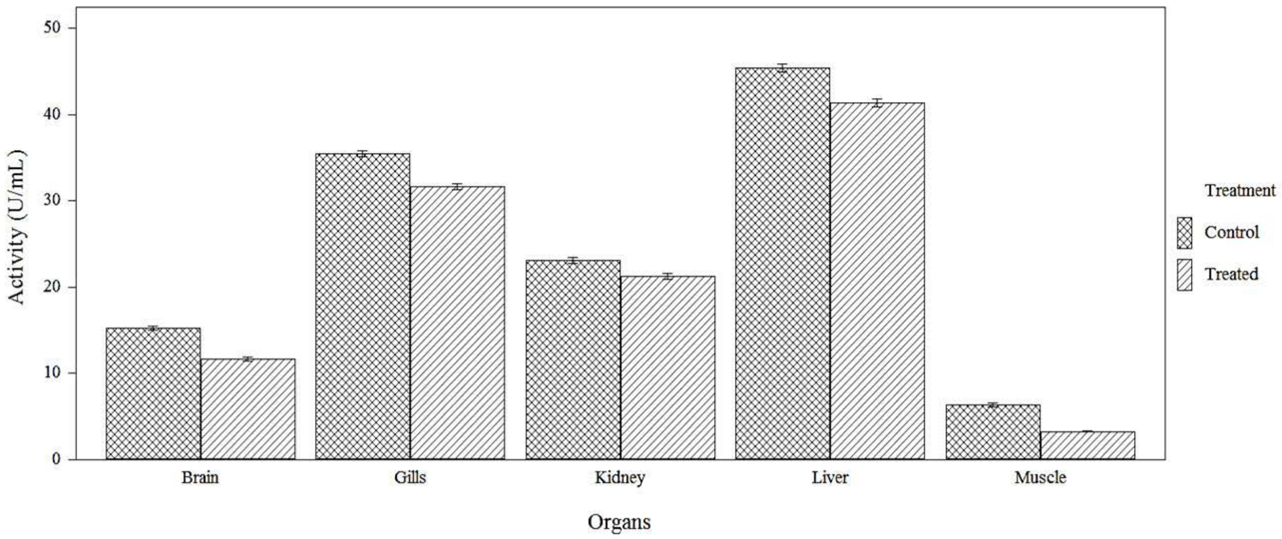

Figure 3: Catalase activity in different organs of *C. mrigala* exposed to 96 h LC_{50} concentration of Fe+Ni+Pb+Zn mixture.

Catalase activity

Figure 3 shows the exposure of Fe+Ni+Pb+Zn mixture at 96 h LC_{50} concentration caused significant depletion in catalase level in all observed organs of *C. mrigala* as compared to control. Results were statistically highly significant (p>0.001). According to Batool *et al.* (2014) acute exposure of metals (chromium and cadmium) to fish caused reduction in catalase activity. Mohanty and Samanta (2016) reported the significantly reduced catalase activity in *Notopterus notopterus* muscle tissues exposed to Fe, Cu, Ni, Cd, Pb and Zn from Mahanadi River. Present work was supported by Atli and Canli (2010), who reported the reduced catalase level in kidney and liver of tilapia under the acute exposure of metals *viz.* Cu, Cd, Zn, Cr and Fe. Heavy metals (Cr, Ni, Pb and Cd) altered the antioxidant enzymes activities in all tissues (gills, brain, kidney and liver) of *Heteropneustes fossilis* and *Channa striatus* inhabiting Kali River of northern India (Fatima and Usmani, 2015). According to Atli and Canli (2010) fluctuation in antioxidant enzymes responses against metal exposures, depend upon nature of toxicants, organ and type of exposure. CAT is primarily a peroxisomal enzyme that converts the hydrogen peroxide into water and oxygen (Fatima and Usmani, 2015). According to the Atli and Canli (2010) the inhibition in catalase may be due to the binding of metal ions to–SH groups on catalase which results in increased O^{-2} and H_2O_2 radicals. According to Orun *et al.* (2008) catalase level dropped off significantly in liver of *Oncorhynchus mykiss* when exposed to metals (Cd+2, Cr+3 and Se).

Conclusion

This study demonstrated that acute toxicity of heavy metals mixture not only affects the behavior of fish but also had negative impact on antioxidant enzyme (catalase) of fish. It was also concluded that catalase enzyme can be taken as a sensitive biomarker in eco-toxicology and fish can be used as a good indicator of water contamination.

Conflicts of interest

The authors declare no conflicts of interest.

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