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Research Article

Acute Nickel Toxicity Responses of *Labeo rohita* and *Cirrhinus mrigala*

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SN conducted the research work. AMMC helped in statistical analysis. SS provided assistance during the manuscript write-up.

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Abstract | The exposure of acute nickel toxicity (96-hr LC_{50} and lethal concentrations) for two fish species viz. *Labeo rohita* and *Cirrhinus mrigala* was determined in this study. During metal stress trials both species were kept at constant temperature (32°C), pH (7) and hardness (250 mg L^{-1}) of water. Physico-chemical parameters of test medium were monitored regularly. The observed mean LC_{50} and lethal concentrations for *Cirrhinus mrigala* were 55.85 ± 2.84 and 128.44 ± 9.25 mg L^{-1}, respectively. On the other hand, for *Labeo rohita*, mean values of LC_{50} and lethal concentrations were calculated as 56.42 ± 2.51 and 120.98 ± 7.18 mg L^{-1}, respectively. *Cirrhinus mrigala* had lower LC_{50} but higher lethal dose. Two fish were different in size as well. A significant positive correlation of metallic ion concentrations was observed with sodium, potassium, carbon dioxide and electrical conductivity whereas an inverse relationship was obtained with dissolved O_2 for both *Labeo rohita* and *Cirrhinus mrigala* test mediums. It is concluded that acute concentration of nickel is lethal for both fish species with a significant difference of LC_{50} and lethal concentrations among two fish species.

Novelty Statement | The study is novel in recommending continuous monitoring of metal contamination in fish species to ensure the sustainability of this food for human consumption.

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Introduction

Pakistan is an agricultural country that relies on the water assets such as rivers, ponds, lakes and streams. The water quality is continuously falling apart due to untreated industrial waste and freshwater becomes more contaminated (Kousar *et al.*, 2020). It causes harmful effects not only for fishes but also shows adverse consequences on other planktonic water bodies due to the presence of heavy metals such as Zn^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+} and Pb^{2+} (Javed and Mahmood, 2001). Aquatic environment becomes affected due to different heavy metal sources for example industrial waste, petroleum, acid rains and metal ions leaching process of soil (Olsvik *et al.*, 2000). The higher concentration of heavy metals creates a major issue for aquatic system and we are pushed back to a contaminated world (Asaolu, 2002). Heavy metals do not show any bending in water and inhabit the sediment quickly because of their specific properties of having more thickness as compared to water (Ghosh *et al.*, 2018). This type of contaminated water with surplus amount of heavy metals causes an increase in the permissible limits of water (Chiu *et al.*, 2011).

Penetration of heavy metals occurs through different ways such as gills, skin (ion exchange), ingestion and tissue adsorption process (Ahmed *et al.*, 2014). Many factors affect the metal accumulation in various body parts such as solubility of water, mode of feeding and ecology. Another most important factor includes the physiology of fish as like age, specie, size, bioavailability, body health,

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reproductive circumstances and varying habitats (Foveau and Dauvin, 2017; Perugini *et al.*, 2014).

Although due to presence of various pollutants, consumption of fish may become a threat for human beings. These chemically metallic substances are highly dangerous even at very low concentrations. Heavy metals are essential components of earth crust that occurs naturally and also measured as natural elements of aquatic system with limited permissible concentration. Now due to intense agricultural and industrial activities, its level of concentration has been increased (Zhang *et al.*, 2011; Martín *et al.*, 2015; Munari *et al.*, 2017).

Specific toxicity tests are performed to check out harmful effects of heavy metals on aquatic creature (Akter *et al.*, 2008; Javed, 2013). It allows to establish a dose-response relationship to find adverse effects of contaminants on the aquatic fauna and discharge rate of pollutants in aquatic organisms (Association *et al.*, 1915). Acute toxicity tests are performed to determine the impact of pollutants on aquatic systems during their life expectancy (Ebrahimpour *et al.*, 2010; Javed, 2012), to analyze metal toxicity on fish biology (Javed and Saeed, 2010), to monitor their ability of adaptation under toxic levels and to estimate various possible toxicity impacts on them (Abdul *et al.*, 2009; Azmat *et al.*, 2012). Low level of essential metals i.e. copper, nickel, zinc, chromium, iron and cobalt causes various infections while their high level may also cause toxicity (Sivaperumal *et al.*, 2007; Abadi *et al.*, 2015). Low concentration of nickel is less damaging when present in fresh water bodies and it may be causes any type of morphological change or any chromosomal aberration within cells (Magyarosy *et al.*, 2002).

Now, fresh water bodies contaminated with pesticides have become a major issue that effect aquatic organisms badly (Indirabai *et al.*, 2010; Iqbal *et al.*, 2012; Naz and Javed, 2012). Heavy metals toxicity in rivers have shown adverse effects on native fish fauna including carps such as *Catla catla, Cirrhinus mrigala* and *Labeo rohita*. (Rauf *et al.*, 2009; Azmat *et al.*, 2012). Although among all pollutants, metals are of vital concern due to their diverse impacts as it causes toxicity in fish (Abdul *et al.*, 2009). Keeping in view of toxic effects of nickel on aquatic habitats, posing threats to the freshwater fish, the present investigation was conducted to study the response of *Labeo rohita* and *Cirrhinus mrigala* to acute (LC_{50} and lethal) toxicity of nickel.

Materials and Methods

The current work was performed in Zoology Laboratory, The Govt. Sadiq College Women University Bahawalpur. To evaluate the acute toxicity effect of nickel on *Labeo rohita* and *Cirrhinus mrigala,* a 96-hr LC_{50} and lethal concentrations trials were carried out at 7 pH, 32˚C temperature and 250 mg L^{-1} hardness of water in 70 liters glass aquaria. Fish were allowed to adapt the laboratory conditions for 12 hours prior to start of the experiment. A desired amount of pure compound $NiCl_2$ was mixed with de-ionized water. Then required metal dilutions was prepared from the stock solution. Different fish used for 96-hr LC_{50} as well as for lethal toxicity tests were mentioned below (Table 1).

Table 1: Average fish weight and total length used for acute toxicity experiments.

Fish age (Days)	Fish species	Wet weight (g ± SD)	Total length (mm ± SD)
84-day	*Cirrhinus mrigala*	2.12 ± 0.11	55.88 ± 0.36
	Labeo rohita	1.64 ± 0.11	51.21 ± 0.09

For each test concentration, 20 fish of each species were kept in each glass aquarium for a period of 96-h. To prevent the fish from stress, metal concentration of each aquarium was gradually increased, 50% test concentration maintained in 3.5 hours along with full toxicant concentration in 7 hours. All test mediums were provided with constant air through air pump installed in each aquarium. Metal concentration for each fish species varied from zero with an addition of 0.05 and 5 mg L^{-1} (as a total concentration) for lower and higher concentrations. Fish mortality and physico-chemical variables (temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium and carbon-dioxide) were recorded at 12 hours interval during 96 hours for each experimental trial (APHA, 1998). The experiment was ended after completion of 96-h of experiment. There were three replications for each experiment. The number of dead fish were counted during the experiment.

Statistical analysis

The complete data obtained after experiment was then statistically analyzed using micro-computer according to Steel *et al.* (1996). To check the statistical variations among different variables, Analysis of Variance (ANOVA) and Duncan's Multiple Range tests were carried out. In addition, correlation was also used to find associations among different factors. Probit Analysis Method (Hamilton *et al.*, 1977) was used to analyze data on percent fish mortality that was attained during 96-h LC_{50} and lethal concentration experiments.

Results and Discussion

An experiment was performed for 84-day age groups of two fish species viz. *Cirrhinus mrigala* and *Labeo rohita*. Separate experimental tests were performed for their sensitivity towards nickel LC_{50} and lethal concentrations with 95% confidence interval. To determine their 96-hr

LC_{50} and lethal responses, both species were exposed to variable water-borne nickel concentrations. Mortality data was analyzed through Probit analysis (Table 2). The obtained mean values of 96-hr Ni LC_{50} for *Labeo rohita* were as 56.42 ± 2.51 mg L^{-1} with confidence interval range of 51.45 - 61.52 mg L^{-1} whereas its mean lethal concentration was monitored as 120.98 ± 7.18 mg L^{-1} among confidence interval of 109.09 - 138.35 mg L^{-1}. High significant results were shown by probability graph of regression coefficient which demonstrates the fillness of regression line (Figure 1). At 95 percent confidence interval, normal distribution of data showed the mean *Cirrhinus mrigala* LC_{50} value as 55.85 ± 2.84 mg L^{-1} among confidence interval range of 50.37 - 61.81 mg L^{-1} whereas the mean lethal concentration was obtained as 128.44 ± 9.25 mg L^{-1} with confidence intervals of 113.47 - 151.57 mg L^{-1} (Figure 2). Statistically different responses (i.e. $p < 0.05$) were observed for both fish species. Significantly more sensitive response for 96-hr LC_{50} was shown by *Cirrhinus mrigala* and significant low lethal response was shown by *Labeo rohita* (Table 2). Complete data concerned with correlation coefficients along with physico-chemical variables and nickel exposure concentrations of test mediums meant for both fish species was mentioned in Tables 3 and 4. A significant positive correlation of metallic ion concentrations was found with CO_2, sodium, potassium, electrical conductivity and total ammonia. However, an inverse relationship was obtained with dissolved O_2 of test mediums, suggesting that different physio-chemical parameters may have significant impact on the obtained results of the experiment hence, their value must be kept on record throughout the experimental duration to keep avoid the unwanted impact of these parameters on actual experimental variables.

Table 2: Mortality rate of fish during acute nickel exposure for 96 hours.

Fish age (at the start of the trial)	Nickel exposure concentrations (mg L^{-1})	Fish mortality (%)							
		Labeo rohita				*Cirrhinus mrigala*			
		R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
	5	0	0	0	0.00	0	0	0	0.00
	10	0	0	0	0.00	10	10	10	10.00
	15	10	10	10	10.00	10	20	20	16.67
	20	10	20	20	16.67	20	20	20	20.00
	30	20	20	20	20.00	30	30	30	30.00
84-day	40	30	30	30	30.00	30	40	40	36.67
	50	30	40	40	36.67	40	40	35	40.00
	60	40	40	40	40.00	50	50	40	46.67
	65	50	50	50	50.00	50	50	50	50.00
	70	60	60	60	60.00	60	60	60	60.00
	75	70	70	70	70.00	80	70	70	73.33
	80	80	80	80	80.00	90	80	90	86.67
	90	90	90	90	90.00	100	100	100	100.00
	100	100	100	100	100.00	-	-	-	-
Factor	Mean Nickel Concentration (mg L^{-1} ± SD)								
	Labeo rohita					*Cirrhinus mrigala*			
LC_{50}	56.42 ± 2.51[a]					55.85 ± 2.84[b]			
Lethal concentration	120.98 ± 7.18[b]					128.44 ± 9.25[a]			

ǂ: The means with different letters (a & b) in a single row are statistically different at $p < 0.05$.

Among different fish species, the acute toxicity (lethal concentration and 96-h LC_{50}) of metals may be varying. A fish may be highly sensitive to a specific metal but at the same time that fish may show high resistant to another metal (Biuki *et al.*, 2010). Due to variable physico-chemical features of metals, the toxicity level of different metals may fluctuate significantly (Azmat *et al.*, 2012). Metals can penetrate into the body through different ways e.g. gills, orally intake of water, foodstuff, non-food particles and skin. Basically, metals are absorbed and transported through blood to any storage organ such as liver for metals transformation or for bioaccumulation in different fish organs (Nussey, 2000; Rauf *et al.*, 2009). Fish skin comes in direct contact with metal ions and usually consumed with muscles so it has more chances of metal contamination (Yousafzai and Shakoori, 2006). Variations in the rate of metals accumulation among different species are species specific (Giguère *et al.*, 2004). An exposure to waterborne heavy metals causes an observable hypersensitivity in fish (Javed, 2012).

Table 3: Correlation coefficients among various parameters of mediums used for *Labeo rohita* for nickel toxicity tests.

	Conc.	Temp	pH	T.H.	$T.NH_3$.	DO	CO2	E.C.	Na	K	Ca
Temp.	0.276										
pH	0.261	0.138									
T.H.	0.250	-0.169	0.274								
$T.NH_3$.	0.830	0.205	0.231	-0.198							
D.O	-0.749	-0.146	-0.179	0.175	-0.986						
CO_2	0.963	0.254	0.261	-.215	0.942	-0.893					
EC	0.948	0.311	0.161	-0.234	0.844	-0.799	0.953				
Na	0.954	0.256	0.238	-0.240	0.952	-0.907	0.998	0.952			
K	0.954	0.367	0.260	-0.236	0.895	-0.846	0.972	0.981	0.970		
Ca	0.473	0.524	-0.282	-0.310	0.378	-0.342	0.470	0.550	0.470	0.537	
Mg	-0.067	-0.759	-0.197	-0.793	-0.096	0.094	-0.087	-0.131	-0.085	-0.204	-0.374

(Critical Value (2 tail 0.05) ± .531; Conc.= Concentration (mg L^{-1}); Temp= Temperature (^{O}C); pH; T.H.= Total hardness (mg L^{-1}); $T.NH_3$=Total ammonia (mg L^{-1}); D.O= Dissolved oxygen (mg L^{-1}); CO_2=Carbon Dioxide; E.C.= Electrical Conductivity (mS cm^{-1}); Na= Sodium (mg L^{-1}); K= Potassium (mg L^{-1}); Ca= Calcium (mg L^{-1}); Mg= Magnesium (mg L^{-1}).

Table 4: Correlation coefficients among various parameters of the test mediums used for *Cirrhinus mrigala* nickel toxicity tests.

	Conc.	Temp	pH	T.H.	$T.NH_3$.	DO	CO2	E.C.	Na	K	Ca
Temp.	-0.146										
pH	0.358	0.465									
T.H.	-0.163	0.211	0.104								
$T.NH_3$.	0.841	-0.154	0.281	-0.169							
D.O	-0.859	0.146	-0.253	0.129	-0.986						
CO_2	0.967	-0171	0.309	-0.169	0.950	-0.959					
EC	0.906	-0.448	0.161	-0.290	0.795	-0.786	0.894				
Na	0.931	-0.176	0.316	-0.137	0.928	-0.943	0.971	0.879			
K	0.943	-0.213	0.310	-0.116	0.880	-0.911	0.958	0.868	0.983		
Ca	0.242	-0.223	-0.214	0.226	0.281	-0.338	0.289	0.232	0.418	0.424	
Mg	-0.249	0.258	0.236	-0.140	-0.297	0.342	-0.299	-0.267	-0.421	-0.423	0.989

(Critical Value (2 tail 0.05) ± .512. Conc.= Concentration (mg L^{-1}); Temp= Temperature (^{O}C); pH; T.H.= Total hardness (mg L^{-1}); $T.NH_3$=Total ammonia (mg L^{-1}); D.O= Dissolved oxygen (mg L^{-1}); CO_2= Carbon Dioxide; E.C.= Electrical Conductivity (mS cm^{-1}); Na= Sodium (mg L^{-1}); K= Potassium (mg L^{-1}); Ca= Calcium (mg L^{-1}); Mg= Magnesium (mg L^{-1}).

Current study indicated that 96-hr LC_{50} nickel concentration varied significantly among the two fish species as *Labeo rohita* and *Cirrhinus mrigala*. *Cirrhinus mrigala* has lower 96-hr LC_{50} (55.85 ± 2.84 mg L^{-1}) than *Labeo rohita* (56.42 ± 2.51 mg L^{-1}). On the other hand, *Labeo rohita* was considerably more sensitive for lethal concentrations of nickel (120.98 ± 7.18 mg L^{-1}) than *Cirrhina mrigala* (128.44 ± 9.25 mg L^{-1}). Kousar *et al.* (2020) examined three fish species viz. *Catla catla, Labeo rohita and Cirrhinus mrigala* which were exposed to chronic sub-lethal levels of nickel concentration of 70.40, 71.99 and 79.9 mg L^{-1} under the duration of 60 days of growth trial. The impact of nickel stress at 10, 20, 30 and 40 mgL^{-1} concentrations on *Channa punctatus* was observed for 30 days of trial (Desai *et al.*, 2002). Kousar and Javed (2014) also determined the acute toxicity and bioaccumulation patterns of heavy metals (As, Ni and Zn) in *Labeo rohita, Cirrhinus mrigala, Ctenopharyngodon idella* and *Catla catla*. The order of metal accumulation was Zn > Ni > As. Compared with other fish species, *Cirrhinus mrigala* had significant ability towards Ni and Zn accumulation as 146.8 ± 149.1 µg g^{-1} and 243.0 ± 190.5 µg g^{-1}, respectively. (Naz and Javed, 2012) described the acute toxicity of metal mixtures (Fe, Zn, Pb, Ni and Mn) in *Catla catla, Labeo rohita* and *Cirrhinus mrigala* and observed high toxicity to fish in terms of 96 hr LC_{50}. According to Yousafzai *et al.* (2010), the order of metal accumulation in *L. dyocheilus* muscles was Zn > Cr > Cu > Pb > Ni > Cd. During present study, significant differences were observed between LC_{50} and lethal concentrations of nickel for *Labeo rohita* and *Cirrhinus mrigala*. Mean nickel concentration LC_{50} for *Labeo rohita* and *Cirrhinus mrigala* was 56.42 ± 2.51 mg L^{-1} and 55.85 ± 2.84 mg L^{-1}, respectively. On the other hand, the calculated mean of lethal concentration of nickel

was 120.98 ± 7.18 mg L^{-1} and 128.44 ± 9.25 mg L^{-1} for *Labeo rohita* and *Cirrhinus mrigala*, respectively. Results showed that *Cirrhinus mrigala* has lower LC_{50} but higher lethal dose compared with *Labeo rohita*.

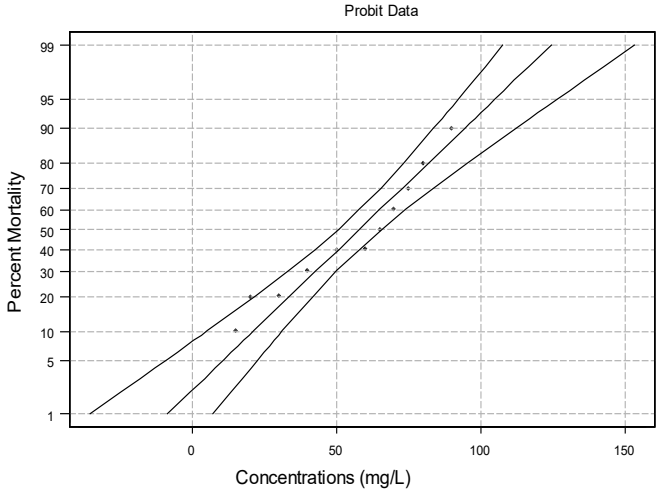

Figure 1: Probability graph for 96-hr LC_{50} and lethal concentrations (mg L^{-1}) determined for *Labeo rohita*.
Normal Distribution (Maximum Likelihood Estimates at 95% Confidence Interval); *Log (Mortality) = -2.0330 + 0.036033 concentration + 27.753; SE= 0.003662;* LC_{50}: 56.4217 mgL^{-1}; SE: 2.5165; Confidence± Interval: 51.4578 – 61.5250; Lethal Concentration: 120.9840 mgL^{-1}; SE: 7.1863; Confidence Interval: 109.0904 – 138.3531; Deviance Chi-Square Value = 5.832; Goodness of fit test, p = 0.924.

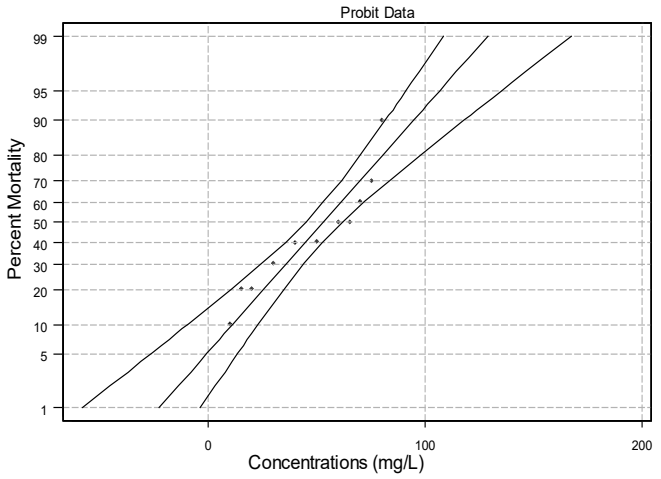

Figure 2: Probability graph for 96-hr LC_{50} and lethal concentrations (mg L^{-1}) determined for *Cirrhinus mrigala*.
Normal Distribution (Maximum Likelihood Estimates at 95% Confidence Interval); *Log (Mortality) = -1.7899 + 0.032048 concentration + 31.203; SE = 0.003664;* LC_{50} : 55.8504 mgL^{-1}; SE : 2.8401; Confidence Interval : 50.3752 – 61.8090; Lethal Concentration: 128.4401 mgL^{-1}; SE: 9.2527; Confidence Interval : 113.4692 – 151.5690; Deviance Chi-Square Value =7.825; Goodness of fit test, p = 0.729.

The correlation of physio-chemical parameters to the metal toxicity and its bioaccumulation is well documented. In our study, except for dissolved oxygen, all studied parameters like CO_2, sodium, potassium, electrical conductivity and total ammonia were directly proportional to the metal toxicity. Similar results were reported earlier stating that these physico-chemical parameters causes increase in metal toxicity for fish (Azmat *et al*., 2016). Yaqub and Javed (2012) also concluded that physio-chemical parameters of fish aquaria have significant effect on heavy metal toxicity studied in these media. Physico-chemical parameters such as, calcium, sodium, dissolved organic matter, and pH are reported to impact metal toxicity of different heavy metals to numerous aquatic organisms, like fish (Niyogi and Wood, 2004). Size and age of fish species also have significant effect on metal accumulation and variations shown in the results.

Conclusions and Recommendations

The current study concluded that nickel is a toxic metal for both the studied fish species when these are exposed to acute concentration of the metal in water bodies. While the LC_{50} of *Labeo rohita* is higher than *Cirrhinus mrigala*, the lethal concentration of nickel for *Cirrhinus mrigala* is slightly higher as compared to the *Labeo rohita*. This study further concluded that physico-chemical parameters have significant impact on acute toxicity of the metal. Hence, it is important to observe these values during experiment on nickel toxicity to obtain more valid results.

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Conflict of interests

The authors have declared no conflict of interest.

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