Research Article

Adverse Impacts of a Mixture of Metals on Two Freshwater Fish Species, *Catla catla* and *Labeo rohita*

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Abstract | The acute toxicity, determined by the LC_{50}+ lethal concentration of a tertiary combination comprising lead (Pb), nickel (Ni), and cadmium (Cd) was assessed on two freshwater fish species, *Catla catla* and *Labeo rohita*, employing probit analysis. Over a period of 90 days, mortality served as the primary toxicity criterion. The experiments were conducted under consistent conditions of pH (7), temperature (28.00.00°C), and water hardness (198.00 mgL^{-1}), with three replicates for each dosage in the tests. Significant variations were noted in the LC_{50} values and lethal responses for both fish species. *Catla catla* exhibited mean LC_{50} and lethal concentrations of 55.78±0.55 and 80.17±0.40mgL^{-1}, respectively, while *Labeo rohita* showed values of 63.92±2.48 and 102.07±0.50mgL^{-1}. *Labeo rohita* demonstrated higher sensitivity in terms of 96-hour LC_{50} compared to *Catla catla*, whereas *Catla catla* exhibited greater sensitivity for lethal responses. Correlation and regression analyses revealed significant positive correlations between metallic ion concentrations in the test mediums and carbon dioxide, sodium, potassium, and electrical conductivity. On the contrary, a negative correlation was established between dissolved oxygen levels and both fish species in the test environment. These findings contribute to understanding the differential responses of *Catla catla* and *Labeo rohita* to the toxic effects of lead, nickel, and cadmium mixture.

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Introduction

Environmental conditions are dynamic, with human influence significantly contributing to environmentally harmful changes through chemical loading into aquatic systems. Globally, researchers have exhibited a notable concern for the contamination of aquatic systems by heavy metals and pesticides, as highlighted by (Al-Saeed *et al*., 2023). Recognized as notable pollutants, these heavy metals exert detrimental impacts on the metabolic, physiological, and structural systems of both aquatic animals and plants. The accumulation of metals within these ecosystems directly threatens their sustainability. The challenge with heavy metals lies in the delicate equilibrium between their essential roles and their potential toxicity to the metabolic activities of organisms, as underscored by (Naz *et al*., 2023c). Agricultural lands irrigated with municipal wastewater and industrial effluents contribute to heavy metal contamination in fish tissues, including the skin, gills, and intestine (Khan *et al*., 2023). Fish, crucial indictors in freshwater systems, are widely studied for assessing metal pollution, especially those hazardous to human health (Alam *et al*., 2023). When commercially important fish species are assessed for heavy metal contamination, they have a tendency to accumulate metals in internal organs such as muscles, liver, and gills as they mature, making them unfit for human consumption (Chatha *et al*., 2023).

Juvenile fish (*Catla catla, Labeo rohita*, and *Cirrhina mrigala)* were subjected to acute toxicity tests in a static water system for 96 hours, exposing them to a broad range of toxicant concentrations. A significant decrease in fish survival rate compared to the control indicated the toxic effects of specific metals (Naz *et al*., 2023b).

Lead (Pb) is a highly hazardous metal in aquatic environments, posing a substantial threat to fish. Fish, being top predators, are particularly susceptible to lead exposure, with potential detrimental effects on their overall well-being and the health of the ecosystem. Given their prevalence and role in the food chain, fish serve as reliable indicators of environmental pollution in aquatic ecosystems (Lee *et al*., 2023). Nickel, classified as a transition metal and an essential micronutrient, is typically found in low concentrations in animal tissues, with well-regulated levels. Both excess and deficiency of nickel have been shown to have adverse effects on fish survival rates, as indicated by (Nielsen, 2021). Cadmium (Cd) induces environmental toxicity in aquatic settings, leading to oxidative stress in fish due to the excessive production of reactive oxygen species within their bodies. Fish, equipped with various antioxidant systems, exhibit changes in antioxidant responses, serving as an assessment parameter for oxidative stress induced by Cd exposure (Lee *et al*., 2019).

While numerous studies delve into the isolated effects of metals on fish, aquatic organisms commonly encounter mixtures of metals. The presence of specific metals can affect the accumulation of others in fish bodies, underscoring the significance of comprehending metal interactions in aquatic environments (Jamil-Emon *et al*., 2023). The freshwater streams in Pakistan's Punjab province confront severe pollution from heavy metals, primarily due to substantial discharges of wastewater and untreated industrial pollutants. This pollution significantly impacts the fish fauna in the region. To safeguard fish in the rivers and lakes of Punjab, understanding their capacity to withstand metal pollution is crucial. This involves discerning the tolerance limits of fish species, such as cyprinids, when exposed to various metal combinations. This knowledge will facilitate the development of policies for the effective conservation of fish species and the anticipation of potential consequences arising from the ongoing metal pollution in Pakistan's aquatic ecosystem. Hence, the objective of this paper is to assess the toxic effects of a metal mixture on fish species.

Materials and Methods

Conducted at the Zoology Department of the Government Sadiq College Women University, this study aimed to investigate the acute (short-term exposure) toxicity of a tertiary mixture comprising equal proportions of cadmium (Cd), lead (Pb), and nickel (Ni) on freshwater fish species, *Catla catla,* and *Labeo rohita*. Toxicity (96 hours) was evaluated using parameters such as the 96-hour LC_{50} and lethal concentrations, while maintaining consistent environmental conditions. Before the experiment, the fish underwent acclimatization to laboratory conditions and were fed twice daily with crumbled feed. Nevertheless, feeding was discontinued 24 hours prior to the initiation of the experiment. To preserve water quality, any remaining feed and fecal waste were meticulously removed from the 70-liter glass aquaria, all filled with de-chlorinated tap water. This rigorous methodology ensured a controlled environment for assessing the impact of the specified metal mixture on the fish species, *Catla catla* and *Labeo rohita*. To conduct toxicity assessments, the fish undergo an acclimation period and were subsequently transferred to glass aquaria. Pure cadmium (CdC12.xH2O), lead

(PbCl2), and nickel (NiCl2.6H2O) compounds were dissolved in deionized water to attain prescribed weights. Subsequently, stock solutions were formulated for diluting the metal mixture (Cd+Pb+Ni) within the range of 0.00 to 120 mgL^{-1} as needed. Table 1 provided details on the average weight and total lengths of the fish used in the experiment.

Table 1: *Measurements of average weights and lengths of fish species during acute toxicity trials.*

Fish age (Days)	Fish species	Wet weight (g±SD)	Total length (mm±SD)
90-day	*Catla catla*	4.21±0.12	56.88±0.37
	Labeo rohita	3.64±0.11	52.22±0.07

Ten (10) fish of each species were placed in individual glass aquariums, with three replicates for each test dose. To reduce stress on the fish, the concentration of the metal mixture in each aquarium was incrementally raised, with 50 percent of the test concentration achieved within 3.5 hours and the complete toxicant concentration reached within 7 hours. A continuous aeration system was upheld in all aquariums. Metal concentrations for each fish species initiated at zero, with incremental increases of 0.05 and 5 mgL^{-1} (as total concentration) for low and high concentrations, respectively. Observations on fish mortality and physico-chemical variables, including temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium, and carbon dioxide, were recorded at 12-hour intervals throughout the 96-hour duration (APAH, 1926).

Metal concentrations in different fish organs, including gills, liver, kidney, fins, bones, muscle, and skin, were assessed both before and after 96-hour acute toxicity tests involving 19 mixtures of LC$_{50}$ and lethal concentrations. The procedures outlined by (Rice *et al*., 2012) were applied for these assessments. The collected data underwent statistical analysis using a Micro-Computer, following the methodologies detailed by (Hair *et al*., 2003). Analysis of variance (Factorial Experiment) and Duncan's Multiple Range tests were employed to identify statistical differences among variables. Additionally, correlation analyses were conducted to reveal relationships among the various parameters under investigation. The data on the percentage of fish mortality during the 96-hour LC$_{50}$ and lethal concentration trials underwent probit analysis, as described by (Hamilton *et al*., 1977).

Results and Discussion

The responsiveness of *Catla catla* and *Labeo rohita* to a combination of three metals was assessed through acute toxicity tests, specifically the 96-hour LC$_{50}$ and lethal concentrations. *Catla catla* and *Labeo rohita*, with average weights of 4.21±0.12 and 3.64±0.12g, respectively, experienced individual exposure tests to assess their sensitivity to lead, nickel, and cadmium in tertiary form. The findings encompass the 96-hour LC$_{50}$, lethal responses, and a 95% confidence interval (Table 2).

Table 2: *Percentage (%) of fish mortality observed during a 96-hour acute exposure to a mixture of Pb+Ni+Cd.*

Fish age	Exposure concentrations (mgL^{-1})	Fish mortality (%)							
		Catla catla				*Labeo rohita*			
		R$_1$	R$_2$	R$_3$	Mean	R$_1$	R$_2$	R$_3$	Mean
	20	10	-	-	3.33	-	-	-	-
	25	10	-	-	3.33	-	-	-	-
	30	10	10	10	10.00	-	10	10	6.67
	35	10	10	10	10.00	-	10	10	6.67
	40	20	10	20	16.67	20	20	30	23.33
90 day	45	30	20	30	33.33	20	20	30	23.33
	50	40	30	40	43.33	30	40	30	33.33
	55	50	50	50	50.00	40	40	50	43.33
	60	60	60	70	63.33	50	50	60	53.33
	65	70	70	80	76.67	60	50	60	56.67
	70	80	90	80	83.33	60	60	70	63.33
	75	80	90	80	83.33	60	60	70	63.33
	80	100	100	100	100	70	70	80	73.33
	85					70	70	80	73.33
	90					80	90	90	86.66
	95					80	90	90	86.66
	100					100	100	100	100

Toxic effects of metals in mixture on fish

For *Catla catla*, the mean 96-hour LC$_{50}$ was determined as 55.78±0.55, with a confidence interval ranging from 50.91 to 60.04 mg/L. The mean lethal concentration was calculated as 80.17±0.40 mg/L, with a confidence interval of 73.46.09–92.64 mg/L. The regression coefficient for the probability graph showed high significance, affirming the precision of the regression line. The model exhibited a Deviance Chi-square value of 5.842, and the goodness of fit test p-value was 0.925, validating the accuracy of the regression line (Figure 1).

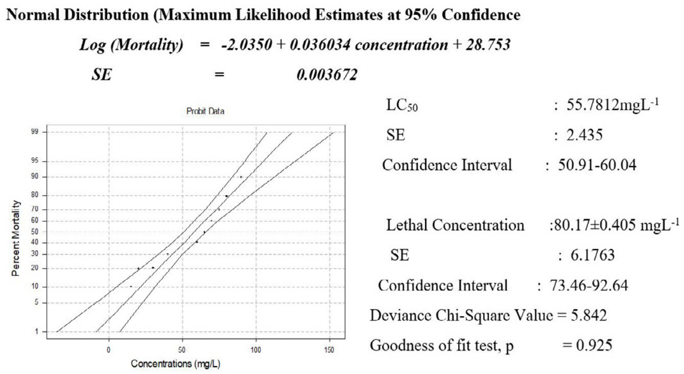

Figure 1: *Graph illustrating the probability of 96-hour LC50 and lethal concentrations (mg/L) determined for Catla catla.*

Similarly, mortality data for *Labeo rohita* during acute toxicity tests with the metal mixture are outlined in Table 2. The normal distribution of data at a 95% confidence interval revealed an LC_{50} value of 63.08±0.86 mg/L, with a confidence interval range of 54.71-69.34 mg/L. The mean lethal concentration of the metal mixture for *Labeo rohita* was computed as 102.07±0.50 mg/L, with confidence intervals of 91.66-123.15 mg/L. The Deviance Chi-square value calculated for this line was 7.825, with a goodness of fit test p-value of 0.729 (Figure 2).

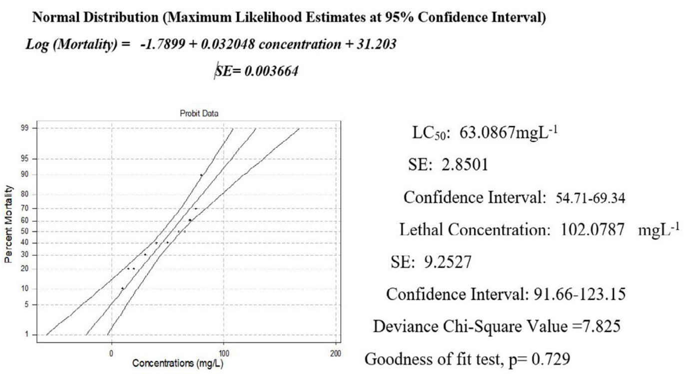

Figure 2: *Probability graph for 96-hr LC50 and lethal concentrations (mgL^{-1}) determined for Labeo rohita.*

Table 2 indicates that there are no noteworthy distinctions among the three replicate mediums utilized for both *Catla catla* and *Labeo rohita* in ascertaining the mean LC_{50} and lethal concentrations of the metal mixture. However, notable distinctions were observed between the LC_{50} and lethal concentrations of the metal mixture for both fish species. Despite maintaining constant temperature, pH, and water hardness across all test trials, Labeo rohita demonstrated higher sensitivity to the tertiary mixture of lead, nickel and cadmium in terms of the 96-hour LC_{50} compared to *Catla catla*. In contrast, *Catla catla* exhibited greater sensitivity than *Labeo rohita* in terms of lethal responses.

Water quality characteristics

Table 3 illustrates correlation coefficients between different physico-chemical variables in the test media and the concentrations of the metal mixture for both *Catla catla* and *Labeo rohita*. In both testing environments, the concentrations of metallic ions showed positive and significant relationships with total ammonia, carbon dioxide, electrical conductivity, sodium, and potassium, while exhibiting an inverse correlation with dissolved oxygen. Noteworthy positive correlations were identified among carbon dioxide, electrical conductivity, sodium, and potassium, with a simultaneous negative yet significant correlation with dissolved oxygen for both fish species. Dissolved oxygen showed a negative correlation with carbon dioxide, electrical conductivity, sodium, and potassium, while carbon dioxide exhibited a positive and significant correlation with electrical conductivity, sodium, and potassium. Moreover, electrical conductivity displayed a positive and significant association with sodium and potassium. Similarly, sodium demonstrated a positive and significant correlation with potassium for both *Catla catla* and *Labeo rohita* in both test environments. Furthermore, in the case of silver carp, calcium exhibited a positive and significant relationship with magnesium. In the test media employed for acute toxicity assessments for *Catla catla*, the relationship between potassium and calcium was negative but significant at $p<0.05$. In summary, the correlation data unveil intricate connections among physico-chemical variables and metal mixture concentrations in the test environments for these fish species, offering valuable insights into the environmental factors influencing their responses during acute toxicity tests.

Bio-accumulation patterns of metals in fish organs during exposure of metal mixture

Table 4 illustrates the patterns of metal accumulation in fish organs during the 96-hour LC_{50} exposure to the Cd+Pb+Ni mixture. *Labeo rohita* demonstrated a notably higher propensity for accumulating cadmium and nickel, whereas *Catla catla* exhibited a significantly greater inclination for lead accumulation. The fish liver emerged as an organ with a significantly higher tendency for accumulating both cadmium and lead, while nickel accumulation was notably higher in fish kidneys. Among the three metals, cadmium accumulation in fish organs was notably higher, followed by nickel and lead. Lethal exposure to this mixture led to a significant increase in both cadmium and nickel in *Labeo rohita*, with *Catla catla* displaying a

Table 3: *Correlation coefficients among different physico-chemical parameters of the test water.*

Catla catla	Conc.	Temp	pH	T.H.	$T.NH_3$.	DO	CO2	E.C.	Na	K	Ca
Temp.	0.27598										
pH	0.26091	0.14754									
T.H.	0.24864	-0.26935	0.27855								
$T.NH_3$.	0.83120	0.21470	0.23224	-0.19917							
D.O	-0.74946	-0.13610	-0.19896	0.17537	-0.98642						
CO_2	0.96890	0.25476	0.26201	-.21469	0.94183	-0.89258					
EC	0.94789	0.31184	0.16257	-0.23461	0.84382	-0.79902	0.95342				
Na	0.95373	0.25105	0.23970	-0.24120	0.95189	-0.90679	0.99902	0.95172			
K	0.95374	0.36764	0.26102	-0.23669	0.89530	-0.84648	0.97643	0.98141	0.96954		
Ca	0.47319	0.52944	-0.29162	-0.30861	0.37769	-0.34166	0.47143	0.55020	0.47012	0.53643	
Mg	-0.06697	-0.75817	-0.19804	-0.7831	-0.09598	0.09431	-0.08694	-0.13085	-0.08461	-0.20635	-0.37239
Critical Value (2 tail 0.05± .53067)											
Labeo rohita											
Temp	-0.14564										
pH	0.35787	0.36527									
T.H.	-0.16316	0.23077	0.10380								
$T.NH_3$.	0.84138	-0.25364	0.28151	-0.16824							
D.O	-0.85855	0.15597	-0.25365	0.12935	-0.98591						
CO_2	0.96663	-016147	0.30920	-0.16795	0.94919	-0.95990					
EC	0.90649	-0.45788	0.16097	-0.28870	0.79526	-0.78547	0.89367				
Na	0.93156	-0.16582	0.31572	-0.13925	0.92760	-0.94249	0.97139	0.87932			
K	0.94313	-0.22319	0.30994	-0.11468	0.87983	-0.91205	0.95848	0.86745	0.98349		
Ca	0.24184	-0.24263	-0.21317	0.22629	0.28198	-0.33650	0.28853	0.23232	0.41797	0.42456	
Mg	-0.24833	0.25864	0.23586	-0.13849	-0.28674	0.34225	-0.29869	-0.26663	-0.42096	-0.42388	0.98893
(Critical Value (2 tail 0.05) ± .51235)											

Table 4: *Accumulation patterns of metals ($\mu g g^{-1}$) in fish organs during lethal exposure of metal mixture # 4 (Cd+Pb+Ni).*

Metals	Fish species	Organs							
		Kidney	Liver	Skin	Muscle	Fins	Gills	Bones	Means
Cd	*C. catla*	558.33±8.33a	554.16±26.00b	109.22±4.06g	116.50±1.81f	287.50±1.07d	471.13±2.86c	182.09±0.81e	325.56±200.07a
	L. rohita	327.22±2.54a	310.00±10.00b	134.17±7.00f	106.42±2.75g	245.56±3.19d	442.42±3.57c	134.47±3.67e	242.89±124.92b
Pb	*C. catla*	186.67±2.89b	71.72±2.30g	119.16±1.67e	134.11±1.54d	145.28±0.24c	115.71±2.48f	440.83±1.04a	173.35±122.91a
	L. rohita	108.33±1.67b	250.00±5.00a	4.90±2.07g	2.55±0.18f	66.13±2.77d	20.38±1.88e	93.24±0.95c	77.93±86.83b
Ni	*C. catla*	350.00±5.00b	506.67±2.89a	88.05±1.27f	52.67±2.02g	133.33±3.81d	150.83±2.60c	129.10±1.24e	201.52±164.65a
	L. rohita	236.67±1.91a	191.66±1.67c	24.64±2.94g	50.40±2.83f	154.17±1.90d	274.99±1.67b	72.91±3.75e	143.63±96.77b

Means sharing identical letters within both a single column and row (representing overall means) are statistically similar at $p < 0.05$. C. catla: Catla catla; L. rohita: Labeo rohita.

significantly higher mean lead concentration. Cadmium accumulation was notably higher in fish liver, whereas both lead and nickel contents were significantly higher in fish kidneys. Fish organs demonstrated a substantially greater tendency for cadmium accumulation, followed by nickel and lead.

In Pakistan, the swift advancements in agriculture and industry have led to aquatic pollution, primarily stemming from the release of untreated industrial wastes and runoff water into water bodies. This has resulted in significant repercussions for the health of fish in these environments (Kumar *et al*., 2023). Despite substantial research exploring the toxic effects of individual metal species (Naz *et al*., 2013, 2023e). It is crucial to acknowledge that organisms in natural waters typically encounter mixtures of metals (Naz *et al*., 2023b; Naz and Javed, 2012). To enhance the role of freshwater fish as an indicator of heavy metal pollution, this study aimed to evaluate the acute toxicity of metal mixtures for two freshwater fish species, *Catla catla* and *Labeo rohita*. The investigation unveiled highly significant differences in tolerance limits, as indicated by LC_{50} and lethal responses, between the two fish species exposed to the tertiary mixture of cadmium, lead, and nickel. Significantly different 96-hour LC_{50} concentrations of the metal mixture were noted, with *Catla catla* demonstrating a considerably higher 96-hour LC50 compared to *Labeo rohita*. Additionally, the lethal concentrations for these two fish species displayed notable differences. These findings align with the results of (Naz and Javed, 2012), confirming the robustness of these observed distinctions.

The researchers carried out a study involving three fish species, namely *Catla catla, Labeo rohita*, and *Cirrhina mrigala*. These fish were exposed to acute levels of 19 mixtures containing lead, nickel, iron, manganese, and zinc.

The results indicated substantial variability in tolerance limits, particularly the 96-hour LC_{50}, among all three fish species when exposed to a combination of the five metals. Significantly, *Labeo rohita* exhibited the lowest sensitivity among the five fish species overall. During the study, observable behavioral changes, including loss of equilibrium, shoaling behavior, swimming with their bellies upwards, and irregular opercular movement, were documented. Earlier studies on the acute toxicity of copper and zinc, both separately and in combination, have provided insights into potential effects on the developmental stages of rainbow trout, *Oncorhynchus mykiss* (Kazlauskiene and Vosyliene, 2008). Moreover, investigations have explored the acute toxicity of heavy metals, such as cadmium, zinc, and copper, either individually or in combination, during the early developmental stages (embryos and larvae) of the Chinese minnow (*Gobiocyprius rarus*). The sensitivity of this fish species to heavy metals varied based on the exposure period and developmental stages, with combinations of Cu+Cd and Cu+Zn showing synergistic lethal effects. Comparative assessments of the susceptibility of species with different phylogenetic positions and developmental stages to toxicants have frequently been

Table 5: *Available studies to metals mixture toxicity.*

Metals in mixture	Effects on different fish species	Author
Fe+Zn+P-b+Ni+Mn	Even at sub-lethal concentrations, heavy metals are lethal to different fish species and affect different biological factors of fish growth including *Catla catla, Cirrhina mrigala, Labeo rohita, Ctenopharyngodon idella, Hypophthalmychthys molitrix*	Naz *et al*., 2023b
Fe+Zn+Pb	Showed substantial impacts on the wet weight gain (g) and length (mm) of fish species, included: *Labeo rohita, Catla catla, , Hypophthalmychthys molitrix Cirrhina mrigala and Ctenopharyngodon idella*	Naz and Chatha, 2022
Ni+Cr+Pb	Showed different histopathological changes in *Oreochromis niloticus*	Rehman, 2020
Cu + Cd	Long term exposure causes adverse hematological and histopathological alterations in *Catla catla*	Naz *et al*. 2021
Pb+Cr+Cd	Increased DNA damage, increased frequency of formation of micronuclei, loss of coordination and tremors were noted in *Labeo rohita*	Yamin *et al*., 2020
Pb+Mn	Regarding overall responses of fish species: *Catla catla, Cirrhina mrigala, Ctenopharyngodon idella, Labeo rohita Hypophthalmichthys molitrix* for their ability to accumulate metals, kidney appeared as an organ to mass significantly higher amounts of metals	Naz *et al*., 2020
Fe+Ni	It was observed that to metal mixture *Hypophthalmichthys molitrix* were significantly more sensitive, followed by that of *Labeo rohita*, *Ctenopharyngodon idella*, *Catla catla* and *Cirrhina mrigala*.	Naz and Javed, 2013a
Fe+Zn+P-b+Ni+Mn	*Labeo rohita* showed significantly least sensitivity to metal mixtures than *Catla catla* and *Cirrhina mrigala*	Naz and Javed, 2012
Pb+Ni	Among two fish species *Hypophthalmichthys molitrix* was found significantly more sensitive in terms of 96-hr LC_{50} than that of *Ctenopharyngodon idella*	Naz and Javed, 2013d
Zn+Pb+Mn	Among treated fish species, the weights were negatively and significantly correlated with their fork and total lengths,	Naz *et al*., 2013
Fe+Zn+Pb+Ni+Mn	*Ctenopharyngodon idella* was less sensitive to mixture of metal as compared to *Hypophthalmichthys molitrix*	Naz and Javed, 2013c
Zn+Ni	Accumulation of all the metals in fish body followed the general order: liver>kidney>gills> skin >muscle> fins >bones. *Catla catla, Cirrhina mrigala, Ctenopharyngodon idella, Labeo rohita Hypophthalmichthys molitrix*	Naz and Javed, 2013b

conducted using acute methods (Sun *et al.*, 1995). Nevertheless, in natural environments, numerous species experience the direct consequences of prolonged exposure to lower concentrations of toxicants or their combinations. In vitro tests that evaluate the effects of sub-lethal toxicity on fish have facilitated the examination of diverse functional changes in physiological systems arising from extended exposure to toxicants (Naz *et al.*, 2023f).

While research on the impacts of individual metals on fish has been extensive, there is limited exploration of fish tolerance limits to metal mixtures, especially those involving more than three metals (Naz *et al.*, 2023a, d). These studies underscore that the impacts of metal mixtures differ in terms of toxicity on living organisms compared to the effects of single metals. The toxicity of a heavy metal mixture to fish depends on factors such as concentration, duration of exposure, and specific composition (Naz and Chatha, 2022).

Aquatic organisms, particularly fish, experience direct and indirect influences from the physical characteristics of the aquatic environment, particularly the water's physico-chemical parameters (Menon *et al.*, 2023). During growth trials, the physico-chemical characteristics of exposure media to metallic ion metal mixtures significantly affected the growth, condition factor, feed intake, and feed conversion efficiency of fish species, including *Catla catla*, *Labeo rohita*, and *Cirrhina mrigala*. The acute toxicity of water-borne and dietary metals to fish is influenced by various abiotic environmental factors such as oxygen, hardness, pH, and temperature (Zaynab *et al.*, 2022).

In the exposure media, positive and significantly variable correlations were observed between metal concentration and total ammonia, carbon dioxide, electrical conductivity, sodium, and potassium, while the correlation was negatively significant with dissolved oxygen. The exposure of zinc, cadmium, and their mixture has been reported to decrease the levels of sodium, chloride, and calcium in the serum of fish, *Oreochromis niloticus* (Firat and Kargın, 2010). Both short and long-term metal toxicity can influence the levels of Na^+, Mg^{+2}, K^+, and Ca^{+2} in various tissues of fish, *Oreochromis niloticus*, with short-term exposure proving more toxic in altering these ionic levels than long-term exposures (Özçelik and Canli, 2023).

The positive and significant correlation coefficient observed between calcium and magnesium aligns with the findings of (Deleebeeck *et al.*, 2007) who conducted a study on the influence of calcium, magnesium, and pH on the toxicity of nickel on juvenile rainbow trout (*Oncorhynchus mykiss*) over a 26-day exposure. They found that the chemical activities of calcium and magnesium contributed to reducing nickel toxicity. Additionally, (Moiseenko and Kudryavtseva, 2001) examined the relationships between nickel and water chemistry, organs, and fish tissues. As a result, water chemistry significantly influences the uptake and accumulation of metals, leading to variable tolerance limits observed in the two fish species during the present investigation.

The fish exhibited a significant decrease in oxygen consumption, coupled with a discernible rise in ammonia, with increasing concentrations of metals in the test media. Changes in the oxygen consumption ratio have been recognized as an indicator of the toxicity of metal mixtures to fish species, such as *Oncorhynchus mykiss* and *Cyprinus carpio*. The study investigated the influence of metals on the oxygen consumption of *Tilapia mossambica*, observing a notable reduction in oxygen consumption in fish exposed to zinc sulfate, cobalt carbonate, lead nitrate, and cadmium carbonate. This decline is likely attributable to the interaction of toxic heavy metals with the fish respiratory system, leading to asphyxiation, abnormalities in gill function, and the inhibition of enzyme systems. Interactions among metals in aquatic media can cause damage to gill tissues, ultimately resulting in fish mortality (Shahjahan *et al.*, 2022; Zaynab *et al.*, 2022).

Disturbances in the aquatic environment can induce a reduced metabolic rate and hindered growth in fish. Consequently, exposure of fish to elevated metal concentrations results in high mortality rates, contributing to the depletion of fish populations in contaminated water bodies (Kayode-Afolayan *et al.*, 2022). In conclusion, significant statistical differences were observed in the metal mixture 96-hour LC_{50} and lethal responses for both *Catla catla* and *Labeo rohita*. *Labeo rohita* demonstrated significantly higher sensitivity in terms of 96-hour LC_{50} compared to *Catla catla*, while *Catla catla* exhibited greater sensitivity than *Labeo rohita* in terms of lethal responses. Positive and significant correlations were identified between metallic ion concentrations and carbon dioxide, sodium, potassium, and electrical conductivity. Conversely, inverse relationships were observed with dissolved oxygen for both fish species.

Conclusions and Recommendations

Catla catla exhibited mean LC_{50} and lethal concentrations of 55.78±0.55 and 80.17±0.40 mg/L, respectively, while *Labeo rohita* showed values of 63.92±2.48 and 102.07±0.50 mg/L. *Labeo rohita* demonstrated higher sensitivity in terms of 96-hour LC_{50} compared to *Catla catla*, whereas *Catla catla* exhibited greater sensitivity for lethal responses. Correlation and regression analyses revealed significant positive correlations between metallic ion concentrations in the test mediums and carbon dioxide, sodium, potassium, and electrical conductivity. On the contrary, a negative correlation was established between dissolved oxygen levels and both fish species in the test environment. These findings contribute to understanding the differential responses of *Catla catla* and *Labeo rohita* to the toxic effects of the lead, nickel, and cadmium mixture.

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Novelty Statement

The current study investigates the acute toxicity in two freshwater fish*Catla catla* and *Labeo rohita* under exposure of metal mixture this study will be highlighting the environmental issue of heavy metal contamination in form of mixture resulting from human activities, which poses a significant threat to aquatic organisms.

Author's Contribution

Moazama Batool: Execution of study, formatting and analysis and reviewed the final version of manuscript.
Saima Naz: Planned research, supervision of the study and arrangement of supplies. Manuscript write-up and reviewed the final version of manuscript.
Ghulam Abbas: Reviewed the manuscript and analysis.
Ahmad Manan Mustafa Chatha: Helped in conducting research and helped in data analysis.
Sheeza Bano and Sadia Nazir: Helped in data compilation and manuscript writing.
Maria Lateef and Fatima Yasmin: Performed the experiment in laboratory.

Conflict of interest

The authors have declared no conflict of interest.

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