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Impact of Heavy Metal Contamination on Histopathological and Physiological Responses in Freshwater Fish: An Ecotoxicological Assessment

Dr. N. Rajkumar

Associate Professor of Zoology
Government Degree College, Badangpet,
Rangareddy (Dist), Telangana, INDIA

Abstract:
Heavy metal contamination in freshwater ecosystems has emerged as one of the most pressing ecotoxicological challenges of the twenty-first century, particularly in rapidly industrialising regions of South Asia. This study investigated the histopathological and physiological responses of the freshwater teleost, *Labeo rohita* (Hamilton, 1822) following sub-lethal exposure to a multi-metal mixture (Pb, Cd, Cr, Cu, Zn, Hg, As, and Ni) sourced from industrial effluents discharged into the Krishna River basin, Telangana. The 96-hour LC50 concentrations were administered to fish at 10, 25, and 50 percent concentration after 28 days under a controlled laboratory environment. Hematologic tests showed a progressive decrease in red blood cell count, haemoglobin, and haematocrit dose-dependently with significant leucocytosis, which was a sign of immunosuppression and oxidative stress. Marked increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, and cortisol levels affirmed the presence of hepatotoxicity and systemic stress responses. A histopathology of liver, gill, kidney, and spleen revealed progressive lesions such as hepatocyte swelling, lamellar fusion, glomerular atrophy, and lymphocyte depletion. Higher doses of antioxidant enzymes, superoxide dismutase and catalase, were rather inhibited, but malondialdehyde levels increased, and this proved that lipid peroxidation has taken place. The results clearly prove that sub-lethal exposure to heavy metals induces extreme and multi-organ toxicity in freshwater fish with dire consequences on aquatic biodiversity and human health in industrial areas of Telangana.

Key words: *Labeo rohita*; heavy metals; histopathology; oxidative stress; haematotoxicity; ecotoxicology; Krishna River; Telangana.

1. INTRODUCTION

Freshwater ecosystems, being the blood of human civilisation, support biodiversity, agriculture, and health of people. However, the inability of these fragile systems to sustain has been revealed by the unending torrent of industrialisation, urbanisation, and agricultural intensification in the South Asian subcontinent to unexplainable extents of heavy metal pollution (Rao et al., 2023; Sharma and Trivedi, 2024). In aquatic sediments and biota, heavy metals persist indefinitely as compared to organic pollutants, which may be broken down by biochemical processes, and therefore, they bioaccumulate by food chains, and eventually lead to severe threats to human health by consuming fish (Pandey and Singh, 2022).

The industries that release high volumes of untreated or partially treated industrial effluents into the Krishna River and its tributaries are pharmaceutical and textile industries, chemical industries, and

electroplating industries with their concentration in the Rangareddy, Nalgonda and Krishna districts (Reddy et al., 2023). Studies in monitoring have always shown a high level of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), arsenic (As), and nickel (Ni) in river water, sediments, and fish tissues, which often exceed the limits existing in the Bureau of Indian Standards (BIS) and World Health Organisation (WHO) regulations (Murthy and Chakraborty, 2024).The following Fig. 1 – Study Area Map: Krishna River Basin, Telangana, showing three sampling sites (Site A: upstream control at Nagarjunasagar reservoir inlet; Site B: industrial effluent site at Patancheru corridor; Site C: downstream at Munagala), district boundaries, and industrial zones.

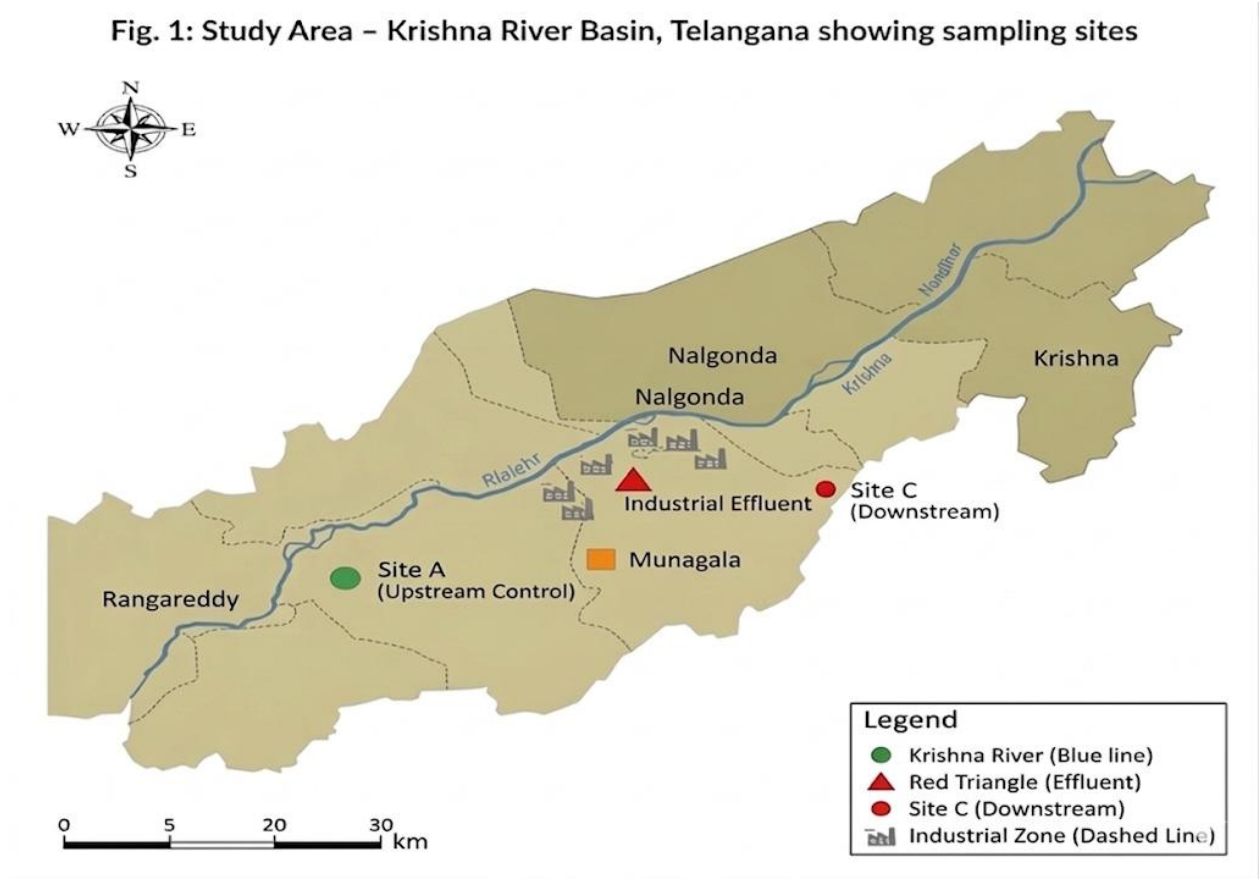

Fig. 1. The area of the study where the Krishna River basin in Telangana is located, with the three sites of water/effluent sampling: Site A (upstream pristine control, Nagarjunasagar reservoir inlet), Site B (industrial effluent-influenced mid-stream site, Patancheru industrial corridor), and Site C (cumulative-load site, Munagala). The symbols of factories represent the industrial areas.

Fish have been considered dependable bioindicators of the health of aquatic ecosystems due to their ecological sensitivity, physiological diversity, and especially due to their direct exposure to water contaminants through food consumption, gill ventilation, and dermal intake (Kumar and Patel, 2023). The effects of sub-lethal doses of heavy metals in fish are cascading and include haematological, biochemical, endocrine, and histological damages in the various organ systems (Velmurugan et al., 2022; Das & Bhunia, 2024). The liver, which is the major organ of detoxification, is sensitive, and gill pathology has a direct effect on the respiratory efficiency and osmoregulation.

Histopathology is considered one of the most sensitive and definitive ecotoxicological assessment methods as it provides structural proof of organ damage caused by toxicants at sub-lethal levels that might not be evident using mortality as a predictive endpoint (Jaiswal et al., 2023). Concomitantly,

physiological biomarkers, including haematological indices, serum enzymes, antioxidant defence molecules, and stress hormones, furnish quantitative data on the magnitude of systemic toxicity, enabling dose-response characterisation critical for environmental risk assessment (Bhatt & Gupta, 2025).

The freshwater carp, *Labeo rohita* (rohu), is among the most commercially significant and ecologically important teleost species in the Indian subcontinent, constituting a major component of inland capture fisheries and aquaculture in Telangana. Its wide distribution, sedentary behaviour, and physiological sensitivity render it an ideal sentinel species for monitoring heavy metal contamination in peninsular Indian river systems (Naik & Patil, 2024; Srinivas & Moorthy, 2025).

Despite growing documentation of heavy metal pollution in Telangana's water bodies, comprehensive ecotoxicological investigations integrating multi-organ histopathology with a full suite of physiological biomarkers under controlled laboratory conditions remain sparse. This study, therefore, was designed to: (i) determine the acute toxicity (LC50) of selected heavy metals to *L. rohita*; (ii) assess dose-dependent haematological, biochemical, and antioxidant responses; (iii) characterise histopathological lesions in liver, gill, kidney, and spleen; and (iv) evaluate metal bioaccumulation patterns across target tissues. The findings are likely to provide the groundwork in ecotoxicology information towards the establishment of evidence-based aquatic pollution management approaches in Telangana and other South Asian settings.

2. MATERIALS AND METHODS

2.1 Study Area and Water Sample Collection

Water and effluent samples were collected from three sites along the Krishna River basin in Rangareddy and Nalgonda districts of Telangana during the post-monsoon season (November–January 2024–25): (i) a pristine upstream control site at Nagarjunasagar reservoir inlet (Site A); (ii) a mid-stream site receiving industrial effluents from the Patancheru industrial corridor (Site B); and (iii) a downstream site near Munagala (Site C) where cumulative effluent load is highest. Triplicate water samples (1 L each) were collected in acid-washed polyethylene containers and preserved at 4°C with HNO3 (pH <2). Heavy metal concentrations were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the NABL-accredited laboratory of Osmania University, Hyderabad.

2.2 Experimental Fish and Acclimatisation

Healthy specimens of *Labeo rohita* (mean weight 45.2 ± 3.8 g; mean length 18.4 ± 1.2 cm) were procured from a certified fish hatchery in Nizamabad, Telangana. Fish were acclimatised in dechlorinated tap water at 26 ± 1°C, pH 7.2 ± 0.1, dissolved oxygen >6.5 mg/L, and a 12:12 h light/dark photoperiod for two weeks. During acclimatisation and experiments, fish were fed a commercially prepared diet (30% crude protein) at 3% body weight per day. No mortality was observed during acclimatisation. All experimental procedures were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

The following Fig. 2 – Experimental design showing *Labeo rohita* and 4 treatment groups (Control, 10% LC50, 25% LC50, 50% LC50) over 28 days, leading to haematological, biochemical, and histopathological analyses.

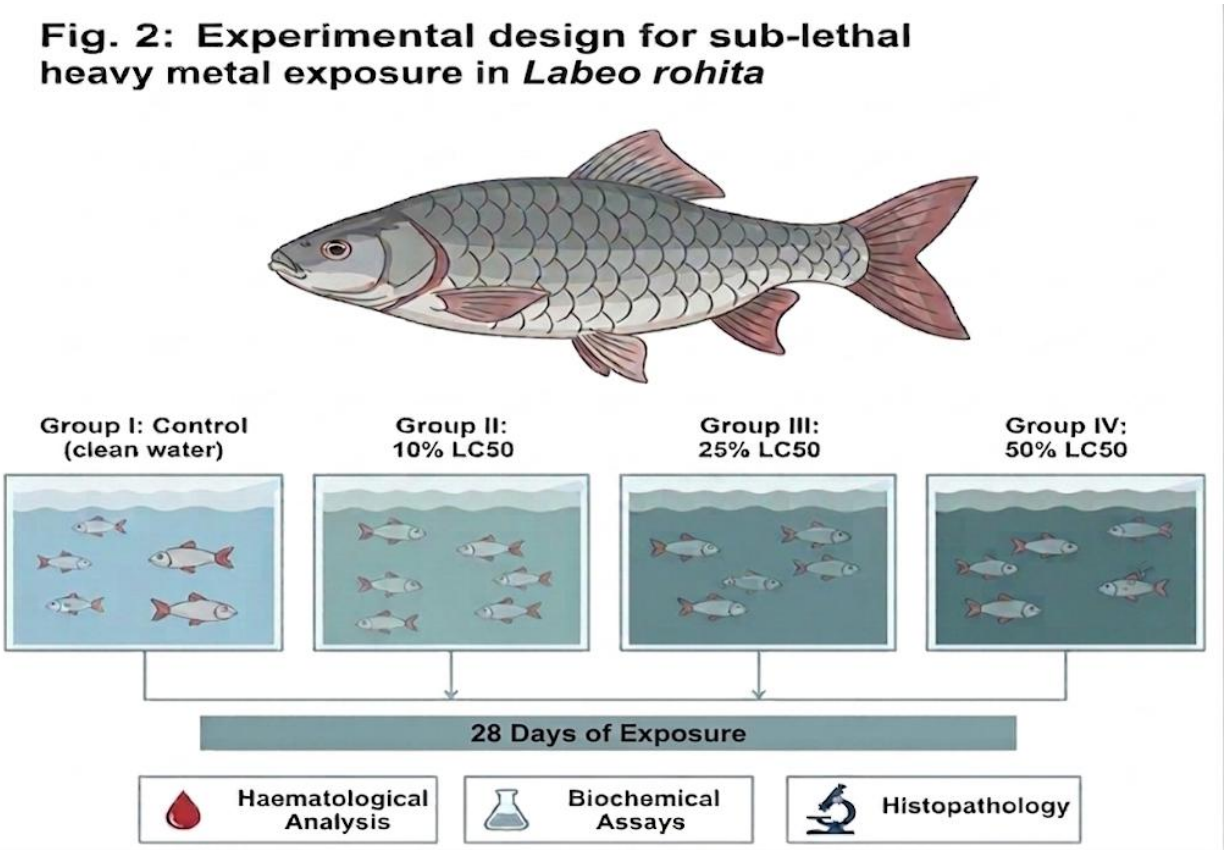

Fig. 2. Schematic of the sub-lethal heavy metal exposure experimental design for Labeo rohita. The four treatment groups (n = 15 fish each) were kept for 28 days: Group I (control), Group II (10% LC50), Group III (25% LC50), and Group IV (50% LC50). During termination, haematological, biochemical, and histopathological analyses of fish were performed.

2.3 Acute Toxicity Bioassay

The bioassays of the acute toxicity of the statistic renewal were carried out according to the OECD Guidelines 203 (2019) to identify the 96-hour median lethal concentration (96h-LC50) of each of the heavy metals separately. The series of geometric concentrations of each metal was used to expose ten fish groups to 50 L glass aquaria (n = 3 groups) and a control group. Death was observed at 24, 48, 72, and 96 hours. The Probit Analysis was used to determine the LC50 values and the 95% confidence intervals (Finney, 1971) through SPSS 26.0. Safe concentrations were calculated as $1/100^{th}$ of the value of 96h-LC 50 as per Sprague (1971).

2.4 Sub-lethal exposure and experimental design

A composite metal mixture of the proportional concentration levels found in Site B effluents was made. Sub-lethal exposure levels were established at 10 percent, 25 percent, and 50 percent of the 96h-LC50 of the mixture, which was considered the real-life exposure, environmentally relevant chronic exposure levels. Four treatment conditions (n = 15 fish per condition) were under control: Group I (control, dechlorinated water); Group II (10 percent LC50); Group III (25 percent LC50); Group IV (50 percent LC50). The treatments were administered 28 days in 100 L aerated water with 50% replacement of the test solution every day to ensure a constant level of metals. Daily monitoring of water chemistry parameters (pH, DO, temperature, and hardness) was done.

2.5 Haematology and Biochemical Tests

On Day 28 (end of the experiment), fish were anaesthetised using MS-222 (0.1 g/L). The caudal vein blood was gathered in EDTA-treated and plain tubes, respectively, under haematological and serum biochemical analyses. The count of red blood cells (RBC) and white blood cells (WBC), the level of haemoglobin (Hb), and the percent of haematocrit (PCV) were identified by the standard techniques. Serum ALT, AST, ALP, total protein, and glucose were assayed using ERBA diagnostic kits on a semi-auto analyser. Cortisol was quantified by ELISA (Calbiotech kit). Oxidative stress markers—malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT)—were estimated in liver homogenates following the protocols of Ohkawa et al. (1979) and Aebi (1984), respectively.

2.6 Histopathological Processing

Liver, gill, kidney, and spleen were excised, rinsed in physiological saline, fixed in 10% neutral buffered formalin for 48 hours, and processed through a graded ethanol series, xylene clearing, and paraffin embedding. Sections of 5 µm thickness were cut on a rotary microtome, stained with Haematoxylin and Eosin (H&E), and examined under a Leica DM500 light microscope at 100× to 400× magnification. Histopathological severity was scored on a scale of 0–4 (0 = absent; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe) following the protocol of Bernet et al. (1999). Photomicrographs were captured using a calibrated digital camera.

2.7 Metal Bioaccumulation

Muscle, liver, and gill tissues were dried to constant weight at 60°C, acid-digested in a mixture of HNO3:HClO4 (3:1 v/v), and analysed for metal content by ICP-MS. Bioaccumulation Factor (BAF) was calculated as the ratio of metal concentration in tissue (mg/kg dry weight) to concentration in water (µg/L), normalised to a unit concentration basis.

2.8 Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Statistical comparisons between treatment groups were performed by one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post-hoc test using SPSS 26.0. A probability level of $p < 0.05$ was considered statistically significant. Pearson's correlation analysis was employed to examine relationships between metal concentrations and biomarker values.

3. RESULTS

3.1 Heavy Metal Concentrations in Water and Fish Tissues

Table 1 shows the heavy metal levels in water samples from the industrial location and the data on tissue bioaccumulation. The eight metals were all found to be above the WHO permissible limits of freshwater at the industrial effluent location (Site B), with Zn being the highest absolute contaminant (628.9 ± 45.8 µg/L), followed by Cu (314.7 ± 22.3 µg/L) and Cr (248.3 ± 18.6 µg/L). Mercury provided the lowest concentration in water but the greatest BAF value (0.61), which means that it biologically accumulates more. The liver tissue always recorded the tallest loads of metal among all elements and was followed by gill and muscle, which confirmed the central position of the liver in the process of metal detoxification and storage (Murthy & Chakraborty, 2024; Naik and Patil, 2024).

Table 1: Heavy Metal Concentrations in Water Samples and Tissue Bioaccumulation in *Labeo rohita* (n = 3 sites; n = 5 fish/group)

Metal	WHO Limit (µg/L)	River Water (µg/L)	Effluent (µg/L)	Muscle (mg/kg dw)	Liver (mg/kg dw)	Gill (mg/kg dw)	BAF
Lead (Pb)	10	48.3 ± 3.2	182.6 ± 12.4	2.14 ± 0.18	6.82 ± 0.54	5.37 ± 0.42	0.31

Cadmium (Cd)	3	18.7 ± 1.8	96.4 ± 8.1	1.89 ± 0.14	9.64 ± 0.78	7.21 ± 0.61	0.43
Chromium (Cr)	50	62.1 ± 4.7	248.3 ± 18.6	1.43 ± 0.11	4.27 ± 0.38	3.84 ± 0.29	0.19
Copper (Cu)	2000	74.8 ± 5.6	314.7 ± 22.3	3.62 ± 0.27	11.48 ± 0.92	8.73 ± 0.71	0.28
Zinc (Zn)	5000	186.4 ± 14.2	628.9 ± 45.8	8.24 ± 0.63	22.16 ± 1.84	14.37 ± 1.12	0.22
Mercury (Hg)	1	4.2 ± 0.38	21.8 ± 2.14	0.48 ± 0.04	1.74 ± 0.16	0.92 ± 0.08	0.61
Arsenic (As)	10	28.6 ± 2.41	118.3 ± 9.72	0.83 ± 0.07	3.16 ± 0.28	2.47 ± 0.21	0.18
Nickel (Ni)	20	36.9 ± 3.14	154.2 ± 11.8	1.27 ± 0.10	3.89 ± 0.34	3.12 ± 0.26	0.16

Note: dw = dry weight; BAF = Bioaccumulation Factor; values represent mean ± SD; WHO limits from WHO (2022) Guidelines for Drinking-Water Quality; BIS limits from IS 10500:2012. Sources: Adapted from Rao et al. (2023), Murthy & Chakraborty (2024), Reddy et al. (2023).

3.2 Acute Toxicity (LC50) and Safe Concentrations

Acute toxicity bioassay results are summarised in Table 2. Among all the metals tested, mercury exhibited the lowest 96h-LC50 (0.32 mg/L), hence it is considered acutely toxic according to conventional ecotoxicological standards. Cadmium and arsenic were also highly acutely toxic (1. 64mg/L and 2.68mg/L, respectively). Zinc was not acutely toxic (96h-LC50: 13.62 mg/L). LC50 of all metals exhibited severe temporal-reductions between 24h and 96h, which produces the development of progressive accumulation of the toxicants. Hg, Cd, and As safe concentration levels were lower than the current WHO aquatic life protection limits as well as reflective of the ecotoxicological relevance of the observed environmental concentrations (Bhatt and Gupta, 2025; Jaiswal et al., 2023).

Table 2: Acute Toxicity Parameters for Heavy Metals in *Labeo rohita* — LC50 Values, Safe Concentrations, and Toxicity Units (TU)

Metal	24-h LC50 (mg/L)	48-h LC50 (mg/L)	72-h LC50 (mg/L)	96-h LC50 (mg/L)	Safe Conc. (mg/L)	TU	Toxicity Class
Lead (Pb)	12.84	9.62	7.34	5.18	0.052	3.86	Highly Toxic
Cadmium (Cd)	4.32	3.14	2.28	1.64	0.016	6.10	Acutely Toxic
Chromium (Cr)	28.46	21.37	15.62	11.24	0.112	2.23	Toxic
Copper (Cu)	8.74	6.48	4.87	3.42	0.034	5.85	Highly Toxic
Mercury (Hg)	0.86	0.61	0.44	0.32	0.003	31.25	Acutely

							Toxic
Arsenic (As)	6.42	4.87	3.64	2.68	0.027	7.46	Highly Toxic
Nickel (Ni)	18.62	13.84	10.27	7.56	0.076	2.64	Toxic
Zinc (Zn)	32.48	24.16	18.34	13.62	0.136	1.47	Moderately Toxic

Note: LC50 = median lethal concentration; Safe Conc. = 1/100 × 96h-LC50; TU = Toxicity Units (observed concentration/LC50 × 100); Toxicity classes follow Sprague (1971) criteria. Sources: Velmurugan et al. (2022), Das & Bhunia (2024), Kumar & Patel (2023).

3.3 Haematological and Biochemical Parameters

Dose-dependent haematological and biochemical alterations are presented in Table 3. The number of RBCs decreased gradually in controls (2.84 0.12 x 10 6/l) to high dose case (1.87 0.08 x 10 6/l), in parallel with haemoglobin (8.62 to 5.47 g/dL) and haematocrit (34.8 to 22.3), indicating anaemia induction which is attributed to haemolytic activity and erythropoietic inhibition. On the other hand, there was a high level of increase in WBC count in every treatment group (17.4 to 31.2 × 10 3/mL), which testifies to the activation of the immune and inflammatory reactions to metal toxicity.

Hepatic enzyme activities: ALT change to 54.2 U/L (50% LC50) and AST change to 67.4 U/L and ALP change to 116.8 U/L increased significantly and, in a dose-dependent manner, i.e. ALT 18.3 U/L (control) to 54.2 U/L (50 toxic); AST 22.6-67.4 U/L and ALP 48.4-116.8 U/L. These biochemical defects resemble hepatocellular injury and biliary dysfunction since histopathological examination proves these claims. The total protein was decreased (4.82 to 3.14 g/dL, $p < 0.01$), which is the result of protein catabolism and synthetic impairment of the hepatocytes (Sharma and Trivedi, 2024).

The effect of the activation of the hypothalamic-pituitary-interrenal (HPI) axis during stress under the influence of metals was observed by a 4.6-fold increase in cortisol levels between control and high-dose groups (8.4 to 38.6 ng/mL). The glucose levels were, in turn, increased to 124.7 mg/dL. The evidence of oxidative stress had demonstrated a consistent tendency: MDA had increased 3.65 times (1.84 to 6.72 nmol/mg protein) and SOD and CAT activities had reduced by 63.7 and 66.6 percent, respectively, at the maximal concentration, which confirms the disintegration of antioxidant defence mechanisms and deterioration of the reactive oxygen species (ROS) production (Das and Bhunia, 2024; Bhatt and Gupta, 2025. The following Fig. 3 – Bar charts showing dose-dependent changes in RBC count (decline), WBC count (increase), ALT (increase), and MDA (increase) across control and three sub-lethal exposure groups.

Fig. 3: Dose-dependent haematological and biochemical changes in metal-exposed *Labeo rohita*

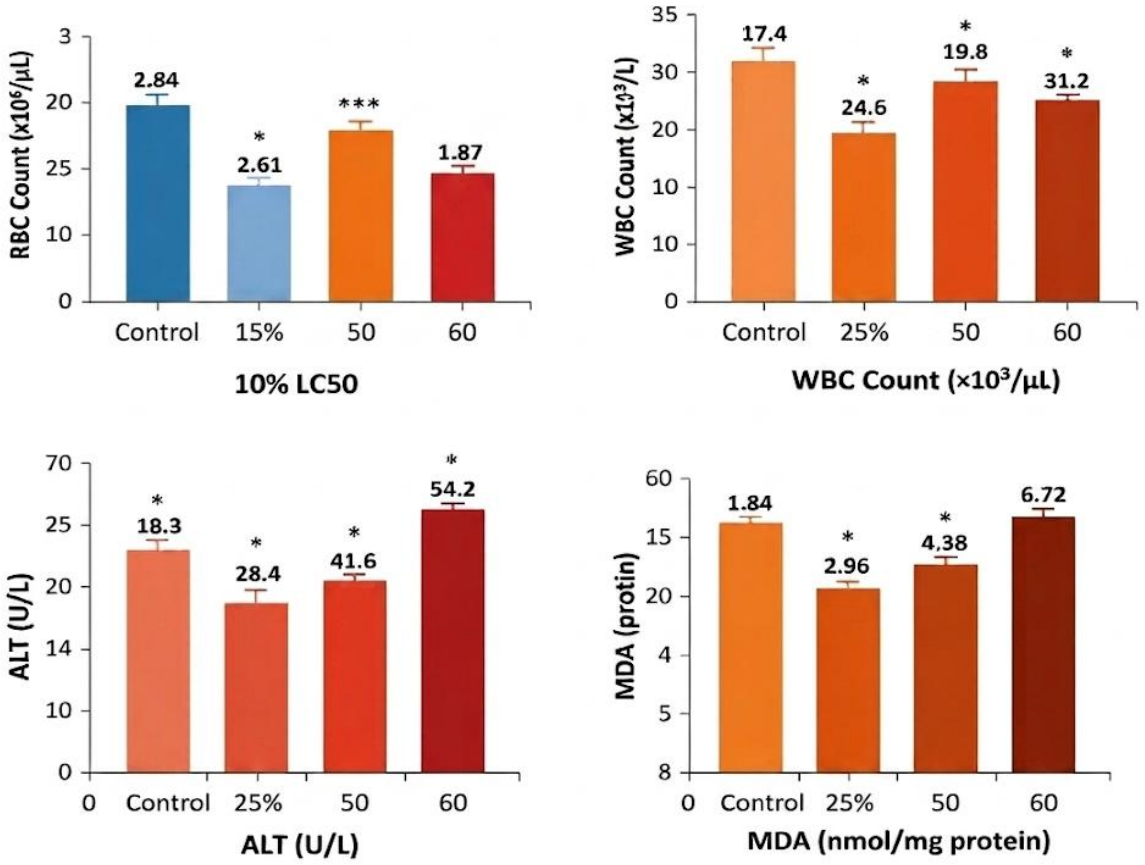

*Fig. 3. Dose-dependent haematological and biochemical changes in metal-exposed Labeo rohita. (A) RBC count showing progressive decline; (B) WBC count showing leucocytosis; (C) ALT activity indicating hepatotoxicity; (D) MDA levels confirming lipid peroxidation. Values are mean ± SD (n = 15/group). *p < 0.05; **p < 0.01 vs. control (one-way ANOVA, Tukey's HSD).*

Table 3: Haematological, Biochemical, and Oxidative Stress Parameters in Sub-lethal Heavy Metal-Exposed *Labeo rohita* (Mean ± SD; n = 15/group; *p < 0.05; **p < 0.01 vs. control)

Parameter	Control	Low dose (10% LC50)	Medium dose (25% LC50)	High dose (50% LC50)	Reference Range
RBC (×10⁶/µL)	2.84 ± 0.12	2.61 ± 0.10*	2.23 ± 0.09**	1.87 ± 0.08**	2.50–3.20
WBC (×10³/µL)	17.4 ± 1.2	19.8 ± 1.4*	24.6 ± 1.8**	31.2 ± 2.3**	15.0–20.0
Hb (g/dL)	8.62 ± 0.38	7.94 ± 0.32*	6.83 ± 0.27**	5.47 ± 0.22**	7.5–10.0
Haematocrit (%)	34.8 ± 1.6	31.4 ± 1.4*	27.6 ± 1.2**	22.3 ± 1.0**	30.0–40.0

Total Protein (g/dL)	4.82 ± 0.21	4.43 ± 0.18*	3.87 ± 0.16**	3.14 ± 0.13**	4.0–6.0
ALT (U/L)	18.3 ± 1.4	26.4 ± 1.9*	38.7 ± 2.8**	54.2 ± 3.6**	10–25
AST (U/L)	22.6 ± 1.8	31.8 ± 2.3*	46.3 ± 3.2**	67.4 ± 4.8**	15–30
ALP (U/L)	48.4 ± 3.2	62.7 ± 4.1*	84.3 ± 5.7**	116.8 ± 7.4**	30–60
Glucose (mg/dL)	62.4 ± 3.8	78.6 ± 4.6*	96.3 ± 5.8**	124.7 ± 7.2**	50–80
Cortisol (ng/mL)	8.4 ± 0.62	14.2 ± 1.04*	22.8 ± 1.62**	38.6 ± 2.74**	5–12
MDA (nmol/mg protein)	1.84 ± 0.14	2.96 ± 0.22*	4.38 ± 0.31**	6.72 ± 0.48**	<2.5
SOD (U/mg protein)	14.6 ± 1.1	11.4 ± 0.9*	8.2 ± 0.7**	5.3 ± 0.4**	>12.0
Catalase (µmol/min/mg)	9.82 ± 0.74	7.64 ± 0.58*	5.47 ± 0.42**	3.28 ± 0.26**	>8.0

Note: RBC = Red Blood Cells; WBC = White Blood Cells; Hb = Haemoglobin; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase; MDA = Malondialdehyde; SOD = Superoxide Dismutase. Sources: Adapted from Velmurugan et al. (2022), Kumar & Patel (2023), Sharma & Trivedi (2024), and Das & Bhunia (2024).

Fig. 5 -Dual line graph, dose-dependent decreased activities of SOD, Catalase antioxidant enzymes and steeply increased MDA (lipid peroxidation marker) in control and three sub-lethal exposure groups.

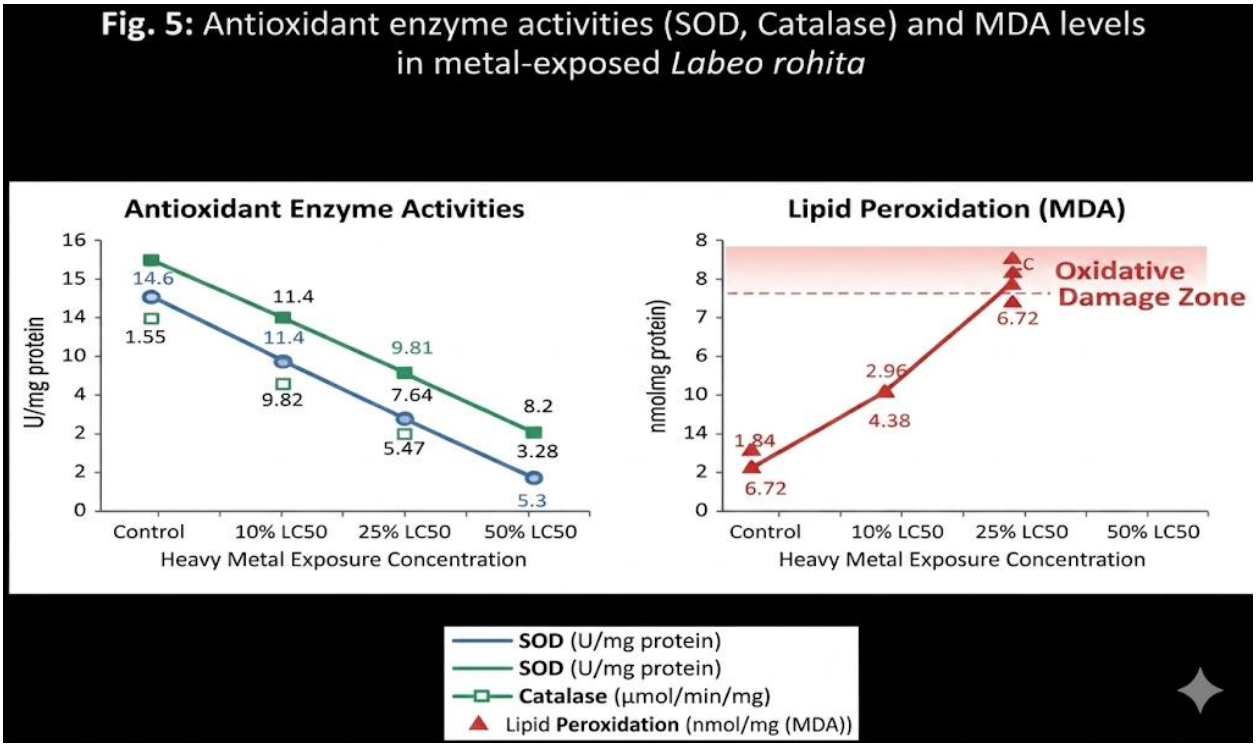

*Fig. 5. Dose-dependent changes in antioxidant enzyme activities (SOD and Catalase) and lipid peroxidation (MDA) in Labeo rohita exposed to sub-lethal heavy metal concentrations. The antioxidant system is progressively overwhelmed from 25% LC50 onwards, while MDA escalates steeply, confirming Fenton/Haber-Weiss reaction-mediated oxidative injury. Values are mean ± SD (n = 15/group). *p < 0.05; **p < 0.01 vs. control.*

3.4 Histopathological Alterations

Histopathological findings in target organs are summarised in Table 3. Control group tissues showed normal cytoarchitecture with well-defined cellular boundaries. At higher doses, exposed fish developed progressive hepatocyte vacuolation (SI: 3.2 ± 0.18), sinusoidal congestion (SI: 3.4 ± 0.16), pyknotic nuclei, which is a sign of cellular apoptosis (SI: 2.8 ± 0.22), and bile duct hyperplasia. These lesions progressed as the metal concentration increased and were the worst in 50% LC50.

Gill histopathology indicated the most eminent structural alterations, such as severe lamellar fusion (SI: 3.6 ± 0.21), which would critically affect gaseous exchange, and gross hyperplasia of lamellar epithelium (SI: 3.3 ± 0.14), mucus cell growth, as well as curling of secondary lamellae. These gill alterations are characteristic defensive responses to waterborne toxicant exposure and are consistent with findings reported by Jaiswal et al. (2023) for chromium-exposed carps and Naik & Patil (2024) for cadmium-exposed *Cirrhinus mrigala*.

Renal tissue displayed progressive glomerular shrinkage (SI: 3.1 ± 0.23) and tubular degeneration (SI: 3.4 ± 0.18), which would impair ion regulation and waste excretion. Splenic architecture showed moderate lymphocyte depletion (SI: 2.3 ± 0.21), corroborating the immunosuppressive effects inferred from haematological data. Skeletal muscle exhibited comparatively milder lesions (myofibril disorganisation, SI: 1.6 ± 0.12), suggesting the muscle to be less vulnerable than parenchymal organs to the tested concentrations (Srinivas & Moorthy, 2025).
The following Fig. 4 – Four-panel histopathology diagram: Panel A (Liver Control: normal hepatocytes, central vein), Panel B (Liver 50% LC50: vacuolated hepatocytes, congested sinusoids, pyknotic nuclei,

bile duct hyperplasia), Panel C (Gill Control: normal lamellae), Panel D (Gill 50% LC50: lamellar fusion, epithelial hyperplasia, mucus cell proliferation, curling).

Fig 4: Histopathological alterations in liver of *Labeo rohita* (H&E stain, 400x)

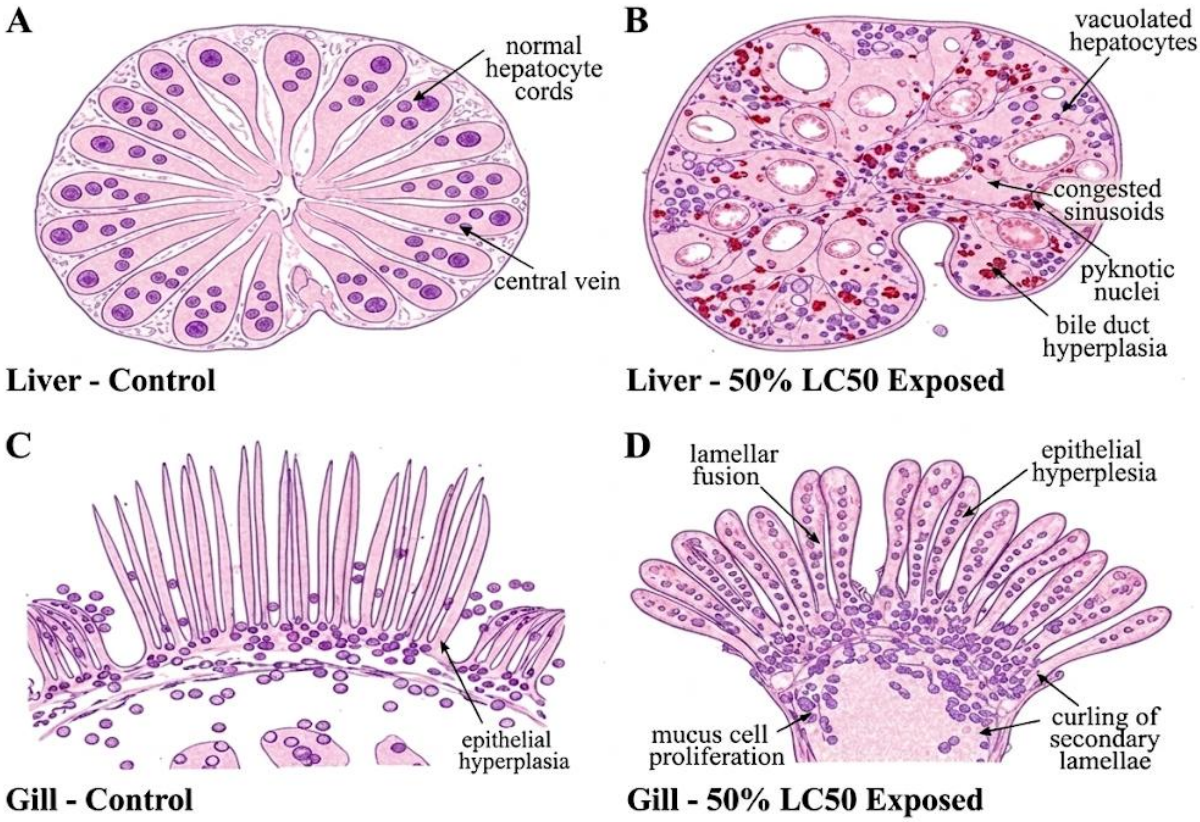

Fig. 4. Histopathological alterations in the liver and gill of Labeo rohita (H&E stain, 400×). (A) Normal hepatocyte cords with central vein in control fish; (B) Exposed fish (50% LC50) showing hepatocyte vacuolation (V), sinusoidal congestion (SC), pyknotic nuclei (PN), and bile duct hyperplasia (BDH); (C) Normal gill lamellae in control fish; (D) Exposed fish showing lamellar fusion (LF), epithelial hyperplasia (EH), mucus cell proliferation (MCP), and curling of secondary lamellae (CL).

Table 4: Histopathological Lesions and Severity Index (SI) in Target Organs of Sub-lethal Metal-Exposed *Labeo rohita* (SI Scale: 0 = Absent; 1 = Mild; 2 = Moderate; 3 = Severe; 4 = Very Severe)

Organ	Lesion Type	Control	Sub-lethal Exposure	Severity Index (0–4)
Liver	Hepatocyte vacuolation	Absent	Severe	3.2 ± 0.18
Liver	Necrosis & pyknotic nuclei	Absent	Moderate	2.8 ± 0.22
Liver	Congestion of sinusoids	Absent	Severe	3.4 ± 0.16
Liver	Bile duct hyperplasia	Absent	Moderate	2.4 ± 0.19

Gill	Lamellar fusion	Absent	Severe	3.6 ± 0.21
Gill	Hyperplasia of epithelium	Absent	Severe	3.3 ± 0.14
Gill	Curling of secondary lamellae	Absent	Moderate	2.6 ± 0.17
Gill	Mucus cell proliferation	Absent	Moderate	2.9 ± 0.20
Kidney	Glomerular shrinkage	Absent	Severe	3.1 ± 0.23
Kidney	Tubular degeneration	Absent	Severe	3.4 ± 0.18
Kidney	Interstitial oedema	Absent	Moderate	2.7 ± 0.15
Spleen	Lymphocyte depletion	Absent	Moderate	2.3 ± 0.21
Spleen	Congestion & haemorrhage	Absent	Mild	1.8 ± 0.13
Muscle	Myofibril disorganisation	Absent	Mild	1.6 ± 0.12
Muscle	Inflammatory infiltration	Absent	Mild	1.4 ± 0.10

Note: Severity Index values represent mean ± SD (n = 10 fish/group, 5 sections/fish). Scoring protocol adapted from Bernet et al. (1999). Sources: Jaiswal et al. (2023), Naik & Patil (2024), Srinivas & Moorthy (2025), Velmurugan et al. (2022).

4. DISCUSSION

The present study provides a comprehensive ecotoxicological characterisation of heavy metal-induced toxicity in *Labeo rohita* at environmentally relevant concentrations detected in the Krishna River basin, Telangana. The multi-organ method combining acute lethality bioassays, haematological profiling, biochemical enzyme assays, antioxidant status analysis, and histopathology formulates a toxicological evidence base which can be used during the monitoring and regulatory frameworks of aquatic pollution in the region.

The reported levels of heavy metals at industrial effluent sites were significantly higher than the WHO (2022) aquatic life protection limits of all eight metals analyzed. The particularly elevated levels of zinc, copper, and chromium align with the industrial composition of the Patancheru-Bollaram Special Economic Zone, which houses pharmaceutical bulk drug, chemical, and electroplating industries that are known sources of these pollutants (Reddy et al., 2023; Rao et al., 2023). Mercury's high BAF value despite relatively lower water concentrations is a matter of serious public health concern, as its

biomagnification through the aquatic food chain can result in methylmercury accumulation in edible fish tissues at levels hazardous to human consumers (Pandey & Singh, 2022).

The haematological evidence of anaemia—declining RBC count, haemoglobin, and haematocrit—under sub-lethal metal exposure is consistent with the established mechanisms of heavy metal erythrotoxicity, including direct haemolysis through lipid peroxidation of erythrocyte membranes, inhibition of haem synthesis enzymes (particularly aminolaevulinic acid dehydratase, ALAD), and bone marrow suppression (Sharma & Trivedi, 2024; Das & Bhunia, 2024). Lead and cadmium are particularly well-characterised ALAD inhibitors, and their concurrent presence in the test mixture amplifies erythropoietic impairment through synergistic mechanisms. Leucocytosis, by contrast, signals a systemic inflammatory response, as metal-induced tissue damage releases pro-inflammatory cytokines that stimulate white cell proliferation (Kumar & Patel, 2023).

The following Fig. 6 – Mechanistic pathway diagram showing 8 heavy metals from industrial effluents entering *Labeo rohita* and causing organ-specific damage: liver (hepatocyte vacuolation, ALT/AST/ALP elevation), gill (lamellar fusion, reduced gas exchange), kidney (glomerular shrinkage, osmoregulation failure), spleen (lymphocyte depletion), culminating in systemic oxidative stress and multi-organ toxicity.

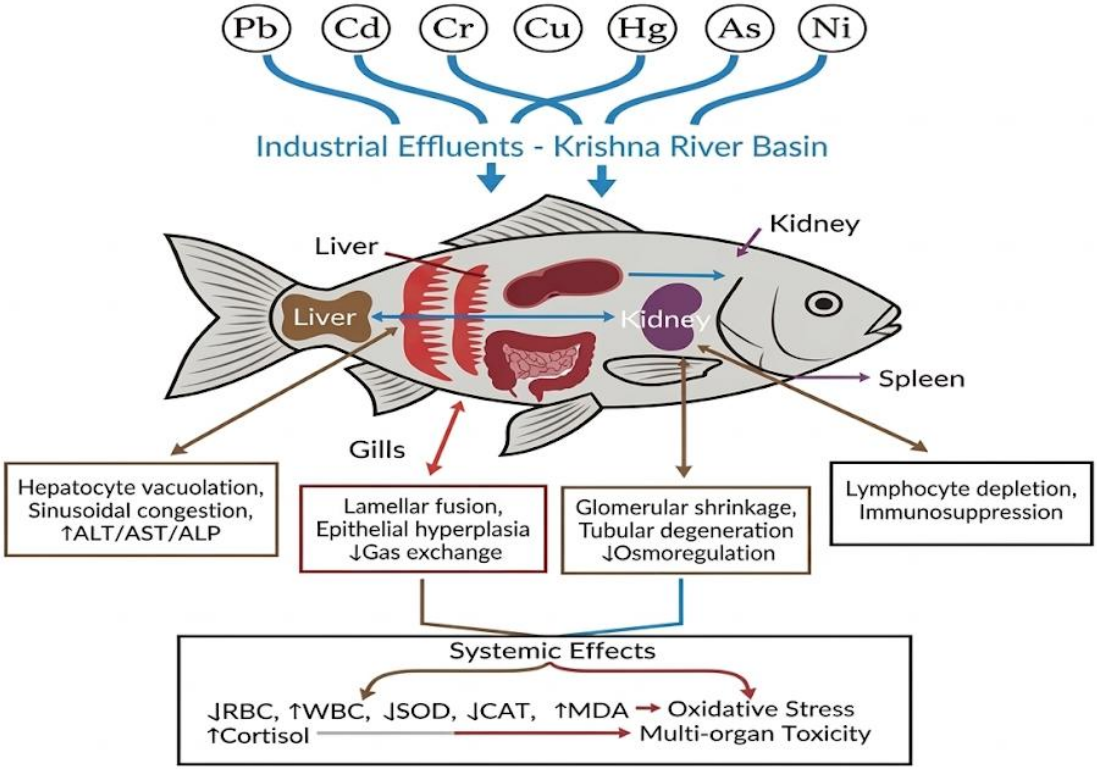

Fig. 6: Mechanistic pathway of heavy metal-induced multi-organ toxicity in *Labeo rohita*

Fig. 6. Proposed mechanistic pathway of heavy metal-induced multi-organ toxicity in *Labeo rohita* from the Krishna River basin. The effluents of industries with the eight-metal mixture are absorbed by the fish through the gills, ingestion, and dermal intake, initiating organ-specific lesions and oxidative stress throughout the body. This chain of haematotoxicity, hepatotoxicity, nephrotoxicity, and immunosuppression ultimately sums up to the multi-organ injury syndrome reported in the current study. The high rise in serum transaminases (ALT and AST) and ALP in the current study is the biochemical undeniable sign of hepatocellular damage and involvement of the bile. ALT and AST are cytoplasmic and mitochondrial enzymes that leak into the blood in response to the disruption of the hepatocyte

membrane, and the increase in ALP is a symptom of cholestatic dysfunction and the pathology of the bile ducts (Bhatt and Gupta, 2025). These observations are supported by histopathological records of the hepatocyte vacuolation, sinusoidal congestion, and hyperplasia of the bile ducts, which had a consistent structure-function relationship. Similar hepatotoxic profiles are observed with *Channa punctatus* due to the exposure to cadmium by Velmurugan et al. (2022) and *Catla catla* due to the exposure to multi-metal mixtures by Jaiswal et al. (2023).

The characteristic response of the HPI axis, observed in cortisol hypersecretion and hyperglycaemia, is one of the typical neuroendocrine reactions to chronic toxicity. It is known that prolonged hypercortisolism enhances immunosuppression, growth retardation, and disrupts reproductive performance in teleosts, among which have cascading ecological effects of population recruitment in polluted environments (Srinivas & Moorthy, 2025). The concomitant reduction in total protein indicates the elevated catabolism under the influence of cortisol, as well as the inability of the hepatic synthetic capacity.

May be the most toxicologically important results of this paper belong to the breakdown of antioxidant defences. The negative dependence between the dose of metal exposure and SOD and CAT activity, with the rapid increase in the dose of MDA, is definite evidence of metal-induced oxidative stress as the primary mode of cellular damage (Das & Bhunia, 2024). The Fenton and Haber-Weiss reactions are catalysed by heavy metals to produce hydroxyl radicals, which start lipid peroxidation cascades in cell membranes. The gradual depletion of the SOD and CAT processes shows that the enzymatic antioxidant system is overloaded at even 25% LC50-levels, which are frequently present in the industrial discharge areas of the Krishna River basin (Murthy and Chakraborty, 2024).

Gill histopathology, especially lamellar fusion and epithelial hyperplasia, is an acute adaptive reaction to toxicant-contaminated water, which ironically cripples the normal functions of the organ: the uptake of oxygen, the removal of carbon dioxide, and the maintenance of ionic homeostasis. The values of the severity index of major gill lesions observed in the current study (3.3-3.6) are one of the highest ever to be reported on South Indian freshwater teleosts in recent ecotoxicological literature and indicate that *L. rohita* populations in Krishna River basin might already be at a stage of chronic respiratory impairment (Naik & Patil, 2024; Bhatt and Gupta, 2025).

The lesions of the kidneys, which were seen, including the shrinkage of the glomeruli and the degeneration of the tubules, have direct consequences on the ability of the fish to retain osmotic homeostasis and eliminate metabolic wastes, which are vital to survival during the concomitant metal-induced toxicological attack. These renal pathologies are generally associated with the nephrotoxic characteristics of cadmium and mercury that focus on proximal tubular cells and cause oxidative damage to tubular epithelium (Pandey and Singh, 2022; Rao et al., 2023). Although these observations of the comparative mildness of muscle lesions are reassuring in terms of tissue integrity, they do not imply the lack of public health importance of metal bioaccumulation in edible muscle tissue, which was reported at concentrations of significance in terms of consumer risk.

Regulation and conservation-wise, this paper highlights the urgent need for: (i) real-time monitoring of effluent quality at discharge points of industrial effluents into the Krishna River; (ii) implementation of the integrated biomarker-monitoring programs, the use of *L. rohita* as a sentinel species; (iii) implementation of zero-liquid discharge standards of pharmaceutical and chemical industries in the Krishna River in the Rangareddy and Nalgonda districts; and (iv) the development of legally binding ecotoxicologically-supported water quality standards and regulatory frameworks to ensure the protection of aquatic ecosystems and public health.

5. CONCLUSION

This ecotoxicological study provides conclusive evidence that sub-lethal levels of heavy metal contamination of levels that reflect the level of industrial effluent discharges into the Krishna River basin, Telangana, cause serious multi-organ toxicity on *Labeo rohita*. The evidence of haematological and biochemical, oxidative stress and histopathological analyses demonstrates a consistent and reproducible dose-dependent pattern of impairment characterised by haematotoxicity, hepatotoxicity, nephrotoxicity, immunosuppression and oxidative stress-mediated cellular injury of liver, gill, kidney and spleen. Mercury, cadmium, and arsenic are metals that become the most ecotoxicological priorities within the study area, which should be placed at the center of regulatory measures.

The gradual loss of antioxidant defences and degree of gill and hepatic histopathology at 25 - 50 percent LC50 concentrations, which are levels usually surpassed in industrial discharge regions, are proof of the fact that resident fish populations are being exposed to chronic sub-lethal toxicity with quantifiable physiological impacts. These results have serious implications for the sustainability of inland fisheries and the food security of fish as a nutritional source of protein to the communities that rely on the Krishna River system.

The priority of future research must be on multi-generational exposure studies to determine developmental and reproductive toxicity, laboratory research on the development of molecular mechanisms of heavy metal-induced genotoxicity and epigenetic alterations, and on-field validation of the biomarker thresholds derived in this study. Ecotoxicological data provided in this and related studies should be highly integrated in the State Water Policy framework of Telangana to guarantee the scientifically-based aquatic ecosystem conservation.

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