Indian Journal of Experimental Biology
Vol. 64, September 2026, pp.823-834
DOI: 10.56042/ijeb.v64i09.27640

Alterations in the activity and isozyme profile of enzymatic stress markers along with histological changes due to lead exposure in *Labeo rohita*

Priya Agrawal & Ajeet Kumar Singh*

Department of Zoology, CMP Degree College, University of Allahabad, 211002, Prayagraj, Uttar Pradesh, India

Received 16 January 2026; revised 17 June 2026

The present study examines the effects of two concentrations of lead acetate (Pb3: 3 mg/L and Pb6: 6 mg/L) on the vital tissues of *Labeo rohita* after 96 hours of exposure. The study focuses on alteration in isozyme profile of enzymatic markers of oxidative stress such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and tissue damage indicator such as lactate dehydrogenase (LDH). Oxidative stress was measured through lipid peroxidation analysis, and corresponding histopathological changes were examined by haematoxylin-eosin staining. In-gel assays detected a single isozyme of CAT in the liver and gill, and GPx in the liver and brain. Four distinct SOD isozymes were detected in the liver and gill, while only two isozymes were found in the brain. Inhibitor assays indicated that the isoform closest to the cathode was Mn-SOD, with the remaining isozymes identified as Cu/Zn-SOD. A total of five LDH isozymes (LDH1 to LDH5) were expressed in the liver, single isozyme in the gill and brain. The isozyme profiles and activities of these enzymes were altered in a tissue-specific manner following lead exposure. Specifically, in the liver, total activities of SOD, GPx and LDH increased, whereas CAT activity decreased in both Pb3 and Pb6-treated groups compared to controls. In the gills, SOD, CAT, and LDH activities were elevated in both lead treatment groups; however, GPx activity was undetectable. In the brain, SOD and GPx activities increased, LDH activity remained unchanged, and CAT activity was not detected in either treatment group. Lipid peroxidation levels were significantly elevated in all tissues in a dose-dependent manner compared to controls. Histopathological examination in tissues revealed degenerative alterations such as vacuolization, necrosis, epithelial hyperplasia and neuronal degeneration in dose dependent manner. The findings demonstrate that lead induced oxidative stress elicits tissue and isozyme-specific antioxidant responses and associated histopathological damage in *Labeo rohita*. These results may contribute to a molecular-level understanding which may help the advancement of aquaculture strategies in environmentally contaminated settings.

Keywords: Lead Toxicity, Oxidative stress, Antioxidant enzymes, LDH, lipid peroxidation

Among the various pollutants associated with aquaculture, heavy metals are of particular significance due to their environmental persistence and propensity for bioaccumulation. The heavy metals most commonly implicated in the contamination of aquaculture products include lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), cadmium (Cd), cobalt (Co), copper (Cu) and iron (Fe)[1].One of the most prevalent heavy metals, lead (Pb) poses a major risk to aquaculture practices and human health[2]. It persists for a long time in environment and bio-accumulated into aquatic ecosystem through intake of water and food or through absorption by skin and gills in fishes[3,4]. Subsequently it can be transferred to human through the food chains.

Heavy metal poisoning disturbs the fish physiology and causes biochemical and histological damage which may be mediated by oxidative stress[1,5-8]. Oxidative stress arises from an imbalance between oxidants and antioxidants, caused by excessive oxidant production, weakened antioxidant defences, or ineffective repair of oxidative damage[9,10]. Hydrogen peroxide (H_2O_2), superoxide radicals, and hydroxyl radicals are examples of ROS that can harm biological molecules such as proteins, lipids, and nucleic acids. Superoxide (O^{2-}), one of the primary forms of intracellular ROS, is a highly reactive molecule[11,12]. However, it can be converted to H_2O_2 by superoxide dismutase (SOD) and subsequently to oxygen and water by several enzymes, including catalase (CAT) and glutathione peroxidase (GPx). Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT, and GPx is an effective method of indicating oxidative stress. Furthermore, there is a greater demand for NAD^+ regeneration during oxidative stress to maintain glycolysis and redox balance[13]. A metabolic enzyme

*correspondence:
E-mail: ajeet.bhuz@gmail.com

lactate dehydrogenase (LDH) reduces the oxidative stress by converting pyruvate to lactate and vice versa to maintain the NAD^+/NADH ratio[14]. Furthermore, LDH isoforms, particularly LDH-A (LDH-5), have been identified as potential ROS sensors and may serve as reliable indicators of tissue damage[15]. Therefore, changes in the activity of LDH isozymes, along with other biomarkers, could be useful tools in aquatic toxicological research.

Labeo rohita is a commercially important freshwater species, widely cultured and consumed across South Asia, especially in India, Pakistan, Bangladesh, and Nepal. *Labeo rohita* is also reported to accumulate higher lead concentration in comparison to other fresh water fishes like *Catla catla* and *Cirrhinus mrigala*[16]. Therefore, it is hypothesized that it might be particularly vulnerable to lead exposure, even during short-term (acute) exposure. In recent years, several studies have investigated the effects of lead exposure in *Labeo rohita*; however, few have assessed enzymatic markers of oxidative stress by spectrophotometric method[17-20]. Moreover, these antioxidant enzymes are expressed in the form of multiple isozymes, therefore conventional spectrophotometric assays of total enzyme activity fail to reveal isozyme profile of these enzymes. Therefore, the present study employed in-gel activity assays to elucidate tissue-specific changes in the isozyme profiles of these enzymes (CAT, SOD, GPx and LDH) and to correlate these changes with histopathological alterations.

Materials and Methods

Chemicals

All the chemicals were of analytical and molecular biology grade. Lead (II) Acetate Trihydrate [$(CH3COO)_2$ $Pb.3H_2O$] was purchased from Sigma Aldrich (CAS no. 6080-56-4). All other chemicals were bought from Loba, Merck and Qualigens.

Fish management and Experimental design

Live specimens of *Labeo rohita* (standard length 12-15 cm, weight 20-22 g) were collected from government fish hatchery at Meja, District-Prayagraj, India, and were maintained in the laboratory at controlled room temperature (25 ± 2 °C) in glass aquaria containing water, aerated continuously. The fish were maintained under laboratory conditions for 15 days acclimatization period prior to the experiment. During this period, they were fed twice daily with commercially available Tokyu® pellets containing 46% protein, 6% fat, 5% fiber, along with essential minerals and vitamins. Water quality parameters were assessed in accordance with APHA[21]. The water quality parameters (mean ± S.D.) of water used for acclimation in controls and for the preparation of test solution were as follows: temperature, 25 ±2 °C; dissolved oxygen, 7.0 ±0.320 mg/L, pH, 7.8±0.07; and alkalinity, 246.78± 3.9 ppm. After acclimatization fish were divided into three groups, three fishes in each group in the capacity of 20 L aquarium. The first group was marked as control and other groups as experimental groups. Short term test of acute toxicity for a period of 96 hours was performed. Lead acetate was dissolved in triple-distilled water to prepare the dosing solutions.

Dose Selection

The doses were determined based on 96 hour LC50 of lead acetate for *Labeo rohita*, reported as 34.30 ppm by Prabha *et al*[22]. Experimental doses were set at approximately $1/10^{th}$ (3 mg/L) and $1/5^{th}$ (6 mg/L) of the LC50 value. Recent studies suggest these concentrations of lead are under the environmentally relevant concentration range[23]. Accordingly, the groups were assigned as follows: Group I remain untreated control (C), group II received 3 mg/L (Pb3), and group III received 6 mg/L (Pb6) of lead acetate. Each experiment was repeated at least three times to determine its reproducibility.

Preparation of Tissue Homogenate

After exposure of 96 hours, fish were sacrificed and tissue samples of liver, gill and brain were excised and washed in normal saline (0.7% NaCl). Tissues were immediately stored in -20ºC until use. Further, homogenised in 50mM Tris buffer (pH7.4) containing 0.1% Triton X-100 with the help of electric operated tissue homogeniser (Universal Biotechnology Private Limited, New Delhi, India) and centrifuged at 12,000g for 20 minutes at 4ºC in high speed bench top centrifuge (Neya 16 R, Remi Elektrotechnik LTD, Mumbai, India). The supernatants were utilized to assess antioxidant enzyme activities and other oxidative stress–related parameters.

Assay of total protein

Total protein content in tissue extracts was determined using a dye binding method of Bradford[24] using a standard curve constructed with Bovine serum albumin (BSA).

Assay of antioxidant enzymes

Assay of Catalase (CAT, EC 1.11.1.6)

The activity of CAT was analyzed by native poly-acrylamide gel electrophoresis (native-PAGE) followed by ferricyanide method of Woodbury *et al*[25]. 20µg sample of protein of each group were mixed with equal amount of 2x native loading buffer and loaded on the wells of 5% stacking gels and resolved on 8% resolving gel. Gels were allowed to run for 5 hours at 4ºC, thereafter soaked in 0.03% hydrogen peroxide (H_2O_2) for 10-25 minutes. Excess H_2O_2 was removed by washing for 1 minute. Gels were stained in a solution containing 1% ferric chloride and 1% potassium ferricyanide for 4-5 min at room temperature. White bands in blue-green background represent CAT activity.

Assay of Superoxide Dismutase (SOD, EC 1.15.1.1)

The in-gel activity assay of SOD was performed according to modified procedure of Beauchamp and Fridovich[26]. 30µg of total protein from each sample was loaded and separated on 10% resolving gel by native PAGE at 4°C. After electrophoresis, the gels were soaked in 1.23mM NBT solution for 20min at room temperature in dark. Gels were washed with distilled water and incubated in 100mM phosphate buffer (pH 7.0) containing 28mM TEMED and 0.28mM riboflavin for 15-20min at 25ºC in dark. Thereafter, the gels were exposed to fluorescent light until achromatic bands became visible on purple-blue background.

Identification of Superoxide dismutase (SOD) isozymes

The identification of different SOD isozymes was performed according the method described by Wang *et al*[27]. SOD enzymes are categorized into three types; Cu/Zn-SOD, Mn-SOD, and Fe-SOD, based on the metal cofactor present at the catalytic site. These isoforms exhibit differential sensitivities to specific chemical inhibitors, facilitating their distinction during analysis. Following native-PAGE, the gel was sectioned and each slab was incubated separately in specific inhibitory solutions: (i) hydrogen peroxide (H_2O_2, 5mM), and (ii) a chloroform: ethanol mixture ($CHCl_3$:CH_2CH_2OH, 3:5 v/v) for 30 minutes. Cu/Zn-SOD and Fe-SOD activities were inhibited upon exposure to H_2O_2, whereas Mn-SOD and Fe-SOD activity bands were eliminated following treatment with the chloroform: ethanol mixture.

Assay of Glutathione Peroxidase (GPx, EC 1.11.1.9)

GPx is thiol based antioxidant enzyme that catalyses the reduction of hydrogen peroxidase using Glutathione (GSH). The activity gel assay of GPx was performed according to the method of Lin et al[28] with minor modifications. 60µg of protein from each sample was loaded and separated on 10% resolving gel by non-denaturing PAGE at 4°C. After completion of electrophoresis, the gel was soaked in 50mM Tris-Cl (pH 7.9) containing 13mM GSH (reduced glutathione) and 0.004% H_2O_2, for 20min at room temperature in dark with gentle shaking. Thereafter, the gels were washed in distilled water and developed in darkness at 30°C containing 1.2mM NBT and 1.6mM PMS for 10min and exposed to light until appearance of clear zone of GPx band on purple background.

Assay of lactate dehydrogenase (LDH, EC 1.1.1.27)

The activity gel assay for LDH was performed according to the method of Dietz et al[29]. 20 µg protein from each sample was loaded and separated on 8% resolving gel at 4°C. After electrophoresis, the gels were soaked in staining solution containing phosphate buffer (pH7.4), 10mM lithium lactate, 150mM sodium chloride, 5mM magnesium chloride,15mM nicotinamide adenine dinucleotide (NAD+), 1.25mM NBT and 3.3mM phenazine methosulphate (PMS) for 10- 45 minutes. Dark brown colour bands were appeared on light surface of gels.

Lipid Peroxidation Assay

Malondialdehyde (MDA) level, an indicator of lipid peroxidation was measured using the method described by Ohkawa *et al*[30], with minor modifications. The MDA concentration was quantified using a standard curve prepared with known concentrations of 1,1,3,3-tetramethoxypropane and expressed as nmol MDA per mg protein.

Histological study

Tissue samples were processed, sectioned, and stained according to standardized protocols[31].The tissues of liver, gill and brain were collected from the treated as well as control fish, washed with normal saline and fixed in Bouin's fixative. After 24 h of fixation, tissues were dehydrated with ascending grades of alcohol and cleared in xylene. Thereafter, tissues were paraffin-embedded, sectioned at 6 µm using a rotary microtome (Model 1120; Medimeas Instruments, Haryana), and mounted on glass slides. The sections were deparaffinized in xylene, rehydrated through descending alcohol grades, and rinsed in distilled water. Staining was performed with

haematoxylin for 5–10 minutes, followed by counterstaining with 1% eosin. The stained slides were observed under compound microscope (Nikon ECLIPSE 80i; Nikon Corp., Japan) at 200X and 400X magnification, and photomicrographs were captured with Moticam 1000 camera (Motic, China) for histological examination.

Densitometry and statistical analysis

Alpha Imager 2200 software (Alpha Innotech) was used for densitometric analysis of the bands. Activity of antioxidant enzymes was measured on the basis of integrated densitometric value (IDV) and presented in arbitrary units (AU). All results were expressed throughout as mean ± SEM. Statistical differences were analysed between data obtained from control and the experimental groups. All the statistical analyses were carried out through Statistical Package for the Social Sciences (SPSS) for windows (standard version 11.5) software. In cases of multiple isoforms, within group and between group analyses was performed by two-way ANOVA followed by Tukey post hoc test. One-way ANOVA followed by Tukey post hoc test was performed in other cases.

Results

Assessment of antioxidant enzyme activity and tissue damage markers in fish liver following lead exposure

The activities of antioxidant enzymes and tissue damage markers in the liver were analysed in both control and lead-exposed groups. In-gel assays revealed the presence of a single CAT isoform in liver tissue. A significant decrease in CAT activity was observed in the Pb3 and Pb6 treatment groups compared to the control (Fig. 1a), indicating a diminished capacity to detoxify hydrogen peroxide (H_2O_2).

Native gel electrophoresis revealed four isoforms of SOD. The isoform closest to the cathode was identified as manganese-dependent SOD (Mn-SOD), while the remaining three were confirmed as copper/zinc-dependent SODs (Cu/Zn-SODs) based on inhibitor assays. All the isoforms were affected similarly in lead treated groups. The combined activity of all SOD isoforms was significantly elevated in both Pb3 and Pb6 groups relative to the control (Fig. 1b), suggesting an up- regulation of antioxidant defence in response to oxidative stress induced by lead exposure.

Similarly, GPx activity, represented by a single band on the gel, was significantly increased in both Pb3 and Pb6 groups compared to the control (Fig. 1C). This increase likely reflects a compensatory mechanism against oxidative stress.

Native PAGE analysis also detected five isoforms of LDH, designated as LDH1 through LDH5, following established nomenclature[32]. Combined densitometric value of all bands revealed a significant increase in LDH activity in lead exposed groups (Pb3 and Pb6) as compared to control. Further, LDH5 was unaffected by lead exposure although other isoforms were showing increased activity dragging the total active towards higher side (Fig. 1D).

Antioxidant enzyme and tissue damage marker activity in fish gills following lead exposure

The total CAT activity, represented by a single band on native PAGE, was significantly elevated in the Pb3 and Pb6 groups compared to the control (Fig. 2A). A similar trend was observed for SOD activity, which also showed a significant increase in both lead-exposed groups, indicating an up-regulated antioxidant defence system in response to lead-induced stress.

Notably, the expression pattern of SOD isoforms differed between the control and treated groups. While four distinct isoforms were present in the control group, only two were mainly active in the Pb3 and Pb6 groups. First band was identified as Mn-SOD and other three were identified as Cu/Zn-SOD, out of which only first Cu/Zn-SOD was expressed in Pb3 and Pb6. Despite this reduction in isoform diversity, the total SOD activity remained significantly higher in the treated groups due to increased activity of the remaining isoforms. Notably, activity of only Cu/Zn-SOD was increased contributed to total activity changes, whereas Mn-SOD was unaffected after lead exposure (Fig. 2B). This result suggest important role of Cu/Zn-SOD in gills during stress condition.

The in-gel assay revealed no detectable GPx activity. However, it is plausible that GPx activity might be detected using a more sensitive spectrophotometric assay, which could uncover changes in enzymatic activity not visible with the current method.

Additionally, LDH activity, represented by a single band, was also elevated in both Pb3 and Pb6 groups (Fig. 2C). This increase in LDH activity may indicate tissue damage in the gills resulting from lead exposure.

Antioxidant and tissue damage marker activity in fish brain following lead exposure

In brain tissues, CAT activity was undetectable by in-gel assay in all experimental groups. However, a

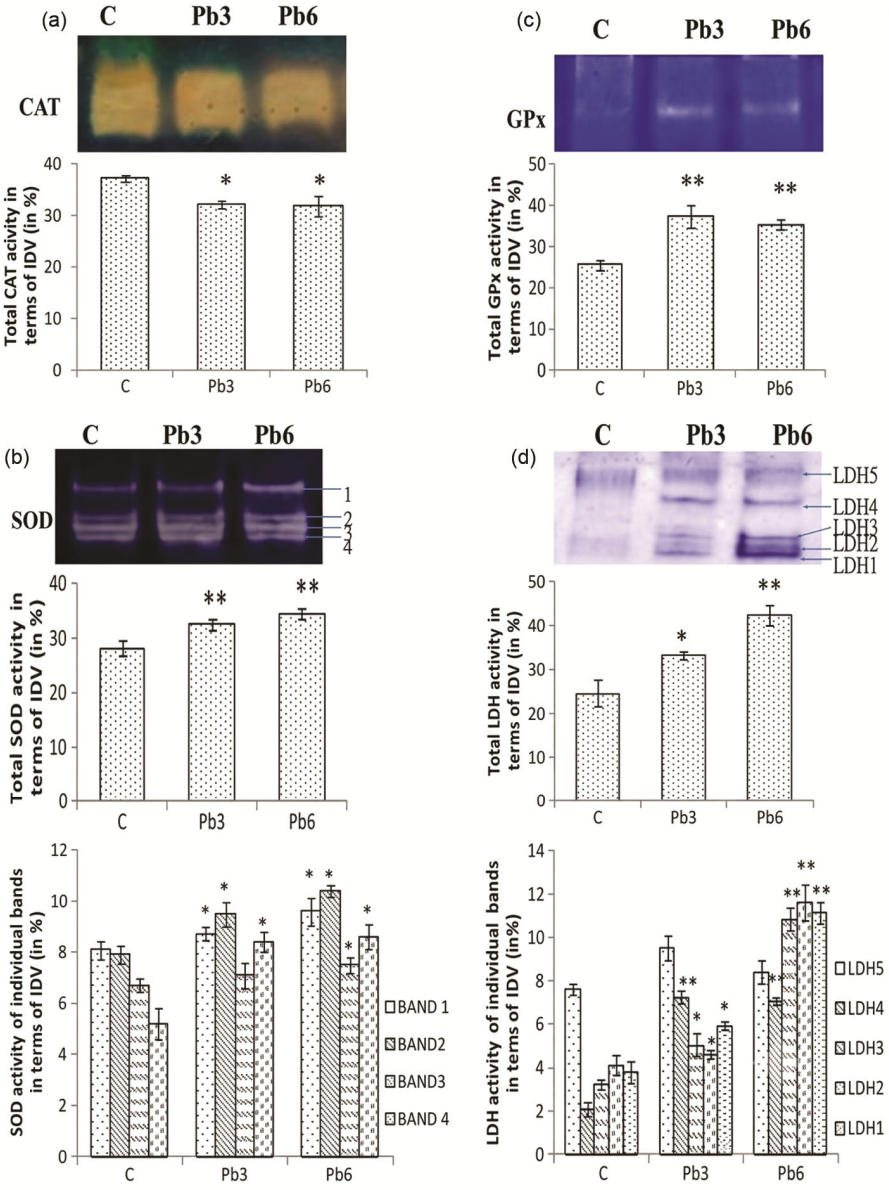

Fig. 1 — Effect of lead exposure on the antioxidant enzymes in the liver of fish *Labeo rohita*.
Activity of **(a)** CAT, **(b)** SOD, **(c)**GPx and **(d)** LDH in the liver of control (C) and two other groups treated with 3 mg/L(Pb3) &6mg/L(Pb6) of lead acetate respectively. Result represents mean ± SEM (n = 9). Asterisk on bar indicates significant difference from corresponding control ($^{*}p<0.05$ and $^{**}p<0.01$).). IDV, Integrated Densitometric value

significant increase in SOD activity was observed in the Pb6 group, while a slight, non-significant elevation was noted in Pb3 compared to the control (Fig. 3A). Two SOD isozymes were detected: the isoform closest to the cathode was identified as Mn-SOD, and the second as Cu/Zn-SOD based on their electrophoretic mobility and inhibitor sensitivity.

GPx activity, represented by a single band, was significantly increased in both Pb3 and Pb6 treatment groups relative to the control (Fig. 3B), indicating an enhanced antioxidant response to lead exposure in brain tissue.

Further, no significant changes in LDH activity were observed among the groups. LDH activity, as represented by a single band on the gel, remained comparable across control and treated fish (Fig. 3C).

Identification of superoxide dismutase (sod) isozymes

Four SOD isoforms were detected in the liver and gill tissues, while only the first two isoforms, located

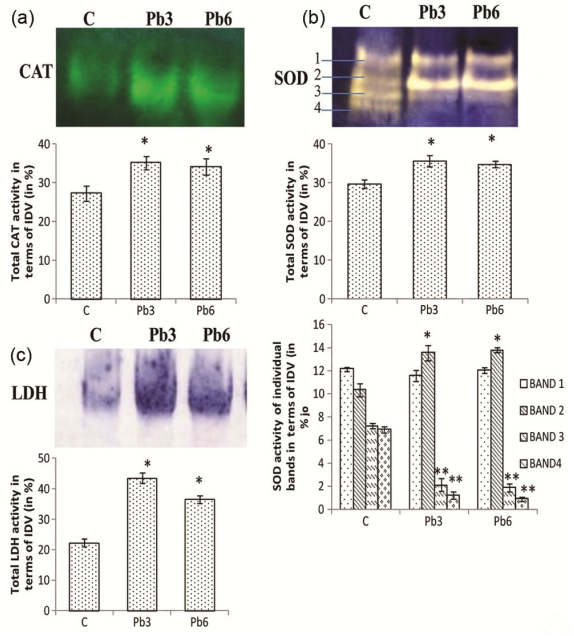

Fig. 2 — Effect of lead exposure on the antioxidant enzymes in the gills of fish *Labeo rohita*.
Activity of (**a)** CAT, (**b)** SOD, and (**c)** LDH in the gills of control (C) and two other groups treated with 3 mg/L(Pb3) & 6mg/L(Pb6) of lead acetate respectively. Result represents mean ± SEM (n = 9). Asterisk on bar indicates significant difference from corresponding control ($^{*}p<0.05$ and $^{**}p<0.01$).). IDV, Integrated Densitometric value

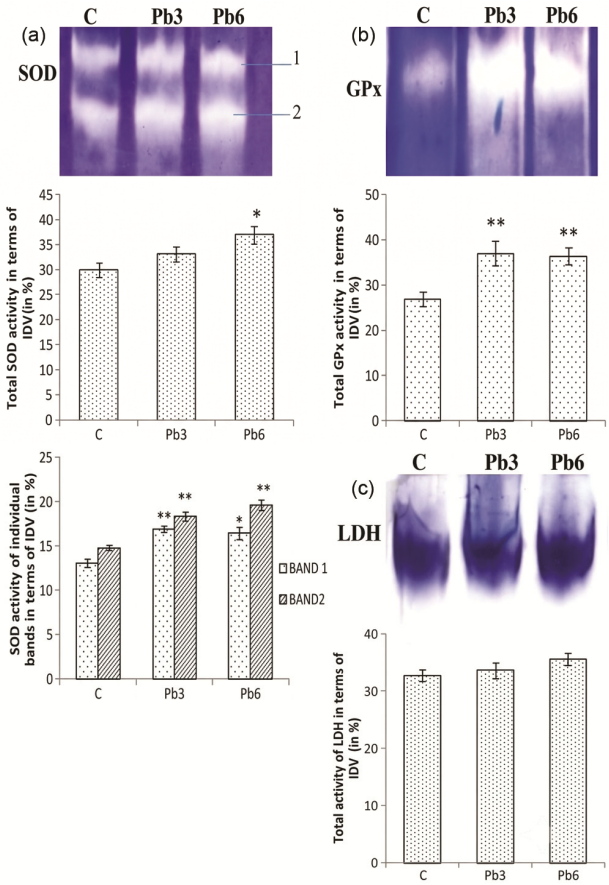

Fig. 3 — Effect of lead exposure on the antioxidant enzymes in the brain of fish *Labeo rohita*.
Activity of (**a)** SOD, (**b)** GPx, and (**c)** LDH in the brain of control (C) and two other groups treated with 3 mg/L(Pb3) & 6mg/L(Pb6) of lead acetate respectively. Result represents mean ± SEM (n = 9). Asterisk on bar indicates significant difference from corresponding control ($^{*}p<0.05$ and $^{**}p<0.01$). IDV, Integrated Densitometric value

closest to the cathode (negative pole), were observed in brain tissue (Fig. 4A, B and C). Upon treatment of the gels with hydrogen peroxide (H_2O_2), the last three bands disappeared, thereby excluding the possibility that these bands correspond to Mn-SOD or Fe-SOD isozymes. This observation suggests that these bands represent Cu/Zn-SOD isoforms. Conversely, when the gels were treated with an ethanol:chloroform mixture, the first band was selectively disappeared. This result rules out the presence of Cu/Zn-SOD or Fe-SOD isoforms in that band, indicating that it corresponds to a Mn-SOD isoform.

Lipid Peroxidation

Lipid peroxidation, as measured by malondialdehyde (MDA) levels, was significantly elevated in the lead treated groups in dose dependent manner as compared to control group across all tissues (Fig. 5A, B and C). This increase in MDA level suggests enhanced oxidative stress and membrane damage induced by lead exposure.

Histological analysis

Histopathological alterations observed in the liver, gills, and brain of *Labeo rohita* clearly demonstrates the toxic effects of lead acetate on vital organs. The liver sections of control fish showed normal histo-architecture of well-arranged polygonal hepatocytes containing centrally located nuclei and distinct cytoplasm (Fig. 6A). In contrast Pb3 group showed prominent degenerative changes such as mild cytoplasmic vacuolization, pyknotic nuclei, hepatic cell haemorrhage and increase in sinusoidal space (Fig. 6B). In Pb6 group severe pathological changes were evident including extensive cytoplasmic vacuolation and necrosis of hepatocytes (Fig. 6C).

Gill tissues of the control group revealed normal architecture with well-defined primary and secondary lamellae, intact epithelial lining, and properly arranged pillar cells (Fig. 7A**)**. However, Pb3 group showed lamellar curling, fusion, and hyperplasia of

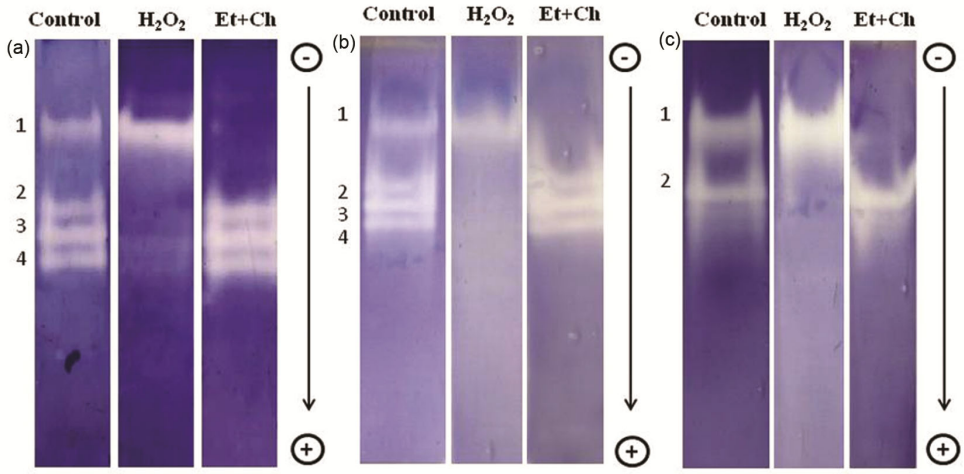

Fig. 4 — Identification of SOD isozymes in *Labeo* ***rohita*****.** **(a)** Liver, **(b)** Gill and **(c)** Brain extracts of control fish were separated on native PAGE. Thereafter, following treatments were conducted and stained with NBT reduction. Lane1: Control (Untreated), Lane 2: H_2O_2 (5mM) treatment, which inhibits Cu/Zn- SOD and Fe- SOD and Lane 3: $CHCl_3$- CH_3CH_2OH (Et-Ch, 3:5 v/v) treatment, which inhibits Mn- SOD and Fe-SOD. Thus, band 1 was identified as Mn-SOD and; Band 2, 3 and 4 were identified as Cu-Zn SOD. The direction of electrophoresis was marked by arrow from cathode (-) to anode (+)

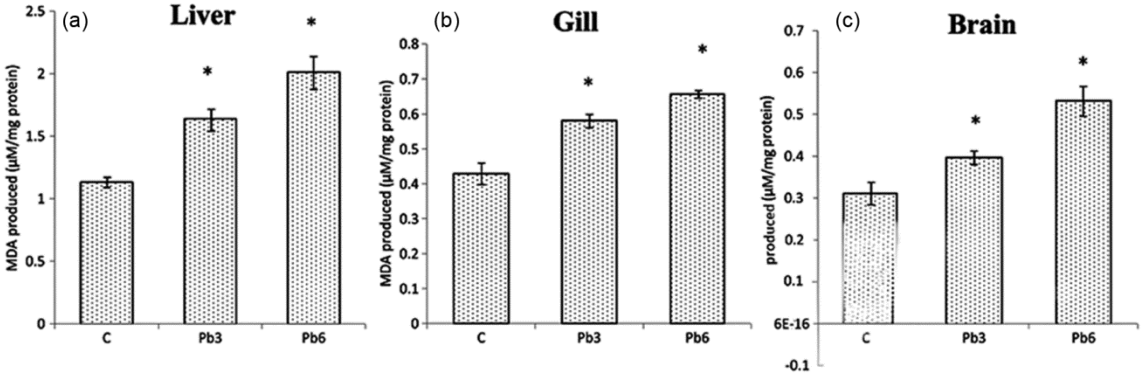

Fig. 5 — Effect of lead exposure on the lipid peroxidation in different organs of fish *Labeo* ***rohita*****.** **(a)** Liver (b) Gill and (c) Brain of control (C) and two other groups treated with 3 mg/L(Pb3) & 6mg/L(Pb6) of lead acetate respectively. Result represents mean ± SEM (*n* = 9). Asterisk on bar indicates significant difference from control ($^{*}p<0.05$ and $^{**}p<0.01$).

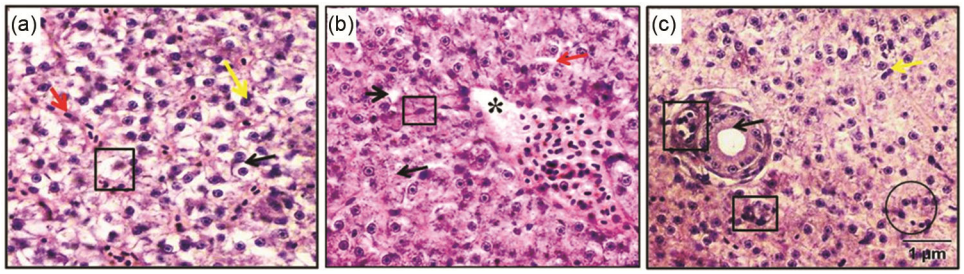

Fig. 6 — Photomicrographs of haematoxylin and eosin stained sections shows histopathological changes in liver of *L. rohita* exposed to lead acetate. (a) Control group showing spherical nucleus (yellow arrow), cytoplasm (square) and polygonal hepatocytes (black arrow), blood sinusoids (red arrow) 40X. (b) Pb3 group showing sinusoidal space (red arrow), pyknotic nuclei (square), degeneration of hepatocyes (black arrow), haemorrhage in central vein (asterisk). (c) Pb6 group showing vacuolation (black arrow), necrotic cells (square), hepatocyte degeneration (circle), pyknotic nuclei (yellow arrow). Scale bar 1µm.

epithelial cells (Fig. 7B), while the Pb6 group displayed severe lamellar necrosis, epithelial sloughing, and complete disorganization of the gill filaments (Fig. 7C).

The brain, particularly the optic tectum region, of control fish exhibited normal neuronal organization with distinct nuclei (Fig. 8A). In Pb3 group exhibited moderate neuronal degeneration, accumulation of

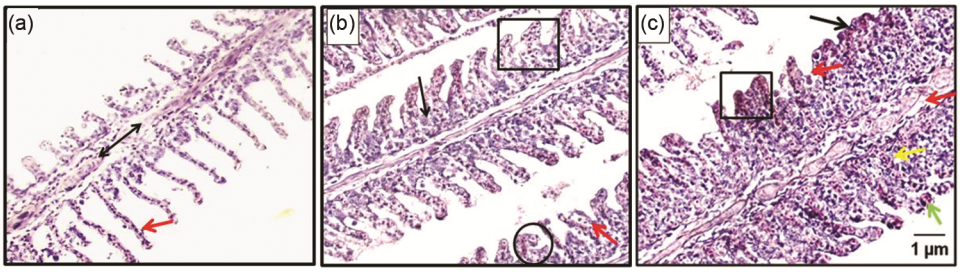

Fig. 7 — Photomicrograph of haematoxylin and eosin stained sections shows histopathological changes in gills of *L. rohita* exposed to lead acetate**.** (a) Gill filament of the control group, Primary gill lamellae (black arrow), secondary gill lamellae (red arrow). (b) Pb3 group showing gill hyperplasia (black arrow), lamellar curling (circle), necrosis (red arrow) and fusion of secondary lamellae (square). (c) Pb6 group showing fusion of secondary lamellae (square), completely collapsed secondary lamellae (black arrow), telengectiasis (green arrow), gill oedema (yellow arrow) and necrosis in lamellae (red arrow). Scale bar 1µm.

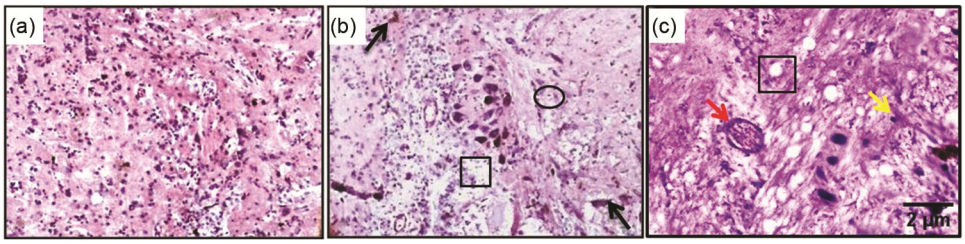

Fig. 8 — Photomicrograph of haematoxylin and eosin stained sections shows histopathological changes in brain of *L. rohita* exposed to lead acetate. a. Control group showing neurons in optic tectum (OT) without any damage. (b) Pb3 group showing pyknotic nuclei (circle), melanin granules (black arrow), and neutrophil loss (square). (c) Pb6 group showing vacuolization (square), inflammation (red arrow) and necrotic cell (yellow arrow). Scale bar 2µm.

melanin granules, cytoplasmic vacuolization, nuclear pyknosis and cellular infiltration (Fig. 8B). Similarly Pb6 group also displayed an increase in vacuolization, cellular infiltration, necrosis and haemorrhage in brain tissue (Fig. 8C).

Discussion

Lead has high affinity for sulfhydryl group (-SH). It disrupts the antioxidant enzymes activity by binding and inhibiting the –SH group in several enzymes such as SOD, CAT and GPx[33]. It can promote oxidative damage by increasing the cellular level of reactive oxygen species, which may lead to disturbance in antioxidant enzymes in fish[34,35]. Further, produced ROS causes DNA strand breaks and replaces the Zn in DNA binding proteins[36]. However, fish can regulate metal concentration to a certain extent, after which bioaccumulation occurs and damages the fish organs[37,38]. Accumulation of lead easily leads to immunosuppression, leading to increased disease susceptibility in fish[39]. High rate of metal accumulation may be observed in gills of mostly fresh water fishes during respiration and osmoregulation[40,41,42] because gills have large surface area and direct contact with water, which is primary medium for heavy metal contamination[43]. Whereas liver plays a significant role in the processing, detoxification, transmission and conversion of toxins and also serves as an active site for adverse effects caused by pollutants in fishes[19].Lead is also a potent neurotoxin that can cause learning and memory impairments as well as brain damage[44].

In the present study, a significant decrease in CAT activity was observed in the liver of both Pb3 and Pb6 groups (Fig. 1A). This reduction in CAT activity may be attributed to its inhibition by the excessive production of its substrate, H_2O_2, resulting from the elevated activity of SOD (Fig. 1B) in both groups. Similar result was also found in heavy metal stressed liver of *Labeo rohita* by Akhtar et al[45]. Similarly, Rehman et al[46] have also reported a significant decrease in CAT activity in rainbow trout, *Oreochromis niloticus* exposed to heavy metals, this decrease in CAT may be due to binding of metal ion to –SH group of enzymes. However, the increased activity of GPx in these groups suggests that this enzyme plays a supportive role in managing the heightened oxidative stress in the liver[47]. The enhanced activity of SOD and CAT in the gills (Fig. 2A and B), as well as SOD and GPx in the brain (Fig. 3A and B), was observed following lead exposure in Pb3 and Pb6. This increase may indicate an adaptive response to counteract mild oxidative stress. Similar

findings have been reported in previous studies[48,49]. The upregulation of antioxidant enzyme activity in response to mild oxidative stress is commonly mediated through the activation of the Nrf2 pathway[50,51]. However, under conditions of elevated oxidative stress, the activity of these enzymes tends to decrease due to oxidative modifications of their active sites[52]. This biphasic response highlights the organism's attempt to manage oxidative damage at lower stress levels and the failure of antioxidant defence mechanisms under higher oxidative stress. These results strongly suggest that oxidative stress levels are higher in the liver compared to the gills and brain, a finding further supported by MDA levels in these tissues. When MDA levels were compared across these tissues, the liver exhibited MDA levels that were approximately 2-3 times higher than those in the gills and brain in all groups (Fig. 5A, B and C). Several studies are in agreement with our finding, where MDA level is significantly increasing in heavy metal exposed fishes[53,54].

LDH is an intracellular enzyme which constitutes a major check point of anaerobic respiration. It facilitates the reversible conversion of pyruvate to lactate, serving an essential role in anaerobic respiration by regenerating NAD^+ required for glycolysis[55]. Therefore, increased LDH activity in liver and gill indicates the impairment of oxidative metabolism in these tissues. It is evident that oxidative stress caused a rise in LDH and this process supplies reducing power against ROS, indicating that LDH functions as an antioxidant[15,56]. Furthermore, elevated LDH activity serves as a marker of tissue damage suggesting potential harm to the liver and gills following lead exposure in both groups (Fig. 1D and 2C). However, no such indication was found in the brain. Consistent with this observation, elevated LDH activity has been observed in the liver of *Clarias gariepinus* when exposed to hexavalent chromium[57] and in the serum of *Labeo rohita* when exposed to copper sulphate[58]. The enzyme harnesses the reducing power of NADH to drive this process[13]. Lactate dehydrogenase (LDH) exists as a tetrameric enzyme composed of varying combinations of two gene products, LDHA and LDHB, whose distinct subunit assemblies generate five isoforms: LDH1 (B_4), LDH2 (AB_3), LDH3 (A_2B_2), LDH4 (A_3B), and LDH5 (A_4). In this study, five isozymes of LDH were found in liver, in which LDH 1 is markedly increased in high level lead treated group. The observed increase in LDH-1 levels in *Labeo rohita* exposed to lead suggests a shift in metabolic enzyme expression likely linked to lead-induced oxidative stress and tissue-specific injury. As LDH-1 is typically associated with aerobic metabolism, its upregulation may reflect a compensatory mechanism to maintain oxidative ATP production or increased enzyme release due to cellular damage[59,60].

An important finding in this study is the tissue-specific differential isozyme profile observed in both control and lead-treated fish. A single isozyme of CAT was detected in the liver and gill, but it was absent in the brain. This finding aligns with studies conducted in rodents, where CAT activity was not observed in control brain tissue lysates in in-gel assays[61,62]. While the presence of CAT activity in brain cannot be entirely excluded, this suggests that peroxide elimination in the brain of *Labeo rohita* is primarily mediated by GPx, as shown in Fig. 3B. A similar result was found for GPx, with a single isozyme detected in both the liver and brain, but not in the gills. Another key observation is that only one peroxide-neutralizing enzyme is active at a time in the gill and brain, while both CAT and GPx are active in the liver. This reflects a higher level of hydrogen peroxide (H_2O_2) in the liver compared to the brain and gill. In most animal tissues, two types of SOD are typically present: Cu/Zn-SOD, which is found in the nucleus and cytoplasm, and Mn-SOD, which is located in the mitochondria[34,63] with some exceptions exhibiting Fe-SOD[60]. In this study, four distinct SOD isozymes were detected in the liver and gill, whereas only two isozymes were observed in the brain (Fig. 4A, B and C). Among the four isozymes identified, the first was identified as Mn-SOD, while the remaining three were identified as Cu/Zn-SOD. The absence of two Cu/Zn SOD isozymes in the brain may be due to the sufficient presence of two isozymes of SOD, which can effectively manage the relatively low oxidative stress in this tissue. Although lead has neurotoxic properties, but it might not cause immediate oxidative stress in brain but may trigger inflammation or mitochondrial dysfunction that becomes apparent later. Additionally, the alteration in the activity of only Cu/Zn-SOD suggests that the primary source of reactive oxygen species (ROS) during acute lead exposure is the cytosol rather than the mitochondria, although further investigation is needed to validate this observation.

Studies in other species have identified five GPx isozymes[64], although we were unable to determine which specific isozyme(s) are expressed in this study. Single isoform of GPx was found elevated in high

dose in both liver and brain (Fig. 1C and 3B), whereas other bands were not clearly visualized. Increase in GPx as well as CAT in high dose is suggesting that GPx might protect CAT against the inactivating effect of H_2O_2[65]. We have documented an increase in GPx in liver and brain but have not excluded the possibility that other isozymes of GPx might be present.

Oxidative stress often leads to damage of several cellular components such as protein, DNA or lipid, which may be reflected in histological alterations[36]. Histopathological study was performed to correlate lead induced oxidative stress with possible tissue damage in vital organs. Histopathological assessment revealed significant alterations in the morphology of the liver, gills, and brain of *L. rohita* exposed to lead acetate compared with the control group. The liver of lead-exposed fish exhibited severe hepatic damage, characterized by necrosis, cytoplasmic vacuolation and degeneration of hepatocytes (Fig. 6B and C). Similar hepatic alterations have been reported in *Oreochromis niloticus* exposed to lead[66] and in *Labeo rohita* exposed to mercury[67]. In the gills, structural changes such as lamellar lifting, curling, and fusion were evident (Fig. 7B and C),which are considered nonspecific defence responses that create a barrier between the external environment and the bloodstream, thereby limiting pollutant entry[18,68]. The observed epithelial hyperplasia and curling of secondary lamellae further indicate an adaptive response of the fish to minimize the direct contact of lead with vital respiratory surfaces[4]. However, the pronounced hyperplasia, necrosis, and epithelial cell sloughing compromised the gill's functional integrity, likely reducing oxygen uptake, impairing carbon dioxide elimination, and disturbing ionic regulation[69]. Lead exposure also induced distinct neuropathological alterations in the brain, particularly in the optic tectum region (Fig. 8B and C), which is responsible for visual processing, motor coordination, and behavioural regulation in fish[70]. Degeneration of neurons, cytoplasmic vacuolization, and necrosis observed in the brain suggest impaired neurophysiological functioning and behavioural disturbances resulting from lead neurotoxicity because Pb has direct toxic effects on neurons, it destroys microtubule that cannot be repaired overtime and limiting brain function[71]. Also presence of melanin granules within the optic tectum appears to be a neuroprotective response, as melanin has a high affinity for binding neurotoxicants, including heavy metals, thereby reducing their bioavailability and mitigating neuronal damage[72]. Similar results are also found in zebra fish exposed to cadmium and mercury[73]. Taken together, it may be concluded that lead exposure leads to significant tissue damage in liver, gill and brain via alteration in enzymatic antioxidant defence system and by producing significant oxidative stress.

Conclusion

The current study determined that lead exposure causes oxidative stress and tissue specific alteration in isozyme profile of antioxidant enzymes, which leads to histological damage in *Labeo rohita*. Further, isozyme-specific increase in hepatic LDH activity suggested metabolic disturbances and a possible shift towards anaerobic energy metabolism under lead-induced stress. Overall, these findings highlight the toxic potential of lead even after short-term exposure and emphasize the need for further molecular and biochemical investigations to better understand its adverse effects in aquaculture species.

Acknowledgement

This work is partially supported by University Grants Commission, India in the form of a Start-up project (F.30-461/2019, BSR) sanctioned to Ajeet Kumar Singh.

Ethical declaration

All experiments were performed following the approval of the Ethics Committee of the institution as per the ethical guidelines for the treatment and maintenance of animals.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1 Javed M, Ahmad I, Usmani N & Ahmad M, Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in *Channa punctatus* from heavy metal polluted canal. *Chemosphere*, 151 (2016) 210.

2 Li X, Kong H, Ji X, Gao Y & Jin X, Zebrafish behavioural phenomics applied for phenotyping aquatic neurotoxicity induced by lead contaminants of environmentally relevant level. *Chemosphere*, 226 (2019) 445.

3 Lee JW, Choi H, Hwang UK, Kang JC, Kang YJ, Kim KI & Kim JH, Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity and immune responses in fish: A review. *Environ Toxicol Pharmacol*, 68 (2019) 101.

4 Mahi TF, Chowdhury G, Hossain MA, Baishnab AK, Schneider P & Iqbal MM, Assessment of lead toxicity in juvenile Nile tilapia (*Oreochromis niloticus*). *Toxics*, 10 (2022) 793.

5 Saliu JK & Bawa-Allah KA, Toxicological effects of lead and zinc on the antioxidant enzyme activities of post-juvenile *Clarias gariepinus*. *Resour Environ*, 2 (2012) 21.

6 Özkan F, Özlüer-Hunt A, Gündüz SG, Berköz M & Yalçın S, Effects of dietary organic selenium against lead toxicity on antioxidant system in *Cyprinus carpio*. *Fish Physiol Biochem*, 40 (2013) 335.

7 Rajeshkumar S, Liu Y, Ma J, Duan HY & Li X, Effects of multiple heavy metals on biochemical and histopathological alterations in common carp. *Fish Shellfish Immunol*, 70 (2017) 461.

8 Zhai Q, Wang H, Tian F, Zhao J, Zhang H & Chen W, Dietary *Lactobacillus plantarum* reduces lead accumulation and alleviates lead toxicity in Nile tilapia. *Aquac Res*, 48 (2017) 5103.

9 Das D, Moniruzzaman M, Sarbajna A & Chakraborty SB, Heavy metal-induced tissue-specific antioxidant responses in Indian major carps. *Environ Sci Pollut Res*, 24 (2017) 21967.

10 Jayakumar V, Senthilmurugan S, Vijayan P & Tamizhazhagan V, Indian major carp *Labeo rohita* (Hamilton 1882), toxicology approaches an overview. *Int. J. Biol. Res*, 3 (2018) 332.

11 Ighodaro OM & Akinloye OA, First-line defence antioxidants: Superoxide dismutase, catalase and glutathione peroxidase. *Alex J Med*, 54 (2018) 287.

12 Paul N, Chakraborty S & Sengupta M, Lead toxicity on non-specific immune mechanisms of *Channa punctatus*. *Aquat Toxicol*, 152 (2014) 105.

13 Luengo A, Li Z, Gui DY, Sullivan LB, Zagourlya M, Do BT, Ferreira R, Naamati A, Ali A, Lewis CA & Vander Heiden MG, Increased demand for NAD^+ relative to ATP drives aerobic glycolysis. *Mol Cell*, 81 (2021) 691.

14 Lagarde D, Jeanson Y, Portais JC, Galinier A, Ader I, Casteilla L & Carrière A, Lactate fluxes and plasticity of adipose tissues in a redox perspective. *Front Physiol*, 12 (2021) 689747.

15 Lin Y, Wang Y & Li PF, Mutual regulation of lactate dehydrogenase and redox robustness. *Front Physiol*, 13 (2022) 1038421.

16 Javid A, Javed M & Al-Z, Bioaccumulation of lead in major carps during 96-h LC50 exposures. *Int J Agric Biol*, 9 (2007) 859.

17 Vaseem H & Banerjee TK, River Ganga contamination and toxic implications on blood parameters of *Labeo rohita*. *Environ Sci Pollut Res*, 20 (2013) 5673.

18 Brraich OS & Kaur M, Histopathological alterations in gills of *Labeo rohita* (Hamilton- Buchanan) due to lead toxicity. *Indian J Exp Biol*, 55 (2017) 576.

19 Shaukat N, Javed M, Ambreen F & Latif F, Oxidative stress biomarkers in assessing lead-induced toxicity in *Labeo rohita*. *Pak J Zool*, 50 (2018) 735.

20 Latif F, Ujan JA, Rind KH, Habib SS, Naz S & Al-Emam A, Effects of lead concentration and exposure duration on accumulation and antioxidant defence in Indian major carps. *Biol Trace Elem Res*, 213 (2025) 1.

21 APHA, Standard methods for the examination of water and wastewater, 16th edn. American Public Health Association, Washington DC (1985)

22 Prabha SP & Rajkumar J, Hepatotoxicity of lead in *Labeo rohita*: An FT-IR study. *J Chem Pharm Sci*, 8 (2015) 193.

23 Dey KK, Mondal P & Chattopadhyay A, Environmentally relevant lead induces oxidative stress and nuclear damage in *Anabas testudineus*. *J Appl Toxicol*, 44 (2024) 260.

24 Bradford MM, A rapid and sensitive method for quantitation of microgram quantities of protein. *Anal Biochem*, 72 (1976) 248.

25 Woodbury W, Spencer AK, Stahman MA, An improved procedure using ferricyanide for detecting catalase isozymes. *Anal Biochem*, 44 (1971) 301.

26 Beauchamp C & Fridovich I, Superoxide dismutase: Improved assays applicable to acrylamide gels. *Anal Biochem*, 44 (1971) 276.

27 Wang J, Liu X & Yu G, Identification of superoxide dismutase isoenzymes. *Front Biol China*, 4 (2009) 442.

28 Lin CL, Chen HJ & Hou WC, Activity staining of glutathione peroxidase after electrophoresis. *Electrophoresis*, 23 (2002) 513.

29 Dietz AA & Lubrano T, Separation and quantitation of lactate dehydrogenase isoenzymes. *Anal Biochem*, 20 (1967) 246.

30 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides by thiobarbituric acid reaction. *Anal Biochem*, 93 (1979) 351.

31 Luna LG, Manual of histologic staining methods of the Armed Forces Institute of Pathology. In Manual of histologic staining methods of the Armed Forces Institute of Pathology 12 (1968) 258.

32 Shaklee JB, Kepes KL & Whitt GS, Specialized lactate dehydrogenase isozymes in teleost fishes. *J Exp Zool,* 185 (1973) 217.

33 Virgolini MB & Aschner M, Molecular mechanisms of lead neurotoxicity. *Adv Neurotoxicol*, 5 (2021) 59.

34 Radi R, Biological antioxidant defences. *Toxicol. Ind. Health*, 9 (1993) 53.

35 Bangeppagari M, Gooty JF, Tirado JO, Mariadoss S, Thangaswamy S, Maddela NR & Ortiz DR, Therapeutic efficiency of spirulina against lead acetate toxicity in *Labeo rohita*. *Am J Life Sci*, 2 (2014) 389.

36 Flora SJS, Role of free radicals and antioxidants in health and disease. *Cell Mol Biol*, 53(2007) 1.

37 Heath AG, Water pollution and fish physiology. Lewis Publishers, Boca Raton (1991)

38 Venkateswarlu V & Venkatrayulu C, Bioaccumulation of lead in tissues of *Mugil cephalus*. *J Appl Biol Biotechnol*, 18 (2020) 1.

39 Dunier M, Water pollution and immunosuppression in freshwater fish. *Ital J Zool*, 63(1996)303.

40 Alsop D, Ng YT, Chowdhury MJ & Wood CM, Interactions of waterborne and diet-borne lead in rainbow trout. *Aquat Toxicol*, 177 (2016) 343.

41 Dai W, Liu S, Fu L, Du H & Xu Z, Dietary lead accumulation induces oxidative stress and DNA damage in tilapia. *Aquac Res*, 43 (2012) 208.

42 Kim JH & Kang JC, Sub-chronic lead exposure and antioxidant responses in juvenile rockfish. *Chemosphere*, 171 (2017) 520.

43 Curcio V, Macirella R, Sesti S, Ahmed AIM, Talarico F, Tagarelli A, Mezzasalma M & Brunelli E, Morphological and Functional Alterations Induced by Two Ecologically Relevant Concentrations of Lead on *Danio rerio* Gills. *Int J Mol Sci*, 23 (2022) 9165.

44 Zhang Y, Zhang P, Yu P, Shang X, Lu Y & Li Y, Transcriptomic insights into lead-induced brain injury in common carp. *Sci Total Environ*, 743 (2020) 140796.

45 Akhtar A, Abdullah S, Saquib MA, Nawaz A, Mehmood T, Yasin S, Akbar H & Areej, Comparative study of catalase activity in normal and heavy-metal-stressed liver of *Labeo rohita*. *J Posit Sch Psychol*, 7 (2023) 119.

46 Rehman T, Naz S, Hussain R, Chatha AMM, Ahmad F, Yamin A, Akram R, Naz H & Shaheen A, Heavy metal exposure alters antioxidant enzymes in *Oreochromis niloticus*. *Asian J Agric Biol*, 9 (2021) 1.

47 Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR & Sadeghi M, Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Front Pharmacol*, 2 (2021) 643972.

48 Vinodhini R & Narayanan M, Bioaccumulation of heavy metals in organs of *Cyprinus carpio*. *Int J Environ Sci Technol*, 5(2009)179.

49 Alak G, Atamanalp M, Topal A, Arslan H, Kocaman EM & Oruç E, Sub-lethal lead toxicity alters antioxidant enzymes in rainbow trout. *Fresenius Environ Bul*, 22 (2013) 1.

50 Ma Q, Role of Nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol*, 53 (2013) 401.

51 Paithankar JG, Saini S, Dwivedi S, Sharma A & Chowdhuri DK, Heavy metal-associated health hazards: Oxidative stress and signalling pathways. *Chemosphere*, 262 (2020) 128350.

52 Zhao L, Zheng YG, Feng YH, Wang GQ, Ma YF & Li MY, Toxic effects of waterborne lead in *Channa argus*. *Chemosphere*, 255 (2020) 127714.

53 Das D, Moniruzzaman M, Sarbajna A & Chakraborty SB, Effect of heavy metals on tissue-specific antioxidant response in Indian major carps. *Environ Sci Pollut Res*, *24* (2017) 18010.

54 Kovacik A, Tvrda E, Miskeje M, Arvay J, Tomka M, Zbynovska K, Andreji J, Hleba L, Kovacikova E, Fik M, & Cupka P, Trace metals in the freshwater fish *Cyprinus carpio*: effect to serum biochemistry and oxidative status markers. *Biol Trace Elem Res*, 188 (2019) 494.

55 Farhana A & Lappin SL, Biochemistry, Lactate dehydrogenase. *Stat Pearls* (2023).

56 Wu H, Wang Y, Ying M, Jin C, Li J & Hu X, Lactate dehydrogenase amplifies reactive oxygen species under oxidative stress. *Signal Transduct Target Ther*, **6** (2023) 242.

57 Aliu C, Ajayi OO, Olawuyi TS, Gbadamosi OK, Barbosa Jr F, Adedire CO & Adeyemi JA, Tissue accumulation, cytotoxicity, oxidative stress, and immunotoxicity in African catfish, *Clarias gariepinus* exposed to sublethal concentrations of hexavalent chromium. *Biol Trace Elem Res*, 202 (2024) 2294

58 Naz S, Hussain R, Guangbin Z, Chatha AMM, Rehman ZU, Jahan S, Liaquat M & Khan A, Copper sulfate induces clinico-hematological, oxidative stress, serum biochemical and histopathological changes in freshwater fish rohu (*Labeo rohita*). *Front Vet Sci*, 10 (2023) 1142042

59 Brancaccio P, Lippi G & Maffulli N, Biochemical markers of muscular damage. *Clin Chem Lab Med*, **48**(2010)757

60 Hsieh YS, Yeh MC, Lin YY, Weng SF, Hsu CH, Huang CL, Lin YP & Han AY, Serum lactate dehydrogenase as a marker for glucose monitoring. *Front Endocrinol*, **13**(2022)1099805.

61 Mokni M, Elkahoui S, Limam F, Amri M & Aouani E, Effect of resveratrol on antioxidant enzymes in rat brain. *Neurochem Res*, **32** (2007) 981.

62 Singh AK & Vinayak M, Resveratrol alleviates inflammatory hyperalgesia by modulation of reactive oxygen species (ROS), antioxidant enzymes and ERK activation. *Inflamm. Res*, 66 (2017) 911.

63 Stutz LA, Diekert K, Jensen LT, Lill R & Culotta VC, Mitochondrial localization of Cu/Zn-SOD. *J Biol Chem*, **276** (2001) 38084.

64 Comhair SAA, Bhathena PR, Farver C, Thunnissen FB & Erzurum SC, Extracellular glutathione peroxidase in asthmatic lungs. *FASEB J*, **15**(2001) 70.

65 Baud O, Greene AE, Li J, Wang H, Volpe JJ & Rosenberg PA, Glutathione peroxidase–catalase cooper ativity in oxidative resistance. *J Neurosci*, **24** (2004) 531.

66 Hossain Z, Hossain MS, Ema NS & Omri A, Heavy metal toxicity in Buriganga river alters the immunology of Nile tilapia (*Oreochromis niloticus* L). *Heliyon*, 28 (2021) 7.

67 Sharma S, Batoye S & Srivastava N, Protective efficacy of quercetin against mercury toxicity in *Labeo rohita*. *Aquacult Int*, **33** (2025) 228.

68 Sharma R, Jindal R, Jhamb N, Banaee M. and Faggio C, Lead Nitrate ($Pb(NO_3)_2$) Toxicity Effects on DNA Structure and Histopathological Damage in Gills of Common Carp (*Cyprinus carpio*). *Microsc Res Tech*, 88 (2024) 81817.

69 Javed M, Ahmad MI, Usmani & Ahmad M, Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in *Channa punctatus* exposed to heavy metal loaded waste water. *Sci Rep*, **7** (2017) 1675.

70 Mishra A & Devi Y, Histopathological alterations in the brain of *Channa punctatus* following chlorpyrifos exposure. *Acta Histo chem*, **116** (2014) 176.

71 Chen L, Liu Y, Jia P, Zhang H, Yin Z, Hu D, Ning H & Ge Y, Lead acetate induces neurotoxicity via synaptic disruption. *Environ Sci Pollut Res*, **29** (2022) 58927.

72 Beltrán-Solís K, García-Mendoza E, Sánchez-Serrano S & López LM, Domoic acid induces neurobehavioral alterations in fish. *Sci Rep*, **13** (2023) 21729.

73 Patel UN, Patel UD, Khadayata AV, Vaja RK, Patel HB & Modi CM, Neurotoxicity following cadmium and mercury co-exposure in zebrafish. *Water Air Soil Pollut*, **232** (2021) 340.