

Tissue-specific variation in antioxidant Defence biomarkers in *Mystus tengara* exposed to pollution

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Abstract

This study examined how antioxidant defence markers and oxidative stress levels differ among tissues in *Mystus tengara* fish collected from various sites along the Chambal River in Nagda, Madhya Pradesh, an area heavily impacted by industrial pollution and human activity. We collected fish samples from one clean reference location (S1) and two polluted sites (S2 and S3). We measured for several key antioxidant markers like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GRed), and lipid peroxidation (LPO). The results showed that polluted areas had noticeably higher levels of antioxidant enzymes, a clear sign that cells were ramping up their defenses in response to the buildup of reactive oxygen species. Among the tissues we examined, the liver stood out as the most effective at combating oxidative stress, particularly in its GPx and GRed activities. That really highlights just how crucial the liver is for cleaning out toxins and keeping things. The gill tissues showed the highest CAT activity and the highest levels of lipid peroxidation, suggesting they're directly affected by water pollutants and that the cells are under severe oxidative stress. Although antioxidant enzymes were significantly increased, elevated LPO levels suggested that oxidative stress surpassed the protective ability of the exposed fish. The findings show that pollution in the Chambal River at Nagda disrupts redox homeostasis in *M. tengara* and underscore the usefulness of antioxidant biomarkers as reliable indicators of aquatic environmental health and contaminant-induced stress.

Keywords: *Mystus tengara*; Chambal River; Nagda; Oxidative stress; Antioxidant enzymes; Lipid peroxidation; Biomonitoring; Aquatic pollution; Fish health; Biomarkers

1 Introduction

Freshwater ecosystems are some of the most biologically rich and productive environments on the planet, but they're also incredibly fragile. They play a vital role in sustaining life, providing essential services such as clean water and habitats for countless species (Reddy, P. B., 2017; Srivastava, B., & Reddy, P. B., 2020). Fast-paced industrial growth, urban expansion, and booming agriculture have all taken a real toll on rivers, increasing pollution and degrading water quality while disrupting the delicate balance of aquatic life (Reddy, P. B., 2016; Singh et al., 2026). Fish are especially useful for gauging the health of freshwater ecosystems. They're found across different levels of the food chain, soak up pollutants from their surroundings, and show clear physical signs when things go wrong, such as pollution or habitat changes (Reddy & Baghel, 2012; Srivastava & Reddy, 2017, 2020). It's like they're nature

The Chambal River runs through central India and plays a crucial role in daily life. It's a lifeline for homes, farms, and factories alike, supplying water where it's needed most. But things aren't looking great for the river near Nagda, Madhya Pradesh. It's under serious pressure from human activity, especially pollution from chemical, textile, and pharma

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factories, plus untreated sewage being dumped straight in (Reddy & Baghel, 2012). When factories keep dumping waste and pollutants into waterways, it can disrupt the delicate balance for fish and other aquatic creatures, causing cellular stress that may weaken them, reduce their chances of survival, or make it harder for them to live well in their habitat.

Oxidative stress happens when your body produces too many reactive oxygen molecules, and its natural antioxidant defences can't keep up with neutralising them. Excessive ROS can wreak havoc on the cell's key components, such as lipids, proteins, and DNA, causing serious problems with how the body functions (Menon et al., 2023; Shrivastav et al., 2026). To fight oxidative stress, aquatic creatures rely on a built-in antioxidant defence system as their natural shield. It's powered by enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GRed). These enzymes play a crucial role in keeping your cells healthy—they help neutralise harmful free radicals, clear out toxic byproducts, and maintain that delicate balance of oxidation and reduction. But if you're exposed to pollutants for too long, those natural defences can get stretched too thin, leading to more oxidative damage and tissue harm (Zahran et al., 2025).

Mystus tengara is a freshwater catfish species commonly found across rivers, reservoirs, and wetlands throughout the Indian subcontinent. Because of its ecological significance, benthic feeding behaviour, and vulnerability to environmental pollution, it is regarded as a highly effective indicator species for biomonitoring research. Assessing antioxidant responses in key organs such as the liver, kidneys, and gills can provide important information about the physiological effects of pollution (Reddy, 2016b). The liver is the main organ responsible for detoxification and metabolism; the kidneys aid in excretion and osmoregulation; and the gills are the initial point of contact for waterborne contaminants. Thus, this study sought to evaluate tissue-specific differences in antioxidant defense biomarkers and lipid peroxidation levels in *Mystus tengara* specimens collected from various locations along the Chambal River near Nagda. The findings elucidate the mechanisms by which pollution triggers oxidative stress and highlight the utility of biochemical biomarkers for assessing the ecological well-being of freshwater ecosystems.

2 Materials and Methods

- **Study Area:** The research was conducted along the Chambal River near Nagda in the state of Madhya Pradesh, India. Three sampling stations were selected based on the level of human impact. Station 1 (S1) was chosen as a reference site with low pollution inputs, whereas Stations 2 (S2) and 3 (S3) were situated downstream of industrial and urban discharge points and thus served as polluted sites. Fish and water samples were gathered at regular intervals throughout the study period.
- **Fish Collection and Tissue Sampling:** Adult specimens of *Mystus tengara*, similar in size and weight, were gathered from each sampling site using cast nets and gill nets. Right after being caught, the fish were moved to the lab in aerated containers. The specimens were identified through standard taxonomic keys and checked for external abnormalities. The fish were humanely euthanised in accordance with the institution's ethical guidelines. Liver, kidney, and gill tissues were carefully removed, rinsed with ice-cold physiological saline (0.85% NaCl), dried with blotting paper, weighed, and kept at 80 °C until biochemical analysis.
- **Tissue Homogenization:** Each piece of tissue was ground in a glass homogeniser in cold 0.1 M phosphate buffer (pH 7.4), about 100 mg. These homogenates were centrifuged for 15 minutes at 10,000 × g at 4 °C. The supernatants obtained were collected and used to evaluate lipid peroxidation and antioxidant biomarkers.
- **Biochemical Analysis:** The activity of superoxide dismutase (SOD) was determined using the method described by Misra and Fridovich (1972), which is based on the inhibition of the auto-oxidation of epinephrine. Catalase (CAT) activity was determined by measuring the decrease of hydrogen peroxide at 240 nm according to Aebi (1984). Glutathione peroxidase (GPx) activity was determined by the method of Rotruck et al. (1973), which is based on the oxidation of reduced glutathione by hydrogen peroxide. Glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, following the method of Habig et al. (1974). Glutathione reductase (GRed) activity was determined using the method of Carlberg and Mannervik (1979), in which the oxidation of NADPH was monitored. Lipid peroxidation (LPO) was determined by measuring malondialdehyde (MDA) production using the thiobarbituric acid reactive substances (TBARS) method, as described by Ohkawa et al. (1979). Tissue homogenates were used to measure total protein content by the method of Lowry et al. (1951), with bovine serum albumin as the standard. The enzyme activity was reported per mg of protein.
- **Data Analysis:** Data are presented as mean ± standard error (SE). Data analyses were carried out using Microsoft Excel for Windows, version 10. The Student's t-test was used to assess differences among the sampling stations. Statistically significant was defined as a p-value of < 0.05. Significance levels were indicated as p < 0.05 (), p < 0.01 (), and p < 0.001.

- **Results:** Pollution-induced oxidative stress produced a significant difference in the antioxidant defence system among the three sampling sites (S1, S2, and S3) in *Mystus tengara*, suggesting that the organism responded differently to the pollution stress at each of the sampling sites (Table 1). When considered collectively, the antioxidant enzyme activities and lipid peroxidation levels in fish from polluted sites (S2 and S3) were markedly higher than at the reference site (S1).
- **Superoxide Dismutase (SOD):** SOD activity was significantly higher across all tissues at the polluted sites. In the liver, SOD activity rose from 12.3 ± 1.1 at S1 to 17.6 ± 1.8 at S2, then declined to 14.3 ± 1.3 at S3. Comparable patterns were seen in kidney tissue (11.2 ± 0.88 at S1 to 15.1 ± 1.4 at S2 and 13.4 ± 1.2 at S3) and gill tissue (12.4 ± 1.4 at S1 to 17.6 ± 1.8 at S2 and 14.3 ± 1.3 at S3). The best induction was at S2, suggesting better SCAS in higher pollution stress.
- **Catalase (CAT):** Levels of CAT activity were significantly increased in the liver and gills. Liver CAT rose from 18.4 ± 0.6 at S1 to 47.6 ± 3.3 at S2 and stayed significantly high at S3 (26.8 ± 2.1). Gill CAT showed the greatest activity across all tissues, rising from 15.6 ± 0.8 at S1 to 53.1 ± 4.4 at S2, then declining to 27.8 ± 1.8 at S3. There was a moderate increase in kidney CAT activity over baseline at S2 (16.6 ± 1.1) and at S3 (14.2 ± 0.6). Glutathione Peroxidase (GPx).

Table 1 Tissue-specific variation in antioxidant defence biomarkers in liver, kidney, and gill of *Mystus tengara* exposed to wastewater of the Chambal River

Biomarker	Tissue	S1 Mean $\pm$ SE	S2 Mean $\pm$ SE	S3 Mean $\pm$ SE
SOD	Liver	12.3 ± 1.1	$17.6 \pm 1.8^{**}$	$14.3 \pm 1.3^*$
	Kidney	11.2 ± 0.88	$15.1 \pm 1.4^{**}$	$13.4 \pm 1.2^*$
	Gill	12.4 ± 1.4	$17.6 \pm 1.8^{**}$	$14.3 \pm 1.3^*$
CAT	Liver	18.4 ± 0.6	$47.6 \pm 3.3^{**}$	$26.8 \pm 2.1^{**}$
	Kidney	12.3 ± 0.9	$16.6 \pm 1.1^*$	$14.2 \pm 0.6^*$
	Gill	15.6 ± 0.8	$53.1 \pm 4.4^{***}$	$27.8 \pm 1.8^{**}$
GPx	Liver	12.3 ± 0.4	$212.7 \pm 8.1^{***}$	$192.3 \pm 6.1^{***}$
	Kidney	32.5 ± 2.2	$102.4 \pm 8.3^{**}$	$89.7 \pm 4.6^{**}$
	Gill	33.4 ± 4.2	$142.3 \pm 5.1^{***}$	$152.4 \pm 7.7^{***}$
GST	Liver	52.3 ± 2.1	$67.6 \pm 4.7^*$	$56.8 \pm 4.1^*$
	Kidney	49.3 ± 2.9	$57.6 \pm 4.4^*$	$54.2 \pm 4.6^*$
	Gill	52.3 ± 3.1	$63.6 \pm 4.3^{**}$	$54.8 \pm 4.2^*$
GRed	Liver	4.93 ± 0.7	$38.4 \pm 0.021^{***}$	$40.3 \pm 2.3^{***}$
	Kidney	2.7 ± 0.7	$17.4 \pm 0.2^{**}$	$13.77 \pm 0.7^{**}$
	Gill	2.4 ± 0.1	$7.9 \pm 0.2^*$	$8.1 \pm 0.2^{**}$
LPO	Liver	8.1 ± 0.01	$39.3 \pm 3.1^{***}$	$37.1 \pm 2.2^{**}$
	Kidney	6.16 ± 0.01	$18.6 \pm 2.1^{***}$	7.14 ± 0.01
	Gill	7.3 ± 0.06	$48.5 \pm 3.8^{***}$	$33.6 \pm 2.1^{**}$

S1, S2 and S3= study stations. SOD, CAT (units mg⁻¹ protein), LPO (nmol/TBARS formed mg protein⁻¹ min⁻¹), GPx (n mol/NADPH oxidized mg⁻¹ protein min⁻¹), GST (nmol/CDNB conjugate formed mg⁻¹ protein min⁻¹) and GSH (m mol g⁻¹ protein min⁻¹). Each value is mean $\pm$ SE (n = 6); N: no significant difference (p > 0.05) compared to the reference site.

Glutathione peroxidase (GPx): GPx showed the greatest change among all biomarkers. Liver GPx levels rose about seventeen times, increasing from 12.3 ± 0.4 at S1 to 212.7 ± 8.1 at S2 and then to 192.3 ± 6.1 at S3. Notable increases were also observed in the kidney (32.5 ± 2.2 at S1, 102.4 ± 8.3 at S2, and 89.7 ± 4.6 at S3) and gills (33.4 ± 4.2 at S1, 142.3 ± 5.1 at S2, and 152.4 ± 7.7 at S3). These results indicate that peroxide detoxification pathways are strongly induced by oxidative stress.

Glutathione S-Transferase (GST): There was a marked increase in GST activity in all the tissues of polluted sites. Liver GST rose from 52.3 ± 2.1 at S1 to 67.6 ± 4.7 at S2, then decreased to 56.8 ± 4.1 at S3. Kidney GST increased from 49.3 ± 2.9 to 57.6 ± 4.4 , then to 54.2 ± 4.6 at S2 and S3, respectively. The Gill GST was high, increasing to 63.6 ± 4.3 at S2, and then falling slightly at S3.

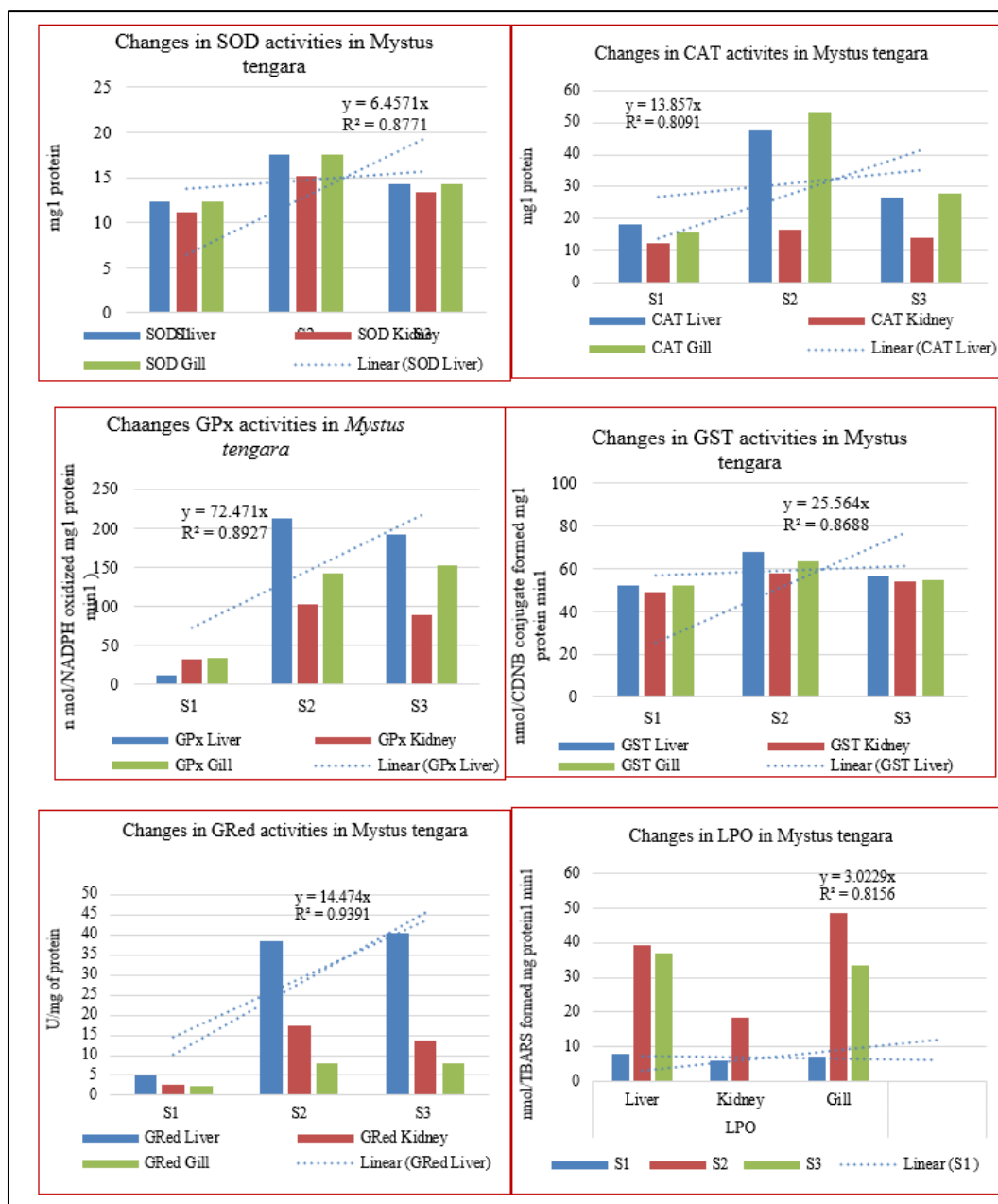


Figure 1 Comparative tissue-specific changes in antioxidant defense enzymes (SOD, CAT, GPx, GST, GRed) and lipid peroxidation (LPO) in *Mystus tengara* collected from three sampling sites (S1-S3)

Glutathione Reductase (GRed): An increase in GRed activity was observed in all tissues. Liver GRed rose from 4.93 ± 0.7 at S1 to 38.4 ± 0.021 at S2 and then to 40.3 ± 2.3 at S3. Kidney GRed rose from 2.7 ± 0.7 to 17.4 ± 0.2 and 13.77 ± 0.7 , while gill GRed increased from 2.4 ± 0.1 to 7.9 ± 0.2 and 8.1 ± 0.2 at S2 and S3, respectively.

Lipid Peroxidation (LPO): LPO increased significantly at polluted sites, indicating greater oxidative damage. Liver LPO rose from 8.1 ± 0.01 at S1 to 39.3 ± 3.1 at S2 and then to 37.1 ± 2.2 at S3. The gills exhibited the highest LPO levels, rising from 7.3 ± 0.06 at S1 to 48.5 ± 3.8 at S2 and then decreasing to 33.6 ± 2.1 at S3. Kidney LPO rose from 6.16 ± 0.01 to 18.6 ± 2.1 at S2 but dropped back close to baseline by S3 (7.14 ± 0.01).

3 Result and Discussion

It is observed that exposure to pollution leads to significant oxidative stress in *Mystus tengara*, as evidenced by elevated antioxidant enzyme activity and increased lipid peroxidation in liver, kidney, and gill tissues. The responses observed indicate activation of enzyme defence mechanisms to counteract the high concentrations of reactive oxygen species (ROS) resulting from a polluted environment (Lushchak, V. I., 2016; Zeng et al., 2025). Increased activity of SOD in all the tissues suggests increased conversion of superoxide radicals to hydrogen peroxide, suggesting the first line of antioxidant defense (Saxena et al., 2022; Zeng et al., 2025). The increased activity of CAT in the liver and gills also indicates improved detoxification of hydrogen peroxide accumulated during oxidative metabolism (Varma et al., 2025; Kumar et al., 2025). The CAT activity was highest in the gills, as they are directly exposed to polluted water and to dissolved pollutants. GPx exhibited the greatest induction among the biomarkers, particularly in the liver. The result highlights the importance of glutathione-dependent antioxidant pathways in defending against peroxide-induced damage to cellular components. The liver is an organ that plays a key role in detoxification and is expected to contain higher concentrations of pollutants, thereby exhibiting stronger antioxidant properties. Results are consistent with those of Parida and Sahoo (2023), who observed that CAT, GPX-1, GST-mu, and CuZnSOD are important enzymes in the antioxidant defence system of rohu under both biotic and abiotic stresses. GST activity increased significantly across different tissues, indicating activation of phase II detoxification pathways. GST is crucial for conjugating xenobiotic metabolites with glutathione, which helps to eliminate them and reduce toxicity. Likewise, Elevated GRed activity suggests continual regeneration of reduced glutathione (GSH), which is crucial for maintaining cellular redox balance under oxidative stress (Oruc et al., 2024). Exposure to fumaronitrile in *Oreochromis mossambicus* revealed oxidative stress and alterations in antioxidant enzyme activity, with significant tissue damage to the gills, liver, and muscle tissues, indicating its toxicity to aquatic organisms (Chinnadurai et al., 2022). While antioxidant defences were induced, the high LPO levels indicate a high degree of oxidative damage in the exposed fish. High LPO levels were observed in gill tissues, indicating lipid oxidation of membrane lipids, likely due to continued exposure to polluted water (Srivastava, B., & Reddy, P. B., 2017; Reddy, P. B., 2018). The liver tissues exhibited high lipid peroxidation, indicating high metabolic activity and pollutant transformation. The relatively lower LPO response in kidney tissues suggests reduced pollutant accumulation, or perhaps better protective mechanisms (UZh, A., & Utegenov, N. U., 2003). In general, the liver and gills were the most responsive tissues, with notable increases in antioxidant enzymes and lipid peroxidation. The findings indicate that pollution disrupts cellular redox homeostasis in *Mystus tengara*, triggering the antioxidant defence mechanism but not completely preventing cellular damage. SOD, CAT, GPx, GST, GRed, and LPO are sensitive biomarkers that, when evaluated together, can be used to assess aquatic pollution and its biological effects on freshwater fish populations.

4 Conclusion

The Chambal River is polluted by industrial and urban waste, causing a large amount of oxidative stress in *Mystus tengara*. The results of this experiment in different activities of the antioxidant enzymes in different tissues suggest that increased activity of SOD, CAT, GPx, GST and GRed is an adaptive response to the presence of reactive oxygen species (ROS). However, the high levels of lipid peroxidation in the gills of *M. tengara* imply that the level of oxidative damage that has occurred is greater than many fish. The liver demonstrated the strongest response with regard to antioxidant enzymes and thus highlights the importance of its role in detoxifying environmental toxins. Overall, *M. tengara* may be used as a reliable bioindicator for assessing aquatic pollution and the health of an ecosystem through the use of antioxidant biomarkers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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