

## Evaluation of Oxidative stress response to detergent in freshwater cat fish *Mystus vittatus* (Bloch)

<sup>1</sup>Kamlesh Rani and <sup>2</sup>Sadguru Prakash\*

<sup>1</sup>Department of Zoology, SPM Govt. P.G. College, Phaphamau, Prayagraj, Uttar Pradesh-211013, India

<sup>2</sup>Department of Zoology, M.L.K.P.G. College, Balrampur, Uttar Pradesh-271201, India

\*Corresponding author:

**Sadguru Prakash**

Department of Zoology, M.L.K.P.G. College, Balrampur, Uttar Pradesh-271201, India  
E-mail: sadguruprakash@gmail.com

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### ABSTRACT:

The present study demonstrated a significant increase in the activities of antioxidant enzymes, including SOD, COD, POD, and GST, in fish exposed to detergent. Results from the one-way ANOVA indicated that sublethal and lethal concentrations of detergent had a pronounced effect on the antioxidant enzyme activities in the fish. Furthermore, the post hoc Tukey test revealed notable alterations in SOD, COD, POD and GST levels in response to both sublethal concentrations and different exposure durations. Overall, these findings suggest that *Mystus vittatus* can serve as a potential biomarker of chemical pollution, as reflected by changes in its antioxidant enzyme responses.

### Keywords:

Tide, Detergent, Antioxidant, *Mystus vittatus*.

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## INTRODUCTION

Fish are widely recognized as valuable bioindicators of aquatic pollution, providing an effective means to assess the impact of anthropogenic activities and to monitor the health of aquatic ecosystems by detecting and predicting the adverse effects of contaminants (Katiyar *et al.*, 2021). Freshwater fish also play a vital role as an affordable and accessible source of dietary protein, particularly for low-income communities. The nutritional quality of fish is largely determined by the quantity and composition of muscle proteins; however, increasing levels of water pollution can gradually diminish these nutrients. Consequently, the degree of water contamination directly influences both the quality and quantity of nutrients in fish tissues.

Detergents, which are primarily synthesized from organic compounds, persist in the

environment for extended periods. Despite their environmental persistence, their widespread use remains unavoidable due to their essential role in household cleaning. These products contain surfactants that lower water's surface tension, thereby enhancing cleaning efficiency. Common surfactants used in detergent production include linear alkylbenzene sulfonate (LAS), dodecylbenzene sulfonate (DBS), and alkylbenzene sulfonate (ABS) (Singh and Pandey, 2023). Once released into aquatic systems, these substances can alter the physical and chemical characteristics of water, adversely affecting non-target aquatic organisms. Such alterations can disrupt cellular structures and physiological functions, ultimately compromising the health of organisms at both population and ecosystem levels.

Fish are highly vulnerable to damage caused by reactive oxygen species (ROS). Under normal physiological conditions, their cells maintain an

efficient antioxidant defense system that balances the production and elimination of ROS. However, when ROS generation exceeds the capacity of the antioxidant defenses, or when these defenses are impaired, oxidative stress occurs, leading to cellular damage (Ali *et al.*, 2014). Therefore, assessing biochemical changes in antioxidant enzyme activities in fish tissues exposed to pollutants provides an effective means of evaluating the toxic effects of household detergents. In this context, the present study aims to investigate variations in the activities of key antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione S-transferase (GST) in the liver and muscle tissues of *Mystus vittatus* following exposure to sublethal concentrations of detergent.

## MATERIALS AND METHODS

### Collection and Acclimatization of Experimental Fish:

Freshwater catfish (*Mystus vittatus*) measuring approximately  $8.5 \pm 0.2$  cm in length and weighing  $9.0 \pm 1$  g were collected from local freshwater bodies and transported to the Ichthyology Laboratory, Department of Zoology, M.L.K.P.G. College, Balrampur (U.P.), in plastic containers. Upon arrival, the fish were disinfected using a 1.0% potassium permanganate ( $\text{KMnO}_4$ ) solution for five minutes to eliminate external infections. The specimens were then acclimatized to laboratory conditions for 15 days at room temperature ( $26 \pm 1.5$  °C) before the start of the experiment.

### Biochemical Assays of Antioxidant Enzymes:

The 96-hour  $\text{LC}_{50}$  value of Tide detergent for *Mystus vittatus* was previously determined to be 17.981 mg/L (Rani and Prakash, 2025). Based on this value, sublethal concentrations equivalent to one-fourth (4.5 mg/L) and one-half (9.0 mg/L) of the  $\text{LC}_{50}$  were selected for chronic exposure experiments lasting 14, 21, and 28 days. The study aimed to examine the effects of these sublethal detergent concentrations on the activities of antioxidant enzymes in *Mystus vittatus*. A control group was maintained under identical environmental conditions without detergent exposure. During the experiment, fish were fed a commercial diet daily, and the water

medium was renewed each day to remove waste and uneaten food, ensuring optimal water quality.

After 14, 21, and 28 days of exposure to sublethal concentrations of detergent, both control and exposed fish were washed and anesthetized using 0.1 g/L benzocaine. The liver and muscle tissues were then carefully dissected for antioxidant enzyme analysis. For enzyme assays, the collected organs were homogenized in phosphate buffer (pH 6.5) at a ratio of 1:4 (w/v). The resulting homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the obtained supernatant was used to determine the activities of various antioxidant enzymes.

**Superoxide dismutase (SOD):** The activity of SOD in the liver and muscle tissues was measured following the indirect method described by Worthington (1988). This method relies on the enzyme's capacity to inhibit the photoreduction of nitro blue tetrazolium (NBT). The absorbance was recorded using a digital spectrophotometer (Model AN-10-03; Zenith Engineers, Agra) at 560 nm, and the enzyme activity was expressed as  $\mu\text{g}$  protein.

**Catalase (COD):** Catalase is a key antioxidant enzyme responsible for breaking down hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) into water and oxygen. The enzyme activity was assessed according to the method of Aebi (1984) by measuring the rate of decrease in  $\text{H}_2\text{O}_2$  concentration at 240 nm. The reaction mixture (2 mL) consisted of 1.95 mL of buffered substrate solution, and the reaction was initiated by adding 0.05 mL of enzyme extract. The change in absorbance was monitored at 240 nm using a digital spectrophotometer over a period of three minutes.

**Peroxidase (POD):** Peroxidase activity was evaluated by assessing its ability to reduce hydrogen peroxide at 470 nm, following the method of Zia *et al.* (2011). After calibrating the spectrophotometer with a blank solution at 470 nm, 0.02 mL of crude enzyme extract was added to 1 mL of buffered substrate solution. The change in absorbance was recorded after three minutes.

**Glutathione-S-transferase (GST):** The activity of GST was determined according to the procedure described by Habig *et al.* (1974), using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates.

**Statistical Analysis:** Biochemical biomarker data were expressed as mean ± standard deviation (SD) for each sample group. Statistical significance was evaluated using the student's t-test in SPSS software.

**RESULTS AND DISCUSSION**

Under normal physiological conditions, antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, and glutathione-S-transferase are activated in

response to mild oxidative stress. These enzymes act as a defense mechanism to neutralize reactive oxygen species (ROS) and protect cells from oxidative damage (Livingstone, 2001). Xenobiotic are known to induce oxidative stress by generating reactive oxygen species (ROS). This oxidative stress can disrupt the antioxidant defense mechanisms and free radical scavenging enzyme systems in aquatic organisms (Ali *et al.*, 2014). However, during chemical exposure, the effectiveness of these antioxidant systems can vary depending on the intensity and duration of stress, as well as the organism's tolerance level (Nahed, 2011). Many xenobiotic compounds exert their toxic effects by inducing oxidative stress in fish, leading to excessive ROS production and subsequent cellular injury (Nazish *et al.*, 2018).

**Table. 1: Effects of sublethal concentrations of Detergent on antioxidant enzyme activity of *Mystus vittatus* (n=4).**

Tissue	Group	Exposure periods in days		
		7	21	28
Superoxide dismutase (SOD) (Unit/mg)				
Liver	Control	10.31±0.12	10.32±0.16	10.33±0.13
	4.5 mg/L	13.25±0.11	15.12±0.14	17.13±0.16
	9.0 mg/L	14.84±0.12	17.15±0.18	20.21±0.12
Muscles	Control	14.81±0.15	14.71±0.17	14.92±0.18
	4.5 mg/L	16.71±0.15	18.91±0.15	20.81±0.13
	9.0 mg/L	18.78±0.14	20.95±0.13	23.11±0.15
Catalase (COD) (n mole/min/mg)				
Liver	Control	2.15±0.07	2.18±0.17	2.17±0.21
	4.5 mg/L	2.72±0.04	3.02±0.21	3.22±0.19
	9.0 mg/L	3.12±0.29	3.38±0.16	3.52±0.14
Muscles	Control	2.02±0.11	2.01±0.12	2.04±0.13
	4.5 mg/L	2.28±0.15	2.48±0.13	2.70±0.11
	9.0 mg/L	2.44±0.18	2.78±0.11	2.92±0.13
Peroxidase (POD) (Unit/mg)				
Liver	Control	0.172±0.006	0.175±0.005	0.180±0.004
	4.5 mg/L	0.193±0.003	0.228±0.005	0.263±0.003
	9.0 mg/L	0.213±0.005	0.268±0.004	0.305±0.006
Muscles	Control	0.078±0.002	0.081±0.004	0.081±0.003
	4.5 mg/L	0.105±0.005	0.130±0.003	0.160±0.004
	9.0 mg/L	0.135±0.004	0.195±0.002	0.218±0.005
Glutathione-S-transferase (GST) (n mole/min/mg)				
Liver	Control	0.735±0.025	0.738±0.024	0.740±0.025
	4.5 mg/L	0.764±0.012	0.812±0.023	0.851±0.072

	9.0 mg/L	0.822±0.013	0.843±0.018	0.871±0.028
Muscles	Control	0.856±0.021	0.858±0.025	0.860±0.024
	4.5 mg/L	0.920±0.122	0.942±0.112	0.962±0.114
	9.0 mg/L	1.122±0.125	1.382±0.112	1.614±0.114

**Table 2: Summary of analysis of variance (ANOVA) showing the level of significance of the antioxidant enzymes activity in *Mystus vittatus* exposed to sub-lethal concentrations of detergent**

Tissue	Source	Sum of Square	Degree of Freedom (df)	Mean Square	F	P (Sig.)
Superoxide dismutase						
Liver	Between Group	78.604	2	39.302	10.698	0.011
	Within Group	22.043	6	3.674		
	Total	100.647	8			
Muscles	Between Group	58.156	2	29.078	9.793	0.013
	Within Group	17.817	6	2.969		
	Total	75.973	8			
Catalase						
Liver	Between Group	2.174	2	1.087	31.126	0.001
	Within Group	0.210	6	0.035		
	Total	2.383	8			
Muscles	Between Group	0.742	2	0.371	10.572	0.011
	Within Group	0.211	6	0.035		
	Total	0.953	8			
Peroxidase (POD) (Unit/mg)						
Liver	Between Group	0.011	2	0.006	5.030	0.052
	Within Group	0.007	6	0.001		
	Total	0.018	8			
Muscles	Between Group	0.016	2	0.008	9.130	0.015
	Within Group	0.005	6	0.001		
	Total	0.021	8			
Glutathione-S-transferase (GST) (n mole/min/mg)						
Liver	Between Group	0.018	2	0.009	10.759	0.010
	Within Group	0.005	6	0.001		
	Total	0.023	8			
Muscles	Between Group	0.458	2	0.229	11.254	0.009
	Within Group	0.122	6	0.020		
	Total	0.580	8			
Significant at < 0.05						

**Table 3: Post hoc test (Tukey) for individual effect of sublethal concentration of detergent in the freshwater fish *Mystus vittatus***

Tissues	Concentration (a)	Concentration (b)	Mean Difference (a-b)	Std. Error (SE)	Sig. (P)
Superoxide dismutase (SOD)					
Liver	Control	4.5 mg/L	-4.84667*	1.56499	0.048
		9.0 mg/L	-7.08000*	1.56499	0.010
	4.5 mg/L	Control	4.84667*	1.56499	0.048
		9.0 mg/L	-2.23333 <sup>NS</sup>	1.56499	0.387

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	9.0 mg/L	Control	7.08000*	1.56499	0.010
		4.5 mg/L	2.23333 <sup>NS</sup>	1.56499	0.387
Muscles	Control	4.5 mg/L	-3.99667 <sup>NS</sup>	1.40699	0.066
		9.0 mg/L	-6.13333*	1.40699	0.011
	4.5 mg/L	Control	3.99667 <sup>NS</sup>	1.40699	0.066
		9.0 mg/L	-2.13667 <sup>NS</sup>	1.40699	0.348
	9.0 mg/L	Control	6.13333*	1.40699	0.011
		4.5 mg/L	2.13667 <sup>NS</sup>	1.40699	0.348
Catalase (COD) (n mole/min/mg)					
Liver	Control	4.5 mg/L	-0.82000*	0.15258	0.004
		9.0 mg/L	-1.17333*	0.15258	0.001
	4.5 mg/L	Control	0.82000*	0.15258	0.004
		9.0 mg/L	-0.35333 <sup>NS</sup>	0.15258	0.129
	9.0 mg/L	Control	1.17333*	0.15258	0.001
		4.5 mg/L	0.35333 <sup>NS</sup>	0.15258	0.129
Muscles	Control	4.5 mg/L	-0.46333 <sup>NS</sup>	0.15297	0.052
		9.0 mg/L	-0.69000*	0.15297	0.010
	4.5 mg/L	Control	0.46333 <sup>NS</sup>	0.15297	0.052
		9.0 mg/L	-0.22667 <sup>NS</sup>	0.15297	0.363
	9.0 mg/L	Control	.69000*	0.15297	0.010
		4.5 mg/L	.22667 <sup>NS</sup>	0.15297	0.363
Peroxidase (POD) (Unit/mg)					
Liver	Control	4.5 mg/L	-.052333 <sup>NS</sup>	0.027424	0.217
		9.0 mg/L	-.086333*	0.027424	0.045
	4.5 mg/L	Control	.052333 <sup>NS</sup>	0.027424	0.217
		9.0 mg/L	-.034000 <sup>NS</sup>	0.027424	0.475
	9.0 mg/L	Control	.086333*	0.027424	0.045
		4.5 mg/L	.034000 <sup>NS</sup>	0.027424	0.475
Muscles	Control	4.5 mg/L	-0.051667	0.024026	0.159
		9.0 mg/L	-0.102667*	0.024026	0.012
	4.5 mg/L	Control	0.051667	0.024026	0.159
		9.0 mg/L	-0.051000	0.024026	0.165
	9.0 mg/L	Control	0.102667*	0.024026	0.012
		4.5 mg/L	0.051000	0.024026	0.165
Glutathione-S-transferase (GST) (n mole/min/mg)					
Liver	Control	4.5 mg/L	-0.071333 <sup>NS</sup>	0.023616	0.053
		9.0 mg/L	-0.107667*	0.023616	0.009
	4.5 mg/L	Control	0.071333 <sup>NS</sup>	0.023616	0.053
		9.0 mg/L	-0.036333 <sup>NS</sup>	0.023616	0.340
	9.0 mg/L	Control	0.107667*	0.023616	0.009
		4.5 mg/L	0.036333 <sup>NS</sup>	0.023616	0.340
Muscles	Control	4.5 mg/L	-0.083333 <sup>NS</sup>	0.116454	0.764
		9.0 mg/L	-0.514667*	0.116454	0.011
	4.5 mg/L	Control	0.083333 <sup>NS</sup>	0.116454	0.764
		9.0 mg/L	-0.431333*	0.116454	0.023
	9.0 mg/L	Control	0.514667*	0.116454	0.011
		4.5 mg/L	0.431333*	0.116454	0.023
*. The mean difference is significant at the 0.05 level. NS = Non Significant					

The present study demonstrated a significant rise ( $P < 0.05$ ) in the activities of antioxidant enzymes in the liver and muscle tissues of *Mystus vittatus* exposed to sublethal concentrations of detergent across all exposure durations (Table 1-3). The enhanced enzyme activity likely reflects a physiological response aimed at neutralizing the excess reactive oxygen species (ROS) generated during detergent exposure.

In the liver and muscle tissues, SOD activity increased from 10.31 to 20.21 units/mg and from 14.71 to 23.11 units/mg, respectively. CAT activity rose from 2.15 to 3.52 nmol/min/mg in the liver and from 2.01 to 2.92 nmol/min/mg in the muscles. POD activity increased from 0.122 to 0.305 units/mg in the liver and from 0.078 to 0.218 units/mg in the muscles. Similarly, GST activity rose from 0.735 to 0.871 nmol/min/mg in the liver and from 0.856 to 1.614 nmol/min/mg in the muscles.

These elevations in antioxidant enzyme activities indicate the onset of oxidative stress. When exposed to toxic substances, fish tend to generate higher levels of ROS, which in turn stimulates the upregulation of antioxidant enzymes as a protective mechanism (Shukla and Prakash, 2022).

Similar elevations in the activities of SOD, CAT, POD, and GST have been observed in freshwater fish exposed to various xenobiotics (Monteiro *et al.*, 2006; Kavitha and Rao, 2007; Kumar *et al.*, 2011; Khare *et al.*, 2019; Shukla and Prakash, 2022). The increased activity of these enzymes is considered a protective mechanism to counteract the reactive oxygen species (ROS) generated during toxicant exposure (Stara *et al.*, 2012).

Statistical analysis of the experimental data (Tables 2-3) indicated that most enzyme activity values showed significant differences ( $P < 0.05$ ), while a few did not, suggesting possible physiological adaptation of the detergent-exposed fish to prolonged stress under chronic toxicity conditions. One-way ANOVA revealed significant alterations in antioxidant enzyme activities across treatments (Table 2). Further,

the post hoc Tukey test confirmed that enzyme activities in exposed fish differed significantly ( $P < 0.05$ ) compared to controls (Table 3). The test also demonstrated that sublethal detergent concentrations and exposure duration had a notable influence on the antioxidant enzyme responses in *Mystus vittatus* (Table 3). Thus, the increased activity of antioxidant enzymes further indicates oxidative damage and the organism's defensive response to detergent stress, reflecting a protective mechanism that aids in mitigating the toxic effects of chemicals and maintaining cellular stability.

## CONCLUSION

In conclusion, fish employ both enzymatic and non-enzymatic defense mechanisms to mitigate oxidative stress generated by reactive oxygen species. The observed variations in the activities of superoxide dismutase, catalase, peroxidase, and glutathione-S-transferase indicate that these enzymes are reliable biomarkers for detecting detergent-induced oxidative stress. The altered antioxidant responses in *Mystus vittatus* suggest potential biochemical disturbances resulting from prolonged exposure to synthetic detergents. Therefore, changes in the activities of antioxidant enzymes in the liver and muscle tissues can serve as valuable indicators of water quality and environmental contamination.

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