

Effects of Sublethal Concentration of Detergent on Serum Enzymatic Activities of Freshwater Fish, *Mystus vittatus* (Bloch)

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ABSTRACT

The toxicity of commercial detergent (Tide), a household cleaning agent, was investigated on some enzymatic activities of catfish (*Mystus vittatus*). The fishes were exposed to sub-lethal concentrations (4.5 and 9.0 mg/L) for 30 days and showed a significant ($p < 0.05$) increase in serum enzymatic activities such as serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminases (GPT), alkaline phosphatases (ALP), Aspartic Aminotransferase (AST) and Alanine Aminotransferase (ALT) under the toxic influence of the detergents. Contrary to this, the activity of acid phosphatases (ACP) was found to be significantly ($p < 0.05$) decreased. Therefore, enzyme activity serves as a sensitive indicator of detergent-induced physiological stress and can be effectively used as a biomarker for early detection of sub-lethal toxicity. Enzymes such as ALP, ACP, AST, and ALT function as important biomarkers for evaluating the effects of detergents on fish blood. Alterations in their activities offer useful information on the health of fish populations and the broader environmental consequences of detergent exposure.

Keywords: Detergent, Tide, *Mystus vittatus*, Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminases (GPT), Alkaline phosphatases (ALP), Aspartic aminotransferase (AST), Alanine aminotransferase (ALT).

INTRODUCTION

Detergents are synthetic organic cleaning agents that persist as environmental contaminants, and their uncontrolled discharge into water bodies without adequate treatment poses serious

risks to freshwater fishes and other aquatic organisms. Detergents are thus emerging as a major environmental problem because of their negative impact on water quality, aquatic flora and fauna and the integrity of the total ecosystem. Detergents in water speed up corrosion, disrupt filtration, sedimentation and coagulation, raise oxygen in water and affect water taste (Vasanthi et al., 2013). The chemicals from detergents also inhibit the exchange of air and water which leads to the reduction of oxygen supply to the submerged plants and animals and is one of the main causes responsible for the decrease of aquatic flora and fauna (Arivizhivendhan et al., 2014).

Synthetic detergents are not only more effective than soaps in hard water but also contain certain additives that make them effective, such as brighteners, softeners, bleaching agents and nutrient-rich compounds like nitrogen and phosphates. They are however rich in phosphate and these nutrients tend to cause eutrophication, which in turn reduces primary and secondary productivity (Maruthanayagam et al., 1994). Detergents have been reported to change the physicochemical properties of aquatic systems such as pH, total alkalinity, free carbon dioxide, dissolved oxygen, photosynthetic activity and increase eutrophication processes (Obiezue et al., 2014).

Enzymatic analysis of fish tissue is useful to gain insight into the internal physiological condition of the fish. Enzymes control a variety of biochemical and biological reactions, and a slight change in their activity can result in abnormal metabolic reactions (Pechiammal and Vasanthi, 2017). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are intracellular enzymes predominantly localized in the liver, heart, gills, kidneys, muscles, and other tissues. Increased concentrations in the blood or serum are known to be good indicators of tissue damage and organ dysfunction (Gabriel and George, 2005). Detergent-induced enzymatic disturbances may impair metabolic pathways, reduce energy availability, and cause respiratory dysfunction through paralysis of respiratory muscles or depression of respiratory centers (Nkpondion et al., 2016). Given the documented toxic effects of detergents on aquatic organisms, the present study was undertaken to evaluate the impact of sub-lethal detergent exposure on serum enzyme activities in the freshwater fish *Mystus vittatus* under controlled laboratory conditions.

MATERIALS AND METHODS

The required number of test fish, *Mystus vittatus* (8.5 ± 0.2 cm; 9.0 ± 1 g in weight), were collected from neighboring freshwater bodies and brought to the Ichthyology Lab of the Zoology Department in plastic containers. The collected fishes were dipped into a 1.0% solution of KMnO_4 for 5 minutes to remove dermal contamination. Fishes were stocked and acclimated to laboratory conditions for 15 days at 26 ± 1.5 °C prior to experimentation. During the first week, the acclimatized fish were provided artificial aeration using an aerator. All of the fish were given commercial food pellets on a regular basis, and the medium (tap water) was changed every two days to get rid of food remnants and feces. Feeding was stopped 24 hours before the toxicity test. The 96 hr LC_{50} value was found to be 17.981 mg/L concentration of the Tide detergent for *Mystus vittatus* (Rani and Prakash, 2025). On the basis of the 96-hour LC_{50} , the fish, *M. vittatus*, were exposed to sublethal concentrations ($1/4^{\text{th}}$ of the 96-hour $\text{LC}_{50} = 4.5$ mg/L and $1/2^{\text{nd}}$ of the 96-hour $\text{LC}_{50} = 9.0$ mg/L) for the period of 10, 20 and 30 days. The chronic toxicity study was conducted to examine the effects of sub-lethal detergent concentrations on the serum enzymatic activity (GOT, GPT, ACP, AST, ALT) of the fish *M. vittatus*. A control group was maintained under similar environmental conditions. The test fish were fed commercial food, and the water was exchanged daily to remove feces and food remnants.

Blood samples were collected from the caudal vein and transferred into glass tubes, then centrifuged at 3500 rpm for 10 minutes. The resulting serum was carefully collected into Eppendorf tubes for further analysis. The enzymatic activities of glutamate oxaloacetate transaminases (GOT) and glutamate pyruvate transaminases (GPT) were estimated by employing the method of Reitman and Frankel (1957), Alkaline phosphatases (ALP), acid phosphatases (ACP), Aspartic aminotransferase (AST), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) by method of Wooten (1964).

One way Analysis of Variance (ANOVA) was used to test for statistical difference in the serum enzymatic activities. Post hoc test (Tukey) and Duncan Multiple Range Test (DMRT) were used in determination of significance at 0.05 level of probability by SPSS version 26.

RESULTS AND DISCUSSION

Enzymes are highly sensitive biomolecules that can lose their structure and function under unfavorable conditions. Physiological or environmental stress that disrupts normal

metabolism is often reflected by measurable changes, either increases or decreases, in enzyme activity. In the present investigation, serum enzyme activities showed significant ($P < 0.05$) alterations following detergent exposure in a concentration and duration-dependent manner in experimental fish, *M. vittatus*, as compared to control groups (Table 1, Figure 1).

Table 1. Effects of sublethal concentrations of detergent on serum enzymatic activities of *Mystus vittatus* (N=3) (Mean±SD)

Biochemical Parameters	Concentration (mg/L)	Exposure Period (Coefficient of variance)		
		14 Days	21 Days	28 Days
Glutamate oxaloacetate transaminases (GOT)(IU/L)	Control	55.08±0.08	56.62±0.09	56.07±0.07
	4.5	60.04±0.08	66.06±0.07	70.12±0.11
	9.0	62.08±0.10	69.47±0.14	72.16±0.19
Glutamate Pyruvate Transaminases (GPT) (IU/L)	Control	20.55±0.5	21.03±0.8	22.23±0.9
	4.5	24.04±0.04	27.12±0.07	30.18±0.12
	9.0	26.08±0.11	29.47±0.14	33.12±0.11
Alkaline Phosphatases (ALP)(IU/L)	Control	38.30±0.08	38.39±0.05	38.45±0.06
	4.5	44.21±0.11	47.24±0.12	50.17±0.14
	9.0	48.14±0.09	52.38±0.07	55.13±0.06
Alanine AminoTransferase (ALT)(IU/L)	Control	28.62±0.12	28.29±0.11	29.12±0.14
	4.5	29.78±0.07	32.12±0.08	33.14±0.16
	9.0	32.17±0.13	35.78±0.18	38.17±0.15
Aspartate Aminotransferase (AST)(IU/L)	Control	69.67±0.15	70.12±0.09	68.78±0.11
	4.5	72.15±1.15	78.19±1.12	83.12±1.16
	9.0	82.17±1.25	88.15±1.22	95.17±1.32
AcidPhosphatases (ACP)(IU/L)	Control	15.25±0.14	15.24±0.12	15.25±0.11
	4.5	13.22±0.08	12.04±0.06	11.14±0.07
	9.0	10.45±0.11	9.15±0.14	8.10±0.04

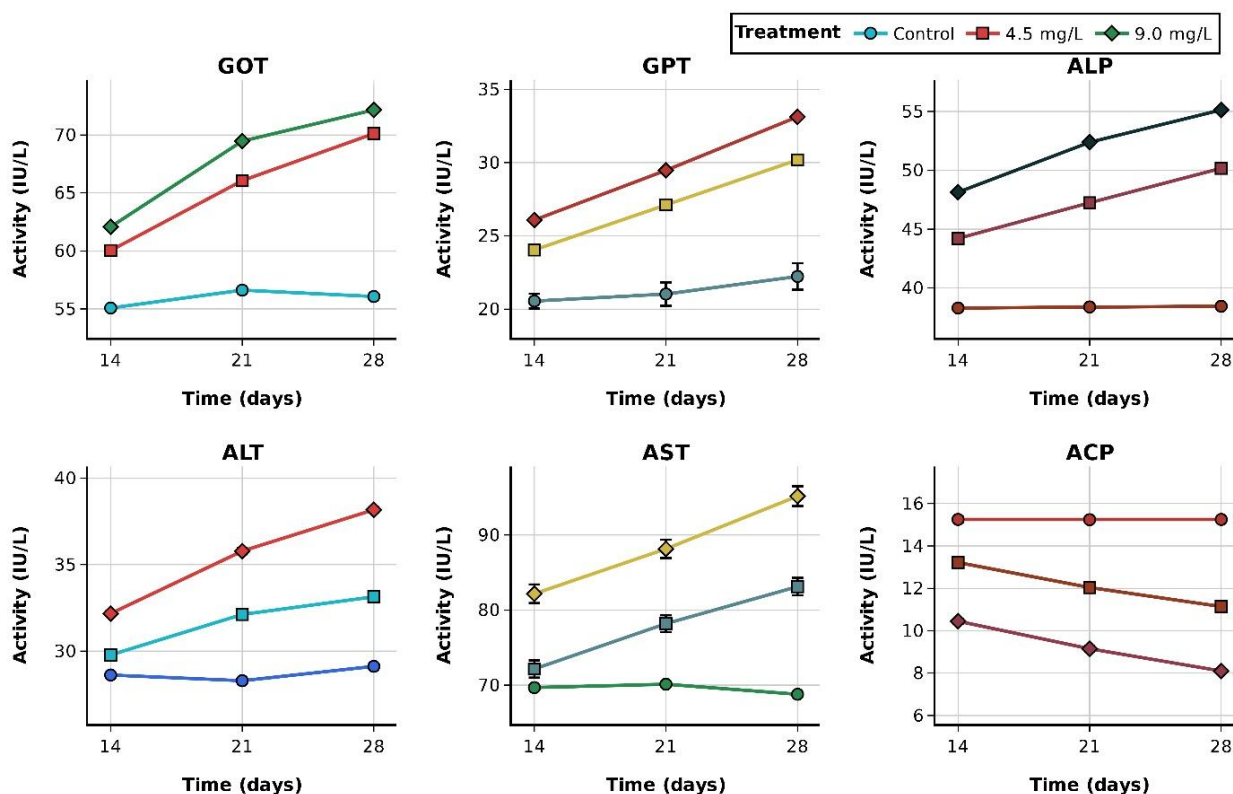


Figure 1. Comparative graphical representation of sublethal exposure of detergent on serum enzymatic activities of freshwater fish, *Mystus vittatus*.

The SGPT (serum glutamate pyruvate transaminase) and SGOT (serum glutamate oxaloacetate transaminase) are synthesized in the liver and help in protein and amino acid metabolism. According to Van der Oost et al. (2003), an increase in serum transaminase enzyme activity is a sensitive indicator of even minor cellular damage. Harper et al. (1981) also reported that the enzymes GOT and GPT are among the most reliable indicators for assessing cellular damage. In the present investigation, GOT and GPT levels increased progressively at both sublethal concentrations, i.e. 4.5 and 9.0 mg/L, after 10, 20, and 30 days, with higher elevations observed at 9.0 mg/L. The increased levels of SGOT and SGPT in detergent-exposed fish indicated liver malfunction. A similar increase in transaminases was shown in detergent-exposed fish, *Channa punctatus* (Choudhary and Jha, 2013). The elevated activities of GOT and GPT observed in the present study can be attributed to detergent-induced damage to cell membranes or altered membrane permeability, resulting in the leakage of these enzymes into the bloodstream. Similar observations linking increased transaminase levels with cellular injury have been reported earlier (Travlos et al., 1996;

Choudhary and Jha, 2013; Summarwar and Lall, 2013). Therefore, changes in the levels of these enzymes serve as clear indicators of the hepatic health of the fish.

Alkaline phosphatase (ALP) is a membrane-bound enzyme localised mainly in hepatocytes, pinocytic vesicles, and Golgi complexes. It is widely distributed in cell membranes involved in active transport and participates in transphosphorylation processes. ALP plays a key role in cellular energy regulation, metabolite transport, and the metabolism of phospholipids, phosphoproteins, nucleotides, and carbohydrates, as well as in protein biosynthesis; therefore, detergent-induced disturbances in membrane integrity can significantly modify its activity (Hedayati et al., 2010). The elevated ALP activity observed in the current study could be due to decreased biliary excretion and/or increased enzyme production (Kaplan, 1986). A similar observation was made by Ogochukwu and Joseph (2009) as well as Choudhary and Jha (2013) in *Clarias gariepinus* and *Channa punctatus*, respectively, when exposed to a detergent, where increased ALP activity was associated with extensive damage to hepatic cells and ruptures of the vascular wall, as a physiological compensation for the stress experienced. Hydrolytic enzymes are closely related in their activity in the synthesis of fibrous proteins; any stimulation or inhibition of their activity may disrupt protein metabolism (Sanisa et al., 1982).

ALT and AST are key enzymes involved in amino acid metabolism. With longer exposure to detergents, ALT and AST activities increased significantly, which reflects increased hepatotoxicity in the present study. ALT and AST were moderately elevated over a period of time at 4.5 mg/L, but also showed significantly elevated levels at 9.0 mg/L, especially after 30 days. In the present investigation, significant changes in AST activity were more pronounced at the higher detergent concentration. The increase in serum AST level in detergent-exposed fish observed in this study suggests an increase in energy demand during stress conditions. A similar increase in AST level was observed in *Channa punctatus* treated with sub-lethal levels of alcoholic extracts of *Nerium indicum* (Tiwari and Singh, 2004). In contrast to the present findings, Luskova et al. (2002) observed a reduction in ALT and AST activities in *Cyprinus carpio* following exposure to diazinon. Similarly, Adedeji et al. (2009) reported that diazinon exposure caused a significant decline in blood alkaline phosphatase and acid phosphatase levels in *Clarias gariepinus*.

Acid phosphatases (ACP) are lysosomal hydrolytic enzymes involved in the breakdown of foreign substances and therefore contribute to detoxification processes. ACP catalyzes the hydrolysis of a wide range of organic phosphate esters in acidic conditions and

is involved in cellular metabolism, producing alcohol and phosphate ions. Its activity is inducible and typically increases in animal tissues in response to toxic stress (Binukumari and Manimegalai, 2012). ACP catalyzes the hydrolysis of phosphate esters.

In the present study, ACP activity declined significantly with increasing exposure duration and with greater reductions at the higher detergent concentration. The reduction in ACP activity may be attributed to the detrimental effects of detergent exposure on cellular structures and organelles, as reported by Jana et al. (1985) and in insecticide exposed fish *Labeo rohita* (Binukumari and Manimegalai, 2012). The alteration in ACP activity may be due to damage caused to the lysosomal membrane (Sherekar and Kulkurni, 1987). The overall, the magnitude of enzymatic changes was greater at higher sublethal concentrations of detergent and after longer exposure periods, reflecting dose- and time-dependent biochemical disturbances, indicating progressive hepatic stress.

ANOVA showed a significant alteration in serum enzymatic levels on the 10th, 20th and 30th day when exposed to sublethal concentrations of detergent as compared to the control ($p < 0.05$). Compared to the fishes exposed to a higher sublethal concentration (9.0 mg/L) of detergent, the serum enzymatic values were significantly lower in fishes exposed to a lower sublethal concentration (4.5 mg/L) (Table 2). The ANOVA results showed a significant difference among the groups for GOT and GPT activities. This indicates that detergent exposure caused a statistically significant alteration in serum GOT and GPT levels in *Mystus vittatus*. ALP showed a highly significant variation among groups. The large F value and low P value indicate that ALP is strongly influenced by detergent exposure and may serve as a sensitive biochemical indicator of detergent-induced stress. The ALT activity showed a significant difference between the treatments, indicating that the detergent exposure does affect hepatic enzyme activity in exposed fish. Statistically significant difference among groups with regard to AST activity. This suggests exposure to detergents caused significant increases in AST levels, suggesting that exposure may have caused tissue damage or disturbance in the metabolism. ACP had the highest level of significance of all enzymes analyzed. Within groups variation was very low and F value was very high, suggesting that detergent had an effect on ACP activity that was strong and consistent. The result of one-way ANOVA shows that there is a significant difference in enzyme activities between the treated groups of *M. vittatus* exposed to sublethal concentrations of detergent (T3, T4, and T5). The results of all the biochemical parameters studied (GOT, GPT, ALP, ALT, AST, and ACP) were found to be statistically significant between the Control and Treated groups. Out of

these, the ACP and ALP seemed to show the most responsive enzyme response to detergent-induced physiologic stress in freshwater fishes and could be considered good biomarkers.

Table 2. A single-factor ANOVA test for the effect of detergents on fish, *Mystus vittatus* exposed to detergents and control fish.

Biochemical Parameters	Source	df	Sum of Squares	Mean sum of squares	F	P
Glutamate Oxaloacetate Transaminases (GOT)	Between Groups	239.687	2	119.844	6.711	0.029
	Within Groups	107.146	6	17.858		
	Total	346.834	8			
Glutamate Pyruvate Transaminases (GPT)	Between Groups	108.783	2	54.392	7.230	0.025
	Within Groups	45.140	6	7.523		
	Total	153.923	8			
Alkaline Phosphatases (ALP)	Between Groups	267.457	2	133.728	18.496	0.003
	Within Groups	43.381	6	7.230		
	Total	310.837	8			
Alanine Aminotransferase (ALT)	Between Groups	67.506	2	33.753	8.255	0.019
	Within Groups	24.533	6	4.089		
	Total	92.039	8			
Aspartate Aminotransferase(AST)	Between Groups	542.813	2	271.407	11.155	0.010
	Within Groups	145.986	6	24.331		
	Total	688.799	8			
Acid Phosphatases (ACP)	Between Groups	54.263	2	27.132	32.900	0.001
	Within Groups	4.948	6	.825		
	Total	59.211	8			

The effects of each sublethal concentrations of detergent (4.5 mg/L and 9.0 mg/L) on various biochemical enzyme parameters of *M. vittatus* fish compared with control group were evaluated by Tukey post hoc test (Table 3). The Tukey test revealed that the serum enzymatic activities were altered by sublethal concentrations of detergent ($P < 0.05$).

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

Biochemical Parameters	Concentration (a) (mg/L)	Concentration (b) (mg/L)	Mean Difference (a-b)	SE	Sig. (P)
Glutamate oxaloacetate transaminases (GOT)	Control	4.5	-9.48333	3.45038	0.074
		9.0	-11.98000*	3.45038	0.031
	4.5	Control	9.48333	3.45038	0.074
		9.0	-2.49667	3.45038	0.759
	9.0	Control	11.98000*	3.45038	0.031
		4.5	2.49667	3.45038	0.759
Glutamate Pyruvate Transaminases (GPT)	Control	4.5	-5.84333	2.23953	0.089
		9.0	-8.28667*	2.23953	0.024
	4.5	Control	5.84333	2.23953	0.089
		9.0	-2.44333	2.23953	0.553
	9.0	Control	8.28667*	2.23953	0.024
		4.5	2.44333	2.23953	0.553
Alkaline Phosphatases (ALP)	Control	4.5	-8.49333*	2.19547	0.019
		9.0	-13.17000*	2.19547	0.002
	4.5	Control	8.49333*	2.19547	0.019
		9.0	-4.67667	2.19547	0.164
	9.0	Control	13.17000*	2.19547	0.002
		4.5	4.67667	2.19547	0.164
Alanine Amino Transferase (ALT)	Control	4.5	-3.00333	1.65101	0.242
		9.0	-6.69667*	1.65101	0.016
	4.5	Control	3.00333	1.65101	0.242
		9.0	-3.69333	1.65101	0.143
	9.0 mg/L	Control	6.69667*	1.65101	0.016
		4.5	3.69333	1.65101	0.143
Aspartate Aminotransferase (AST)	Control	4.5	-8.29667	4.02749	0.179
		9.0	-18.97333*	4.02749	0.008
	4.5 mg/L	Control	8.29667	4.02749	0.179
		9.0	-10.67667	4.02749	0.084
	9.0 mg/L	Control	18.97333*	4.02749	0.008
		4.5	10.67667	4.02749	0.084
Acid Phosphatases (ACP)	Control	4.5	3.11333*	0.74147	0.013
		9.0	6.01333*	0.74147	0.000
	4.5 mg/L	Control	-3.11333*	0.74147	0.013
		9.0	2.90000*	0.74147	0.018
	9.0 mg/L	Control	-6.01333*	0.74147	0.000
		4.5	-2.90000*	0.74147	0.018

SE, Standard Error; *The mean difference is significant at the 0.05 level.

A significant increase in GOT activity was observed at 9.0 mg/L when compared with the control group ($P = 0.031$), indicating enzyme alteration at the higher detergent concentration. However, the difference between the control and 4.5 mg/L as well as between 4.5 mg/L and 9.0 mg/L was not statistically significant. GPT activity showed a significant difference between the control and 9.0 mg/L concentration ($P = 0.024$), suggesting a marked effect of higher detergent exposure. No significant variation was observed between the control and 4.5 mg/L or between 4.5 mg/L and 9.0 mg/L. ALP exhibited significant changes at both detergent concentrations. The differences between the control and 4.5 mg/L ($P = 0.019$) and between the control and 9.0 mg/L ($P = 0.002$) were statistically significant. However, the comparison between 4.5 mg/L and 9.0 mg/L was not significant, indicating that even the lower sublethal concentration was sufficient to affect ALP activity. A significant difference in ALT activity was observed between the control and 9.0 mg/L concentration ($P = 0.016$). No significant difference was found between the control and 4.5 mg/L, or between 4.5 mg/L and 9.0 mg/L, suggesting that ALT responded primarily to higher detergent exposure. AST activity showed a significant increase only at 9.0 mg/L compared to the control ($P = 0.008$). The variations between the control and 4.5 mg/L and between 4.5 mg/L and 9.0 mg/L, were not statistically significant. ACP activity showed highly significant differences across all comparisons. Both 4.5 mg/L and 9.0 mg/L concentrations differed significantly from the control ($P < 0.05$), and the difference between 4.5 mg/L and 9.0 mg/L was also significant ($P = 0.018$). This indicates a strong, dose-dependent response of ACP to detergent exposure.

Tukey post hoc analysis indicates that sub-lethal concentrations of the detergent produce significant differences in some of the metabolic and phosphatase enzymes in *Mystus vittatus* and have a more pronounced effect at higher concentrations of the detergent. In comparison, ACP and ALP proved to be the most sensitive enzymes studied; transaminases (GOT, GPT, ALT and AST) were significantly changed primarily at high concentrations, indicating the physiological or tissue stress caused by the detergents in the fish.

CONCLUSION

Detergents are one of the significant sources of pollution in the aquatic environment and are a great hindrance to the biochemical balance of freshwater fishes. In response to these stresses, fish will initiate metabolic changes that may ultimately negatively affect fish health, growth,

and survival, with impacts to aquatic biodiversity and ecosystem stability. The present study revealed that even sub-lethal exposure to sub-lethal concentrations of detergents can cause toxicological effects that have resulted in significant changes in the enzymatic parameters in *Mystus vittatus*. These disturbances can cause aquatic organisms to become more susceptible to disease and can ultimately result in their death. Thus, enzyme activity can be used as a sensitive marker of the physiological stress caused by detergents and can be used as a useful biomarker to detect sub-lethal toxicity early. Water quality and biochemical parameters should be routinely monitored to detect environmental stress and to prevent extensive ecological damage in a timely manner. In conclusion, the results indicate that detergents induce a high level of metabolic stress in fish and that the enzymatic parameters studied are good bio-indicators of physiological disturbance in fish.

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