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Acute Toxicity and Sublethal Effects of Lauryl Alcohol Ethoxylate on Oxidative Stress and Antioxidant Defense Parameters in Benthic Oligochaete Worm, *Tubifex Tubifex*

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Abstract: ***The present study aimed to assess the acute toxicity of Lauryl alcohol ethoxylate (LAE) and its sublethal effects on oxidative stress enzymes in Tubifex tubifex, a benthic oligochaete worm. The results indicated that the 96-hour median lethal concentration (LC_{50}) of LAE is 0.77 mg/l for Tubifex tubifex. The model fit performance depicted that GUTS-SD model can better predict the survival rate of Tubifex tubifex. Sublethal concentrations of LAE (10% and 20% of the 96h LC_{50}) significantly altered the oxidative stress enzymes. Reduced glutathione (GSH), glutathione S-transferase (GST), and glutathione peroxidase (GPx) all displayed a significant initial increase followed by a subsequent decline, whereas catalase (CAT) activity and malondialdehyde (MDA) levels increased significantly at all exposure periods with increasing concentrations of LAE. Moreover, the effects of LAE on Tubifex tubifex were demonstrated by the establishment of potency index, integrated biomarker response (IBR) and biomarker response index (BRI) assessment. These findings suggest that exposure of Tubifex tubifex to LAE influences the survival of Tubifex tubifex at the acute stage and modifies alterations in oxidative stress enzymes at the sublethal level.***

Keywords: Lauryl alcohol ethoxylate, Tubifex tubifex, acute toxicity, oxidative stress, integrated biomarker response, biomarker response index

I. INTRODUCTION

Surfactants are a wide family of chemical compounds with both hydrophobic and hydrophilic sites required for organic pollutant solubilization.[1]. Surfactants introduced into freshwater can have a substantial impact on the biological system [2]. The total annual use of surfactants is increasing at a steady rate [3]. Although the majority of surfactants are biodegradable, their prolonged use in groundwater and constant dumping on the surface contribute to the aquatic environment's ongoing and repetitive occurrences. [4]. When surfactants cling to macromolecules, they are poisonous to them and interfere with their efficient function in biological systems [5]. Surfactants are toxic to aquatic organisms, according to numerous studies [4], [6]–[8]. There are four types of surfactants: anionic, cationic, non-ionic and zwitterionic. [9]. Out of these, cationic surfactants are compounds that have a lengthy, hydrophobic chain that connects to a positive nitrogen atom. [10]. When compared to anionic surfactants, these are more hazardous and, in particular, are not substituted for several industrial uses [11]. This class of surfactants is widely utilised in a variety of industries, including textiles, emulsifiers, wetting agents, disinfectants, and cosmetics [10], [11]. One such non ionic surfactant with antimicrobial properties is Lauryl Alcohol Ethoxylate [12].

Tubifex tubifex is a freshwater sediment-dwelling benthic oligochaete worm. It is a massive species with a global distribution that is robust to a wide range of environmental conditions. It is easily cultivable in laboratories and serves as a valuable food source for fish [13].

While the preliminary toxicity research employs a lethal endpoint such as the LC50, sublethal toxicity studies are far more judicious because the species is exposed to significantly lower, biologically relevant hazardous quantities of toxic compounds [14]–[17]. Moreover, the use of general unified survival models (GUTS) has been recommended as a suitable strategy for evaluating toxicant risk in the environment. The damage-related mortality process is defined by two survival strategies: stochastic death (SD) and individual tolerance (IT).

Individuals are comparable in the SD model, and the risk of death from chemical stress increases as damage grows when a specific level of impairment is reached. Individuals, on the other hand, vary in their vulnerability to chemical stress, and once the damage exceeds an individual's threshold, it dies instantly [18], [19].

The metabolism of xenobiotics in organisms significantly contributes to the formation of reactive oxygen species (ROS) [20]. These reactive oxygen species (ROS) effectively start lipid peroxidation (LPO) and cause severe oxidative stress damage to biomolecules like DNA, proteins, and membranes [21].

When there is an imbalance between the production of reactive oxygen species (ROS) and their neutralisation by antioxidant enzymes such as CAT, SOD, GPx, and GSH, oxidative stress occurs [22]. As a result, an effective and secondary technique for evaluating antioxidant enzyme activity may be relevant in aquatic toxicology studies (Bhattacharya et al., 2021). A few observations addressing oxidative stress alterations in *Tubifex tubifex* following pesticide exposure have been presented [24]–[29]. However, there are few data on the negative effects of surfactants on oxidative stress in these worms [2].

Because single biomarkers cannot give an appropriate and practical assessment of a toxicant's toxicity on aquatic life forms, an amalgamated biomarker analysis is recommended to better understand an organism's reaction to toxic substances [30]. As a result, IBR provides a comprehensive methodology that incorporates all biomarker reactions and plays an important role in determining the toxicity of contaminants [31]. Moreover, BRI has been widely utilized in recent years to integrate multiple biomarker responses. It is rudimentarily focused on the evaluation of the organism's overall health status [32].

As a result, the goal of this study is to assess the acute toxicity of LAE to *Tubifex tubifex* in terms of LC50 values after acute exposure, as well as to investigate the possible toxicity of LAE at sublethal concentrations by monitoring changes in oxidative stress indicators. Then, IBR and BRI are used to determine the toxicity of LAE in *Tubifex tubifex*. The GUTS-SD and IT models were used to assess aquatic species' acute responses to surfactants, anticipate toxicity, and determine which model, SD or IT, best matched the toxicity data.

II. MATERIALS &METHODS

The appropriate quality assurance procedures for sample processing, storage, and preservation were followed, as specified by the US EPA.

A. Test Organism and Maintenance Condition

Adult *Tubifex tubifex* (Phylum: Annelida, Class: Clitellata, Order: Oligochaeta, and Family: Naididae) were collected from a local aquarium shop in Burdwan, West Bengal, India and acclimatized in unchlorinated water for 24 h (temperature 25.9 ± 0.4 °C, pH 7.2± 0.6, free CO_2 16.9 ±0.7 mg/l, dissolved oxygen 7.1 ±0.5 mg/l). Then, organisms averaging 11.4 ± 0.2 mm in length were added to the experimental setup. The physiochemical characteristics of the test water were maintained during the exposure duration (temperature 27.2 ±0.3 °C, pH 7.2 ±0.3, free CO_2 17.8 ±0.3 mg/l, dissolved oxygen 6.7 ±0.5 mg/l, total alkalinity 177 ±5.2 mg/l as $CaCO_3$, hardness 120 ± 4.1 mg/l as $CaCO_3$).

B. Test Chemicals

The technical grade of LAE was obtained from the chemistry department, The university of Burdwan and other reagents were procured from Sisco Research Laboratories Pvt. Ltd. (SRL), India. The stock solution of LAE (1% w/v) and subsequent dilutions were made following a standard protocol [33].

C. Bioassay for Acute Toxicity and Survival rate Projection

A static renewal acute toxicity bioassay was carried out in 250 mL glass beakers containing 200 mL water and ten *Tubifex tubifex*. Each experiment was repeated three times. Initially, a range detection test was performed to determine the range of mortality levels. Following that, a final test was conducted by exposing the worms to various nominal concentrations of LAE (0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.10, 1.20, 1.30, 1.40, 1.50) for 96 hours, each with a control containing water free of the toxicant. The worms were counted for mortality at 24, 48, 72, and 96 hours. The LC_{50} values were determined at 24, 48, 72, and 96 h using Finney's probit analysis, with log concentration as the dependent variable and probit as the independent variable [34]. The survival rate pattern of *Tubifex tubifex* in response to LAE was evaluated using GUTS modeling, which was accomplished using the standalone software OpenGUTS. kd (the dominant rate constant), mw (the median of the threshold distribution), hb (the background hazard rate), and bw (the killing rate that is exclusively used for SD) are the model parameters employed [18], [19].

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D. Determination Of Oxidative Stress Parameters At Sublethal Levels

To analyze oxidative stress enzyme parameters at a sublethal level, 2 g of *Tubifex tubifex* is transferred from the stock tank to glass beakers, each holding 1 liter of unchlorinated tap water. Two sublethal concentrations of LAE (10% of 96h LC50 values, i.e., 0.07 mg/l and 20% of 96h LC_{50} values, i.e. 0.15 mg/l) were delivered over periods of 1d, 7d, and 14d. The control worms were placed in another glass beaker with 1l of sterile water free of any toxicant. On day 1, LAE was administered into the experiment (initial treatment). Then, 10% of the test medium was renewed every two days and was replaced with LAE at 10% of the initial nominal concentration. Perpetual aeration was provided during the exposure times. The operation was repeated three times. 1 g of worms were collected and homogenised from each replicate at each exposure period in a 0,1 M phosphate buffer (pH 7.6). Centrifugation at 10000 g for 10 minutes was conducted using a cold centrifuge (Hermle Labortechnik), and the supernatant was kept at -20^0 C until further analysis. The protein content was evaluated using the Bradford technique [35]. Standard techniques have been utilized to quantify the activities of CAT (Beers and Sizer, 1952), SOD [37], GST [38], GPx [39], MDA [40], and GSH [41]. The effects of CAT, SOD, GSH, GST, and GPx were quantified in units per milligram of protein (U/mg protein). In contrast, MDA levels were quantified in nanomoles of thiobarbituric acid reactive substance (TBARS) per minute per milligram of protein (nmol TBARS/min/mg protein).

E. Determination of IBR and BRI

The data on oxidative stress biomarkers were articulated utilizing an IBR system based on the protocol of Beliaeff and Burgeot (2002) and expressed in radar plots. Moreover, the biomarker response index (BRI) for determining the health status of the organism using standard protocol [32]

F. Statistical Analysis

The LC_{50} values were calculated using Finney's probit analysis in Microsoft Excel 2013. Survival curves were established using Kaplan-Meier analysis. A two-way ANOVA followed by the Tukey post hoc test was used to identify the comparisons between controls and exposed worms. The analyses are summarised as mean ± standard deviation. Mean values with a $p<0.05$ significance level is considered statistically significant. A correlation matrix plot was used to determine the associations between oxidative stress indicators.

III. RESULT AND DISCUSSION

The LC_{50} values of LAE to *Tubifex tubifex* associated with 95% confidence intervals are depicted in Table 1 and are reported to be 1.00, 0.93, 0.87 and 0.77 mg/l, respectively. Hence based on the LC_{50} values, LAE is considered moderately toxic to *Tubifex tubifex*

Table 1: The LC_{50} values and 95% confidence limits of LAE to *Tubifex tubifex* at different exposure periods (24, 48, 72 and 96 h).

Exposure period (h)	LC_{50} ± SE (mg/l)	95% confidence limit	
		Lower	Upper
24	1.00 ± 0.020	0.920	1.099
48	0.93 ± 0.021	0.852	1.032
72	0.87 ± 0.030	0.767	1.003
96	0.77 ± 0.025	0.688	0.861

Moreover, the survivability curve also depicts that LAE significantly affected the overall survival rates of *Tubifex tubifex* in a dose and duration-dependent manner with respect to control *(*Mantel log-rank test; $p < 0.05$) (Fig 1). The 100 % survivability of *Tubifex tubifex* is observed in control at all exposure periods (24, 48, 72 and 96 h).

However, with the increase of concentration of LAE as well as periods of exposure (24, 48, 72 and 96 h), the survivability rate of *Tubifex tubifex* decremented significantly (Mantel log-rank test; $p < 0.05$).

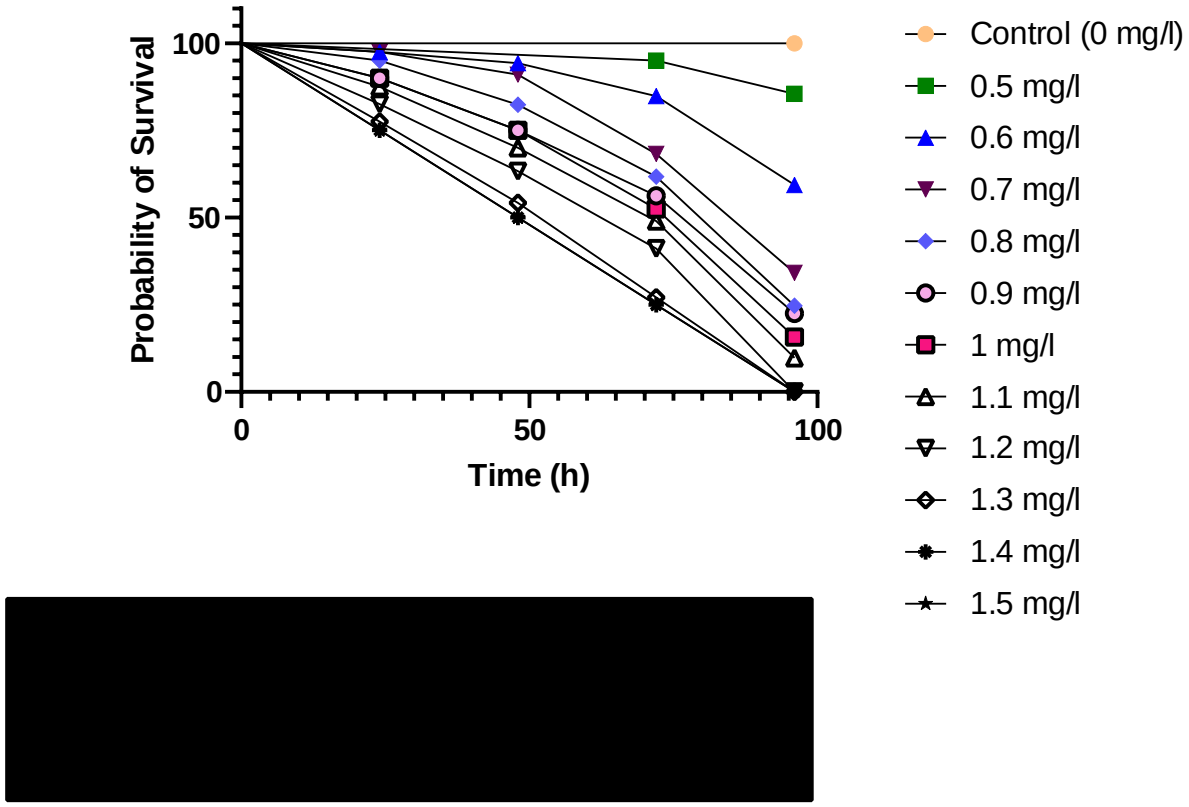

Fig 1: Kaplan-Meier survival curves of *Tubifex tubifex* exposed to different concentrations of LAE (0.5, 0.6, 0.7, 0.8, 0.9, 1.0,1.1, 1.2, 1.3, 1.4 and 1.5 mg/l) at different exposure periods (24, 48, 72 and 96 h).

The model parameters and the fitted performance of GUTS (SD or IT) are given in Table 2. The fitted performance of GUTS–IT was better than that of GUTS–SD in the case of LAE based on AIC values (a smaller AIC value indicates the best fit). Thus, the model simulation illustrated that the GUTS-IT model could better predict the survival rate observed in *Tubifex tubifex* for surfactant exposure than the GUTS-SD model at an acute level. The survival model output demonstrates that the model deducing SD or IT should be chosen wisely to determine the toxic effects of various toxicant exposure patterns. It is clear that such mechanistic modeling has significant potential for enhancing the accuracy of environmental risk management in the future and can significantly help in effective decision-making. Based on the GUTS-IT model, the 100d LC_{50} value was determined and depicted in Table 3.

Table 2: Model parameters in case of LAE [Kd indicates Dominant rate constant; m_w indicates Threshold for mortality; b_w indicates Killing rate; h_b indicates background hazard rate & F_s indicates Spread factor of the threshold distribution]

Symbol	**GUTS-RED**		**unit**	**AIC Value**	
	SD	IT		SD	IT
k_d	143.8 (0.001641 - 143.8)	1.075 (0.001641 – 143.8)	d^{-1}	**247.18**	251.13
m_w	0.4856 (0.001368 – 1.485)	0.701 (0.001368 - 3)	mg/l		

b_w	1.04 (0.01756 – 222967)	-	L/mg/d
h_b	$1e^{-6}$	$1e^{-6}$	d^{-1}
F_s	1	2.269 (1.05 – 20)	

Moreover, the forecasted LC_{50} values from GUTS-SD models are given in Table 3

Table 3. The forecasted LC_{50} values of LAE to *Tubifex tubifex*

Time [d]	LC_{50} GUTS-SD (mg/l)
1	1.159 (1.024 – 1.356)
2	0.821 (0.7537 – 0.9142)
3	0.7088 (0.6593 – 0.7705)
4	0.6529 (0.6089 – 0.6992)
7	0.5811 (0.5364 – 0.6103)
14	0.5333 (0.4836 – 0.5526)
100	0.4923 (0.437 – 0.5058)

Antioxidant enzymes are direct biomarkers of oxidative stress, capable of neutralizing reactive oxygen species (ROS) and other pro-oxidative enzymes in cells under typical conditions [43], [44]. The effect of LAE on different antioxidant enzymes is depicted in Fig. 2 CAT is a critical enzyme that effectively neutralizes reactive oxygen species (ROS) in the antioxidant system and degrades H_2O_2 to molecular oxygen and water [45], [46]. Catalase activity increased significantly at 0.07 mg/l and 0.15 mg/l of LAE during the 1, 7 and 14 d exposure period compared to the control group ($p< 0.05$). This increase in CAT results in increased nuclear Nrf2 expression, which protects cells from H_2O_2-induced stress [47]. Mosleh et al. (2014) observed a uniform increase in CAT activity in *Tubifex tubifex* following exposure to pyrimethinal fungicides.

SOD is the most important oxidative stress enzyme because it provides significant resistance against oxidative stress by converting reactive oxygen radicals to hydrogen peroxide [48]–[51]. SOD activity increased significantly at 0.07 mg/l and 0.15 mg/l of LAE during the 1 and 7 d exposure period but decreased considerably at 0.07 mg/l and 0.15 mg/l of LAE during 14 d exposure period in comparison to the control group ($p< 0.05$). This increase in SOD activity could be related to the stimulation of superoxide ions, which activate the formation of SOD, which protects cells from oxidative damage [52]. On day 14, however, the decrease in SOD activity is probably related to the excessive formation of ROS as a result of toxic pollution, which harmed or inactivated SOD's action by oxidizing the cysteine in SOD or by reducing the expression of SOD-related genes [53].

GPx alleviates possible oxidative stress by accelerating the conversion of hydrogen peroxide to water and oxygen. When GPx is blocked, more hydrogen peroxide is accessible, resulting in tissue degradation and oxidative stress. GPx activity is always specifically linked to GSH concentration. This is because it promotes the synthesis of oxidized glutathione by utilizing reduced glutathione to remove hydrogen peroxide [54]. In the present study, GPx activity increased significantly on 1 and 7 d at all LAE concentrations (0.07 mg/l and 0.15 mg/l, respectively) and appeared to play a significant role in antioxidant protection [55]. At the end of the 14-day exposure period, GPx activity reduced significantly compared to the control level at all LAE concentrations (0.07 mg/l and 0.15 mg/l, respectively) ($p <0.05$). This decrease in GPx could result from the antioxidant defense system's failure to inhibit toxicant-induced ROS generation [56]. This decrease may indicate that the amount of hydroperoxide formed during lipid peroxidation exceeds the antioxidant capability [57]. Additionally, these changes in GPx activity are most likely due to changes in GPx mRNA expression [58]. Similar results were observed in *Tubifex tubifex* following a 15-day thallium exposure (Kiliç and Kiliç, 2017).

GSH is a significant non-protein thiol that protects cells from lipid peroxidation [60], [61]. In the current study, GSH increased gradually on 1 and 7 d at LAE concentrations of 0.07 mg/l and 0.15 mg/l. This increased level of GSH may be a protective mechanism against toxicant exposure in the presence of low levels of oxidative stress [62]. However, on day 14, GSH activity decreased considerably at all doses (0.07 mg/l and 0.15 mg/l). This is because it utilizes reduced glutathione to eliminate hydrogen peroxide and stimulate the production of oxidized glutathione. [62], [63]. This GSH depletion is most likely due to glutathione peroxidase oxidation in response to increased free radicals. GSH levels may fluctuate due to sudden variations in glycolysis rate and Krebs cycle activity, resulting in mitochondrial malfunction [64].

GST is a critical biotransformation enzyme in the phase II phase that regulates the accumulation of GSH and xenobiotics and is widely regarded as a crucial component of the detoxification mechanism [65]. GST activity was significantly increased on 1 and 7 d when *Tubifex tubifex* was subjected to different dosages of LAE (0.07 mg/l and 0.15 mg/l) ($p<0.05$). This increase in GST activity could result from a faster rate of glutathione disulfide (GSSG) synthesis (Li et al., 2010). However, a significant decrease in GST activity in *Tubifex tubifex* at different LAE doses (0.07 mg/l and 0.15 mg/l) during the 14d of exposure time as compared to control ($p< 0.05$) indicated that the worm's detoxification mechanism was impaired under long-term exposure [62]. This considerable decrease in GST activity could be due to the downregulation of GST-related gene expression [66]. Due to the downregulation of GST genes in response to toxicant exposure, nuclear transcription factors cannot bind to the relevant promoter region, resulting in increased ROS generation [67]. Similar variations in the GST level were seen in *Tubifex tubifex* treated with chitosan [25].

ROS interacts with unsaturated fatty acids in membranes during oxidative stress, resulting in LPO. Increased LPO levels imply an increase in ROS production [61], [68]. MDA, a sensitive and delicate oxidative cell damage marker, is the culminating product of LPO [69]. In the present study, a substantial increase in MDA activity at all exposure periods was observed, along with an increase in LAE concentrations (0.07 mg/l and 0.15 mg/l) as compared to control ($p<0.05$), indicating increased ROS production [70]. This increase in MDA could result from the interaction of LAE with polyunsaturated fatty acids resulting in oxidative stress [64]. Increased MDA levels affect the permeability of the cell membrane, allowing toxicants to enter the cell, resulting in DNA damage and eventually apoptosis [57]. A similar effect on MDA activity was observed in *Tubifex tubifex* following 15d exposure to thallium [59]

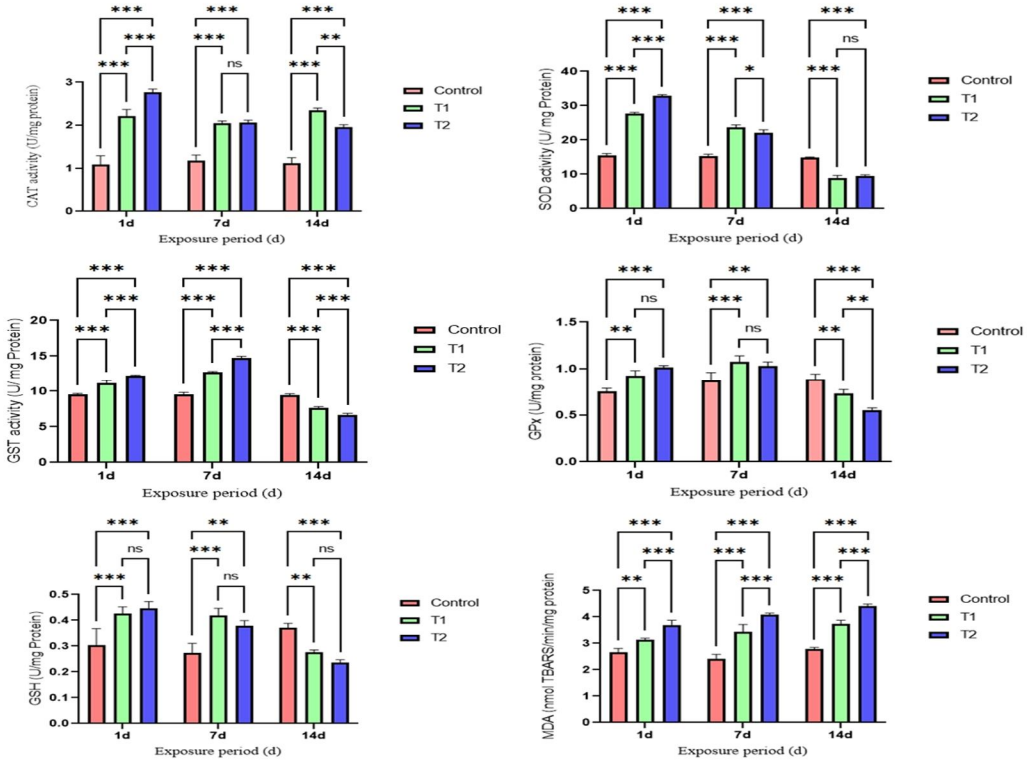

Fig 2: Effects of different sublethal concentrations of LAE on a) CAT, b) SOD, c) GPx, d) GSH, e) GST and f) MDA levels in *Tubifex tubifex* at different exposure periods. Different letters (a-c) indicate significant difference within the same exposure period ($p < 0.05$). T1 and T2 indicate LAE concentration at 10% of 96h LC_{50} value (0.07 mg/l) and 20% of 96h LC_{50} value (0.15 mg/l).

Two-way ANOVA observation depicted that LAE concentration, time of exposure, and their interactions vigorously impacted all of the oxidative stress biomarkers being investigated (CAT, SOD, GST, GPx, GSH, and MDA or LPO) (p<005) (Table 4).

Table 4: Two-way ANOVA for LAE concentration in mg/l (LAE) and period of exposure in days (exposure period) on oxidative stress biomarkers in *Tubifex tubifex* after sublethal exposure to LAE.

Source	Sum of Squares	DF	F	Sig.
CAT				
Exposure period	7.349	2	F (2, 18) = 287.4	P<0.001
LAE	0.3387	2	F (2, 18) = 13.24	P<0.001
Exposure period x LAE	0.9683	4	F (4, 18) = 18.93	P<0.001
SOD				
Exposure period	193.4	2	F (2, 18) = 276.9	P<0.001
LAE	927.5	2	F (2, 18) = 1328	P<0.001
Exposure period x LAE	467.2	4	F (4, 18) = 334.4	P<0.001
GST				
Exposure period	13.28	2	F (2, 18) = 175.2	P<0.001
LAE	91.99	2	F (2, 18) = 1214	P<0.001
Exposure period x LAE	51.50	4	F (4, 18) = 339.8	P<0.001
GPx				
Exposure period	0.02450	2	F (2, 18) = 4.576	P=0.025
LAE	0.3437	2	F (2, 18) = 64.18	P<0.001
Exposure period x LAE	0.3235	4	F (4, 18) = 30.21	P<0.001
GSH				
Exposure period	0.01588	2	F (2, 18) = 8.268	P=0.003
LAE	0.04395	2	F (2, 18) = 22.88	P<0.001
Exposure period x LAE	0.07108	4	F (4, 18) = 21.48	P<0.001
MDA				
Exposure period	9.513	2	F (2, 18) = 215.5	P<0.001
LAE	1.100	2	F (2, 18) = 24.93	P<0.001
Exposure period x LAE	0.4526	4	F (4, 18) = 5.126	P=0.006

To investigate the overall correlations between surfactants and indicators of oxidative stress, a correlation matrix plot was constructed. According to the plot LAE is positively correlated with CAT and MDA.

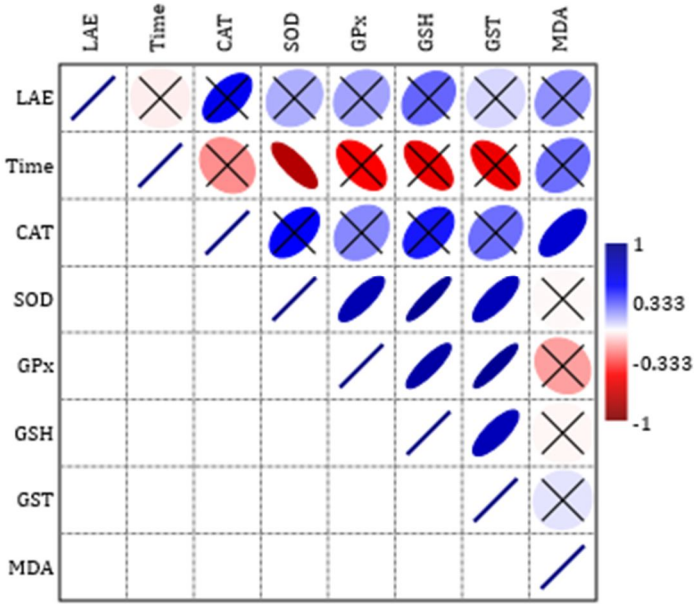

Fig. 3: Pearson correlation matrix between LAE concentration (mg/l), exposure period (d) and oxidative stress biomarkers in *Tubifex tubifex*. Cross indicates p>0.05

The IBR index was applied to quantify the overall stress of LAE on *Tubifex tubifex*. The IBR portrays the inclusion of several biomarkers in a single value conveniently [42]. It is a puissant technique and an efficacious strategy to evaluate the health status of living organisms by coordinating and amalgamating biomarkers [71]. Higher IBR values commonly indicate the more distressing ecological condition for the organisms, whereas low scores of IBR demonstrate favorable environmental conditions [72]. Based on concentration and exposure periods, the present study shows that T2-14d is the highest affected group, followed by T1-14d, T2- 1d, T1-1d, T2-7d, C-1d, C-7d, T1-7d and C-14d (Fig. 4). In integration, biomarker weights and scores for exposed worm parameters are used to calculate BRI, which conventionally reflects the general health condition of worm [32]. The BRI values of LAE are within 0-2.6 (Fig. 5), which portrays paramount alterations from the normal [32]. Thus, it is conspicuous from our finding that LAE adversely impacts worm health.

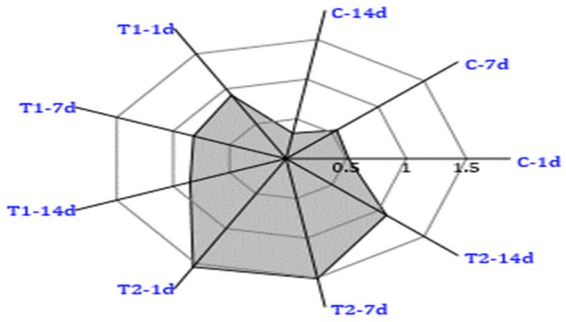

Fig. 4: IBR of oxidative stress parameters measured in *Tubifex tubifex* after chronic exposure to LAE. C indicates control (0 mg/l), T1 indicates LAE concentration at 10% of its 96h LC_{50} value (0.07 mg/l); T2 indicates LAE concentration at 20% of its 96h LC_{50} value (0.15 mg/l).

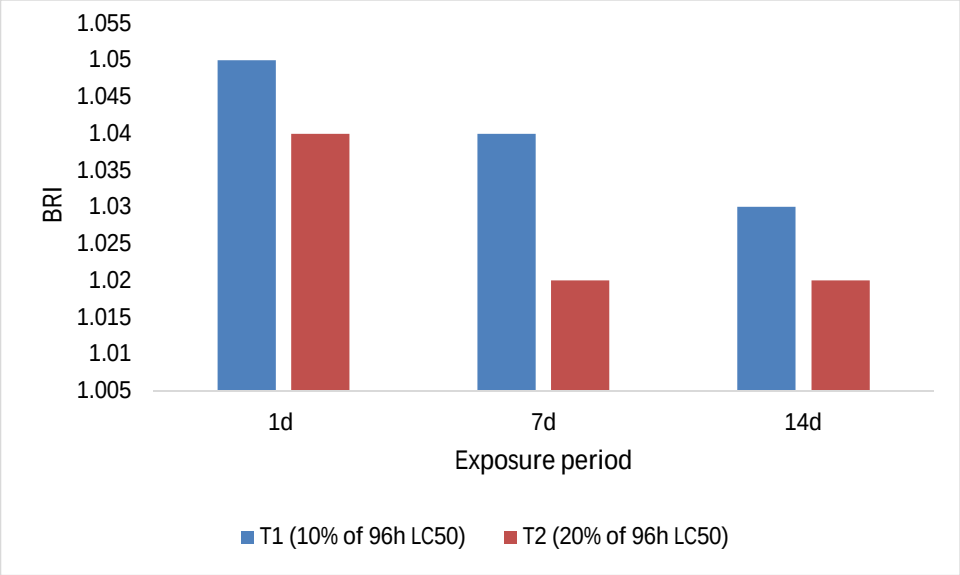

Fig. 5: BRI values representing the health status of *Tubifex tubifex*. upon exposure to LAE

IV. CONCLUSION

The finding of this study revealed that *Tubifex tubifex* showed alterations in survivability and ethological changes at the acute level and modifications in oxidative stress parameters at the sublethal level by incorporating surfactant LAE. Consequently, the present study on the toxic effects of LAE against *Tubifex tubifex* implicatively indicates that oxidative stress biomarkers are the critical attributes for ascertaining aquatic species' intricate health status. However, further studies are needed to extract LAE toxicity on tubificid worms at the ultrastructural level and to reduce their toxicity by utilizing adequate plant extract.

A. Ethical Approval

This study does not include animal experiments by the authors that require the ethics committee's permission. In particular, no ethical approval is needed for invertebrates such as *Tubifex tubifex.*

B. Funding

The research did not receive any specific grant from funding agencies in public, commercial or nonprofit sectors.

C. Conflict of Interest

The authors declare that they have no conflict of interest.

V. ACKNOWLEDGMENT

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REFERENCES

[1] M. Lechuga, M. Fernández-serrano, E. Jurado, and F. Ríos, "Ecotoxicology and Environmental Safety Acute toxicity of anionic and non-ionic surfactants to aquatic organisms," Ecotoxicol. Environ. Saf., vol. 125, pp. 1–8, 2016, doi: 10.1016/j.ecoenv.2015.11.027.
[2] R. Bhattacharya, A. Chatterjee, S. Chatterjee, and N. C. Saha, "Oxidative stress in benthic oligochaete worm, Tubifex tubifex induced by sublethal exposure to a cationic surfactant cetylpyridinium chloride and an anionic surfactant sodium dodecyl sulfate," Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol., vol. 240, 2021, doi: 10.1016/j.cbpc.2020.108906.
[3] A. Kumar Dutta, "Introductory Chapter: Surfactants in Household and Personal Care Formulations - An Overview," in Surfactants and Detergents, IntechOpen, 2019. doi: 10.5772/intechopen.89245.
[4] D. S. Mustapha and K. A. Bawa-Allah, "Differential toxicities of anionic and nonionic surfactants in fish," Environ. Sci. Pollut. Res., vol. 27, no. 14, pp. 16754–16762, May 2020, doi: 10.1007/s11356-020-08212-6.
[5] T. Ivanković and J. Hrenović, "Surfactants in the environment," Arhiv za Higijenu Rada i Toksikologiju, vol. 61, no. 1. pp. 95–110, Mar. 01, 2010. doi: 10.2478/10004-1254-61-2010-1943.
[6] R. Freitas et al., "Biochemical and physiological responses induced in Mytilus galloprovincialis after a chronic exposure to salicylic acid," Aquat. Toxicol., vol. 214, Sep. 2019. doi:10.1016/j.aquatox.2019.105258.

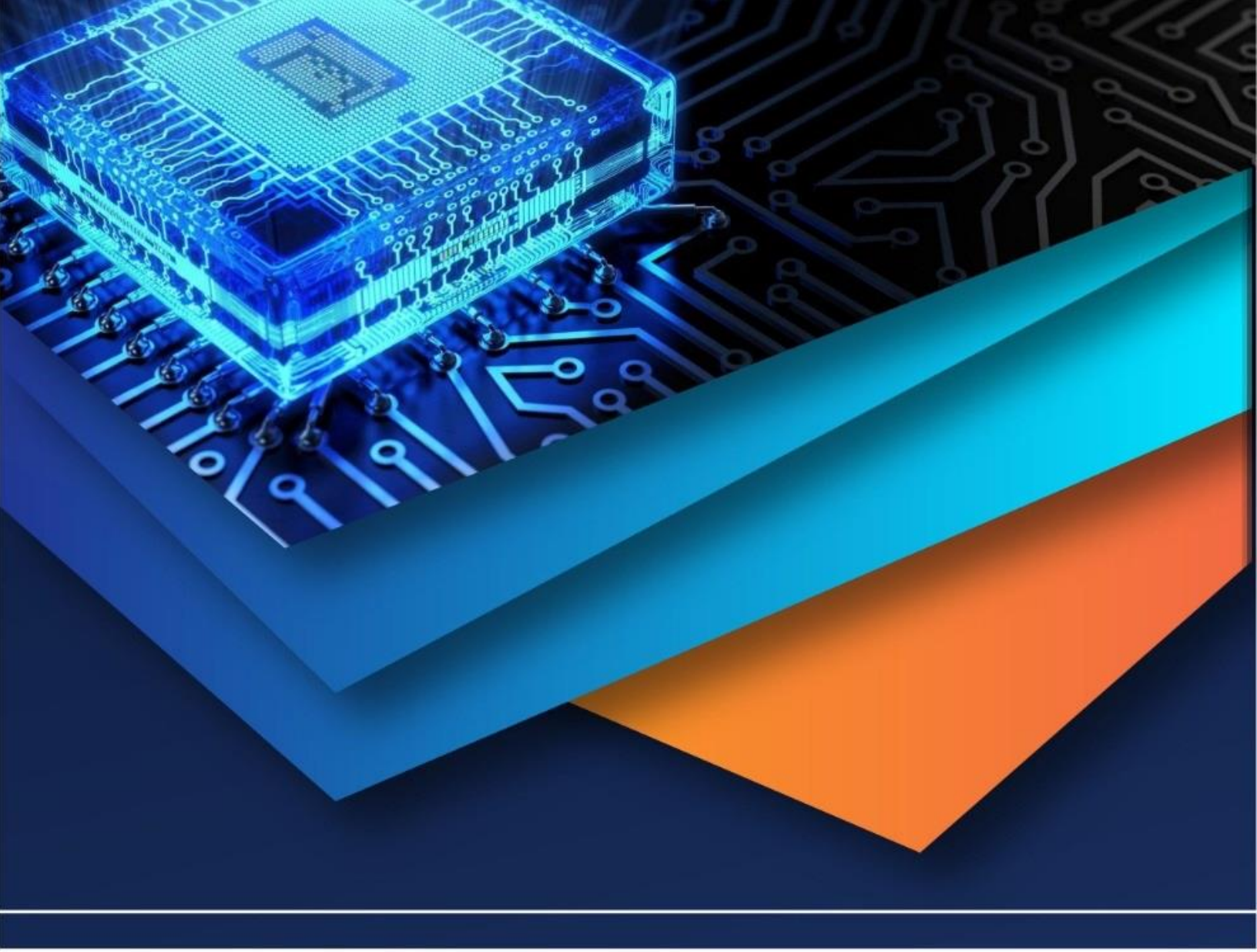
[7] R. Freitas et al., "Toxic impacts induced by Sodium lauryl sulfate in Mytilus galloprovincialis," Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol., vol. 242, Apr. 2020, doi: 10.1016/j.cbpa.2020.110656.
[8] I. Hering et al., "Evaluation of potential environmental toxicity of polymeric nanomaterials and surfactants," Environ. Toxicol. Pharmacol., vol. 76, May 2020, doi: 10.1016/j.etap.2020.103353.
[9] M. Jackson, C. Eadsforth, D. Schowanek, T. Delfosse, A. Riddle, and N. Budgen, "Comprehensive review of several surfactants in marine environments: Fate and ecotoxicity," Environmental Toxicology and Chemistry, vol. 35, no. 5. Wiley Blackwell, pp. 1077–1086, May 01, 2016. doi: 10.1002/etc.3297.
[10] R. Puchta, "Cationic surfactants in laundry detergents and laundry aftertreatment aids," J. Am. Oil Chem. Soc., vol. 61, no. 2, pp. 367–376, Feb. 1984, doi: 10.1007/BF02678796.
[11] K. Jardak, P. Drogui, and R. Daghrir, "Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes," Environ. Sci. Pollut. Res., vol. 23, no. 4, pp. 3195–3216, Feb. 2016, doi: 10.1007/s11356-015-5803-x.
[12] L. Intorre, V. Meucci, D. Di Bello, G. Monni, G. Soldani, and C. Pretti, "Tolerance of benzalkonium chloride, formalin, malachite green, and potassium permanganate in goldfish and zebrafish," J. Am. Vet. Med. Assoc., vol. 231, no. 4, 2007, doi: 10.2460/javma.231.4.590.
[13] R. Bhattacharya, A. Chatterjee, and N. C. Saha, "Acute toxicity and ethological responses of oligochaete worm Tubifex tubifex (Muller) exposed to a cationic surfactant Cetylpyridinium chloride," Int. J. Sci. Res. Biol. Sci., vol. 6, no. 2, pp. 8–14, 2019, doi: 10.26438/ijsrbs/v6i2.814.
[14] V. Aliko, M. Qirjo, E. Sula, V. Morina, and C. Faggio, "Antioxidant defense system, immune response and erythron profile modulation in gold fish, Carassius auratus, after acute manganese treatment," Fish Shellfish Immunol., vol. 76, pp. 101–109, May 2018, doi: 10.1016/j.fsi.2018.02.042.
[15] N. Brahma and A. Gupta, "Acute toxicity of lead in fresh water bivalves Lamellidens jenkinsianus obesa and Parreysia (Parreysia) corrugata with evaluation of sublethal effects on acetylcholinesterase and catalase activity, lipid peroxidation, and behavior," Ecotoxicol. Environ. Saf., vol. 189, Feb. 2020, doi: 10.1016/j.ecoenv.2019.109939.
[16] A. Petrovici, S. A. Strungaru, M. Nicoara, M. A. Robea, C. Solcan, and C. Faggio, "Toxicity of deltamethrin to zebrafish gonads revealed by cellular biomarkers," J. Mar. Sci. Eng., vol. 8, no. 2, Feb. 2020, doi: 10.3390/jmse8020073.
[17] M. D. Prokić, T. B. Radovanović, J. P. Gavrić, and C. Faggio, "Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives," TrAC - Trends in Analytical Chemistry, vol. 111. Elsevier B.V., pp. 37–46, Feb. 01, 2019. doi: 10.1016/j.trac.2018.12.001.
[18] T. Jager, C. Albert, T. G. Preuss, and R. Ashauer, "General unified threshold model of survival - A toxicokinetic-toxicodynamic framework for ecotoxicology," Environmental Science and Technology, vol. 45, no. 7. pp. 2529–2540, Apr. 01, 2011. doi: 10.1021/es103092a.
[19] T. Jager and R. Ashauer, "Modelling survival under chemical stress," A Compr. Guid. to GUTS Framew., no. January, 2018
[20] M. A. Burgos-Aceves et al., "Modulation of mitochondrial functions by xenobiotic-induced microRNA: From environmental sentinel organisms to mammals," Science of the Total Environment, vol. 645. Elsevier B.V., pp. 79–88, Dec. 15, 2018. doi: 10.1016/j.scitotenv.2018.07.109.
[21] M. O. Ansari et al., "Evaluation of DNA interaction, genotoxicity and oxidative stress induced by iron oxide nanoparticles both in vitro and in vivo: attenuation by thymoquinone," Sci. Rep., vol. 9, no. 1, Dec. 2019, doi: 10.1038/s41598-019-43188-5.
[22] E. B. Kurutas, "The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state," Nutrition Journal. 2016. doi: 10.1186/s12937-016-0186-5.
[23] R. Bhattacharya, I. Daoud, A. Chatterjee, S. Chatterjee, and N. C. Saha, "An integrated in silico and in vivo approach to determine the effects of three commonly used surfactants sodium dodecyl sulphate, cetylpyridinium chloride and sodium laureth sulphate on growth rate and hematology in Cyprinus carpio L," Toxicol. Mech. Methods, 2021, doi: 10.1080/15376516.2021.1973633.
[24] Y. Y. Mosleh, J. Mofeed, M. Afifi, and O. A. Almaghrabi, "Biological effects of pyrimethinal on aquatic worms (tubifex tubifex) under laboratory conditions," Bull. Environ. Contam. Toxicol., vol. 92, no. 1, pp. 85–89, 2014, doi: 10.1007/s00128-013-1153-x.
[25] Y. Y. Mosleh, S. Paris-Palacios, M. T. Ahmed, F. M. Mahmoud, M. A. Osman, and S. Biagianti-Risbourg, "Effects of chitosan on oxidative stress and metallothioneins in aquatic worm Tubifex tubifex (Oligochaeta, Tubificidae)," Chemosphere, vol. 67, no. 1, pp. 167–175, Feb. 2007, doi: 10.1016/j.chemosphere.2006.09.019.
[26] Y. Y. Mosleh, S. Paris-Palacios, and S. Biagianti-Risbourg, "Metallothioneins induction and antioxidative response in aquatic worms Tubifex tubifex (Oligochaeta, Tubificidae) exposed to copper," Chemosphere, vol. 64, no. 1, pp. 121–128, Jun. 2006, doi: 10.1016/j.chemosphere.2005.10.045.
[27] Y. Y. Mosleh, S. Paris-Palacios, M. Couderchet, S. Biagianti-Risbourg, and G. Vernet, "Effects of the herbicide isoproturon on metallothioneins, growth, and antioxidative defenses in the aquatic worm Tubifex tubifex (Oligochaeta, Tubificidae)," Ecotoxicology, vol. 14, no. 5, pp. 559–571, Jul. 2005, doi: 10.1007/s10646-005-0008-6.
[28] Y. Y. Mosleh, S. Paris-Palacios, M. Couderchet, and G. Vernet, "Effects of the herbicide isoproturon on survival, growth rate, and protein content of mature earthworms (Lumbricus terrestris L.) and its fate in the soil," Appl. Soil Ecol., vol. 23, no. 1, pp. 69–77, 2003, doi: 10.1016/S0929-1393(02)00161-0.
[29] S. Paris-Palacios et al., "Toxic effects and bioaccumulation of the herbicide isoproturon in Tubifex tubifex (Oligocheate, Tubificidae): A study of significance of autotomy and its utility as a biomarker," Aquat. Toxicol., vol. 98, no. 1, pp. 8–14, Jun. 2010, doi: 10.1016/j.aquatox.2010.01.006.
[30] W. Sanchez, T. Burgeot, and O. Perceval, "Perspectives from the French workshop on the development and validation of biomarkers and bioassays for the monitoring of aquatic environments," Environ. Sci. Pollut. Res., vol. 19, no. 4, pp. 1345–1347, May 2012, doi: 10.1007/s11356-012-0789-0.
[31] A. Serafim et al., "Application of an integrated biomarker response index (IBR) to assess temporal variation of environmental quality in two Portuguese aquatic systems," Ecol. Indic., vol. 19, pp. 215–225, Aug. 2012, doi: 10.1016/j.ecolind.2011.08.009.
[32] J. A. Hagger, M. B. Jones, D. Lowe, D. R. P. Leonard, R. Owen, and T. S. Galloway, "Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: A case study," Mar. Pollut. Bull., vol. 56, no. 6, pp. 1111–1118, Jun. 2008, doi: 10.1016/j.marpolbul.2008.03.040.
[33] APHA, "Standard Methods for the Examination of Water and Wastewater, 21st ed.; APHA:Washington, DC, USA, 2005.," Am. Water Work. Assoc. Public Work. Assoc. Environ. Fed., vol. 552, 2005, doi: 10.2105/AJPH.51.6.940-a.
[34] D. J. Finney, "Probit Analysis, Cambridge University Press," J. Pharm. Sci., vol. 60, no. 9, p. 333, 1971, doi: 07161417.
[35] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," Anal. Biochem., vol. 72, no. 1–2, pp. 248–254, May 1976, doi: 10.1016/0003-2697(76)90527-3.
[36] R. F. BEERS and I. W. SIZER, "A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase.," J. Biol. Chem., vol. 195, no. 1, pp. 133–140, Mar. 1952, doi: 10.1093/jxb/48.2.181.
[37] C. Beauchamp and I. Fridovich, "Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels," Anal. Biochem., vol. 44, no. 1, pp. 276–287, 1971, doi: 10.1016/0003-2697(71)90370-8.
[38] W. H. Habig, M. J. Pabst, and W. B. Jakoby, "Glutathione S transferases. The first enzymatic step in mercapturic acid formation," J. Biol. Chem., vol. 249, no. 22, pp. 7130–7139, 1974.
[39] R. A. Lawrence and R. F. Burk, "Glutathione peroxidase activity in selenium-deficient rat liver," Biochem. Biophys. Res. Commun., vol. 71, no. 4, pp. 952–

958, Aug. 1976, doi: 10.1016/0006-291X(76)90747-6.
[40] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Anal. Biochem., vol. 95, no. 2, pp. 351–358, 1979, doi: 10.1016/0003-2697(79)90738-3.
[41] T. P. M. Akerboom and H. Sies, "Assay of Glutathione, Glutathione Disulfide, and Glutathione Mixed Disulfides in Biological Samples," Methods Enzymol., vol. 77, no. C, pp. 373–382, Jan. 1981, doi: 10.1016/S0076-6879(81)77050-2.
[42] B. Beliaeff and T. Burgeot, "Integrated biomarker response: A useful tool for ecological risk assessment," in Environmental Toxicology and Chemistry, 2002, vol. 21, no. 6, pp. 1316–1322. doi: 10.1002/etc.5620210629.
[43] M. N. O. Ajima, P. K. Pandey, K. Kumar, and N. Poojary, "Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) exposed to verapamil," Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol., vol. 196, pp. 44–52, Jun. 2017, doi: 10.1016/j.cbpc.2017.03.009.
[44] S. C. Iheanacho and G. E. Odo, "Neurotoxicity, oxidative stress biomarkers and haematological responses in African catfish (Clarias gariepinus) exposed to polyvinyl chloride microparticles," Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol., vol. 232, Jun. 2020, doi: 10.1016/j.cbpc.2020.108741.
[45] S. Pandey, I. Ahmad, S. Parvez, B. Bin-Hafeez, R. Haque, and S. Raisuddin, "Effect of endosulfan on antioxidants of freshwater fish Channa punctatus bloch: 1. Protection against lipid peroxidation in liver by copper preexposure," Arch. Environ. Contam. Toxicol., vol. 41, no. 3, pp. 345–352, 2001, doi: 10.1007/s002440010258.
[46] T. Usman, S. Abdullah, H. Naz, K. Abbas, L. Shafique, and Q. Siddique, "Acute toxic effect of technical grade insecticides on behavior, catalase activity and total protein contents of fish, ctenopharyngodon idella," Pak. J. Zool., vol. 52, no. 5, pp. 2023–2026, Oct. 2020, doi: 10.17582/JOURNAL.PJZ/20181103091108.
[47] Q. Ma, "Role of Nrf2 in oxidative stress and toxicity," Annual Review of Pharmacology and Toxicology, vol. 53. pp. 401–426, Jan. 2013. doi: 10.1146/annurev-pharmtox-011112-140320.
[48] C. Faggio, M. Pagano, R. Alampi, I. Vazzana, and M. R. Felice, "Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternium-15 in Mytilus galloprovincialis," Aquat. Toxicol., vol. 180, pp. 258–265, Nov. 2016, doi: 10.1016/j.aquatox.2016.10.010.
[49] M. El Hajam et al., "Evaluation of softwood and hardwood sawmill wastes impact on the common carp 'Cyprinus carpio' and its aquatic environment: An oxidative stress study," Environ. Toxicol. Pharmacol., vol. 75, Apr. 2020, doi: 10.1016/j.etap.2020.103327.
[50] J. H. Kim et al., "Growth performance, oxidative stress, and non-specific immune responses in juvenile sablefish, Anoplopoma fimbria, by changes of water temperature and salinity," Fish Physiol. Biochem., vol. 43, no. 5, pp. 1421–1431, Oct. 2017, doi: 10.1007/s10695-017-0382-z.
[51] J. H. Kim et al., "Toxic effects on hematological parameters and oxidative stress in juvenile olive flounder, Paralichthys olivaceus exposed to waterborne zinc," Aquac. Reports, vol. 15, Nov. 2019, doi: 10.1016/j.aqrep.2019.100225.
[52] Q. Zhang et al., "Oxidative stress and lipid peroxidation in the earthworm Eisenia fetida induced by low doses of fomesafen," Environ. Sci. Pollut. Res., vol. 20, no. 1, pp. 201–208, 2013, doi: 10.1007/s11356-012-0962-5.
[53] Q. Cao, A. D. Steinman, L. Yao, and L. Xie, "Toxicological and biochemical responses of the earthworm Eisenia fetida to cyanobacteria toxins," Sci. Rep., vol. 7, no. 1, Dec. 2017, doi: 10.1038/s41598-017-16267-8.
[54] E. Ogueji, C. Nwani, C. Mbah, S. Iheanacho, and F. Nweke, "Oxidative stress, biochemical, lipid peroxidation, and antioxidant responses in Clarias gariepinus exposed to acute concentrations of ivermectin," Environ. Sci. Pollut. Res., vol. 27, no. 14, pp. 16806–16815, May 2020, doi: 10.1007/s11356-019-07035-4.
[55] W. Xiong, X. Ding, Y. Zhang, and Y. Sun, "Ecotoxicological effects of a veterinary food additive, copper sulphate, on antioxidant enzymes and mRNA expression in earthworms," Environ. Toxicol. Pharmacol., vol. 37, no. 1, pp. 134–140, Jan. 2014, doi: 10.1016/j.etap.2013.11.014.
[56] N. Özok, "Effects of cypermethrin on antioxidant enzymes and lipid peroxidation of Lake Van fish (Alburnus tarichi)," Drug Chem. Toxicol., vol. 43, no. 1, pp. 51–56, Jan. 2020, doi: 10.1080/01480545.2019.1660363.
[57] O. Serdar, "The effect of dimethoate pesticide on some biochemical biomarkers in Gammarus pulex," Environ. Sci. Pollut. Res., vol. 26, no. 21, pp. 21905–21914, Jul. 2019, doi: 10.1007/s11356-019-04629-w.
[58] S. Selmi, K. Rtibi, D. Grami, H. Sebai, and L. Marzouki, "Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice," Toxicol. Reports, vol. 5, pp. 189–195, Jan. 2018, doi: 10.1016/j.toxrep.2017.12.021.
[59] V. KILIÇ and G. AYDOĞAN KILIÇ, "Antioxidative responses in Tubifex tubifex against thallium induced oxidative stress," ANADOLU Univ. J. Sci. Technol. –C Life Sci. Biotechnol., pp. 1–1, Aug. 2017, doi: 10.18036/aubtdc.288885.
[60] S. Anjum et al., "Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse," Food Chem. Toxicol., vol. 49, no. 11, pp. 2849–2854, Nov. 2011, doi: 10.1016/j.fct.2011.07.062.
[61] M. Faheem and K. P. Lone, "Oxidative stress and histopathologic biomarkers of exposure to bisphenol-A in the freshwater fish, Ctenopharyngodon idella," Brazilian J. Pharm. Sci., vol. 53, no. 3, Feb. 2018, doi: 10.1590/s2175-97902017000317003.
[62] R. Sreejai and D. S. Jaya, "Studies on the changes in lipid peroxidation and antioxidants in fishes exposed to hydrogen sulfide," Toxicol. Int., vol. 17, no. 2, pp. 71–77, Dec. 2010, doi: 10.4103/0971-6580.72674.
[63] D. K. Sharma and B. A. Ansari, "Effects of deltamethrin on CAT, LPO and GSH in Tissues of Zebrafish Danio rerio," Res. J. Environ. Toxicol., vol. 7, no. 1, pp. 38–46, 2013, doi: 10.3923/rjet.2013.38.46.
[64] S. Gheorghe et al., "Evaluation of sub-lethal toxicity of benzethonium chloride in cyprinus carpio liver," Appl. Sci., vol. 10, no. 23, pp. 1–15, Dec. 2020, doi: 10.3390/app10238485.
[65] D. R. Livingstone, "The fate of organic xenobiotics in aquatic ecosystems: Quantitative and qualitative differences in biotransformation by invertebrates and fish," in Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, May 1998, vol. 120, no. 1, pp. 43–49. doi: 10.1016/S1095-6433(98)10008-9.
[66] A. L. F. Destro et al., "Effects of subchronic exposure to environmentally relevant concentrations of the herbicide atrazine in the Neotropical fish Astyanax altiparanae," Ecotoxicol. Environ. Saf., vol. 208, Jan. 2021, doi: 10.1016/j.ecoenv.2020.111601.
[67] S. Maity, R. Banerjee, P. Goswami, M. Chakrabarti, and A. Mukherjee, "Oxidative stress responses of two different ecophysiological species of earthworms (Eutyphoeus waltoni and Eisenia fetida) exposed to Cd-contaminated soil," Chemosphere, vol. 203, pp. 307–317, Jul. 2018, doi: 10.1016/j.chemosphere.2018.03.189.
[68] R. J. Huggett, R. A. Klmerle, P. M. Mehrle, and T. W. LaPoint, Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress. CRC Press, 2018. doi: 10.1201/9781351070270.
[69] A. A. Sharbidre, V. Metkari, and P. Patode, "Effect of diazinon on acetylcholinesterase activity and lipid peroxidation of poecilia reticulata," Res. J. Environ. Toxicol., vol. 5, no. 2, pp. 152–161, 2011, doi: 10.3923/rjet.2011.152.161.
[70] S. Shukla, R. C. Jhamtani, M. S. Dahiya, and R. Agarwal, "Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate

and herbicide in zebrafish," Toxicol. Reports, vol. 4, pp. 240–244, 2017, doi: 10.1016/j.toxrep.2017.05.002.

[71] Z. H. Li, J. Velisek, R. Grabic, P. Li, J. Kolarova, and T. Randak, "Use of hematological and plasma biochemical parameters to assess the chronic effects of a fungicide propiconazole on a freshwater teleost," Chemosphere, vol. 83, no. 4, pp. 572–578, Apr. 2011, doi: 10.1016/j.chemosphere.2010.12.024.

[72] A. J. Madu and M. D. Ughasoro, "Anaemia of Chronic Disease: An In-Depth Review," Medical Principles and Practice, vol. 26, no. 1. 2017. doi: 10.1159/000452104.



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