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The Impact of Surfactant on Aquatic Ecosystems: A Study on Biochemical Alterations in *Clarias gariepinus* Induced with Linear Alkylbenzene Sulfonates

Ikpesu, Thomas Ohwofasa

Department of Biology, Federal University Otuoke, Nigeria.

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ABSTRACT

The toxicity of linear alkylbenzene sulfonates (LABs) to *Clarias gariepinus* was investigated. For 30 days, the fish were exposed to LABs at 0.00, 0.50, 1.00, 1.50, and 2.00 mg/L. After each trial period, one fish from each plastic tub was chosen and its heart was punctured for blood samples. The blood samples were then collected and deposited in pre-designated bottles for analysis. Following blood collection, a fish was dissected and its organs were extracted. The organs were preserved in liquid nitrogen at -25°C until they were analyzed. A portable refractometer was used to quantify total serum protein content. A microplate reader was used to measure reduced glutathione (GSH). Albumin was quantified using the Bromocresol Green albumin assay kit, whereas alanine aminotransferase activity was assessed colorimetrically. Subtracting albumin from protein concentration yielded the globulin content. On days 23 and 30, protein content corresponds positively with exposure length and differs significantly ($p < 0.05$) between the control and treatment groups. The activity of GSH reduced slightly but not significantly ($p > 0.05$). Significant variations in albumin and globulin ($p < 0.05$) only on day 30. AAS activity differs significantly ($p < 0.05$) between treatments and the control. This study demonstrated that LAB exposure can be harmful to human health. Because anthropogenic sources are the primary source of LAB exposure, authorities must implement strict mitigation measures to limit this risk.

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INTRODUCTION

Detergents are frequently employed in both industrial and domestic contexts to cleanse various items, such as equipment, installations, heavy-duty machinery, automobiles, and materials that have become soiled with oil or grease. The production, usage, and exposure of detergents are unavoidable because they are widespread environmental contaminants, most likely as a result of their usage in the formulation of cleaning agents and for dispersing oil spills. The most significant sources of contamination in water are sewage treatment plants, runoff from industrial waste, and direct home use of water (Adewoye and Lateef, 2004). The release of secondary or tertiary sewage effluents, including the products of laundry detergents, which may be low in concentration, has the potential to cause acute aquatic hazardous effects (Céspedes *et al.,* 2016). Several studies, including Adewoye and Fawole (2002) and Adewoye and Fawole (2005), have found that the uncontrolled discharge of detergent into an aquatic ecosystem has the potential to decrease the concentration of dissolved oxygen and hinder

*Corresponding Author Email: *ikpesuto@fuotuoke.edu.ng*

respiratory processes, leading to asphyxiation, a state of unconsciousness or death caused by inadequate oxygenation of the blood in the lungs. Additionally, this can result in structural deterioration of organs, such as liver dysfunction.. Similarly, Ademuyiwa *et al*. (2007) found that detergents have a depraved effect on all kinds of fish and other aquatic life.

When detergent concentrations reach 15 parts per million, the vast majority of fish will die (Alaa Adewolu *et al.,* 2008). Surfactants lower the surface tension of water, and they have been linked to lowering the breeding capabilities of aquatic species. Surfactants in concentrations as low as 5 are known to kill salmon eggs (Adewoye *et al.,* 2005). The breakdown of alkylphenol polyethoxylated results in the creation of alkyl (especially) nonylphenols, which are endocrine disruptors in animals (Adewoye and Lateef, 2004). All detergents degrade the exterior mucus layers that protect fish from bacteria and parasites, with the gills of the fish being the most severely affected (Gomez, 2011). This can result in significant damage to the gills of the fish (Fernandes *et al*., 2007).

Organic pollutants such as insecticides and phenols, for example, are considerably more readily absorbed by fish than by humans (Adewoye *et al.,* 2005). A detergent concentration of only 2 parts per million (ppm) can cause fish to absorb twice the quantity of chemicals that they would normally have absorbed. However, the concentration itself is not high enough to have an adverse effect on fish directly. Phosphates in detergents can cause freshwater algal blooms, which release pollutants into the environment and deplete oxygen levels in aquatic bodies. When algae decompose, they consume the oxygen that would otherwise be accessible to aquatic life (Abhijit *et al*., 2016).

Linear alkylbenzene sulfonate (LAS) is the most commonly used anionic surfactant in institutional and commercial laundry because of its outstanding cleaning qualities and strong cleaning potential (Mungray and Kumar, 2009). There has been a worrisome increase in the use of linear Akly sulfonate in many industrial and household items. Overuse and subsequent disposal of waste in rivers have major effects, notably on aquatic security. As a result, before discharging into the water, all detergents, no matter how light or strong, should be rendered harmless.

Due to the inexpensive cost of linear, it is the most popular detergent on the market today. The most extensively used detergent is made with LAS as its primary raw element.

Surfactants are extremely toxic and dangerous to aquatic organisms, and their widespread use in domestic and industrial settings encourages quantitative and qualitative research into their effects on aquatic organisms, as most of the studies on surfactant toxicity that are currently available are based on nominal rather than measured concentrations. Therefore, it could be necessary for wider investigations that are inclusive and concentration-dependent and can be used as a baseline for surfactant ecotoxicity.

MATERIALS AND METHODS

Pre-Analytical Stage

Fish experiments are conducted in accordance with the relevant norms and regulations. In this study, *C. gariepinus* that were self bred were utilized for this study. The organisms in question exhibit a notable capacity for growth, displaying heightened resilience to conditions of low dissolved oxygen levels and suboptimal water quality. Additionally, they possess a strong inclination towards plant consumption, accompanied by a robust and insatiable appetite. Periodically, they underwent examinations to check on their health, maturity development, and immunity to disease and parasite infection. To maintain the water quality, the water in the brood fish pond is periodically replaced and refilled.

Experimentation

The fish was transported carefully to the biology lab at Federal University Otuoke, where it

was acclimated for two weeks. After acclimating for 14 days, the fish were moved into 10-liter plastic tubs, ten fish per tub. The linear alkylbenzene sulfonates concentration ranges reported in the field (0.00, 0.50, 1.00, 1.50, and 2.00 mg/L) were given to the fish for 30 days. Fish were fed twice daily, both the control and experimental groups at 3% body weight throughout the experiment.

The treatments were replaced completely every other day, and the plastic tubs were kept as clean as possible. Throughout the investigation, daily measurements of the water's physicochemical properties were taken. Following each experimental period, a fish was removed from each plastic tub, and its heart was punctured to collect blood samples. A blood sample was drawn and placed in labelled sample bottles for a respective investigation.

Immediately after the collection of the blood, a fish was dissected and the organs (liver, kidney, and gills) were excised, transferred to liquid nitrogen, and kept at -25^{o} C until the analysis.

Analysis of Serum Protein

Blood proteins are a significant indicator of health, and their evaluation serves as a foundation in the general biochemistry of an organism. The initial stage in protein pattern analysis is the determination of total serum or plasma protein concentrations (Lundblad, 2003). Because of its convenience, quickness, and the small amount of material required, the refractometry technique of analysis was employed to analyze the total protein concentration in fish, following Bigbee,s (2004) procedure.

The blood sample was centrifuged at 600 × *g* for 5 minutes to separate the serum. A portable refractometer (JSCP-Uridens(r), Sao Paulo, Brazil) was used to quantify total serum protein content. A drop (10µl) of serum was placed in the portable refractometer, and the total serum protein content expressed as grams per deciliter (gdL^{-1}) was read. Before each series of measurements, the refractometer's calibration was confirmed using purified water. All measurements were taken at room temperature (about 20^{o}C). The concentration of serum protein was reported as a mean ± standard error ($g\ dL^{-1}$).

GraphPad InStat (version 3.00, GraphPad InStat Software Inc. 200) was used for the analysis. A paired t-test was used to compare the statistical differences between the control and the various treatments. A $P < 0.05$ was deemed significant.

Analyzing Reduced Glutathione (GSH)

Glutathione (GSH), a tripeptide with thiols (glutamyl-cysteinyl-glycine), is a crucial antioxidant in many species. It has been connected to both the maintenance of protein sulfhydryl group oxidation states and the detoxification/elimination of xenobiotics. In addition, GSH plays a role in the development of some human diseases, including as cancer and heart disease. Both reduced (GSH) and oxidised (GSSG) forms of glutathione are present in cells, with GSH being the more prevalent form under physiologically normal circumstances. A modified version of Koyuncu *et al.* (2017). formula .'s was used to calculate the amount of GSH Using a microplate reader (Spectra max M5, USA) with excitation at 345 nm and emission at 425 nm as the reference, GSH samples were found. The serum values were displayed as nmol/ml.

Alanine Amino Transferase Assay

Colorimetric analysis was used to determine alanine aminotransferase activity. Blood plasma was centrifuged to remove red blood cells and then used as an enzyme supply. Specifically, the enzyme reaction mixture had 400 mM alanine, 210 mM a-keto glutarate, 2.5 mM arsenate, and 20 mM TRIS-HCl pH7.5. Dinitrophenyl hydrazine diluted to 1% in 2N hydrochloric acid was used to stop the reaction. The absorbance was then measured at 440 nm after an appropriate quantity was added to NaOH 1.3N. The Activities of ALAT is expressed in UI

Determination of Albumin and Globulin the Blood

Albumin is the protein found in the serum in the highest concentration. It is made in the liver and is renowned for changing the arrangement. Albumin may transport a wide range of substances, including bilirubin, fatty acids, uric acid, many medications, and antibiotics because of its steric affinity. To maintain a healthy osmotic pressure, albumin is also necessary. Elevated serum albumin concentrations are associated with dehydration. Low serum and albumin levels can indicate rheumatoid arthritis, liver disease, kidney issues, and malnutrition.

The amount of albumin in the fish serum was determined using the BCG (Bromocresol Green) albumin assay kit, and absorbance was measured at 628 nm in comparison to a blank. Albumin was subtracted from the total protein content to get the globulin content.

Statistical Analysis

Data were analyzed using the Minitab Statistical Computing System, SAS (SAS Institute Inc, 1985), SPSS version 16.0 (Chicago IL, USA), Microsoft Excel 2010 (Roselle, IL, USA), and SPSS, version 10 (SPSS, 2016) software were used for the statistical and graphical evaluations. The least significant difference (LSD) was determined using post hoc testing for treatments and control group comparisons at a probability level of 0.05% and 0.01%.

RESULTS & DISCUSSION

The protein

The protein level on day 2 in the treated fish irrespective of the treatment in comparison with the control (Fig.1). On days 9, 16, 23, and 30, the protein level decreases with an increase in the concentration of LABS. The protein concentration varies significantly ($p<0.05$) between the control and all the treatments on days 23 and 30.

Responses of the GSH in the Fish Tissue

The responses of the GSH in the tissue of *C.gariepinus* stimulated with various concentrations of linear alkylbenzene sulfonates is shown in Figure 3. Forty-eight hours of exposure showed a slight inhibition in the activity of the enzyme, and its activities in the liver at 0.05,0.1, 0.15, and 0.20mg/l of the toxicant were 110.10,105.82, 101.32, and 100.39 nanomoles/100 fwt respectively. In the gills, at the same concentrations, the activities were;94.30,92.10,92.56 and 86.17 nanomoles/100 fwt. In the kidney, the activities were; 88.19,82.70,81.18 and 79.89 nanomoles/100 fwt. No significant ($p \geq 0.05$) changes in the enzyme activities in all the treatments when compared with the control.

Inhibition on day 30th followed a similar pattern as that of day 2nd, the highest inhibition (72 nanomoles/100 fwt) was observed at 0.20mg/l of LABS, in the liver while the least inhibition was detected in the kidney (83.19 nanomoles/100 fwt), at 0.05mg/l LABS. No significant ($p \geq 0.05$) between the control and various treatments at the lower concentrations of 0.05mg/l and 0.1mg/l LABS. Nevertheless, the inhibition was highly significant ($p \leq 0.05$, $p \leq 0.01$) at 0.15 and 0.20mg/l LABS only in the liver's tissue (Figure)

Considering the three tissues, in the control group GSH activities was highest in the liver and least in the kidney, and the percentage inhibition followed the same pattern. Liver (63.21%), gills (52.56%) and the kidney (42.19%)

Alanine Aminotransferase Activity

The activity of alanine aminotransferase in the plasma of *C.gariepinus* exposed to sublethal concentrations of linear alkylbenzene sulfonates is shown in Figure 3. The changes in the activity of the enzyme is time and concentration-dependent. At 0.05mg/l of LABS, on day 2nd, 9th ,16th 23rd and 30th, the activity of the enzyme in (U/ml) are 10.40 ± 0.30, 5.11,15.50 ± 0.41,

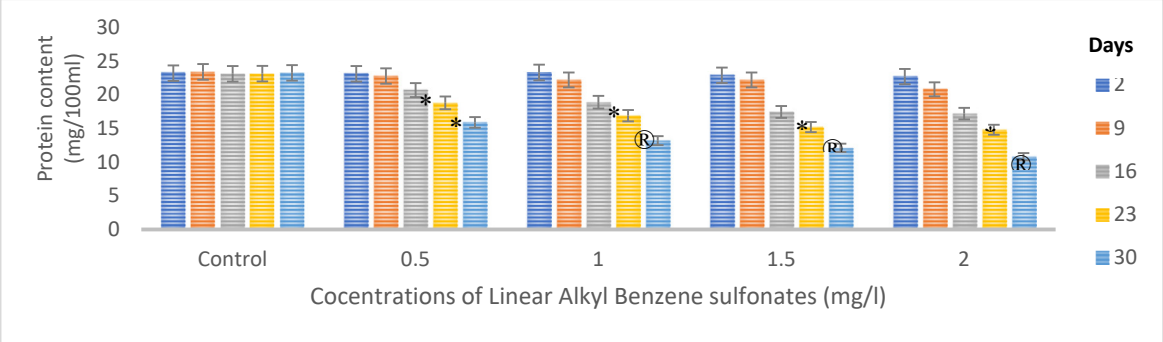

Fig. 1. Protein content in the sera of *C.gariepinus* exposed to different concentrations of Linear Alkyl Benzene sulfonates.

Table 1. Responses of albumin and globulin to linear alkylbenzene sulfonates intoxication in the serum of C. gariepinus

	Albumin (mg/100ml)		Globulin(mg/100ml)	
	Day 2	Day 30	Day 2	Day 30
Cons (mg/l)	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
0.00	$9.10 \pm 0.30^{*}$	$9.00 \pm 0.20^{*}$	$4.20 \pm 0.10^{*}$	$4.00 \pm 0.20^{*}$
0.05	$8.90 \pm 0.00^{*}$	$8.10 \pm 0.02^{*}$	$3.90 \pm 0.02^{*}$	$3.20 \pm 0.73^{*}$
0.10	$8.60 \pm 0.20^{*}$	$8.00 \pm 0.01^{*}$	$3.50 \pm 0.01^{*}$	$2.0 \pm 0.02^{*}$
0.15	$8.20 \pm 0.30^{*}$	$3.08 \pm 0.20^{\beta}$	$3.40 \pm 0.40^{\beta}$	$1.70 \pm 0.01^{\beta}$
0.20	$8.50 \pm 0.30^{*}$	$3.00 \pm 0.01^{\beta}$	$3.10 \pm 0.03^{\beta}$	$1.20 \pm 0.03^{\beta}$

*Not significant ($p \leq 0.05$) *; β: significant ($p \leq 0.05$) $^{\beta}$

21.40 ± 1.70, 32.90 ± 2.11, and 43.50 ± 1.89 respectively. At 0.10mg/l, the activity on day 2nd to 30th are; 10.80 ± 1.10,18.40 ± 0.60, 24.20 ±3.10, 42.10 ± 0.70 and, 57.32 ± 2.47. at 0.15mg/l of the toxicant, the enzyme activity on the days are 11.90 ± 1.12, 22.40 ± 0.04, 32.10 ± 0.50,54.20 ± 0.50, and 63.80 ± 1.13. At 0.20 mg/l of the inducer chemical, ALAT activity on days 2nd to 30th are 12.40 ± 2.60, 28.12 ± 3.10, 36.10 ± 0.58, 59.10 ± 3.10, and 70.23 ± 4.60 (Figure 1).

On days 2 and 9th at 0.05mg/l, the enzyme activity varies significantly ($p < 0.05$), between the various treatments and the control, while in other treatments and irrespective of the day, the activity was highly significant ($p < 0.01$), when compared with the control (Fig.1)

Responses of Albumin and Globulin in the blood of the fish to LABs Treatments

The fish exposed to varying concentrations of LABs show a decreasing level of albumin and globulin with an increase in concentrations and exposure duration.

On day 2, the albumin content slightly decreases with concentrations and no significant ($p \geq 0.05$) difference between the treatments and the control. In the long exposure (day 30th), the obvious changes in albumin level were recorded at 0.15mg/ and 2.0mg/l of LABS, and were (3.00±0.01) mg/100ml (3.08±0.20) mg/100ml respectively, and were significant ($p \leq 0.05$), when compared with the control

The globulin contents for both days were inhibited and varied significantly ($p \leq 0.05$) at 0.15 and 0.20mg/l of the toxicant when compared with the control (Table 1)

The present study examines the impact of exposure period and surfactant concentrations on the protein content of fish treated with Linear alkylbenzene sulfonates. It was observed that both exposure duration and surfactant concentrations were associated with a decrease in protein content. This decrease may be linked to metabolic degradation. It is possible that exposure to toxicants, which induce stress in the fish, stimulates proteolysis rather than protein synthesis.

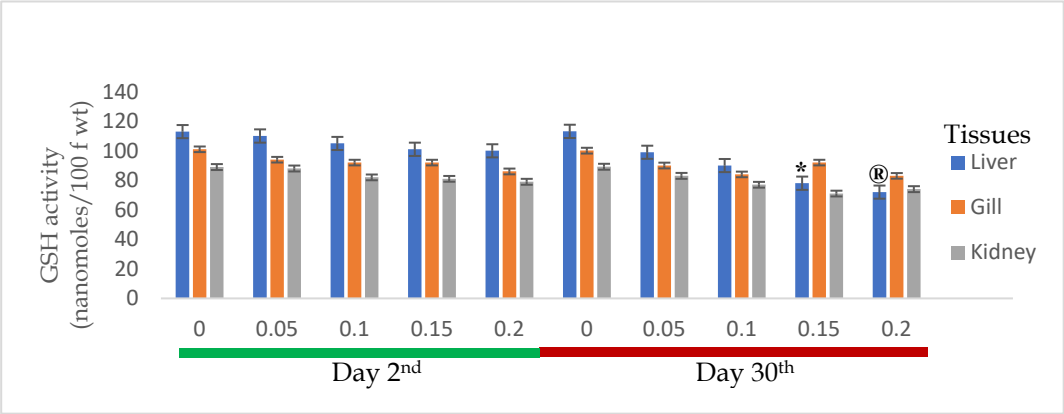

Fig. 2. Activities of the GSH in the tissues of *C.gariepinus* exposed to sublethal concentrations of linear alkylbenzene sulfonates (mg/l); A symbol above the bars indicates significant $^{*}(p \leq 0.05)$; $^{®}$ $(p \leq 0.01)$ different between the control and various exposures

This could be attributed to the absence of transcription processes mediated by nucleic acids. In a similar vein, the need for increased energy synthesis through proteolysis and gluconeogenesis contributed to a reduction in protein content, mirroring the findings of the current study. Anitha *et al*. (2010) made a comparable discovery, observing a reduction in the overall protein content of *Labeo rohita, Catla catla,* and *Cirrhinus mrigala* fish species when exposed to the toxicant fenvalerate. According to the findings of Sirohi (2007), the protein content in the muscles and liver of *Channa punctata* fish exhibited a decline after a 30-day exposure to permethrin. The fish were subjected to concentrations of the toxicant at 0.15 ppm, 0.20 ppm, and 0.25 ppm. This resulted in a reduction of 20.69%, 31.81%, and 43.94% in liver tissue, and 26.22%, 33.07%, and 49.33% in muscle tissue, respectively. These findings align with the current investigation. Similarly, in a study conducted by Adeyemi (2014), the author examined the biochemical responses of *C. gariepinus* to cypermethrin and discovered a decrease in the total proteins of the muscle and liver as a result of the exposure to cypermethrin.

The activity of glutathione in the fish examined was influenced by the presence of Linear Alkylbenzene Sulfonates in the liver, gill, and kidney, with the liver exhibiting the most pronounced effect. The potential impact of the toxicant on the liver may arise from its essential role in the detoxification process of harmful substances. GSH is a prominent sulfhydryl molecule with low molecular weight found in the cytoplasm. It functions as a cellular reductant and protective agent against various contaminants using its SH-group (Sayeed *et al*., 2003). According to Li *et al.* (2010), it functions as a scavenger of oxyradicals and serves as a substrate for antioxidant enzymes. Glutathione plays a crucial role in safeguarding cells against the harmful consequences of reactive oxygen species by engaging in a reaction with them, resulting in the formation of glutathione disulphide (GSSG). The antioxidant defense effect is observed to occur spontaneously by the action of glutathione or through the involvement of glutathione S-transferase (Zhang *et al.,* 2004) Glutathione transferase is facilitated by this compound, serving as a cofactor, to aid in the elimination of certain chemicals and reactive compounds from cellular environments (Cheung *et al.,* 2001). Therefore, alterations in the levels of glutathione may serve as a significant signal of an organism's capacity for detoxification. The depletion of GSH signifies the progression into phase II biotransformation, leading to an increased susceptibility to oxidative stress as a result of diminished cellular protective activity (Monteiro *et al*., 2006). In the current experiment, it was observed that the depletion of GSH was most pronounced in the liver tissue compared to the gills and renal tissues. This depletion of GSH in

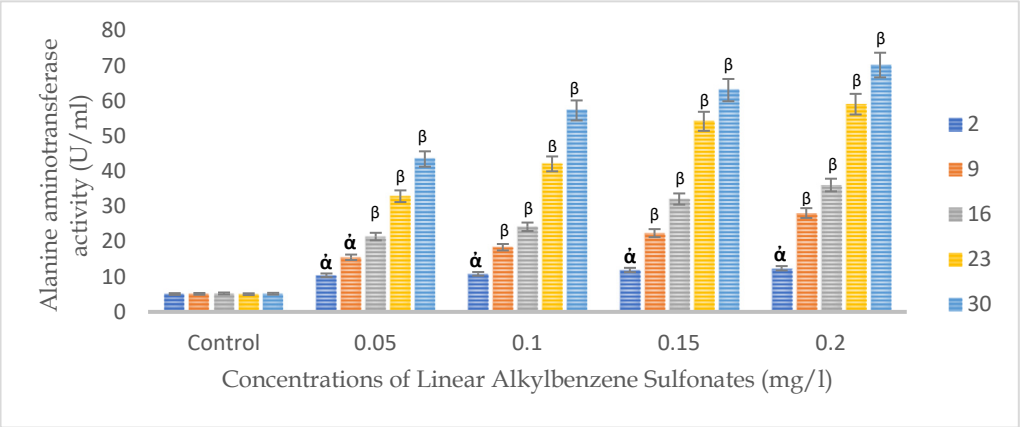

Fig. 3. Plasma alanine aminotransferase activity in *C.gariepinus* subjected to sublethal linear alkylbenzene sulfonates. Data reported as mean ± SE. Bars with symbols above them show significant differences between control and experimental groups.($p<0.05$); β ($p < 0.01$

the liver tissue led to cellular deterioration (Yonar and Sakin, 2011). A decline in liver and gill function in Cyprinus carpio (Rao, 2006) and Carassius auratus (Sayeed *et al.,* 2003) has been documented as a result of pesticide exposure.

The enzyme alanine aminotransferase is crucial in the process of amino acid synthesis and deamination, particularly during periods of stress, to meet the organism's heightened energy requirements (Fallah *et al.,* 2005). In a similar vein, hepatic cells possess a significant abundance of aminotransferase enzymes due to the liver's pivotal role as the primary organ responsible for the interconversion of dietary substances. The findings of this study demonstrate a statistically significant elevation in alanine aminotransferase levels in the serum of fish exposed to sodium lauryl ether sulphate, as compared to the control group. The observed elevation in alanine aminotransferase activity suggests the presence of tissue injury, potentially resulting from disruptions in typical physiological and biochemical pathways, such as the Krebs cycle and tricarboxylic acid cycle. Consequently, this enzymatic leakage from the liver cytosol over the membrane into the bloodstream may occur. The current findings align with the observations made by Jee *et al.* (2005), wherein elevated serum ALT activity was noted in *Sebastes schlegeli* following exposure to cypermethrin. The heightened activity of alanine aminotransferase in teleostean fishes due to pesticide exposure has been noted by multiple authors (Begum, 2004). The increased activity of alanine aminotransferase facilitated the production of oxaloacetic acid and pyruvate, enabling the fish to satisfy the augmented energy requirements induced by the stress caused by carbofuran as concluded by Samanta *et al*. (2013) in their investigation on *Clarias batrachus*

Albumin is the primary protein responsible for maintaining the essential colloid osmotic or oncotic pressure, which governs the movement of water and diffusible solutes across capillaries. It constitutes approximately 70% of the colloid osmotic pressure. The function of this entity encompasses the transportation of bilirubin, hormones, metals, vitamins, and medications. Additionally, it plays a crucial part in the metabolism of fats by effectively binding fatty acids and maintaining their solubility within the plasma. Moreover, under normal blood pH conditions, albumin exhibits a negative charge and an affinity for cations, particularly Na+ within the vascular compartment. Additionally, owing to its negative charge, albumin is capable of providing some anions required to maintain a balance with the plasma cations. This study examines the impact of sublethal doses of linear alkyl benzene sulfonates on the albumin level in *C.gariepinus*. The results indicate a significant decrease in albumin levels, with a clear gradient observed in both doses and concentrations ($p < 0.05$). The reduction in albumin synthesis may

be ascribed to the stress induced by the detergent on the fish's normal physiological functions, exhibiting similarities to other forms of distress such as end-stage liver disease, intestinal malabsorption syndromes, and protein-calorie malnutrition (Saparuddin *et al.,* 2020).

The treated fish exhibited lower quantities of globulin compared to the control group, and a similar pattern was observed for albumin levels. The observed decline in globulin levels during consecutive days of exposure may potentially be attributed to external causes, such as the impact of a hazardous environment. According to Reddy (2002), the reduced levels of globulin and serum enzyme activity observed in *C.gariepinus* exposed to sub-lethal concentrations of detergent may be attributed to the pollution-induced stress experienced by the fish. This stress could lead to the mobilization of proteins from muscle tissue to the bloodstream, as a compensatory mechanism to counteract the acidosis resulting from lactate accumulation (Fogarty *et al*., 2019).

CONCLUSION

These findings support the notion that LABs modify fish physiology and have a significant impact on catfish hormonal profiles and particular growth rates. This study had shown that exposure to LABs can have major negative impacts on human health as well as cause serious harm to human life. Since anthropogenic sources are the primary causes of LAB exposure, responsible authorities must enact stringent mitigation measures to manage this hazard.

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CONFLICT OF INTEREST

The author confirms that no conflicts of interest related to the submission of this paper for publication. The author has also avoided any unethical practices including plagiarism, lack of informed consent, misconduct, fabricated or falsified data, multiple submissions, or unnecessary repetition of work.

LIFE SCIENCE REPORTING

This research did not involve the application of any life science threat.

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