Chronic impact of linear alkyl benzene sulphonate (LAS) on some biochemical enzymes in the gills of Clarias gariepinus adult.

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**Abstract**

Sub lethal impacts of linear alkyl benzene sulphonate (LAS) on some biochemical enzymes in the gills (organ for gas exchange, ionic regulation, acid-base balance, and nitrogenous waste excretion by fishes) of Clarias gariepinus adult were studied. Aspartate aminotransferase (AST), Alanin aminotrasminase (ALT), Acid phosphatase (ACP) and Alkaline phosphatase(ALP) were measured in the gills during a 30-day exposure to chronic concentrations (10.00, 20.00, 30.00, 40.00 and 50.00 mg/l) of linear alkyl benzene Sulphonate (LAS). Generally, the impact of LAS on the selected enzymes in the gill decreased Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities and raised Alanin aminotrasminase (ALT) and Acid phosphatase (ACP) activities with increase in detergent concentration when compared to their respective controls.

**Key words: Acid phosphatase (ACP), Alanin aminotrasminase (ALT), Alkaline phosphatase(ALP), Aspartate aminotransferase (AST), Linear- alkyl benzene Sulphonate (LAS)**

**Introduction**

Low-level mixture of various toxic chemical has unpredictable effects on fish. Sometimes they are additive, antagonistic or synergistic. Detergents are among those chemicals that can act synergistically with other chemicals in the aquatic environment, making aquatic organisms more vulnerable to other more toxic compounds such as petroleum products, pesticides, chloramines and heavy metals (Jobling and Sumpter, 1993). It should be noted that fish live in equilibrium with their external environment and must continually adjust to natural changes in the environment through a variety of adaptive physiological and biochemical mechanism to maintain their internal equilibrium (Henderson et al., 1981). The capacity to adapt to human disruptive changes in the environment is limited and the toxic effects of pollutants may affect all levels of animal organization from the cell to entire organism including biochemical processes, cell integrity, organ physiology, growth and reproduction (Stephanuo and Giger, 1982). Surfactants generally impact on higher aquatic organisms via their respiratory structures. In higher organisms such as fish, the respiratory structures (gills), consists of epithelial membranes that may be extensively folded to provide large surface areas for gaseous exchanges. Destabilization of these epithelial membranes, as may occur when exposed to surfactants, results in changes in membrane permeability, cellular lysis and impairment of cellular respiration (Mc Williams and Payne, 2001). Surfactants can be toxic to aquatic life at concentrations as low as 0.025mg/l (Chattopadhyay and Konar, 1985; Whitehouse et al., 1998). However, the quality of linear alkylbenzene sulphonate (LAS) in the environment as well as the duration of degradation (1-5 days) is useful in determining the effects.

**Materials And Methods**

Thirty adults (mean weight, 850.00± 10.22g SD; mean length 29.20± 7.12 cm SD.) of C. gariepinus were obtained from Abdul’s Fish Farm Rukpakulusi and transported by car in a 20L trough covered with a perforated cover to the Fisheries laboratory of the Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, Port Harcourt. On arrival, the 30 specimens were acclimated in 30 rectangular plastic aquaria filled with twenty litre of water each for 7 days. The aquaria were washed with a piece of foam and fish fed once daily with a 42% crude protein diet at 2% body weight. A range finding test (trial test) was carried out using Linear alkyl benzene Sulphonate detergent for 10 days at varied (0.00, 10.00, 18.00, 25.00, 37.00, 50.00 mg/l) concentration levels. Sub-lethal
concentrations of 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l for the definitive test were obtained based on the range finding test. Five graded concentrations - 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l of the solutions were prepared and thoroughly mixed to avoid hot spot in five replicates each. A fish was introduced into each aquarium and covered with a perforated plastic lid to prevent escape of fish. To avoid injuries or bruises on experimental fish, a scoop net was used daily to collect and transfer fish to buckets low level water until aquaria with solution were ready. Ammonia-nitrogen, alkalinity, conductivity, dissolved oxygen, water pH, temperature and turbidity were the physicochemical parameters considered in this study. At the end of the investigation period, fish were killed with a blow on the head and dissected in order to collect 0.5g of gill with the aid of penknife. Samples were macerated with pestle and mortar. Alkaline phosphatase, Alanineaminotransferase (ALT), Aspartate aminotransferase (AST), Acid Phosphatase (ACP) were determined in the gills using standard methods.

Results

The mean values of alkalinity, conductivity, turbidity, ammonia and temperature were raised while pH and dissolved oxygen decreased with increase in detergent concentration when compared with control (Table 1). Linear alkyl benzene Sulphonate (LAS) declined aspartate aminotransferase (AST) activities with increase in detergent concentration in the gill when compared with control (Table 2). At 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l, LAS respectively decreased AST activities by 18.32% (195.00±8.24 IU/L), 24. 82% (179.50±10.00 IU/L), 32.77% (160.50±7.00 IU/L), 37.49% (149.25±7.50 IU/L) and 54.03% (109.75±8.04 IU/L) when compared with control, 100% (238.75±10.11 IU/L). ALP activities in the gill were also decreased by 51.86% (287.50±9.24 IU/L) at 10.00mg/l, 58.14% (250.00±9.22) at 20.00mg/l, 64.42% (212.50±10.00) at 30.00mg/l, 69.86% (180.00±6.11) at 40.00mg/l and 74.47% (152.50±7.12) at 50.00mg/l with increase in detergent concentration when compared with control, 100% (597.25±10.24). Generally, LAS, respectively raised: ALT activities in the adult fish gill by 7.69%, 21.79%, 30.77%, 51.28% and 64.10% when compared with control and ACP activities by 20.00%, 35.20%, 58.00%, 68.00% and 101.20% when compared with control. Generally, the impact of LAS on: AST activities at 20.00, 30.00, 40.00 and 50.00mg/l were respectively, 6.5% 14.45%, 19.17% and 35.71% < that at 10.00mg/l (18.32%); ALP activities at 20.00, 30.00, 40.00 and 50.00mg/l were respectively, 6.28%, 12.56%, 18.00% and 22.86% < that at 10.00mg/l. Also, the impact of LAS on: ALT activities at 20.00, 30.00, 40.00 and 50.00mg/l were respectively, 14.1%, 23.08%, 43.59% and 56.41% > that 10.00(7.69%); ACP activities at 20.00, 30.00, 40.00 and 50.00mg/l were respectively, 15.20%, 38.00%, 48.00% and 81.20% > that at 10.00mg/l(20.00%).

Discussion

The water parameters considered in this work were within the tolerance ranges of warm water fish species (Adeniji and Ovie, 1989; Boyd, 1979; EIFAC, 1977; EPA, 1976; Okey, et al., 2013). This suggests that the parameters did not seem to alter the chronic impact of LAS to test fish; hence, it might not have contributed to the observed enzymatic changes of the test fish species in this study. This agrees with the work of Onusiriuka and Ufodike (1994) who exposed C. gariepinus to Akee apple and sausage plant extracts and reported no significant difference (p > 0.05) in the water quality parameters analyzed. Low AST and ALP activities suggest a decrease in energy demand, metabolic pathway and amino acids. Decrease in ALP activity in the organ could be attributed to a fall in the synthesis of glycogen caused by lowered metabolic demands and also due to electrolyte imbalance caused by tissue overhydration (Shaffi, 1975). However, in other studies (Ayalogu et al., 2001; Svoboda et al., 2001; Tiwari and Singh, 2004) an increase in the activities of AST and ALT was recorded indicating that there was an increased demand for energy due to tissue impairment. The increase in ALT activity in this study suggests a shift towards anaerobiosis as a consequence of hypoxia under toxicant impact leading to respiratory distress (Siva, 1980) as the enzyme is involved in osmoregulation (Tiwari and Sigh, 2004). Depending on the level, toxicants or pesticides inhibit energy production by suppressing aerobic oxidation of carbohydrate leading to energy crisis in animals (Kohli 1995; Obomanu, 2009). In the gill, under the influence of the toxicant, both aerobic and anaerobic conditions are likely to operate depending on the availability of molecular oxygen and other physiological needs. In this study, the general inhibition of ALT and ALP imply that there was no cell damage but a disruption of the activity of the TCA cycle, respiratory process and glycolytic pathways. The pattern of response of AST, ALT, ACP and ALP activities in the gill to the toxicant seem to suggest that the excitation or inhibition of their activities is organ specific. They are concentration dependent. Besides, the trends (mostly inhibition in AST and ACP) below the control level suggest the fish was not able to detoxify, bio-transform nor excrete the toxin within the 30 days. The major biochemical response to the effect of detergents in fishes is the inhibition of the activities of a number of enzymes such as AST, ALT, ACP, and ALP (Abel, 2006). Detergents disrupts the nervous structure of fish by reducing the activity of enzymes at nicotinic and musculanic receptors (Misra et al., 1991). Dropping the activity of these enzymes allows a protracted effect of acetylcholine, a neurotransmitter, on the receptors. The disabling of enzymes can have a toxic effect leading to harmful physiological effects or even fatality at a certain concentrations. Detergent surfactants restrain acetylcholine esterase in the brain of Clarias gariepinus (SETAC, 1997) and also 80% inhibition in the neural tissue of Tilapia guineensis following acute effect of detergent (Ezemonye et al., 2007). Zaccane et al. (1985) checked the toxic effects of ionic active detergent, LAS on fish for some days and observed a decrease in the activity of the enzymes in the plasma. In this study, the activities of AST and ALP decreased significantly which indicate cellular toxicity of the detergent even at low doses under prolonged period of exposure. This disagrees with the findings of Swisher (1975) who reported that the activity levels of
AST and total adenosine triphosphate (ATPase) in muscles, gills, liver and brain of T. mossambica exposed to detergent showed that transaminases were elevated in all the tissues in addition to a shift in aminotransferases reaction due to the impact of surfactants. Transamination is one of the principal pathways for the synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during irregular energy demands under various adaptive conditions (Chetty et al., 1980). Maintenance of internal homeostasis through biochemical processes in the Krebs cycle may be reflected in varying levels of enzymes AST, ALT, ALP in the serum (plasma) occasioned by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Heath, 1991; Uedeme-Naa, 2015.). This work partially agrees with the work done by Gabriel and George (2005) who reported that serum AST and ALT are raised when disease process affects cell integrity in that LAS in this work depressed serum AST and ALP. Giboney, (2005) observed that Phosphates (ALP and ACP) and transferases (AST and ALT) tests are part of standard laboratory tests to detect health abnormalities in animals. Alterations in these enzymes (protein that regulate the rate of a chemical reaction in the body) activities of fish resulting from toxicant effects in various organs of fish have been reported (Begun, 2004). Importantly, biochemical changes in fishes are aimed at stabilizing equilibrium in the presence of toxicants, which are known to disrupt physiological and biological processes (Wedemeyer and Mcleay, 1981). The transaminases are a group of enzymes catalyzing interconversion of amino acids and α - ketoacids by transfer of amino groups and elevate activities of these tissues. The exposure of gill AST, ALT, ACP and ALP enzymes to detergent in this work resulted in alterations in their activities which agree with the findings of Ozoret et al., 2008, who reported that when hepatocytes are damaged, enzymes normally located in cytosol are liberated into the extra cellular space and enter into circulation due to membrane defects causing elevated permeability. Detergent induced alterations in aspartate aminotransferase and alkaline phosphatases activities have been reported in fish and this elevation was directly attributed to toxic action of detergent on gill (Agrahari et al., 2007). Datta et al., (2009) observed elevated level of AST and ALT in C. batrachus exposed to detergent at sublethal concentration. This is also in partial agreement with this work, where AST was decreased and ALT was raised with increase in detergent concentration. Detergent can inhibit the activities of many enzymes especially those involved in the cellular glucose uptake, glucogenesis, fatty acid oxidation and production of glutathione due to its sulfhydryl group binding capability (Arthur, 1970).

### Table 1: Water Quality Variables (Mean ± S.D) in the Experimental tanks for adult fish during exposure period.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Concentrations (mg/l)</th>
<th>10.00</th>
<th>20.00</th>
<th>30.00</th>
<th>40.00</th>
<th>50.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia (mg/l)</td>
<td>0.8±0.15a</td>
<td>1.17±0.17b</td>
<td>1.49±0.23c</td>
<td>1.20±0.08b</td>
<td>1.28±0.19a</td>
<td>1.37±0.21c</td>
</tr>
<tr>
<td>Alkalinit(mg/l)</td>
<td>52.0±10.00b</td>
<td>77.00±11.20b</td>
<td>87.00±13.02b</td>
<td>93.75±16.00a</td>
<td>99.50±28.82ae</td>
<td>105.00±20.03e</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>3.4±2.31a</td>
<td>3.02±1.31b</td>
<td>3.45±0.36c</td>
<td>3.55±2.31a</td>
<td>3.70±2.33a</td>
<td>3.70±1.91a</td>
</tr>
<tr>
<td>pH</td>
<td>6.6±0.57b</td>
<td>6.21±0.22a</td>
<td>6.13±0.20c</td>
<td>5.84±0.23b</td>
<td>5.56±0.21b</td>
<td>4.89±0.24b</td>
</tr>
<tr>
<td>Conductivity(S/m)</td>
<td>25.1±5.17c</td>
<td>26.50±18.32b</td>
<td>34.50±21.22c</td>
<td>37.00±23.01c</td>
<td>340.75±23.23c</td>
<td>472.75±25.00c</td>
</tr>
<tr>
<td>Turbidity (mg/l)</td>
<td>23.0±6.11a</td>
<td>42.75±6.20b</td>
<td>67.50±7.01b</td>
<td>77.25±7.04b</td>
<td>120.0±8.02c</td>
<td>130.0±9.23c</td>
</tr>
<tr>
<td>D/O (mg/l)</td>
<td>5.98±0.99b</td>
<td>4.32±0.54a</td>
<td>3.58±0.34e</td>
<td>3.21±0.30c</td>
<td>2.56±0.28b</td>
<td>2.34±0.25b</td>
</tr>
</tbody>
</table>

Means within the same column with different superscripts differ significantly (P<0.05).

### Table 2: Activities of selected enzymes in the gills of C. gariepinus adult exposed to jumbo detergent for 30 days (Mean ± S.D)

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>AST (IU/L) % Control</th>
<th>ALT (IU/L) % control</th>
<th>ACP (IU/L) % control</th>
<th>ALP (IU/L) % Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>238.75±10.11a</td>
<td>100</td>
<td>19.50±2.00a</td>
<td>100</td>
</tr>
<tr>
<td>10.00</td>
<td>195.00±8.24a</td>
<td>-18.32</td>
<td>21.00±1.05a</td>
<td>+7.69</td>
</tr>
<tr>
<td>20.00</td>
<td>179.50±10.00b</td>
<td>-24.82</td>
<td>23.75±2.01a</td>
<td>+21.79</td>
</tr>
<tr>
<td>30.00</td>
<td>160.50±7.00b</td>
<td>-32.77</td>
<td>25.50±5.10b</td>
<td>+30.77</td>
</tr>
<tr>
<td>40.00</td>
<td>149.25±5.00b</td>
<td>-37.49</td>
<td>29.50±3.22b</td>
<td>+51.28</td>
</tr>
<tr>
<td>50.00</td>
<td>109.75±8.04a</td>
<td>-54.03</td>
<td>32.00±4.32c</td>
<td>+64.10</td>
</tr>
</tbody>
</table>

Means within the row with different superscripts (a,b,ab,c,d) are significantly different (P<0.05)

### Conclusion

The present work indicates that linear alkylbenzene sulfonate causes considerable alterations in enzymes activities and is likely to induce tissue damage in C. gariepinus. Therefore, detergents should be handled with care and prevent its entrance into aquatic surroundings.

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