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Comparative impacts of linear alkyl benzene sulfonate (LAS) in the liver enzymes of *Clarias gariepinus* juvenile and adult

Uedeme-Naa B and George ADI

Abstract
The impact of a commercial detergent containing Linear alkyl benzene Sulfonate, a household cleaning agent was investigated to determine to what extent it alters liver enzymes of *Clarias gariepinus* with mean weight of 246.30± 14.12g SD and mean length of 16.15±1.40cm SD for juveniles and mean weight of 850.00±10.22g SD and mean length 29.20± 7.12 cm SD for adult. After series of range finding test, the two life stages were exposed to chronic concentrations of 10.00, 20.00, 30.00, 40.00 and 50.00mg/l of detergent for 30 days in a renewable bio assay. It was observed that detergent respectively raised AST activities in the liver of juvenile fish by 35.52 and 33.20% at 10.00 and 20.00mg/l higher than that of adult fish while the reverse was observed at 40.00mg/l where that of adult fish was 138.47% higher than that of juvenile fish. At 30.00 and 50.00mg/l, AST activities in juvenile fish were respectively 27.85 and 6.98% less than control whereas that of adult fish was 93.18 and 190.90% higher than control. ALT activities in adult fish were respectively 27.52, 49.08, 55.96, 63.30 and 63.30% lower than control at 10.00, 20.00, 30.00, 40.00 and 50.00mg/l and that of adult was higher than that of control by 45.00% at 10.00mg/l; 10.00% at 30.00mg/l, lower than control by 60.00% at 20.00mg/l and same with control at 40.00mg/l. At 10.00, 20.00 and 30.00mg/l, ACP activities were respectively 90.93, 31.97 and 2.95% while at 40.00 and 50.00mg/l, it was 40.44 and 42.30% higher than that of control in adult fish. ACP activities in juvenile fish was same with control at 40.00 and 50.00mg/l. ALP activities in the liver of juvenile fish was 31.90 and 46.68% higher than that of adult fish whereas at 30.00, 40.00 and 50.00mg/l, it was respectively 34.65, 42.07 and 48.26% higher than control in the adult fish and 49.97, 50.00 and 49.64% lower than control in juvenile fish.

Keywords: Linear alkyl benzene Sulfonate (LAS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP)

Introduction
Many studies have shown that biochemical changes occurred in fishes that were exposed to environmental contaminants (Rao, 1989) [30]. Changes due to these environmental pollutants including detergents and their metabolites have necessitated studies to determine the effects of detergents in the aquatic environment on biochemical parameters in fish (Adams *et al*., 1996) [1]. According to Hinton and Lauren (2010) [16], fish liver microscopic structure is an indicator of physiological and biochemical function which, when altered, may produce biomarkers of prior exposure to toxicants. The liver has a key role in xenobiotic metabolism and excretion, digestion and storage, and the production of yolk protein. Thus, alterations in structure are expected under certain toxic conditions. These specific functions were summarized in more detail by Hinton *et al.* (1992) [17] as follows: 1. the liver of teleosts is the major site of the Cytochrome P450-mediated, mixed- function oxidase system (Stegeman *et al.*, 2009) [34]. This system inactivates some xenobiotics, while activating others to their toxic forms; 2. Nutrients derived from the gastro-intestinal absorption are stored in hepatocytes and released for further catabolism by other tissues (Walton and Cowey, 1982; Moon *et al*., 1985) [41, 20]; 3. Bile synthesised by the hepatocytes (Schmidt and Weber, 1973; Boyer *et al*., 1976) [35, 3] aids in the digestion of fatty acids and carries conjugated metabolites of toxicants (Gingerich, 1982) [12] into the intestine for excretion or entero-hepatic recirculation; 4. The yolk protein vitellogenin, destined for incorporation into the ovum, is synthesized entirely within the liver. Receptors in the liver must bind the hormone, estradiol, for initiation of the signal to begin synthesis of this reproductive component. According to Brusle and Gonzalez (2004) [5], many environmental

parameters can alter liver structure and metabolism including pollutants (e.g. metals, pesticides, hydrocarbons, PCB), food (quantity and quality), biotoxins (algae and fungi), parasites, infectious germs (virus and bacteria) and physiochemical parameters (e.g. pH, oxygen, temperature etc.). Liver histology has been proven to be indicative of exposure to pollution (e.g. Barnhoorn *et al*., 2004) [6]. Pathological changes that are associated with fish liver according to Takashima and Hibiya (2011) [37] include cloudy swelling atrophy, necrosis, vacuolar degeneration, fatty degeneration, bile stagnation, hepatitis cirrhosis, congestion and tumours. The main substances stored in fish liver are glycogen and to a lesser extent lipid. Glycogen particles may be found scattered in the cytoplasm or aggregated in large concentrations. Though lipid materials are not uncommon in fish liver, intensive accumulation is more often found in aqua-cultured fish, revealing nutritional inadequacy of artificial feeds. Lipids can be found in small to medium-sized droplets interspersed with the organelles in the parenchymal cells, or nearly filling the cytoplasm of so-called fat-storing cells. In general, the presence of toxicants in aquatic media exerts its effect at cellular or molecular level which results in significant changes in biochemical parameters. Due to metal complex formation, normal functioning of cell is disturbed and that in turn may result in variation on physiological and biochemical mechanisms of animals (Gagnon *et al*., 2006) [11]. Bielinska (1987) [7] also reported that Sodium hypochlorite; a common bleaching agent of detergents enhances Lipid Peroxidation in blood lipoproteins and phospholipid liposomes. A comparative study conducted by Panasenko *et al*. (1995) [26] on the toxicity of some commercial detergents on Nile Tilapia, *Oreochromis niloticus* revealed their adversity to survival of the species. Study on the role of linear alkyl benzene sulfonate using the common detergent "Henko" on *Puntius ticto* revealed the histopathological lesions on gill archs,rakers and gill filaments (Omotoso and Fagbenro. 2005) [23]. Study on Toxicological impact of house hold detergent "Surf" on digestive tissue on fresh-water fish *Clarius batrachus* (Linn) marked the large scale destruction of the tissues of gastro-intestinal mucosa and liver (Jain *et al*. 2011) [18]

Clarias gariepinus live and survive in captivity (ponds and reservoirs) with other fishes without any disturbance, feed on natural and artificial diet, grow at a faster rate and attain marketable size in short span of time, breed successfully and prolifically in confinement at maturity, hardy and able to tolerate climatic as well as environmental or ecological changes in culturable waters, resist parasites and diseases, palatable and highly nutritive (Butler, 1971 and Gupta and Gupta, 2008) [8, 14]. The genus *Clarias* is unique in its ability to survive a wide variety of environmental extremes (Gorman, 2000) [13]. Fishes in this group have high efficient air breathing organs which allow them to survive in oxygen depleted water (Gui, 2003) [15], migrate over and for distances as long as 1.2 km and aestivate in damp burrows (Chandi, 1992) [9]. *Clarias* spp. is able to survive excessive crowding, feeds on natural and compounded feed for fast growth, and rigours of transport (Si-fa and Chenhong, 2003) [32]. *Clarias* spp. survives in habitats where few or no other fishes can (Phillips, *et al*., 2003) [28] and have proved a serious threat to native populations in areas where they have been introduced, because of their superior adaptability (Kapuscinki and Miller, 1993) [19]. *Clarias gariepinus* has fast growth rate in the natural and cultured environments and has proved to be successful aquaculture species (Paul, 2007) [27].

Materials and Methods

The Linear alkyl benzene sulfonate was used in this study because: (a) it is widely used as the main active substance in detergents and (b) there is little or no studies on the chronic impact of this element in the fresh water in Nigeria (where this study was carried out). Detergent definitive tests of 10.00, 20.00, 30.00, 40.00 and 50.00mg/l were obtained through a serial dilution process according to Santanu (2013) [31] after series of trial test on Juveniles (mean weight, 246.30± 14.12g SD; mean length 16.15±1.40cm SD) and adults (mean weight, 850.00±10.22g SD; mean length 29.20± 7.12 cm SD.) of *C. gariepinus* obtained from a Fish Farm in Port Harcourt, Nigeria. The five different concentrations tested10.00, 20.00, 30.00, 40.00 and 50.00mg/l as well as control (water without) were most suitable because at anything above 50.00mg/l, specimens were jumping out of aquaria. As a renewable bio assay, specimens were cared for on regular basis by exchanging water solution (water with detergent) daily for 30 days and to prevent escape, aquaria were covered with perforated cover. The containers were washed daily and fish fed once with a 35% crude protein diet at 2% body weight for juvenile fish and 1% for adult fish (Gabriel *et al*., 2005) [10].

Physico-chemical parameters such as Ammonia, alkalinity, temperature, pH, conductivity, turbidity and dissolved oxygen were adequately monitored. Ammonia - Nitrogen (NH_3 - N) with the phenate method of ammonia determination (APHA, 1998) [2], temperature measurements was determined with a mercury-in glass thermometer, pH with291 Mk 2 pH meter, conductivity with Horiba water checker, turbidity with a probe was inserted in water and the turbidity values obtained were read using the Horiba water checker measured by standard methods according to APHA, 1998.Dissolved Oxygen (DO) with Winkler's method (APHA, 1998) [2].

Blood samples were also collected from the fish (behind the anal fin) with 21G size needle and syringe. Blood for enzyme analysis was stored in heparinized bottles. Fish were killed with a blow on the head after blood collection and dissected in order to collect samples (0.5g) of liver tissues with the aid of penknife. Sample was macerated with pestle and mortar. To prepare samples for enzyme, 5mlof physiological saline was used. After the addition of diluent, the samples were centrifuged at the rate of 300 rounds per minutes for 10 minutes. The supernatants were then removed and stored in plain bottles at – 4ºC for analysis

Result

Ammonia, temperature,pH, turbidity and dissolve oxygen in the control aquaria for juvenile and adult fish showed no significant difference except in alkalinity and conductivity where the two life stages significantly differed (P<0.05). At 10.00, 20.00, 30.00, 40.00 and 50.00mg/l, water quality of juvenile and adult fish significantly differed (P<0.05) in alkalinity, conductivity and turbidity (Table 1 and 2). Conductivity peaked at 50.00mg/l in adult fish followed by that of juvenile fish while ammonia and pH were minimal (Figure1). Detergent respectively raised AST activities in the liver (Table 3 and 4)of juvenile fish by 35.52 and 33.20% at 10.00 and 20.00mg/l higher than that of adult fish while the reverse was observed at 40.00mg/l where that of adult fish was 138.47% higher than that of juvenile fish. At 30.00 and 50.00mg/l, AST activities in juvenile fish were respectively 27.85 and 6.98% less than control while that of adult fish was

93.18 and 190.90% higher than control. ALT activities in adult fish were 27.52, 49.08, 55.96, 63.30 and 63.30% lower than control at 10.00, 20.00, 30.00, 40.00 and 50.00mg/l while that of adult was higher than that of control by 45.00% at 10.00mg/l; 10.00% at 30.00mg/l, lower than control by 60.00% at 20.00mg/l and same with control at 40.00mg/l. At 10.00, 20.00 and 30.00mg/l. ACP activities were respectively 90.93, 31.97 and 2.95% while at 40.00 and 50.00mg/l, it was 40.44 and 42.30% higher than control in adult fish. ACP activities in juvenile fish was same with control at 40.00 and 50.00mg/l. ALP activities in the liver of juvenile fish was 31.90 and 46.68% higher than that of adult fish whereas at 30.00, 40.00 and 50.00mg/l, it was respectively 34.65, 42.07 and 48.26% higher than control in the adult fish and 49.97, 50.00 and 49.64% lower than control in juvenile fish (Table 3 and 4). ALP activities peaked at 20.00mg/l, followed by 10.00mg/l while that of adult was concentration dependent (Figure 2).

Table 1: Water Quality Variables (Mean ± S.D) in the Experimental Tanks for Juveniles during the Exposure Period.

Variables	Concentrations (mg/l)					
	0.00	**10.00**	**20.00**	**30.00**	**40.00**	**50.00**
Ammonia. (mg/l)	1.76±0.28^{a}	1.59±0.37^{a}	1.44±0.43^{a}	1.54±0.62^{a}	1.49±0.20^{a}	1.44±0.40^{a}
Alkalinity(mg/l)	77.00±12.76^{a}	87.00±21.64^{a}	96.00±21.14^{ab}	115.00±21.66^{b}	133.00±21.12^{b}	141.12±21.11^{b}
Temperature (^{o}C)	30.42±2.33^{a}	30.27±0.48^{a}	30.45±0.63^{a}	30.55±0.59^{a}	30.70±0.73^{a}	30.71±0.84^{a}
Ph	6.39±0.57^{a}	6.45±0.65^{a}	6.68±0.30^{a}	6.60±0.14^{a}	6.60±0.16^{a}	6.40±0.21^{a}
Conductivity(S/m)	211.50±16.71^{a}	270.25±18.61^{ab}	303.75±28.11^{b}	343.25±19.621^{b}	345.25±14.11^{b}	360.11±28.11^{c}
Turbidity (mg/l)	21.50±6.23^{a}	25.00±7.16^{b}	27.00±6.21^{b}	26.11±7.11^{b}	45.00±7.11^{c}	73.00±8.11^{d}
DO (mg/l)	5.59±0.98^{c}	3.81±0.68^{ab}	3.20±0.72^{ab}	2.95±0.64^{b}	2.55±0.33^{b}	2.33±0.13^{b}

Means within the same row with different superscripts (a, b, ab, c, d) differ significantly (*P*<0.05).

Table 2: Water Quality Variables (Mean ± S.D) in the Experimental Tanks for Adult Fish during Exposure Period.

Variable	Concentrations (mg/l)					
	0.00	**10.00**	**20.00**	**30.00**	**40.00**	**50.00**
Ammonia. (mg/l)	0.83±0.15^{a}	1.17±0.17^{b}	1.49±0.23^{c}	1.20±0.0.8^{b}	1.28±0.19^{b}	1.37±0.21^{c}
Alkalinity(mg/l)	52.00±10.00^{a}	77.00±11.20^{b}	87.00±13.02^{b}	93.75±16.00^{b}	99.50±28.82^{ab}	105.00±20.03^{ab}
Temperature (^{o}C)	30.43±2.31^{a}	30.28±1.31^{a}	30.45±0.36^{a}	30.55±2.31^{a}	30.70±2.33^{a}	30.70±0.91^{a}
pH	6.65±0.57^{a}	6.21±0.22^{a}	6.13±0.20^{a}	5.84±0.23^{ab}	5.56±0.21^{ab}	4.89±0.24^{b}
Conductivity(S/m)	251.25±17.17^{a}	262.50±18.32^{a}	345.50±21.22^{b}	378.00±23.01^{b}	340.75±23.23^{b}	472.75±25.00^{c}
Turbidity (mg/l)	23.00±6.11^{a}	42.75±6.20^{a}	67.50±7.01^{ab}	77.25±7.04^{ab}	120.00±8.02^{c}	133.00±9.23^{d}
DO (mg/l)	5.98±0.99^{b}	4.32±0.54^{b}	3.58±0.34^{ab}	3.21±0.30^{ab}	2.56±0.28^{a}	2.34±0.25^{a}

Means within the same row with different superscripts (a, b, ab, c, d) differ significantly (*P*<0.05).

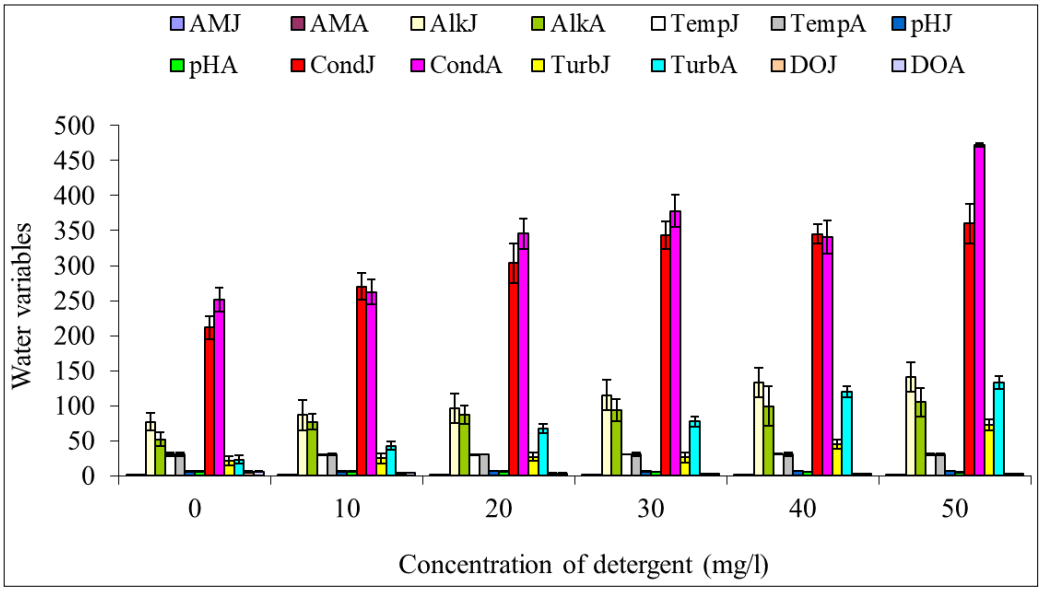

Fig 1: Water quality variables (Mean ± S.D) in the experimental aquaria for juvenile and adult *C. gariepinus.* Key: AMJ = Ammonia for juvenile, AMA = Ammonia for adult, AlkJ = Alkalinity for juvenile, AlkA = Alkalinity for adult, TempJ = Temperature for juvenile, TempA = Temperature for adult, pHJ = - log. of hydrogen ion concentration for juvenile, pHA = - log. of hydrogen ion concentration for adult, CondJ = Conductivity for juvenile, CondA = Conductivity for adult, TurJ = Turbidity for juvenile, TurA = Turbidity for adult, DOJ = Dissolved oxygen for juvenile, DOA = Dissolved oxygen for adult.

Table 3: Activities of Selected Enzymes in the Liver of *C. gariepinus* Juveniles Exposed to Jumbo Detergent for 30 Days (Mean ± S.D)

Conc (mg/l)	AST (IU/L)	% Control	ALT (IU/L)	% Control	ACP (IU/L)	% Control	ALP (IU/L)	% Control
0.00	53.75±14.36^{a}	100	50.00±11.55^{b}	100	10.00±1.21^{a}	100	95.50±28.87^{ab}	100
10.00	87.50±8.66^{b}	+62.79	72.50±14.43^{d}	+45.00	20.00±1.11^{c}	+100.00	142.50±25.98^{c}	+49.22
20.00	97.25±18.87^{b}	+80.93	20.00±1.21^{a}	-60.00	15.00±1.22^{ab}	+50.00	167.50±95.26^{c}	+75.39
30.00	38.78±7.51^{ab}	-27.85	55.00±10.00^{b}	+10.00	12.50±2.89^{a}	+25.00	47.78±14.14^{a}	-49.97
40.00	57.50±25.98^{a}	+6.98	50.00. ±11.55^{b}	0.00	10.00±0.01^{a}	0.00	47.75±14.21^{a}	-50.00
50.00	50.00±21.21^{a}	-6.98	60.00±3.21^{c}	+20.00	10.00±0.00^{a}	0.00	50.00±0.12^{a}	-49.64

Means within the same column with different superscripts (a,b,ab,c) differ significantly (P<0.05)
Key: AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ACP=Acid phosphatase, ALP=Alkaline phosphatase.

Table 4: Activities of Selected Enzymes in the Liver of *C. gariepinus* Adults Exposed to Jumbo Detergent for 30 Days (Mean ± S.D)

Concentration (mg/l)	AST (IU/L)	% control	ALT (IU/L)	% Control	ACP (IU/L)	% Control	ALP (IU/L)	% Control
0.00	11.00±1.21^{a}	100	54.50±1.12^{c}	100	20.40±1.31^{a}	100	101.00±2.40^{a}	100
10.00	14.00±1.32^{ab}	+27.27	39.50±1.10^{b}	-27.52	22.25±1.41^{a}	+9.07	118.50±1.30^{a}	+17.32
20.00	16.25±1.33^{ab}	+47.73	27.75±0.90^{ab}	-49.08	24.08±1.12^{ab}	+18.03	130.00±2.20^{ab}	+28.71
30.00	21.25±2.01^{b}	+93.18	24.00±1.11^{a}	-55.96	24.90±2.39^{ab}	+22.05	136.00±2.50^{ab}	+34.65
40.00	27.00±2.43^{b}	+145.45	20.00±1.21^{a}	-63.30	28.65±0.21^{b}	+40.44	143.50±3.20^{b}	+42.07
50.00	32.00±2.50^{c}	+190.90	20.00±1.21^{a}	-63.30	29.03±0.01^{b}	+42.30	149.75±4.02^{c}	+48.26

Means within the same column with different superscripts (a,b,ab,c) differ significantly (P<0.05).
Key: AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ACP=Acid phosphatase, ALT=Alkaline phosphatase.

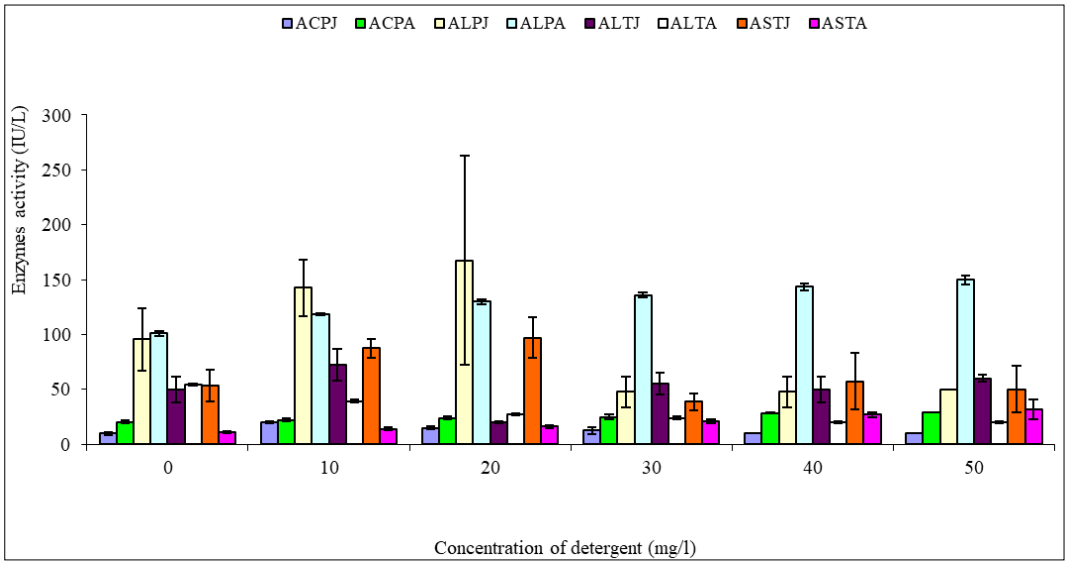

Fig 2: Comparative activities of enzymes in the liver of juvenile and adult *C. gariepinus* exposed to detergent for 30 days (Mean ± S.D) Key: ACPJ=Acid phosphatase juvenile, ACPA=Acid phosphatas adult, ALPJ= Alanin phosphatase juvenile, ALPA=Alanin phosphatase adult, ALTJ=Alanin aminotransferase juvenile, ALTA=Alanine aminotransferase adult, ASTJ=Aspartate aminotransferase juvenile, ASTA=Aspartate aminotransferase adult.

Discussion

Water physical and chemical characteristics remained within the species comfort limits along the experimental period (Viveen *et al*.,1985, Boyd,1979; Ovie and Adeniji, 1990) [40, 4, 24] as optimal requirements for African catfishes and did not vary significantly (P>0.05) between the two life stages under consideration. This suggests that the parameters did not seem to negatively influence the test fish in this study.

The liver contains numerous enzymes, some of which are also present in the plasma in very low concentrations. These enzymes have no other major known functions in the liver other than to provide information about hepatic state and disorders. These disorders could be as a result of injury or liver disease. The injury could be caused by reactive metabolites, resulting from xenobiotic metabolism in the liver. Hence, the need to monitor the enzymes to ascertain the physiological state of the liver of *C. gariepinus* exposed to detergent. Considering hepatoxicity induced by carbon tetrachloride, Madgy and Rogers (1993) [21] observed that during the initial 48 hours period, liver enzymes such as aspartate transaminase and alamine transaminase appeared and then receded from the serum. This is in line with this work as detergent respectively raised AST activities in the liver of juvenile fish by 35.52 and 33.20% at 10.00 and 20.00mg/l higher than that of adult fish while the reverse was observed at 40.00mg/l where that of adult fish was 138.47% higher than that of juvenile fish. At 30.00 and 50.00mg/l, AST activities in juvenile fish were respectively 27.85 and 6.98% less than control while that of adult fish was 93.18 and 190.90% higher than control. Venkateswara and Rao, 2006 and Tietz, 1995 [39, 36] reported that reductions in transminations occurs due to amino acid input into the TCA cycle in order to cope with energy crisis during toxicant – based stress. This agrees with with this work in that ALT activities in adult fish were 27.52, 49.08, 55.96, 63.30 and 63.30% lower than control at 10.00, 20.00, 30.00, 40.00 and 50.00mg/l while that of juvenile was higher than that of control by 45.00% at 10.00mg/l; 10.00% at 30.00mg/l, lower

than control by 60.00% at 20.00mg/l and same with control at 40.00mg/l. Enzymes alterations in the organs of lower organisms are used as a measure of the extent of hepatic damage (Uedeme-Naa and George, 2017) [38]. ALP activities in the liver of juvenile fish was 31.90 and 46.68% higher than that of adult fish whereas at 30.00, 40.00 and 50.00mg/l, it was respectively 34.65, 42.07 and 48.26% higher than control in the adult fish and 49.97, 50.00 and 49.64% lower than control in juvenile fish. Decreased level of ALP activity in the liver tissue of exposed fish, depictsit might have been impaired. This view was also opined by Ovuru and Mgbere (2000) [25]. Acid phosphatase activities reflect a change in endoplasmic reticulum mass; it is also known to take place in the cell membrane and may be involved in metabolic movements (Uedeme-Naa and George, 2017) [38]. Gabriel and George (2005) [10] observed that enzymes activities under toxicant exposure could be affected by the concentration and mode of action of the toxicant, mode and duration of exposure, and species specific response. This could be responsible for ACP response to detergent where its activities were respectively 90.93, 31.97 and 2.95% at 10.00, 20.00 and 30.00mg/l while at 40.00 and 50.00mg/l, it was 40.44 and 42.30% higher than control in adult fish. ACP activities in juvenile fish was same with control at 40.00 and 50.00mg/l. Zaccone *et al.* (1985) [42] evaluated the toxic effects of ionic active detergent (LAS) on fish liver for some days and observed that activities of ACP, ALP, AST and ALT increased significantly or marginally with increase in concentration in the liver, which thus indicated cellular toxicity of these detergents even after their administration in low doses for a long period. The activity levels of AST and ALT studied in muscles, gills, liver and brain of *T. mozambica* exposed to detergent, showed that transaminases were elevated in all the tissues in addition to a shift in aminotransferases reaction under surfactants impacts (Sprague, 2003) [33]. This is not absolute in this work as opined above due to the fact that ALP activities in the liver of juvenile fish was 31.90 and 46.68% higher than that of adult fish whereas at 30.00, 40.00 and 50.00mg/l, it was respectively 34.65, 42.07 and 48.26% higher than control in the adult fish; and 49.97, 50.00 and 49.64% lower than control in juvenile fish. In both the juvenile and adult fish, ALT, AST, ACP and ALP activities were elevated with increase in detergent concentrations. This is similar to the findings by Naveed *et al.* (2010) [22] in *Channa punctatus*, under exposure to detergent. Disrupted activities of both aminotransferases which was more noticeable in adult fish, indicated a diminished transamination process, which have been reported in mosquito fish (Park *et al*., 2006**)** [29].

Conclusion

The level of liver enzymes alterations in both life stages of *Clarias gariepinus* in this study shows that some basic physiological functions must have been affected and this could lead to species paralysis or extinction over time. So, the rate at which xenobiotics are dumped in our water bodies must be under strict control by agencies saddled with the responsibility.

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