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Relative Enzymes Activities in Selected Organs and Serum Plasma of *Clarias gariepinus* Juveniles and Adults Exposed to Chronic Levels of Detergent – Linear Alkylbenzene Sulfonate (LAS)

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Abstract The effect of chronic concentrations of detergent on serum enzymes in the gills, muscle, kidney, liver and plasma of juvenile and adult mudfish, *Clarias gariepinus* (Burchell) was investigated under static bio-assays during a 30 days' exposure period. Concentrations of detergent used were 0.00 (control), 10.00, 20.00, 30.00, 40.00 and 50.00mg/l. Relatively, it was observed that on exposure of serum enzymes in the gills, muscle, kidney, liver and plasma of juvenile and adult *Clarias gariepinus*, detergent altered: Aspartate aminotransferase (AST) activities in all the organs of both life stages except in the muscle, liver and plasma of adult fish; Alanine aminotransferase (ALT) activities in both juvenile and adult fish in all the organs except in the muscle, gill and plasma of juvenile fish; Acid phosphatase (ACP) activities in all the organs except in the muscle, gill and plasma of adult fish; Alkaline phosphatase (ALP) activities in all the organs of juvenile and adult fish. This implies that detergent did not impact significantly ($P>0.05$) on AST activities in the muscle, liver and plasma of adult fish; that of the ALT activities in the muscle, gill and plasma of juvenile fish and that of the ACP activities in the muscle, gill and plasma of adult fish while it impacted significantly ($P<0.05$) on the ALP activities in all the organs of both life stages.

Keywords Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Acid phosphatase (ACP), Alkaline phosphatase (ALP)

Introduction

Contamination of natural water by detergents has become a matter of concern in recent years because of their large scale use in home and industrial applications such as washing powders, dye fasteners, formulation of shampoos, industrial and household cleansing agents such as toothpaste, tooth powder and in dispersing oil spills [1]. Available reports indicate that entry of detergents into aquatic system build up in the food-chain and are responsible for many hazardous effects and even death of the aquatic organisms, including fishes [2]. [3] noted that detergents are cleaning products derived from synthetic organic compounds. It is cheap to produce detergent from biochemical sources and besides, its ability to foam in acid or hard water gives it an advantage over soaps. Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactant component is between 10-20%. The other components include bleach, filler, foam stabilizer, builders, perfume, soil-suspending agents, enzymes, dyes, optical brighteners and other materials designed to enhance the cleaning action of the surfactants [3-4]. [5] observed generally that detergents are xenobiotic compounds which are usually washed into water bodies and are made up of several compounds of which the active components are the surface-active

agents or surfactants. Acute toxicity studies in surfactants as well as exposure to sub lethal concentrations gave sufficient opportunity to monitor the behavioural changes. Avoidance reactions were the most commonly observed in fishes. [6] observed avoidance reaction of salmonids at very low concentrations of alkyl benzene sulphonate. It was also observed that the avoidance reaction was more pronounced in case of exposure to anionic surfactants. [7] studied the responses of fish, mussels, clams and crustaceans on exposure to various anionic and non-ionic surfactants. The swimming activity was affected in case of fishes and it was observed that more active fish species were affected the most. The ability for valve closure was affected in *Mytitus edulis* on exposure to the non-ionic nonyl phenol 10 ethoxylate. The siphon retraction was adversely affected in *Mya arneria.* The burrowing activity of the cockle was inhibited by the surfactants. Crustaceans like prawns, *Leander sp*. exhibited violent movements of abdomen and extremities on surfactant exposure. In barnacle the beat of cirri and shell closure were affected, the cirri beat gradually decreased on exposure to increasing concentrations of nonyl phenol 10 ethoxylate. Swimming of the nauplius larvae of *Balanus* and zoea of *Hya* were also seriously affected on exposure to surfactants.

Avoidance reactions for anionic surfactants were studied on a large scale by many workers [8]. Concentrations between 0.002 – 0.011 ppm of linear alkyl benzene sulfonate and alkyl sulfates elicited avoidance reactions [9] in Plecoglassus. In case of medaka, the concentration required was 0.027 ppm [10]. For alkyl benzene sulfonate the concentration eliciting the avoidance reaction was 0.001 for *Salmo gairdneri* and 0.02 ppm for *Gadus, norrhua*. A higher concentration of non-ionic surfactants was required for eliciting avoidance reactions but for alkyl phenol 10 ethoxylates it was 2-4 ppm. The responses to the surfactants were erratic in most of the cases. Swimming and feeding responses were affected at higher concentrations [11].

Many studies have shown that biochemical changes occurred in fishes that were exposed to environmental contaminants [12]. 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 [13]. Biochemical characteristics of blood are among the important indices of the status of internal environment of fish under any pollutant exposure [14]. Change in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, making it possible to study the mechanisms of the effects of these substances [15].

Detergent surfactants are complex organic chemicals where hydrophilic and hydrophobic groups are joined together in the same molecules [16]. There are various types of surfactants used in detergents formulations. Examples are Linear alkyl sulfonates, branched-chain alkyl benzene sulfonate, alpha olefin sulfonates, alkyl sulphates, alkyl ether sulphates and others. The linear alkyl benzene sulfonate (LAS) - ionic surfactants is the most widely used [17]. [18] noted that it was introduced as a biodegradable alternative to the non-biodegradable branched-chained alkylbenzene sulfonates. Linear alkylbenzene sulfonate (LAS) according to [19] has been reported by World Health Organization, [20] to have a high adsorption coefficient, which is attributed to the physicochemical properties of the surfactants. The LAS molecules adsorb to the suspended solid in water bodies and hence end up in sediments along the water course or sludge in treatment plants [21].

[22] reported that there are two kinds of detergents with different characteristics: Phosphate detergents and surfactant detergents. Detergents that contain phosphates are highly caustic, while those with surfactants are very toxic. Surfactant detergents are used to enhance the wetting, foaming, dispersing and emulsifying properties of detergents while phosphate detergents are used to soften water and help suspend dirt in water [23].

Gill tissue has its own importance while assessing the toxicity of surfactants. The large surface area of the tissue coupled with its important role in respiration and osmoregulation make it ideal for examining the toxic effects. Gill viability in presence of surfactants linear alkyl benzene sulfonate and nonyl phenol was studied in rainbow trout by [24]. The viability of gills deteriorated rapidly during 60 mm of exposure to 100 micromoles/litre of linear alkyl benzene sulfonate and to nonyl phenol. Linear alkyl benzene sulfonate was also found to decrease cadmium (Cd) transfer whereas nonyl phenol increased Cd retention. When tested at environmentally relevant concentrations (0.05 ppm), linear alkyl benzene sulfonate doubled the Cd transfer whereas nonyl phenol had no effect.

The effects of surfactants on gill osmoregulatory function were studied by monitoring the changes in the activity of the gill Na^+- K^+ ATPase. It was reported by many workers that low concentrations of surfactants activated this

membrane- bound enzyme while high concentrations had an inhibitory effect. The effects of syndets like Idet 5L and Swanic 6L (SLS) on ATPase activity was studied by [25] in the fish *Channa punctatus*. They exposed the animals to sub lethal levels of these syndets for 25 and 50 days. The analysis of the enzyme activity revealed that enzyme inhibitions were highest in the gill and brain homogenates for oligomycin-sensitive Mg^{2+} ATPase with pronounced effects (65%) after 50 days of exposure to 7.5ppm of Swanic. Fish exposed to lower concentrations showed an insignificant activation of Na -K ATPase and Mg^2 ATPasc in the gills. A similar study on in vivo responses of ATPase was done on *Mystus vitrofus* [25] exposed to Swascofix (alkyl benzene sulfonate). The brain, gill, liver and kidney tissues were sampled. After a period of 60 days the highest inhibition was noted in the brain followed by gill, kidney and liver and it was observed that low concentrations in some cases enhanced the activity. [26] also reported enhanced Mg^{2+}- Ca^{2+} ATPase activity in the microsomal fraction of the bovine brain cortex treated with sodium deoxycholate and Lubrol-WX. The ATPase is concerned with the active transport of sodium ions out of the cell and potassium ions into the cell. Hence it is fundamental to functions like regulation of cell volume and electrolyte balance. Thus an inhibition of the enzyme would result in alterations in membrane/nerve transmission and uncoupling of oxidative phosphorylation. The surfactant is supposed to exert its toxic effect probably at the active site of the enzyme.

[27] studied the effects of sub lethal concentrations of sodium alkyl aryl sulfonate on 21day exposure in *Ctenopharyngodon idella* at 3, 5 and 8 ppm. It was noted that plasma sodium levels were decreased below the normal levels of 150 mmol significantly after 15 days. An increase in opercula movements was also noted.

Materials and Methods

One hundred and eighty *Clarias gariepinus* of known weights were obtained from a standard fish farm and transported in four 50 litre containers to the wet laboratory of the Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, Port Harcourt were acclimation was done for 7 days, juvenile and adult fish respectively fed at 2% and 1% body weight with 42% crude protein diet. Series of trial tests with detergent solution were carried out before the definitive test of 10,20,30,40 and 50mg/l were obtained. The experiment was a renewable bio assay where detergent of varied concentrations was renewed on daily basis for 30 days. At the end of the experiment, specimens were weighed and measured by standard method. Blood samples were collected from the fish (behind the anal fin) with 21G size needle and syringe and preserved in EDTA for haematological studies. Fish were killed with a blow on the head after blood collection and dissected to collect 0.5g of gill, 0.5g of liver, 0.5g of kidney, 0.5g of muscle and 0.5g of spleen with penknife. Sample was macerated independently with pestle and mortar. To prepare samples for enzyme, 5mlsof physiological was used. After the addition of diluent, the sample was centrifuged at the rate of 300 rounds per minutes for 10 minutes. The supernatant was removed and stored in plain bottles at $-4^{o}C$ for analysis. The experimental design was a completely randomized design (CRD) at five treatments levels with five replicates each and control.

Obtained data were collated and analyzed using statistics software 8.0 for windows after being tested for normality (Kolmogorov - Smirnov test) of variance (Bartetts test). When necessary conditions were satisfied, a two-way analysis of variance (ANOVA) was employed to show significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatment were significantly different [28].

Result

In the juvenile fish (Figure 1), it was observed that aspartate amino transferase (AST) activities was raised by detergent at 10.00 and 20.00 mg/l and suddenly dropped at 30.00mg/l in the muscle. In the gill, liver and kidney, activities were within the same range, though high level of fluctuations wert observed. In adult fish (Figure 8), a sharp decrease in AST activities were observed in the kidney and gills. Detergent had no significant impact on AST activities in the muscle, liver and plasma. This implies that in juvenile fish, AST activities are most impacted upon in the muscle while that of the adult fish is in the both kidney and gills. Alkaline aminotransferase (ALT) activities in juvenile fish (Figure 2) was observed more in the kidney and liver where sharp decrease was recorded and no

significant impact was observed in that of muscle, gill and plasma while in that of adult fish (Figure 7), it was observed that significant activities were recorded ALT of all the organs – kidney, liver, gills, muscle and plasma. This means that ALT in all the organs of adult fish are vulnerable to any stress causing substance introduced to water bodies. Acid phosphatase (ACP) activities in all the organs - kidney, liver, gills, muscle and plasma of juvenile fish (Figure 3) were significantly affected while that of adult fish (Figure 5) it was observed that enzymes activities were only influenced in the liver and kidney. In adult fish, ACP activities in the muscle, gill and plasma were not impacted upon by detergent. Alkaline phosphatase activity (ALP) activities in juvenile fish (Figure 4) peaked at 10.00 and 20.00mg/l and suddenly crashed at 30.00 – 50.00mg/l in the kidney. Detergent caused the decrease of ALP activities in the muscle, liver, gills and plasma of juvenile fish and in all the organs of adult fish (Figure 6) with increase in detergent concentration.

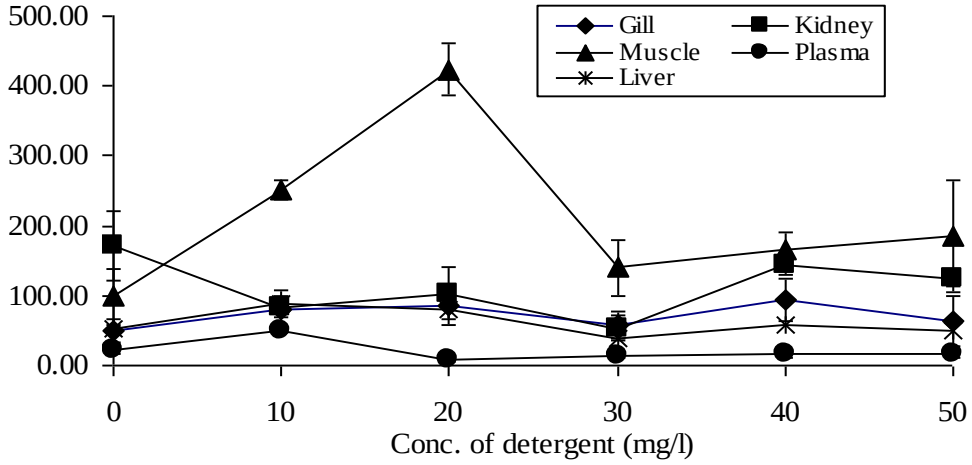

Figure 1: Relative aspartate aminotransferase (AST) activity in the tissues of C. gariepinus juveniles exposed to chronic levels of Jumbo detergent (Bars=SD)

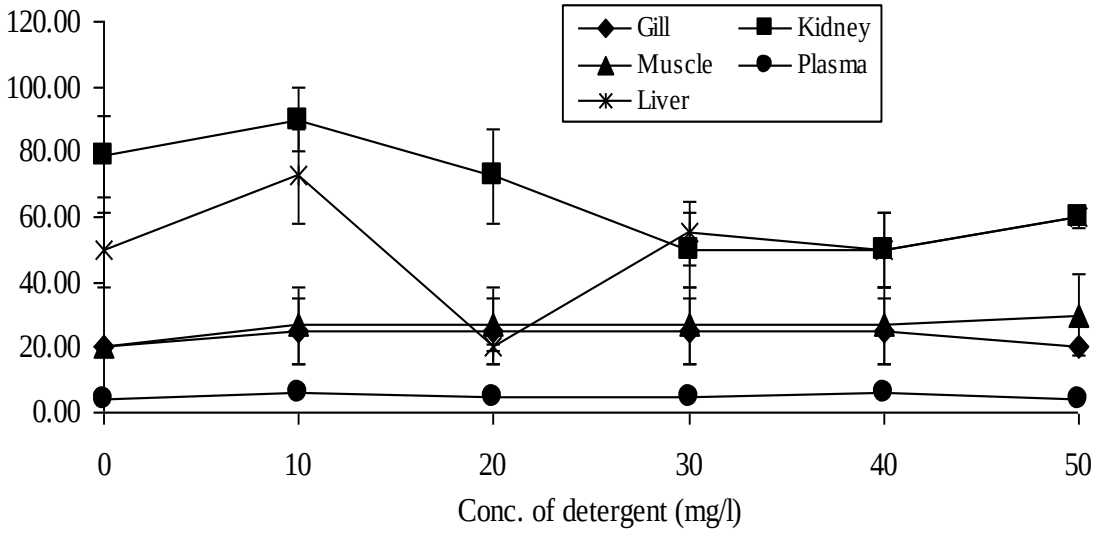

Figure 2: Relative alanine aminotransferase (ALT) activity in the tissues of C. gariepinus juveniles exposed to chronic levels of jumbo detergent (Bars=SD)

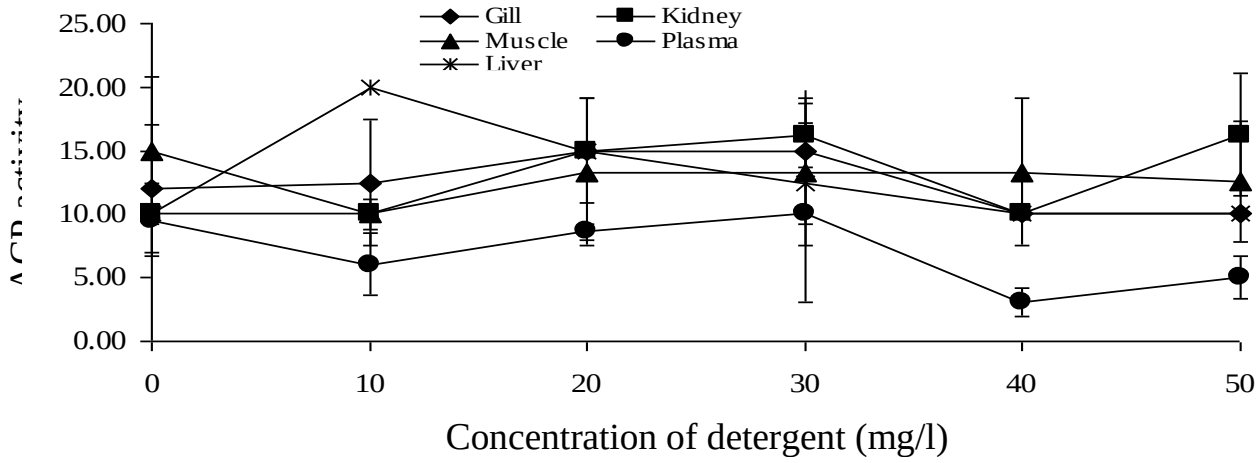

Figure 3: Relative acid phosphatase (ACP) activity in the tissues of C. gariepinus juveniles exposed to chronic levels of jumbo detergent (Bars=SD)

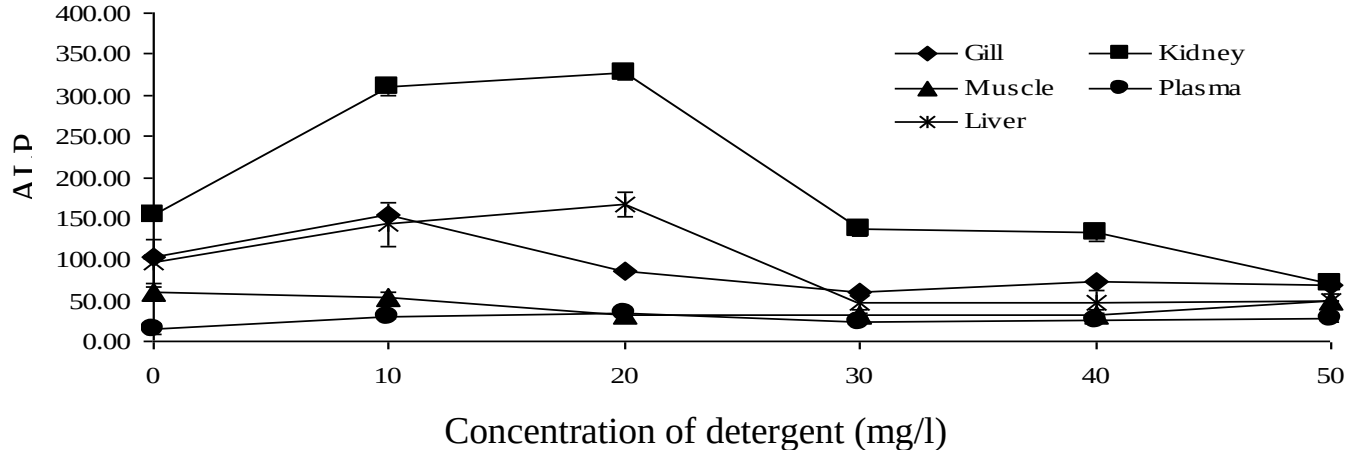

Figure 4: Relative alkaline phosphatase (ALP) activity in the tissues of C. gariepinus juveniles exposed to chronic levels of jumbo detergent (Bars=SD)

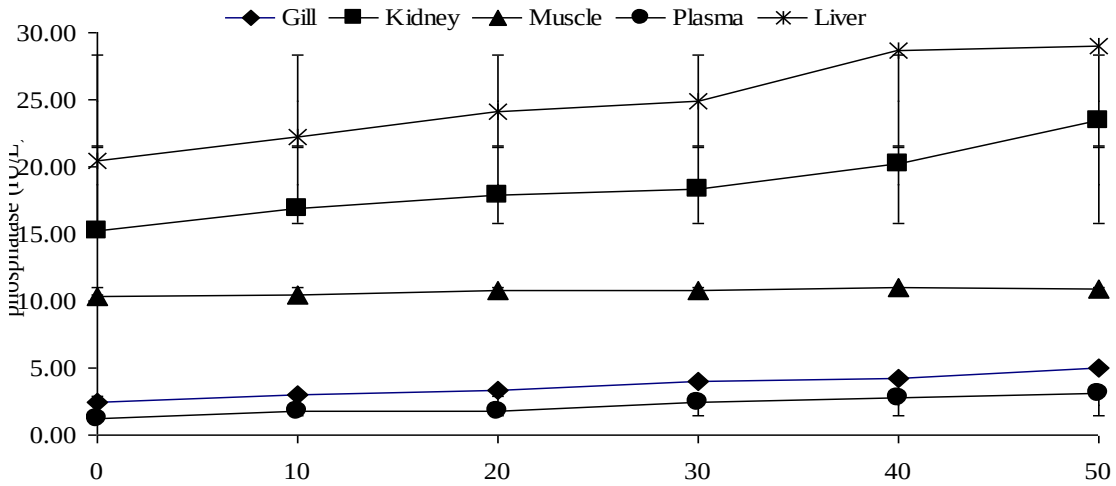

Figure 5: Relative acid phosphatase (ACP) activity in the tissues of C. gariepinus adult exposed to chronic levels of jumbo detergent (Bars=SD)

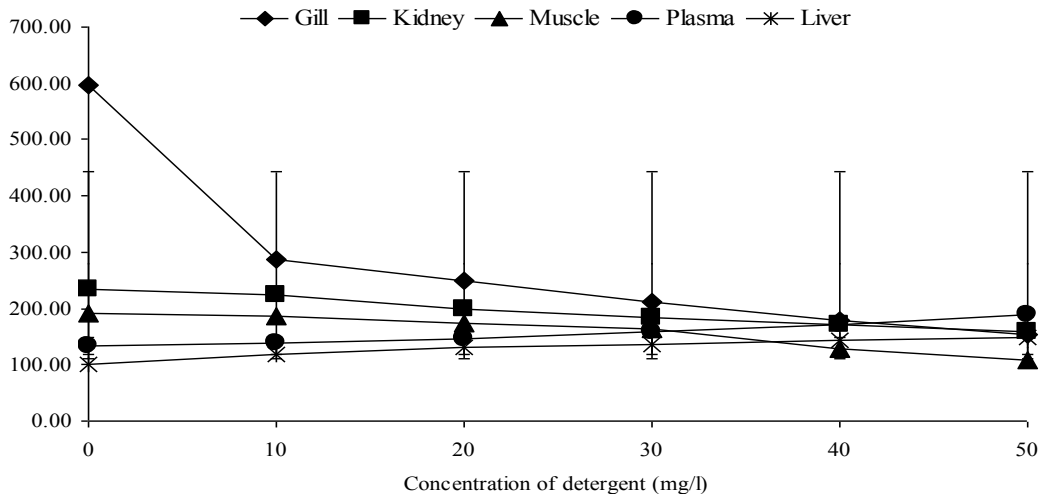

Figure 6: Relative alkaline phosphatase (ALP) activity in the tissues of C. gariepinus adult exposed to chronic levels of jumbo detergent (Bars=SD)

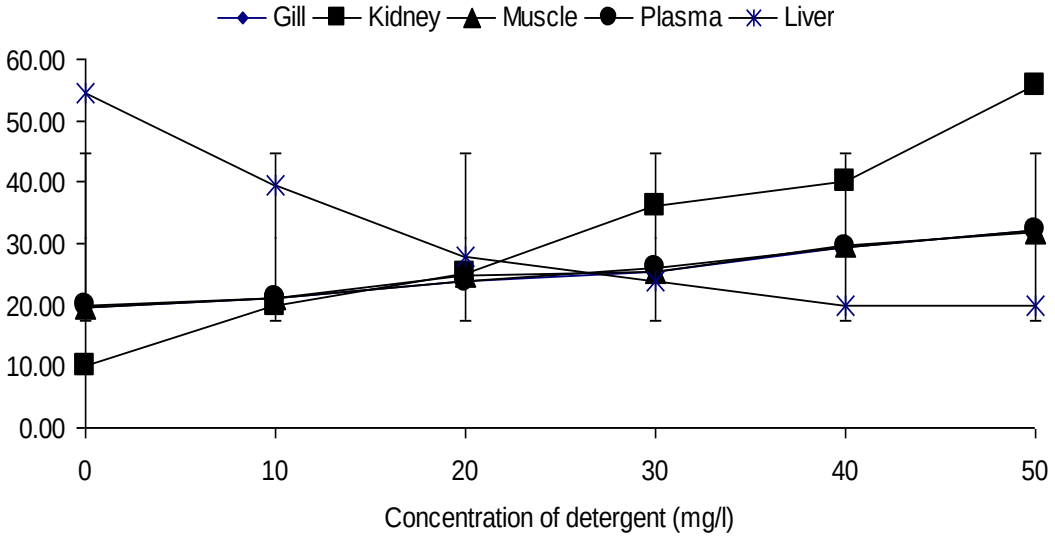

Figure 7: Relative alkaline aminotransferase (ALT) activity in the tissues of C. gariepinus adult exposed to chronic levels of jumbo detergent (Bars=SD)

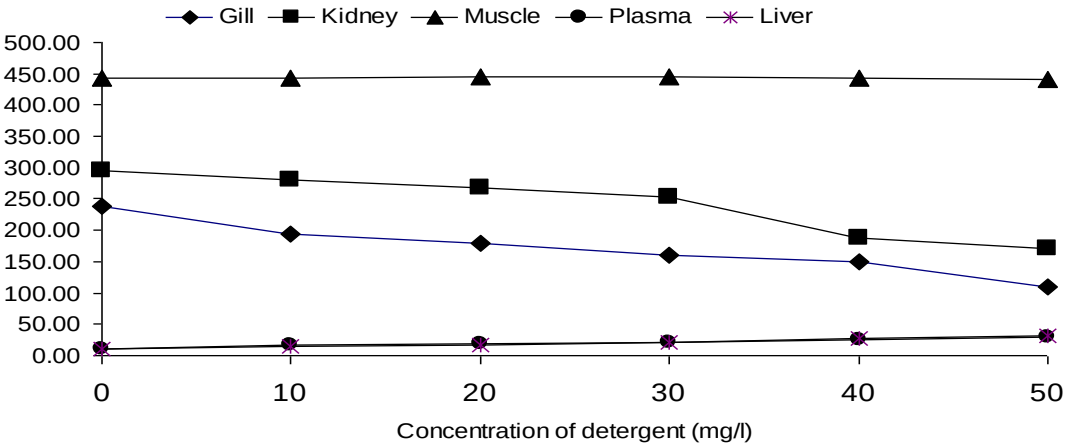

Figure 8: Relative aspartate aminotransferase (AST) activity in the tissues of C. gariepinus adult exposed to chronic levels of jumbo detergent (Bars=SD)

Discussion

[29] noted that the activity of transaminase in fish may be significantly changed under the influence of different toxic agent's cell destructive processes and size of the fish. They observed that oxidative stress caused by different toxic chemicals is a function of size which results into damaged tissues and liberated various transaminases into the plasma for circulation. This in agreement with this work in that alkaline phosphatase (ALP) activities in juvenile fish peaked at 10.00 and 20.00mg/l and suddenly crashed at 30.00 – 50.00mg/l in the kidney due to the influence of detergent on an enzyme responsible for the control of the formation of biochemical intermediates essential for physiological functions. Detergent caused the decrease of ALP activities in the muscle, liver, gills and plasma of juvenile fish and in all the organs of adult fish with increase in detergent concentration. Phosphatase is an important constituent of many biological processes involving genetic transduction because it can regulate the proteins to which they are attached [30]. ACP serves as a biochemical marker for lysosomal activity while ALP indicates membrane transport and integrity in the neuronal architecture. ACP exhibited gradual elevation in all the tissues at all concentrations. ALP also exhibited similar trend as ACP. The Alkaline phosphates (ALP) and Acid phosphatase (ACP) were elevated during toxic exposure period and under stress condition in both sizes which may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, which is more pronounced in adult size fish [31-33]. The increase could also result in a shift in biosynthetic, mixed-function oxidase and energy metabolism pathways, which are found to be more advanced in adult fish [34].

[35] noted that in intoxication, (such as detergent intoxication) free radicals ($H0^-$ and OH^+) are formed which in turn bring lysis of the lipid bi-layer of the cell membrane by oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which methylene $-CH_2-$ groups lie, that possess especially reactive hydrogen molecules. This phenomenon is known as "lipid peroxidation". Consequently, the cells of soft tissues like liver, kidney and gill etc. are destroyed and the contents of the cell are released to the body fluid (serum). The activity of the primary transaminase enzymes AST and ALT are mainly localized in the hepatocytes. These are the main catalysts of conversion of nonessential amino acids from essential amino acids in this main metabolic organ. Hepatocyte destruction increases their level of activity in blood serum. Reduction of these enzyme-activities in tissues like liver may hamper the normal process of transamination in the organ. Acid phosphatase activity was found to alter moderately in the tissues; this could be due to the inhibition of synthesis or increased turn over in presence of the pollutant. At lower doses of 3.25 ppm there was activation whereas at higher concentrations of 6.5 and 9.75 ppm there was inhibition. Alkaline phosphatase activity was significantly decreased in the liver of *Cirrhina mrigala* after 30 days. Aspartate aminotransferase activity showed a decrease possibly due to impairment of aerobic metabolism. It was inferred that the depletion of phosphatases could be due to uncoupling of oxidative phosphorylation followed by intoxication [36].

Conclusion

Municipalities should treat sewage for the removal of anthropogenic substances that are harmful to aquatic organisms. Detergents contain chemicals that can harm the human body as well as the natural environment. There is a need of multiple controls on use of phosphorus to achieve the desirable water quality conditions. The Nigerian government has not taken enough steps to address these problems. Without mandatory legislation, industry does nothing to reduce the levels of harmful chemicals, or to inform the consumer about potential damage. This is even true of multinational firms who are forced to follow much stricter norms in Western Countries like Canada and United States. Made laws in this regard should be implemented to the latter by Ministry of Environment (Federal and States). The recommended standard for linear alkyl benzene sulfonate (LAS) limit in detergent should be 0.5% of the total weight. In all municipalities sewage treatment, removal of Nitrogen and Phosphate should be made compulsory by separate legislation. Awareness needed should be made at consumer level regarding the linear alkyl benzene sulfonate (LAS) contents in detergents and its impact on the environment.

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