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Relative impact of detergent on selected electrolytes in some organs of *Clarias gariepinus* juveniles and adults

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Abstract
Selected electrolytes as Sodium ion (Na^+), potassium ion(K^+), calcium ion (Ca^{2+}), chloride ion (Cl^-, hydrogen carbonate ion (HCO_3^-) in the gill, muscle, liver, kidney and blood plasma of *Clarias gariepinus* of mean weight of 246.30± 14.12g SD; mean length 16.15±1.40cm SD for juvenile and mean weight of 850.00± 10.22g SD**;** mean length 29.20± 7.12 cm SD for adult were compared after exposure to chronic levels of linear alkylbenzene sulfonate. Results from the study indicated that in both life stages detergent: raised the Na^+, K^+, HCO_3^- in the muscles and decreased that of Ca^{2+}, and Cl^-; raised Ca^{2+}, K^+ and HCO_3^- in the gill; decreased HCO_3^- in the liver; raised K^+ in the kidney; raised Cl^- in the blood plasma when compared with control. It was also observed that Na^+ in juvenile fish was within the same range as control in all the organs except that of muscle whereas it fluctuated in adult fish with increase in concentration. In all the organs of juvenile fish, K^+ and Ca^{2+} were raised except Ca^{2+} in muscle and decreased in all the organs of adult fish except K^+ in gill, kidney and blood plasma when compared with control. Chloride ion (Cl^-) in the muscle, gill, liver and kidney of juvenile fish decreased significantly (P<0.05) when compared with control while that of adult fish was same as control. However, in blood plasma, Cl^- was raised above control in the two life stages and HCO_3^- fluctuated when compared with control.

Keywords: Electrolytes, sodium ion (Na^+), potassium ion (K^+), calcium ion (Ca^{2+}), chloride ion (Cl^-), hydrogen carbonate ion (HCO_3^-) and *Clarias gariepinus*

Introduction
Many studies have shown that biochemical changes occurred in fishes that were exposed to environmental contaminants (Rao, 1989) [17]. 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) [5]. Biochemical characteristics of blood are among the important indices of the status of internal environment of fish under any pollutant exposure (Edquisit *et al*., 1992) [11]. 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 (Luskova *et al*., 2001) [15]. 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 Pierterse *et al.* (2005) [8]. 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. Nero *et al.* (2005) [9] 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 opercular movements was also noted. Sub cellular studies using surfactants were done mainly at the level of cell membrane. Red cell membrane was the commonly used because a release of haemoglobin could serve as a criterion of stability. The differential release of red cell membrane components was done by Nilmi (2008) [6].

The surfactants used were SDS, Triton X-100 and deoxycholate. It was found that SDS extracted lipids and proteins separately whereas Triton was found to initially affect membrane labilisation by interacting with the membrane proteins. It was also found that SDS bound to all components of the RBC membrane and that the release of membrane components roughly paralleled their water solubility.

The effects of a cationic compound Zephiran were studied in the gills. It induced cremation of the red cell membrane (Price *et al.,* 2007) [7]. Dielectric haematological and biochemical investigations on detergent toxicity in fish blood were done by Bielinska (1987) [13] and Bansal *et al*., 1979 [24] in *Cyprinus carpio*. The sub lethal exposure resulted in decrease in the RBC count, hemoglobin and hematocrit. Also, there was an increase uptake of sodium into the cells and intra cellular potassium was also elevated. The differential release of proteins and lipids from the cell membrane was studied in trout gill epithelium by Roberts (2001) [18]. These studies highlighted the significance of critical unicellar concentration in the solubilisation of the membrane components which has great applications in the extraction and study of a large number of membranes bound enzymes.

Pre incubation of cells in the presence of detergents at low concentrations strongly inhibited the release of phospholipase A_2 about 65% and 55% inhibition was affected by $l0^{-8}$gm/l of sodium dodecyl sulfate and 10^{-7} gm/litre of cetyl tri-methyl ammonium bromide respectively. Also, uptake of thymidine precursors was affected by sodium dodecyl sulfate at 10^{-2} gm/litre. The release of liver acid phosphatase was studied from rat lysosomes in presence of sodium dodecyl sulfate, benzalkonium chloride and tween. The release rate was increased by all the chemicals. It was $>10^{-6}$M for cationics, $>10^{-5}$ M for anionics and tween (Da Silva *et al.,* 2004) [12]. Interaction of non- ionics with the cell membrane has been studied in detail by Regen *et al.* (1989) [3]. It was deduced that non ionics interacted with the cell membrane phospholipids and this led to modification of membrane structure and permeability. This in turn caused leakage of ions, amino acids and enzymes from the cell resulting in cell damage. Supramolecular surfactants like polyethylene glycol (PEG) as well as Triton readily disrupted the cell membrane of egg yolk. Studies by Calland (2004) [14] have shown that Triton and other surfactants caused leakage from palmitoyl oleoyl phosphatidyl choline/cholesterol large unilamellar vesicles. Several studies on model membranes revealed that the effect of synthetic surfactants depend upon the cholesterol concentration of the lipid bilayer whereas the effect of Triton was not affected by the same (Grier 2007) [10]. The interaction of surfactants with the artificial membranes was found to modify many physico-chemical properties of the cell membrane phospholipids. A fluorescence depolarisation study indicated that alkanoyl-N-methyl glucamide surfactants decreased the fluidity of the dipalmitoyl phosphatidyl choline membranes (Leino *et al.,* 2005) [4].

Materials and Methods

Experimental fish: Juvenile (150) and adult (30) of *Clarias gariepinus* respectively having mean weight of 246.30± 14.12g SD; mean length 16.15±1.40cm SD and mean weight of 850.00± 10.22g SD**;** mean length 29.20± 7.12 cm SD were obtained from a diseased free fish farm and acclimated to laboratory condition for 7 days before the commencement of experiment. The study was carried out in aquaria in a static system. Juvenile and adult fish were respectively fed at 2% and 1% body weight with a commercial feed of 42% crude protein.

Test solution preparation: Trial test to prepare chronic detergent solution for the experiment was done using Santanu (2013) [16] method. The final definitive test of 50.00mg/l was reached where for juvenile and adult fish, any quantity of linear alkylbenzene sulfonate that exceeded 50.00mg/l resulted in to erratic swimming, jumping or death. The decisions on 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l were reached after confirmation. Based on the definitive test result, juveniles and adults of *Clarias gariepinus* (African mudfish) were exposed to 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l detergent solution for 30 days.

Treatment: Following acclimation, specimens were divided in to six groups: a control group was maintained in detergent free water and the other groups were exposed for 30 days to 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l. To maintain a constant detergent concentration, water and detergent were renewed. Control and treated specimens were transferred every 24 h to aquaria with clean water and linear alkylbenzene sulfonate (detergent) were added to the water containing the treated fish to the normal concentration.

Collection of fish samples: Fish specimens were killed with a blow on the head and dissected in order to collect samples of gill, liver, kidney, muscle and spleen tissues with the aid of penknife. Sample was macerated with pestle and mortar. 5ml of de-ionized water was used to prepare the electrolyte. After the addition of this 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 – 4 ^{o}C for analysis.

Statistical analysis: Analysis of variance (ANOVA) was used for data analysis and Duncan Multiple Range Test (DMRT) used to test for difference between different levels of treatments and to separate means where applicable. Test of significance was at 95% probability.

Results

In all the organs in juvenile fish, sodium ion fell within the same range as control except at 30.00mg/l where sodium ion in the muscle peaked much more than control but later dropped as concentration increased while in adult fish, sodium ion in the plasma, liver and muscle was raised above control while that of gill and kidney dropped below control with increase in detergent concentration (Fig. 1 and 10). Potassium ion in the gill, kidney and liver (Fig.2) was gradually raised above control while that of muscle dropped with increase in concentration up to at 30.00mg/l where it rose again and peaked above control at 50.00mg/l in juvenile fish. It was within the same range in the liver as concentration increased. In adult fish, potassium ion in the plasma, kidney and gill increased while that of muscle and liver dropped with increase in detergent concentration. Potassium ion in the gill and kidney peaked at 50.00mg/l and that of muscle and liver least at 50.00mg/l in adult fish (Fig. 9). Detergent slightly raised the calcium ion in all the organs above control and crashed that in the gill of juvenile fish far below control with increase in concentration. Calcium ion in the gill, liver and muscle of adult fish decreased while that in the plasma and

kidney was within the same range with control with increase in concentration (Figs. 3 and 6). Chloride ion in all the organs of juvenile fish dropped except that of plasma which peaked at 10.00mg/l and later dropped at 40.00mg/l control as concentration increased (Fig. 4). In adult fish organs, chloride ion in all the organs was the same as control except that of the plasma which was consistently raised as concentration increased (Fig. 7). Hydrogen ion concentration in the gill and plasma was slightly raised above control while that of muscle, kidney and liver decreased with increase in detergent concentration in juvenile fish (Fig. 5). Detergent consistently raised hydrogen in all the organs of adult fish except in the liver which dropped at 20.00mg/l and plummeted at 50.00mg/l as concetration increased (Fig. 8).

Discussion

Troubled aquatic environment can be considered a potential source of stress as it creates a number of responses in the animal to deal with the physiological changes triggered by exterior changes. These responses can be found in fish and in other vertebrates in the form of alterations in the biochemical changes in the enzyme and electrolyte activity (Donaldson, 1981) [34]. Since measurement of biochemical parameters reflect the poor or good condition of fish (condition factor), more quietly than other commonly measured parameters and they react quickly to changes in the environmental condition (Atkinson and Judd, 1978) [33]. Electrolytes are needed for osmo-regulatory purposes in the body system of living organisms, thus electrolyte balance in the body of organism is necessary for the normal function of cells and organs. Gabriel *et al.*, 2011 [29], revealed that the basic function of electrolytes in the body include the control of fluid distribution, intracellular and extracellular acido-basic equilibrium so as to achieve proper maintenance of osmotic pressure of body fluids and normal neuro-muscular irritability. Therefore, alterations of the electrolyte balance of an organism would adversely affect the organism concerned. Electrolyte ($Na^{+,}$ K^{+}, P^{+}, and Ca^{2+}) levels indicate the operation of a variety of homeostatic mechanisms in the body (Clarke, 1998 [31], Kulkarni, 2015) [35]. Sodium (Na^{+}), potassium (K^{+}) and Chloride (Cl^{-}) play an important role in osmoregulation and homeostasis (Kulkarni, 2015) [35]. In vertebrates, the Na+ concentration in the extracellular fluid surpasses that in the cytosol, whereas K^{+} is higher in the intracellular fluid compared to the plasma. This is in line with this work in that Na^{+}, in the muscle, liver and plasma of adult fish increased above control and decreased below control in the gill and kidney whereas in the juvenile fish Na+ in the muscle was raised while that in gill, liver, kidney and plasma was same with control as concentration increased. In fish, Na^{+} enters the gill cells from the blood; co-transported with K+ and $Cl^{-}$and driven by an electrochemical gradient favorable to Na^{+}. Chloride ion exits the apical portion of the cell through a channel that is very similar to the defective structure that produces cystic fibrosis in animals. Na^{+} is moved back across the basolateral membrane into the blood by Na^{+}/ K^{+} activated ATPase. Furthermore, the Na+/ K^{+} ratio is vital for the ion permeability barriers in the cell membrane. (Evans, 1993) [32]. One of the divalent ions, calcium (Ca^{2+}), serves a number of functions in fish. It combines with phosphorus (P^{+}) for the deposition of bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Ca^{2+} appears to be important in the reproduction and mitochondrial functions. It is generally recognized that Ca^{2+} has an important role in osmoregulation (Wurst and Stickney, 1989) [21]. The significant dose-dependent alterations of electrolytes in the muscle, gill, liver, kidney and plasma observed in *Clarias gariepinus* juvenile and adult exposed to chronic detergent indicate that these fish were severely stressed. The suppressive effect on sodium ion (Na^{+}), potassium ion(K^{+}), calcium ion (Ca^{2+}), chloride ion (Cl^{-}), hydrogen carbonate ion (HCO_3^{-}) in all the organs of the two life stages due the toxicant cannot be ruled out. Ghatak and Konar, 1991 [2] reported that the feeding rate of tilapia was reduced significantly when exposed to pesticides at various concentrations. Any type of deviation in electrolytes level can be used as indicator for the assessment of kidneys function. In this study, in toxicant exposed fish, an alteration in electrolytes is an indication of loss of renal function. This was confirmed by Ates, 2008 [30] in rainbow trout exposed to some heavy metals in the laboratory. It was reported that selected heavy metal acts as neuro-toxicant, as changes in form, frequency, or posture of swimming movements of treated groups of fish was observed, with changes often occurring much earlier than mortality.

Ajai. *et al.*, 2013 [1] reported that lead exposure to *H.fossilis* provoked hypocalcemia. This derives support from the studies of Rogers *et al*. (2003) [28] who have also reported hypocalcemia in lead-exposed rainbow trout. Other investigators have also noticed decreased blood/plasma calcium content of fish treated either with aldrin (Bano 1982 [27]; Singh *et al.* 1996) [26], cadmium (Muramoto 1981) [25], cypermethrin (Mishra *et al*. 2005) [19]. Contrary to it, an elevation of plasma calcium concentrations has also been reported by other workers from the fish exposed to various toxicants (Uedeme-Naa and George 2019) [20].

The hypocalcemia observed in lead-exposed *H. fossilis* may be attributed to the impairment of either net electrolyte influx at the gill or renal function. Rogers *et al.* (2003) [28] have reported reduced calcium uptake in lead-exposed rainbow trout. Several investigators have reported degenerative changes in the gills of fishes after exposure to various pesticides (Adeyemo 2008) [23]. Degeneration of gills may affect the ionic permeability and cause decreased ionic levels in the blood. Tubular necrosis may be the other possible reason for the hypocalcemia and hypophosphatemia observed in lead-exposed *H. fossilis*. Kidney degeneration has been reported by several workers after the exposure of the fish to toxicants (Rabitto *et al*. 2005) [22].

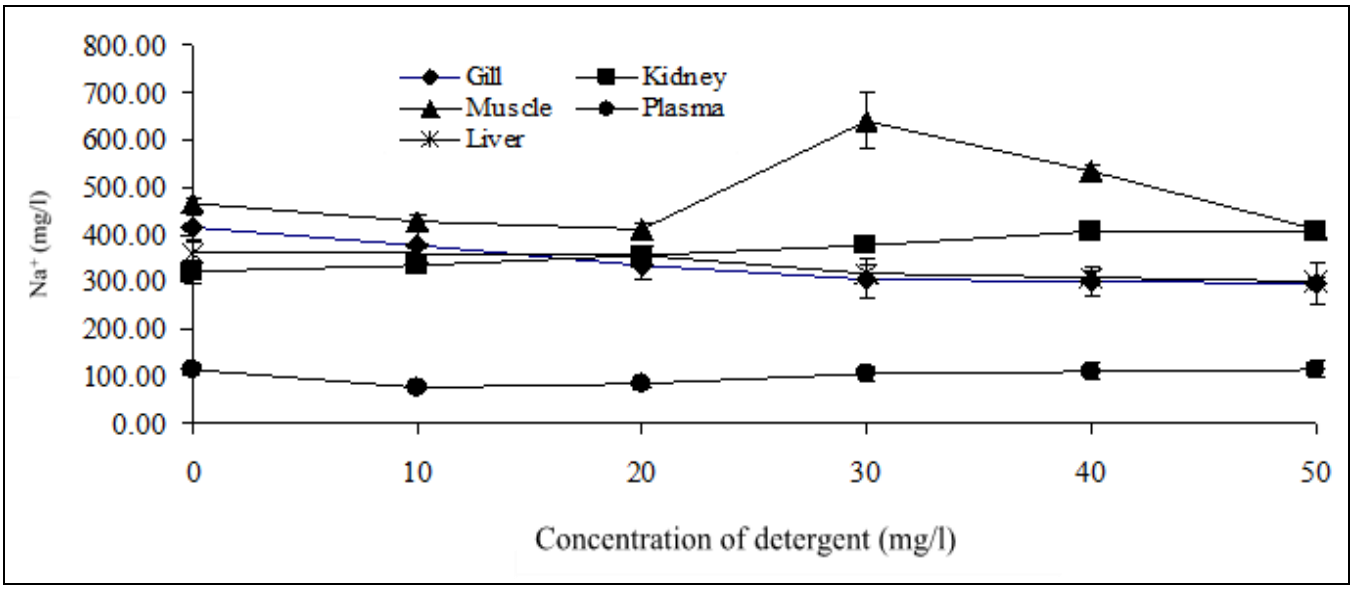

Fig 1: Relative levels of Na^+ in the organs of *C. gariepinus* juveniles exposed to chronic levels of jumbo detergent

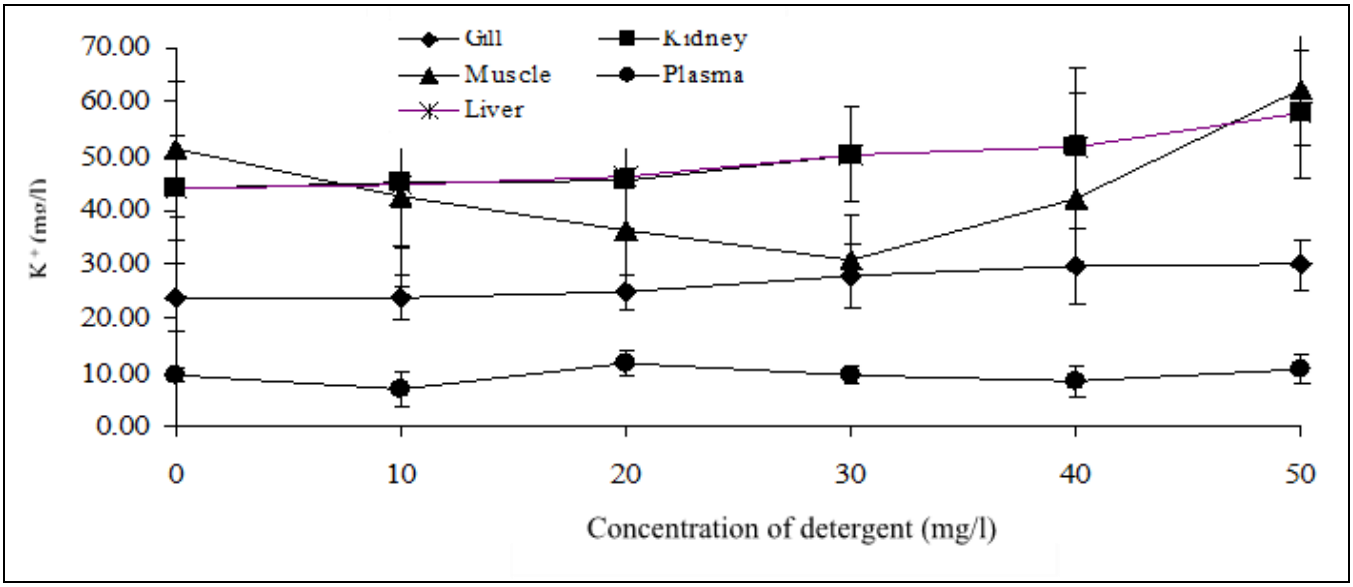

Fig 2: Relative levels of K^+ in the organs of *C. gariepinus* juveniles exposed to chronic levels of jumbo detergent.

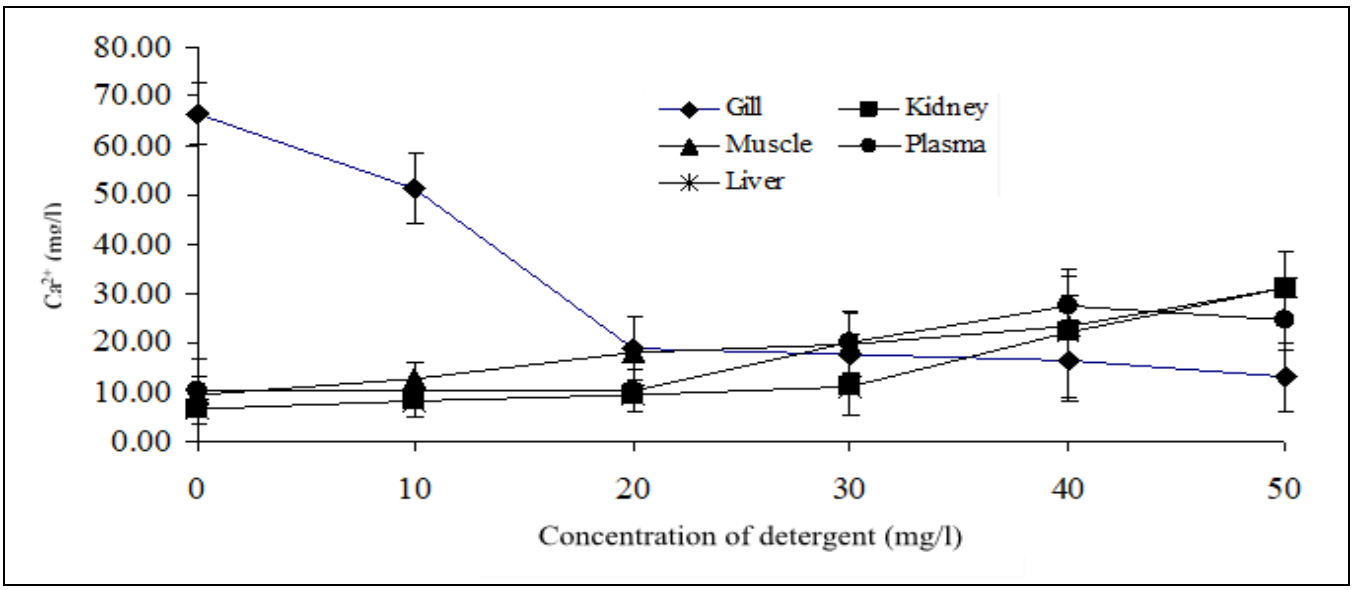

Fig 3: Relative levels of Ca^{2+} in the organs of *C. gariepinus* juveniles exposed to chronic levels of Jumbo detergent

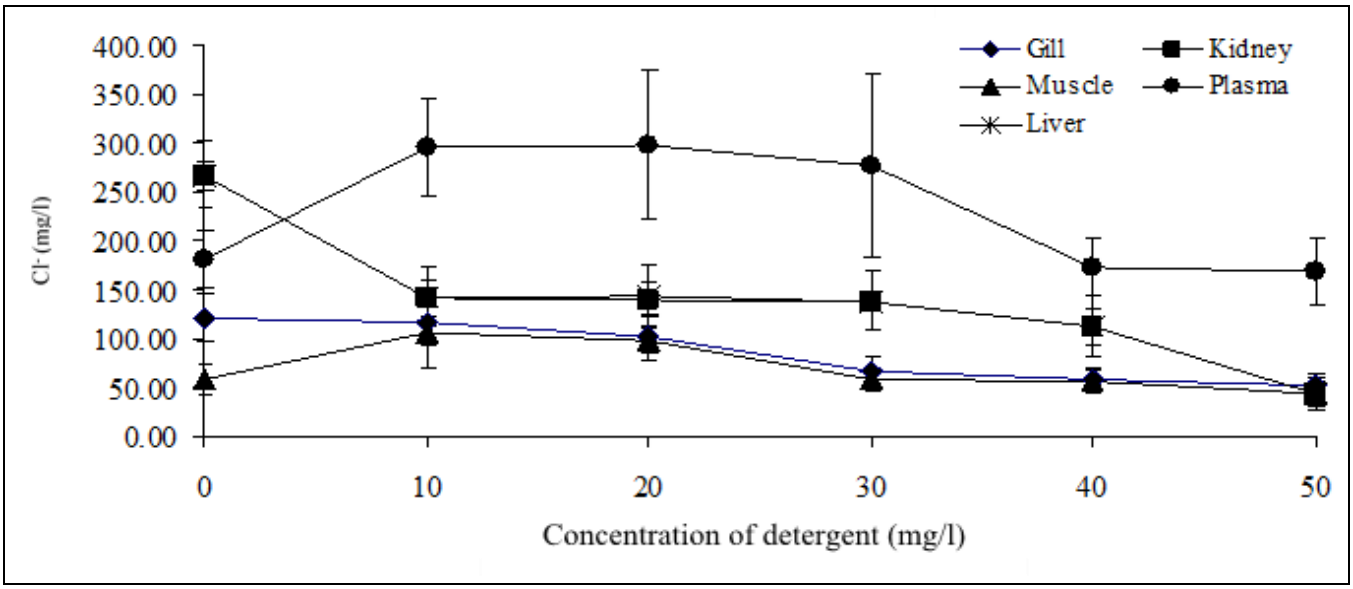

Fig 4: Relative levels of Cl^- in the organs of *C. gariepinus* juveniles exposed to chronic levels of jumbo detergent

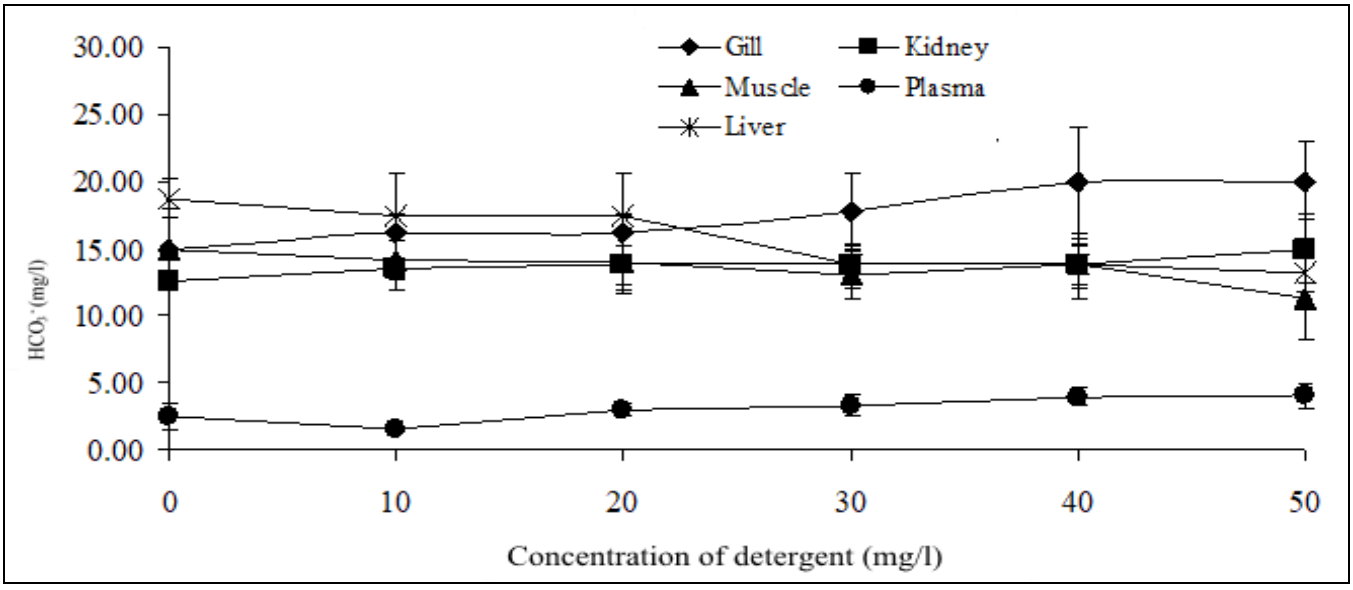

Fig 5: Relative levels of HCO_3^- in the organs of *C. gariepinus* juveniles exposed to chronic levels of jumbo detergent

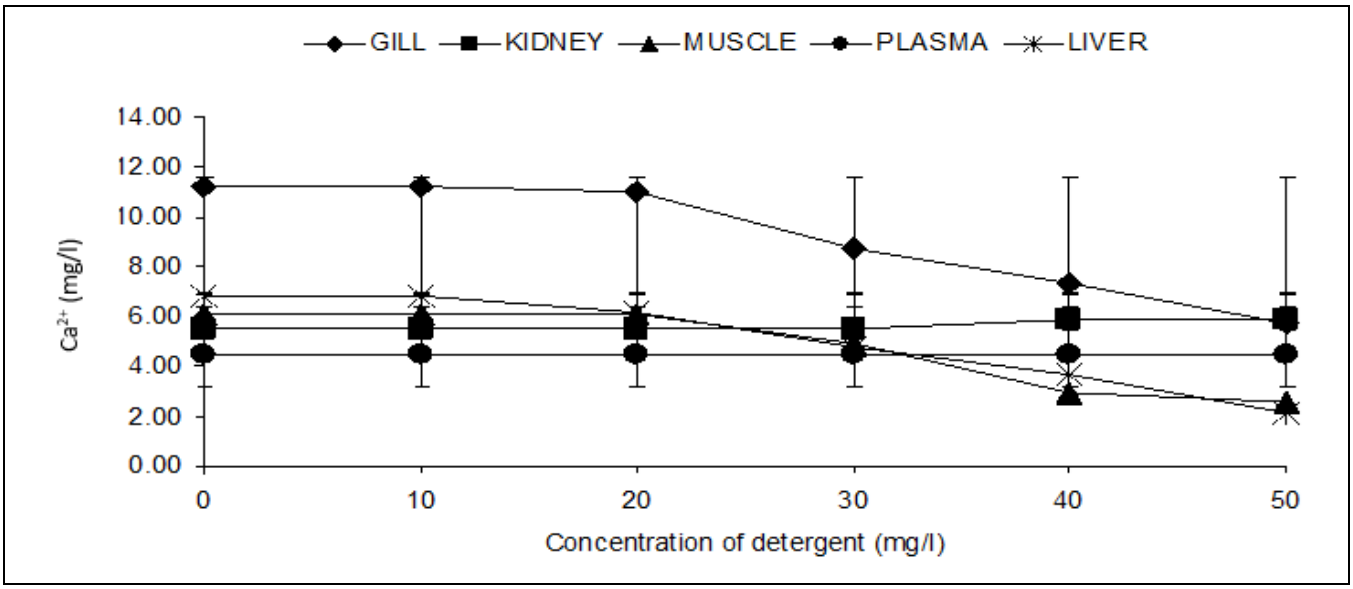

Fig 6: Relative levels of Ca^{2+} in the tissues of *C. gariepinus* adults exposed to chronic levels of jumbo detergent

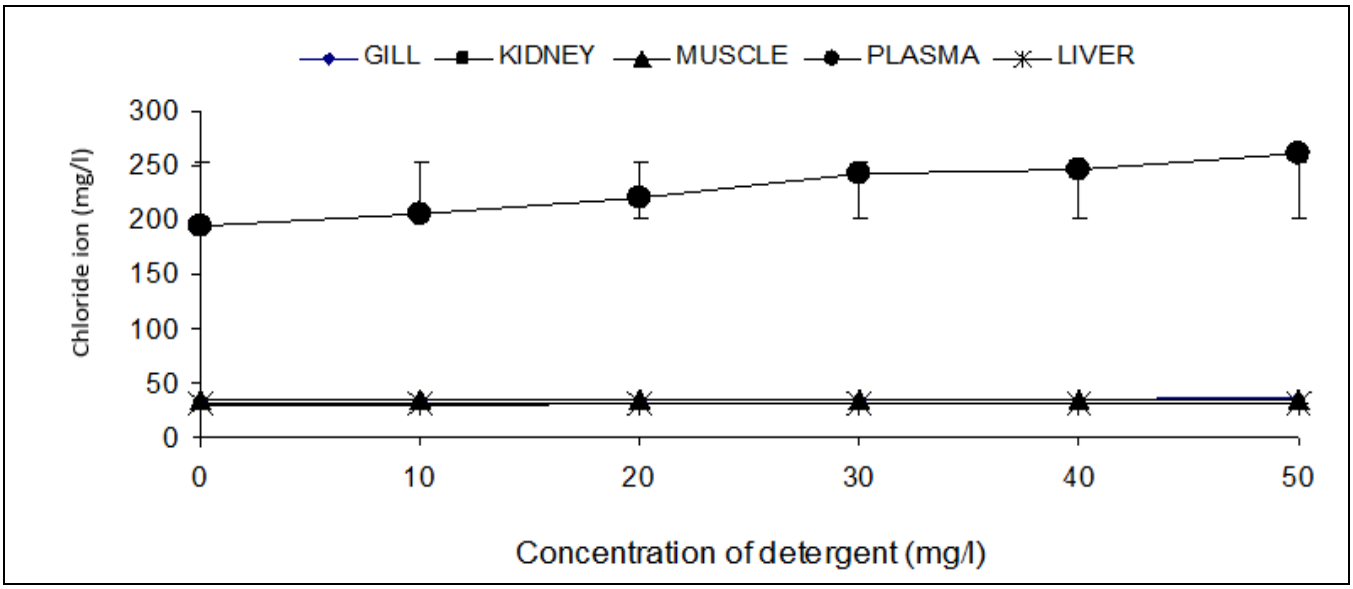

Fig 7: Relative levels of Cl^- in the tissues of *C. gariepinus* adults exposed to chronic levels of jumbo detergent

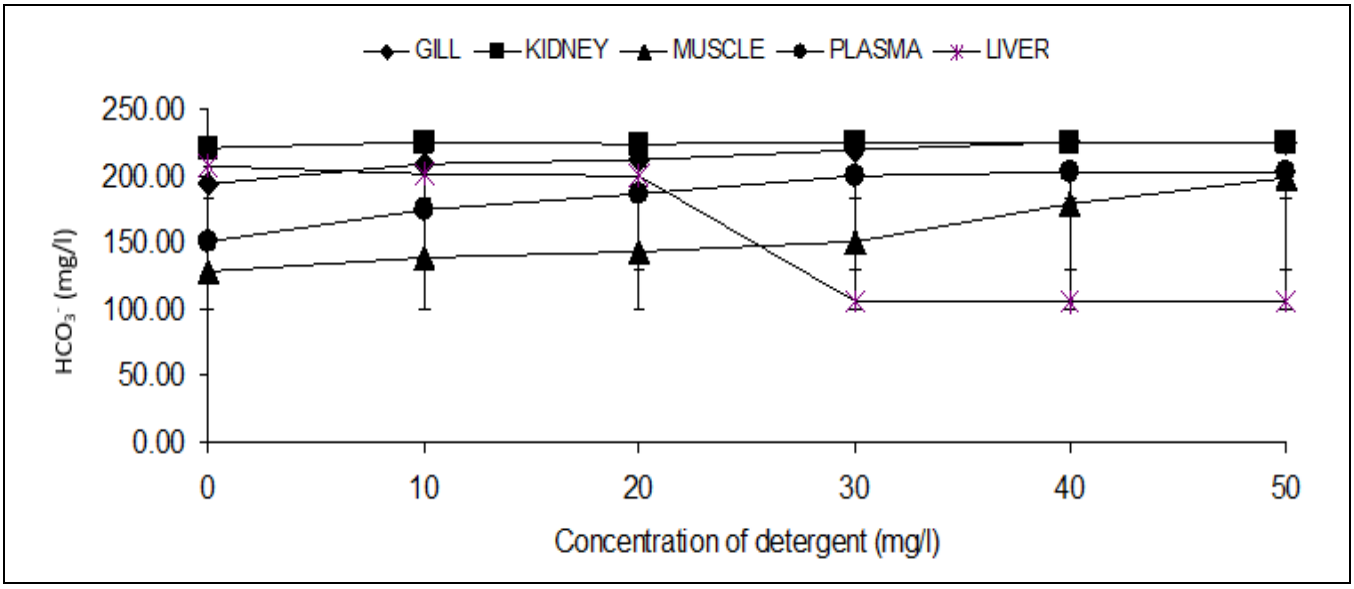

Fig 8: Relative levels of HCO_3^- in the tissues of *C. gariepinus* adults exposed to chronic levels of jumbo detergent

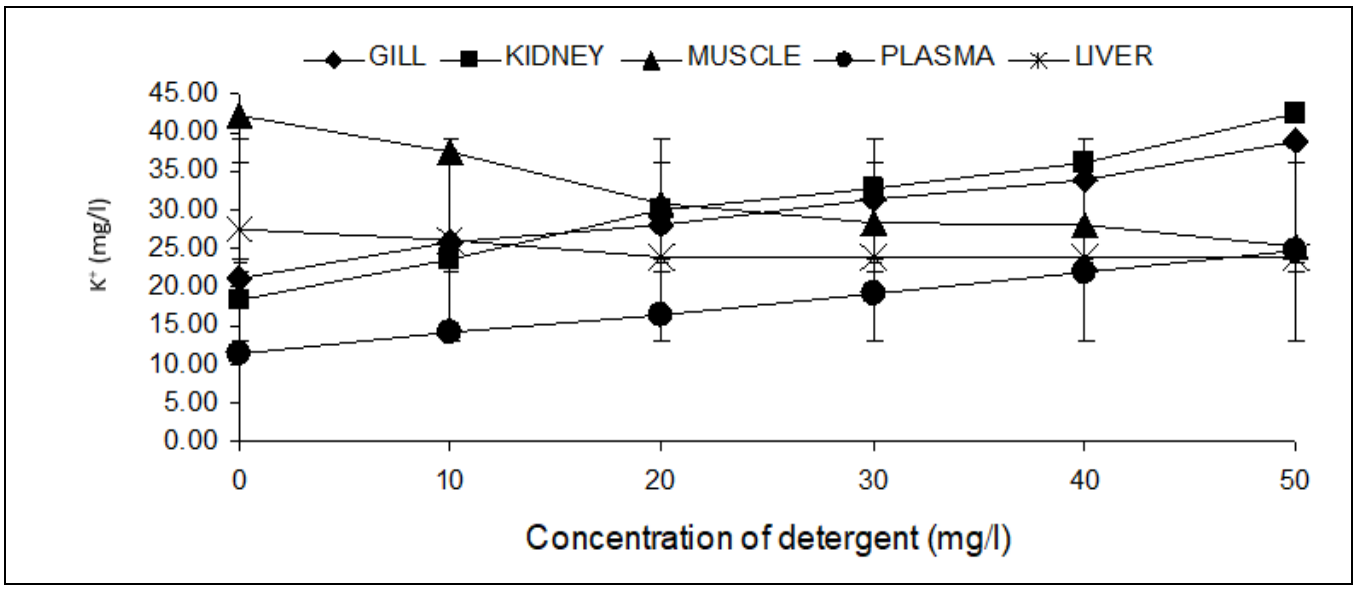

Fig 9: Relative levels of K^+ in the tissues of *C. gariepinus* adults exposed to chronic levels of jumbo detergent

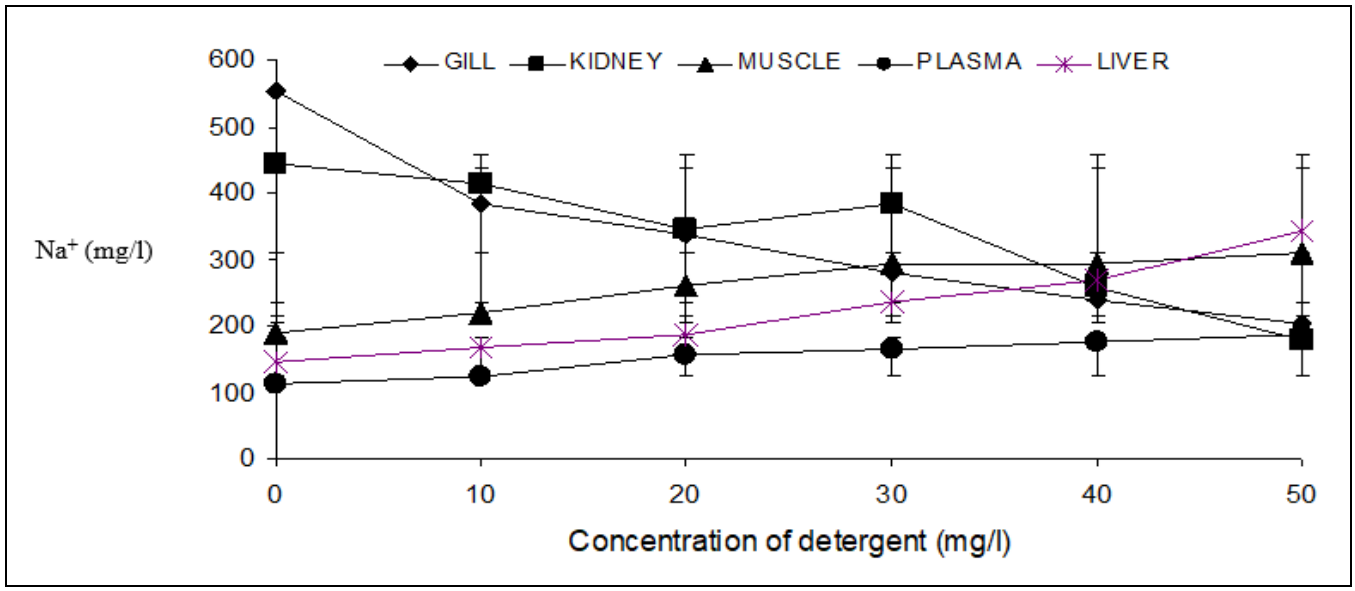

Fig 10: Relative levels of Na^+ in the tissues of *C. gariepinus* adults exposed to chronic levels of jumbo detergent

Conclusion

This work confirms the fact that detergent relatively affects the electrolytic functions of important organs in *C. gariepinus*. Alterations in the electrolytes of the gill, muscle, liver, kidney and plasma would greatly influence the physiology of the fish as sodium, potassium, calcium, chloride and hydrogen carbonate ions are vital for several biological actions including reproduction in the two life stages of *C. gariepinus*. So, care should be taken to avoid disposal of detergent wastes near fish and other aquatic organisms culture environment.

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