Journal of Agriculture and Social Research, Vol. 14, No. 1, 2014

INFLUENCE OF LINEAR ALKYLBENZENE SULPHONATE ON SOME SERUM ELECTROLYTES OF *CLARIAS GARIEPINUS* (BURCHELL 1822) JUVENILES

UEDEME-NAA, B. AND ERONDU, E.S
Department of Fisheries, Faculty of Agriculture,
University of Port Harcourt, Nigeria
Email: barikpoau@gmail.com. Tel: +2348037781164

ABSTRACTS

One hundred and fifty juveniles of C. gariepinus (mean weight 246.30± 14.12g SD/mean length 16.15±1.40cm SD) were exposed to 5 chronic levels (10.00, 20.00, 30.00, 40.00 and 50.00 mg/l.) of Linear alkyl benzene Sulphonate for 30 days to assess the effect on biochemical parameters. Electrolytes activities increased with the increase in detergent concentration in all the tissues under consideration, except Na^+, Ca^{2+} and Cl^- in the gill; Cl^- in the kidney; Cl^- and HCO_3^- in the muscle and liver. The magnitude of alteration in the plasma biochemical parameters were evidently greater in the gill and kidney. This study concludes that Linear alkylbenzene Sulphonate (LAS), even at safe doses, are quite potent to manifest biochemical crisis in fish and that all the electrolytic indices used are viable bioindicators for detergent toxicity in fish.

Keywords: detergent, serum electrolytes, bioindicators, clarias

INTRODUCTION

Detergents are widely used in industrial and domestic premises to wash equipment, installations, heavy duty machines, vehicles and oil soiled materials. It is a common environmental contaminant probably due to its use in the formulation of cleaning agents, pesticides and for dispersing oil spills at seas; so the use, production and exposure, of detergents is unavoidable. The major entry points into water are through sewage works, run off from industrial wastes and directly from domestic usage (Adewoye and Lateef, 2004). Warren (1977); Adewoye and Fawole (2002) and Adewoye *et al.* (2005) reported that the indiscriminate dumping of detergent into an aquatic system might decrease the dissolved oxygen concentration and impair respiration leading to asphyxiation (which is an indication of unconsciousness or death produced by failure of the blood to become properly oxygenated in the lungs) and may ultimately result into organ architectural degradation such as liver dysfunction.

Detergents are also used in pesticide formulations and for dispersing oil spills at sea with deleterious effects on target organisms (Abel, 2006). Ezemonye *et al.* (2007) noted that detergents generally have poisonous effect on all types of aquatic life as a result of degradation of alkylphenol polyethoxylates (non-ionic) which leads to the formation of alkyl (particularly) nonylphenols, that acts as endocrine disruptors. All detergents destroy the external mucus layers that protect fish from bacteria and parasites and affect the fish gills most particularly (Kolak, 1999).

Fishes are very good bio-indicators of aquatic contamination and as bio-indicator species respond with great sensitivity to changes in the aquatic environment. Scanning of pertinent literatures reveals that detergent related works on fish are still very scanty and limited to acute toxicity determination (Lal, 1983). This has informed this present study which reflects changes in serum Na^+, K^+, Cl^-, Ca^{2+} and HCO_3^- in gill, kidney,muscle,plasma,

and liver of fresh water fish (*C. gariepinus*) exposed for 30 days to chronic concentrations of Linear alkylbenzene solphonate (LAS).

METHODOLOGY

One hundred and fifty juveniles of *C. gariepinus* (mean weight, 246.30± 14.12g SD; mean length 16.15±1.40cm SD) was obtained from Abduls Fish Farm Rukpakulusi, Port Harcourt, Rivers State. They were transported by car in a 20L trough covered with a perforated cover to the laboratory in the Department of Fisheries and Aquatic Environment Laboratory (Rivers State University of Science and Technology, Port Harcourt). On arrival, five pieces per aquarium were acclimated in thirty rectangular plastic aquaria containing twenty litre of water each for 7 days. The top of the aquaria were covered with perforated plastic cover to prevent fish from escape and the water was exchanged daily. The aquaria were washed with a piece of foam and the fish was fed once daily with a 42% crude protein diet at 3% body weight. A range finding test (trial test) was carried out using local Jumbo detergent (Surfactant i.e Linear alkylbenzene surfactant, LAS obtained from Rivers State Vegetable Oil Company (RIVOC), Port Harcourt. Five fish were exposed to each concentration (0.00, 10.00, 18.00, 25.00, 37.00, 50.00 mg/l) of detergent for 10 days. Management of water in aquaria were as in acclimation period. The test solutions were renewed daily immediately after washing and fish were fed as in acclimation period. The use of juveniles was due to the sensitivity nature of juveniles to toxicity tests than adults.

Sub-lethal concentration for the definitive test was done based on the range finding test. Five graded concentrations (0.00, 10.00, 20.00, 30.00, 40.00 and 50.00 mg/l) of the solutions were prepared (thoroughly mixed to avoid hot spot) in five replicates each. Five fish were introduced into each aquarium and covered with a perforated plastic lid to prevent escape of the fish. To avoid injuries or bruises on experimental fish, a scoop net was used daily to collect and transfer fish to empty buckets without 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 experimental period, Fish were killed with a blow on the head and dissected in order to collect samples, 0.5g each of gill, liver, kidney, muscle and spleen tissues with the aid of penknife. Sample was macerated with pestle and mortar. Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) were determined in the gill, kidney, muscle, plasma and liver.

RESULTS

Table 1 shows that there was no significant difference ($P>0.05$) in water quality variables when compared with control except in conductivity and turbidity. It was observed that sodium ion (Na^+), Ca^{2+} and Cl^- decreased while K^+ and HCO_3^- (Table 2) increased in activities in the gill as concentration increased when compared with control. In the kidney, Na^+, K^+, Ca^{2+} and HCO_3^- increased in activities while Cl^- decreased in activities (Table 3) as concentration increased when compared with control. There was no definite trend in the activities of Na^+ in that as concentration of LAS increased, activities also increased and dropped sharply at the highest concentration (Table 4). The activities of Na^+ at 20.00 - 30 mg/l were greater than control while others were less when compared with control. Potassium ion and HCO_3^- decreased in activities due to the influence of LAS as concentration increased when compared with control except at 50.00mg/l in K^+. In the Muscle above, the activities of Ca^{2+} increased at regular interval above control as concentration increased while more Cl^-

activities occurred at 10.00-20.00mg/l when compared with control. At 30.00-50.00mg/l, Cl^- activity dropped when compared with control (Table 4). The activities of Na^+ (Table 5) dropped at 10.00mg/l and rose on a regular basis as concentration increased to the end when compared with control where the highest activity was observed. Potassium ion (K^+) had the highest activities at 20.00mg/l, followed by 50.00mg/l and least at 10.00mg/l when compared with control. This was an irregular trend. The activities of Calcium ion (Ca^{2+}) initially dropped below control at 10.00mg/l and increased much more than the control from 20.00mg/l to 50.00mg/l as concentration increased. Initially, chloride ion (Cl^-) activities rose sharply above control at 10.00-30.00mg/l and suddenly dropped below it as concentration increased. The activities of HCO_3^- increased progressively from 10.00 - 50.00mg/l when compared with control which was lower except at 10.00mg/l. Sodium ion, Cl^- and HCO_3^- (Table 6) decreased in activities following a definite trend as concentration increased while K^+ and Ca^{2+} were the reverse when compared with control which ranked much more in all except K^+ and Ca^{2+}.

Table 1: Water Quality Variables (Mean ± S.D) in the Experimental tanks 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 (^oC)	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]
P^H	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]
D/O (mg/l)	5.59±0.98[c]	3.81±0.68[ab]	3.2±0.72[ab]	2.95±0.64[b]	2.55±0.33[b]	2.33±0.13[b]

Means within the same column with different super scripts differ significantly ($P<0.05$).

Table 2: Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) in the Gill of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean ± S.D).

5	Na^+ (mg/l)	K^+ (mg/l)	Ca^+ (mg/l)	Cl^- (mg/l)	HCO_3^- (mg/l)
0.00	415.00±25.77[d]	23.87±6.12[a]	66.50±6.14[c]	121.25±24.11[b]	15.00±2.88[a]
10.00	373.00±51.96[c]	23.89±4.12[a]	51.37±7.21[b]	116.00±23.23[b]	16.25±4.33[a]
20.00	335.00±28.86[b]	24.75±3.4[ab]	18.87±6.37[ab]	101.12±24.12[b]	16.25±4.33[a]
30.00	306.10±40.12[ab]	27.75±6.84[b]	17.61±4.22[ab]	67.62±14.11[ab]	17.75±2.89[a]

40.00	300.21±31.11^{ab}	29.71±7.11^{b}	16.38±8.21^{a}	57.61±10.12^{a}	19.88±4.11^{a}
50.00	296.31±41.11^{a}	29.89±4.53^{b}	13.01±6.92^{a}	52.50±12.11^{a}	20.00±2.87^{b}

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

Table 3: Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) in the Kidney of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean ± S.D).

Conc. (mg/l)	Na^+ (mg/l)	K^+ (mg/l)	Ca^+ (mg/l)	Cl^- (mg/l)	HCO_3^- (mg/l)
0.00	320.00±23.09^{a}	44.12±9.61^{a}	6.50±0.11^{a}	267.00±14.61^{d}	12.50±0.11^{a}
10.00	335.14±17.32^{a}	44.95±11.71^{a}	8.01±3.01^{a}	142.12±18.61^{c}	13.51±0.86^{b}
20.00	3565.14±15.77^{a}	45.61±9.61^{a}	9.51±1.01^{a}	140.22±17.61^{c}	13.75±1.44^{b}
30.00	377.50±17.07^{ab}	50.25±8.71^{b}	11.12±5.96^{ab}	138.41±31.11^{b}	13.75±1.61^{b}
40.00	405.00±15.61^{b}	51.50±10.11^{b}	22.00±6.89^{b}	112.51±18.11^{bd}	13.75±1.71^{b}
50.00	407.51±19.58^{b}	57.76±11.83^{b}	31.25±7.21^{c}	43.75±6.98^{a}	15.00±2.55^{ab}

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

Table 4: Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) in the Muscle of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean ± S.D).

Conc.(mg/l)	Na^+ (mg/l)	K^+ (mg/l)	Ca^+ (mg/l)	Cl^- (mg/l)	HCO_3^- (mg/l)
0.00	465.00±28.86^{a}	51.37±12.49^{a}	9.51±3.46^{a}	57.51±16.11^{a}	15.00±2.88^{b}
10.00	427.00±15.16^{a}	42.37±16.35^{b}	12.61±3.41^{ab}	107.12±35.75^{c}	14.11±1.44^{b}
20.00	412.51±12.88^{a}	36.37±11.31^{a}	18.11±7.21^{ab}	97.75±12.61^{b}	14.00±2.44^{b}
30.00	638.75±59.21^{b}	30.62±8.61^{a}	19.61±6.21^{ab}	57.50±12.64^{a}	13.00.±1.71^{a}
40.00	533.75±11.08^{ab}	42.25±11.72^{b}	23.37±6.25^{b}	57.25±12.71^{a}	13.75±2.50^{ab}
50.00	412.50±12.88^{a}	62.37±10.25^{c}	31.25±7.31^{c}	43.75±12.91^{a}	11.25±3.16

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

Table 5: Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) in the Plasma of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean ± S.D).

Conc.(mg/l)	Na^+ (mg/l)	K^+ (mg/l)	Ca^+ (mg/l)	Cl^- (mg/l)	HCO_3^- (mg/l)
0.00	116.50±6.57^{b}	9.67±1.21^{b}	10.27±6.71^{a}	181.50±28.40^{a}	2.51±1.01^{a}
10.00	78.25±5.31^{a}	6.92±3.21^{a}	10.22±1.19^{a}	296.00±56.70^{b}	1.56±0.16^{a}
20.00	84.75±10.18^{a}	11.60±2.19^{c}	10.40±4.21^{a}	298.25±76.07^{b}	3.00±0.51^{b}
30.00	103.75±15.88^{b}	9.63±1.61^{b}	20.02±6.14^{b}	276.50±94.20^{b}	3.25±0.81^{b}
40.00	111.75±19.15^{b}	8.57±2.95^{b}	21.52±6.14^{b}	172.50±29.55^{a}	4.00±0.74^{c}
50.00	115.25±19.90^{b}	10.57±2.64^{c}	24.54±6.22^{b}	169.25±33.05^{a}	4.01±0.81^{c}

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

Table 6: Sodium ion (Na^+), Potassium ion (K^+), Calcium ion (Ca^{2+}), Chloride ion (Cl^-) and Hydrogen carbonate ion (HCO_3^-) in the Liver of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean ± S.D).

Conc. (mg/l)	Na^+ (mg/l)	K^+ (mg/l)	Ca^+ (mg/l)	Cl^- (mg/l)	HCO_3^- (mg/l)
0.00	360.00 ± 23.09^c	44.12 ± 9.61^a	6.51 ± 0.11^a	267.00 ± 34.29^c	18.75 ± 1.44^b
10.00	359.00 ± 17.32^c	44.75 ± 11.62^a	8.01 ± 3.12^a	142.12 ± 30.11^b	17.51 ± 0.01^b
20.00	355.00 ± 5.77^c	46.21 ± 9.62^a	9.51 ± 0.37^a	143.31 ± 31.11^b	17.51 ± 0.01^b
30.00	317.00 ± 17.07^b	50.25 ± 8.71^b	11.12 ± 5.96^b	138.37 ± 30.52^b	13.75 ± 1.44^a
40.00	310 ± 10.01^b	51.50 ± 14.88^b	22.01 ± 12.89^c	112.51 ± 31.61^{ab}	13.75 ± 1.48^a
50.00	307 ± 9.57^a	57.75 ± 11.83^b	31.25 ± 7.21^c	43.75 ± 17.51^a	13.25 ± 1.43^a

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

DISCUSSION

In an attempt to define and measure the effects of pollutants on aquatic organisms, biomakers and bioindicators are employed in several studies on xenobiotics. The principle of this approach is the analysis of electrolytes (Jee and Kang, 2005). According to Adhikari *et al.*, (2004), toxicants act by the disruption of cell membrane permeability replacing the structural or electro-chemical important element in the cell which causes functional failure.The basic function of electrolytes in the body lies in controlling fluid distribution, inter and extra cellular acids, basic equilibrium, maintaining osmotic pressure of body fluids and normal neuro muscular irritability (Adeyemo, 2005 and Lal *et al*., 1983). Sodium (Na^+); Potassium (K^+) and Calcium (Ca^{2+}) are the major cations of the extra cellular fluid (Celik *et al.*, 2005; Adeoye, 2007). Electrolyte variations in fish exposed to toxicants including detergents influence their performance in diverse ways such as reproduction, size of eggs among others. (Fu et al., 1990; Chowdhurry et al., 2004). Since fresh water fish take up most of the ions necessary for homeostasis from the water via their gills, the drop of plasma electrolytes apparently result in the increase of efflux of ions across these organs and an impairment of active ion uptake by the chloride cells of the gill (Wendelaar-bonga and Luck, 2007).

The decrease and increase of electrolyte activities as LAS concentration increases in all the organs concerned depicts that liver tissue of the exposed fish might have been impaired. This was in agreement with Thophon *et al.*, (2003) that LAS influenced the volume of electrolytes (Na^+, K^+, Ca^{2+}, Cl^- and HCO_3^-) in gill, kidney, muscle, plasma, and liver with increase in concentration. This could result in instability of fish in terms of osmoregulation over time because electrolyte concentrations are indicative of the ability of organisms to osmoregulate. Stability is often compromised with stress, disease of gill lesions that increase gill permeability to ions (Al-Kahem, 1994), osmoregulation dysfunction which is an inherent part of stress in fish (Harrel and Moline, 1992, Weirich *et al*., 1992). The decrease in Na^+ activities in all the organs except kidney and plasma in this study with increasing concentration of LAS could be due to the epinephrine released during the stress responses which increased oxygen demand, dilation of gill blood vessels and increased use of vessels

that are not normally used at rest. This increased the surface area of the gills that is available for gas exchange. Water influxes and ion losses are reduced, a phenomenon known as osmoregulatory compromise (Calamari and Marcetti, 1973).

Minerals are mainly responsible for the maintenance of osmotic pressure in blood and proper functioning of all types of tissues (Hoffman and Lammel, 1984). The presence of activator ion of alkali metal series of Na^+ and K^+ are essential for the activity of many enzymes in fish (Kimerle, 1989). Toxic metals, detergents and pesticides can alter the electrolytes in the blood (Ludwig and Serekan, 1988). ATP and its related systems are known to participate in several metabolic processes, whilst Na^+ and K^+ ATPase located in the cell membrane has been implicated in the active transport of Na^+ and K^+ across the cell membrane (Taylor *et al.,* 2003). The changes in the levels of Na^+ in this study might be due to gill damage and inhibition of electrolytic activity. Changes in plasma ion content (decrease or increase above control) have been attributed to kidney damage and altered electrolytic activity which is in line with the findings of Blaxhall, 1999.

CONCLUSION AND RECOMMENDATION

In conclusion, the increased electrolytic activities in this study may be correlated with cell membrane damage or changed permeability caused by detergent leading to selective leakage of electrolyte to blood stream as suggested by Travlos (1996) and Summarwar and Lall, (2013). This contention also gets support from the view of Harper (1981), that electrolytes are diagnostically most useful to detect cellular damage. The decreased profile of these electrolyte estimated in this study is attributed to adverse effect of detergent on cell and its organelles (Jana *et al*., 1985). Since all electrolytes are basically membrane bound and hence any perturbation in membrane property as a result of interaction with surfactants could lead to alteration in its activities, this study has shown that Linear alkylbenzene solphonate(LAS) induced severe metabolic crisis in *Clarias gariepinus* juvenile and each of the parameters investigated can safely be used as a marker or bio-indicator of stressed physiological state of the fish. In view of the toxicity effect of this detergent, it can be inferred that, indiscriminate discharge of detergent effluents can induce damages to the tissues and organs, which might make all the living entities in polluted environment vulnerable to diseases, and eventually leads to death. Therefore there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluents prior to their discharge into the environment.

REFERENCES

Abel, P.D. (2006). Toxicity of synthetic detergents to fish and aquatic invertebrates. *Journal of Fishery Biology* 6(3), 279 – 298.

Adhikari, S., Sarkar, B. and Chatterjee, A. (2004). Effects of cypermethrin and carbofuran on haematolgical parameters and prediction of their recovery in fresh water fish *Labeo rohita.* Ecotoxicology and Safety, 58:220-226.

Al-Kahem, H.F. (1994). The toxicity of nickel and the effects of sublethal levels on haematological parameters and behaviour of the fish *Oreochomis niloticus. J. Univ. Kwait, Sc.,* 21(2):243-252.

Adeyemo, O.K. (2005). Haematological and Histopathological effects of cassava mill effluent in *Clarias gariepinus: African Journal of Biomedical Research*. 6(3):179 – 183.

Adeoye, A. (2007). *Medical Laboratory Practice.* 1st edition 238p.

Adewoye, S.O., Fawole, O.O., Owolabi, O.D. & Omotosho, J.S. (2005). Toxicity of cassava waste water effluents on African Catfish (*Clarias gariepinus).Ethiopian Journal of Science* 28 (7),189 -194.

Adewoye, S.O. & Lateef, A. (2004). Evaluation of the Microbiological characteristics of Oyun river – A polluted river in North-Central Nigeria. *Pollution Ressearch* 23(14), 587 – 591.

Adewoye, S.O. & Fawole, O.O. (2002). Acute toxicity of soap and detergent effluent to fresh water *Clarias gariepinus* fingerlings. A*frican Journal of Science* 22,112-118

Blaxhall, P.C. (1999). The haematological assessment of the health of fresh water fish. A review of selected literature. *J. Fish. Biol.,* 4:593-603.

Calamari, D and Marcetti, R.(1973). The toxicity of LAS to rainbow trout (*Salmo gairdneri*). Water Res.,7: 1453-1463.

Celik, A., Momarci, B. and Askin, A. (2005). Evaluation of Cytogene effects of lambdancyhalothrin on wister rat bone marrow. *Ecotoxicol. Environ Safety*, 61(1):128-133.

Chowdhurry, M.J., Pare, E. F. and Wood, C.M. (2004). Physiological effects of detergent on rainbow trout. *Comp. Biochem. Physol. Toxicol. Pharmacol.,*139: 163-173.

Ezemonye, L.I., Ogeleka, D.F. & Okiemen, F.E. (2007). Acute toxicity of industrial detergent (Neatex O and corrossium inhibitor (Norust, R486) to early stages of cichlids. *Tilapia guineensis, Environmental Ecology* 23(2), 131-138.

Fu, H., Steinbach, O.M. and Balm P.H.M. (1990). Involvement of Cortisol and Metaliothionein proteins in the physiological responses of *Oreochromis mossambicus* to sublethal stress. *J. Aqua.Toxicol.,* 16:257-270.

Harper, H.A., Rodwell, V.W., and Mayer, P.A. (1981). Review of physiological chemistry. Lange Medical publication. California.

Harrel, R.M. and Moline, M.A. (1992). "Comparative stress dynamics of Broodstock striped Bass, *Morone saxatilis*, associated with two capture techniques". *Journal of the World Aquaculture Society,* 23:58-76.

Hoffman, R. & Lammel, R. (1984). Effects of repeated blood sampling on some blood parameters in freshwater fish. *Journal Fishery Biology* 24,245-251.

Jana, S., Sahana,S.S., Chaudhuri,M.A. and Chaudhuri, D.K. (1985). Effect of mercury on inorganic phosphorous and activities of acid and alkaline phosphatases in fresh water fish. 3: 2-4.

Jee, H.J. and Kang, C.J. (2005). Biochemical challenges of enzymatic defence system after detergent exposure in olive flounder. *Physio. Res.,* 54:585 – 591.

Kolak, A.S. (1999). Inter individual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Journal of Fishery and Aquatic* Science 56,708 – 710.

Kimerle, R.A. (1989). Aquatic and terrestrial ecotoxicology of LAS. Tenside, surfactants. Deterg., 26: 167-176.

Ludwig, H. F and Serekan, A.S. (1988). Evaluation of use of anionic detergents(ABS) in Malasia. *Water Res.,* 22: 257-263.

Lal, H., Mishra, V., Vishwanatha, P.N. and Krishnamurthi, C.R.(1983). Comparative studies on ecotoxicology of synthetic detergents. 8: 447-450.

Taylor, L.N., Wood, C.M. and Medonale D.G. (2003). An evaluation of sodium loss and gill metal binding properties in rainbow trout. *Environment Toxicology and Chemistry,* 22:2159 – 2166.

Thophon, S., Kruatrachue, M. and Sahaphory, S. (2003). Histopathological alterations of white sea bass. *Environment. Pollut.,* 121:307-320.

Travlos, G.S.,Morris, R.W., Elewell, M.R., Duke, A., Rosenblum, S. and Thompson, M.B. (1996). Frequency and relationship of clinical chemistry and liver and kidney histopathology finding in 13 week 13 study of rats. 107:17-29.

Summarwar, S. and Lall, D. 2013. Effects of toxins on blood plasma of battrachus. 3:133-136.

Warren, C.E. (1977). Biology and water pollution. *W.B.Sanders and Company, Philadelphia. U.S.A.,* 434 p.

Weirich, C.R., Tomasso, J.R. and Smith, T.J. (1992). "Confinement and transportation induced stress in white Bass *Morone chrysops*, stripped Bass, *M. saxatilis*, Hybrids, Effect of calcium and salinity". *Journal of the World Aquaculture Society.,* 23:49-59.

Wendelaar–bonga, S.E. and Luck, R.A.C (2007). Toxicants and Osmoregulation in fish. Netherlands *Journal of Zoology.* 42,478-493.