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ORIGINAL ARTICLE

Histopathological Alterations in Gills of Catfish and Changes in Aquarium pH, Temperature and Dissolved Oxygen due to Lead Nitrate and Crude Oil

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ABSTRACT
Histopathological changes in fish tissues have been used to assess effects of pollutants in aquatic environments. Gills of fishes are continuously bathed in water and are vulnerable to pollutants. Histopathological alterations in gill tissues of Catfish Clarias gariepinus exposed in aquaria treated with varying concentrations of lead nitrate and crude oil were therefore studied. One hundred Clarias gariepinus juveniles of mean weight 138±12gand length 28±1.5cmdivided into 5 groups of 20 fish each which comprised the control group, groups treated with lead nitrate 20mg/L and 35mg/L;and groups treated with crude oil 300ppm and600ppm.Observation lasted for 10 weeks during which pH, temperature and dissolved oxygen of aquaria containing each group were monitored. Histological sections of gills from each group were subsequently prepared, and histopathological alterations observed under light microscopy and demonstrated in photomicrographs of treated groups included hyperemia, oedema, necrosis, fibrosis, degeneration and lymphocyte infiltrations which occurred with increasing concentrations of pollutants studied. There was no marked differences in pH and temperatures of treated and control aquaria but dissolved oxygen in control was significantly higher than the treated aquaria.
Keywords: *Lead Nitrate, Crude Oil, Clarias gariepinus, Gill tissue, Histopathology.*

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INTRODUCTION

The most commonly observed features prior to fish kill during exposure of fish to solutions of heavy metals has been respiratory distress [1]. Fish gills serve a variety of physiological functions including respiratory gas exchange, osmo-regulation, nitrogen excretion and control of acid-base balance [2].Trace amounts of metals occur naturally in water, however waste water from mining, chemical industries and from Agriculture, for example, are the main source of water pollution[3, 4]. Additionally, rain contaminated by burning fossil fuels is another source of pollution [5]. In fish, the route of heavy metals entry is through the gills, mouth and skin. Lead, a biologically non-essential metal is relatively abundant in nature and of extensive use in modern times [6]. The build-up and transportation of lead in water, atmosphere and sediment result in bio-accumulation of the metal in various pockets of food chain[7]. The residues of Lead in room paints from old houses pose a risk from chips and dust [8].

Pollution from crude oil is common all over the world and particularly endemic in countries whose economies are dependent on the oil industry. In Nigeria oil industry operations are both onshore and offshore and all the oil terminals and most refineries in the country are located in the Niger Delta region and hence more than 90% of oil-related activities take place in this region [9]. According to [10] most of

the spills occur in the coastal areas and swamps of the Niger Delta. Exposure of aquatic organisms to crude oil have been shown to impact on various aspects of fish physiology and sometimes leading to large scale mortality [11, 12, 13].Histopathological changes in fish exposed to pollutants have been used as sensitive biomarkers for assessing the effects of several environmental contaminants including petroleum products [14]. Investigations by several workers have revealed histopathological changes in various organs of fish (Ovaries, gill and liver) exposed to hydrocarbons [15].

The gills, being continuously bathed in the surrounding water are most vulnerable organs to the various aquatic pollutants [16]. It leads to alterations in the normal respiratory surface and would lower down the diffusing capacity of gases through the gills [17]. Since gills have a key role in the transport of oxygen for metabolism, they offer a favourable material for studies on effects of toxic pollutants on respiration therefore [18] recommended the studies of gill histopathology to understand the biological responses to a variety of aquatic pollutants. The toxicity of any chemicals alters the physiological state of the animals, thereby impairing the various metabolic activities. Therefore to have a clear understanding as to how these chemicals cause injury to the tissues, it is essential to study the histopathological changes that could take place in gill tissues in response to such chemicals [19]. This study was undertaken to investigate the Histopathological Alterations in Gills of Catfish and Changes in Aquarium pH, Temperature and Dissolved Oxygen due to Lead Nitrate and Crude Oil

MATERIALS AND METHODS

Acute toxicity tests lasting 96 hours were performed on *Clarias gariepinus* of mean weight 138±15g and mean length 28±1.5cm taken according to Adeyemo *et al*. (2008) to determine the LD_{50} of lead nitrate and crude oil. Thereafter sub-lethal concentrations of lead nitrate and crude oil were used to treat the fish in 5 experimental aquaria A, B, C, D and E. *Clarias gariepinus* collected from Rehoboth Farms Nkwelle-Ezunaka, Oyi Local Government Area, Anambra State, Nigeria, were acclimatized for 14 days before being divided into the five experimental groups. They were fed with fish pellets; Skretting(R) fish feeds at 1% biomass half at 900 hours and 1700 hours respectively. Group A consisted 20 fish kept as control, Group B consisted of 20 fish treated with 20mg/L Lead Nitrate, Group C consisted of 20 fish treated with 35mg/L Lead Nitrate, Group D consisted of 20 fish treated with 300ppm crude oil while Group E consisted of 20 fish treated with 600ppm crude oil.Water change was done every four days to maintain the pollutant strength and dissolved oxygen (DO) level as well as minimize the level of ammonia during the experiment which lasted 70 days. Water quality parameters DO, pH, and temperature were monitored during the experiment.The DO was measured using a Dissolved Oxygen Analyzer Model JPB 607. The pH was measured with a portable pH meter P Hep(R) a registered trade marks of "Hanna instruments".www.hannainst.com. The temperature was measured with a mercury bulb, water thermometer with a range of O^oC-400^oC. The data were statistically analyzed using ANOVA (MS-Excel 2001: MS USA).

Representative samples of live *Clarias gariepinus* fishes from each group were euthanized, dissected and the gills eviscerated for histological examination according to [3] as follows: The organs were labeled and fixed separately in small plastic containers in 10% Formal-Saline solution at room temperature for 24hrs to prevent autolysis and putrefaction. 1000mls of Formal-Saline solution was formulated with 100ml of concentrated formaldehyde, 900ml of tap water, and 8.5g of sodium chloride crystals. The preserved tissues were left in running water for thirty minutes to remove all traces of fixative before being sectioned into pieces of 2mm diameter and then dehydrated for 30 minutes in ascending series70, 90, and100%concentration of ethanol respectively. The tissues were cleared of ethanol overnight in chloroform and then immersed in molten paraffin wax for infiltration at 60ºC in an electric oven for one hour. The tissues were then embedded in fresh molten paraffin wax at room temperature. The embedded tissues were sectioned, each 5µm in thickness, with a microtome knife with a manual rotary microtome (American optical comp. No. 800). The serial sections were mounted on clean microscope glass slides previously subdued with egg albumen to enhance proper adhesion of the tissue on the slides. The slides were properly labeled, cleared of paraffin in twochanges of Xylene for thirty minutes; and hydrated in descending grades 90, 70, and 50% concentration of ethanol for ten minutes respectively. Staining was done using Harris H&E stain in a glass staining trough. The stained sections were dehydrated in ascending grades 50, 70 and 90% concentration of ethanol, cleared in chloroform solution, and covered with cover slides using Canada balsam for proper adhesion. The permanent slides were produced and photomicrographs of the sections taken with photomicrograph microscope Model-Gallen III No. Bm 600 at varying magnifications [20].

RESULTS AND DISCUSSION

Gill sections from the control group were normal with core of fibro-muscles and cartilages surrounded by columnar epithelium forming papillary projections on their surface and were richly vascularized (Plate 1). Primary and secondary lamellae of gills of fish exposed to Lead Nitrate were hyperaemic, oedematous and degenerated (Plates 2 & 3). The primary and secondary gill lamellae of those exposed to crude oil showed oedema, necrosis, increased vascular congestion (hyperaemia) with infiltration of the sub-mucosa by fibrinous exudates with increasing concentration of the pollutant (Plates 4 & 5). Degrees of the pathological changes in the gills were therefore directly dependent on concentrations of the pollutants studied. Helminth parasites of fishes [21] may also be responsible for pathologic changes in fish tissues but the fishes used in this study were juveniles and were not considered to be infected at that age.

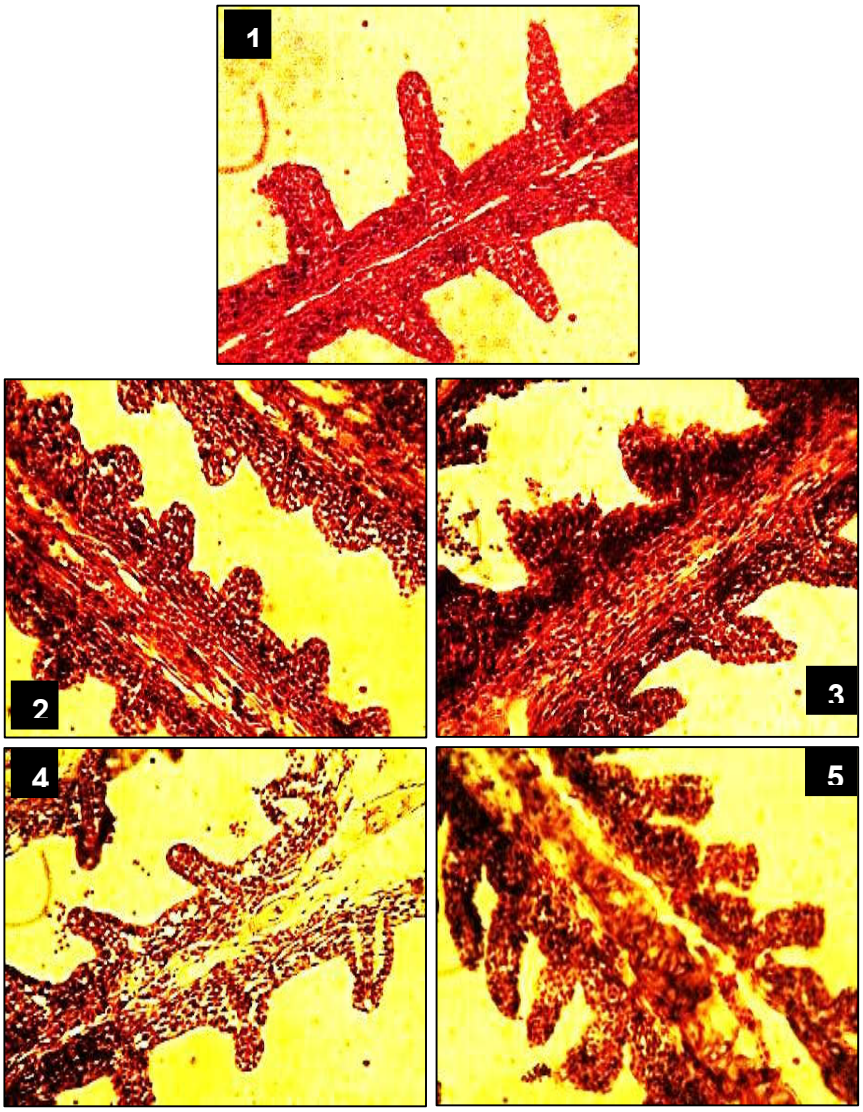

Plate 1: Photomicrograph of thehistological section of normal Gill section of control fish showing normal primary and secondary lamellae. H&E stain x 400.

Plate 2: Photomicrograph of thehistopathological sectionofGill section of fish treated with 20mg/l lead nitrate, showing hyperaemic primary and secondary lamellae. H&E Stain x 400

Plate 3: Photomicrograph of thehistopathological sectionofGill section of fish treated with 35mg/l lead nitrate, showing hyperaemic primary and secondary lamellae, oedema and degeneration of secondary lamellae. H&E stain x 400.

Plate 4: Photomicrograph of the histopathological section of Gill section of fish exposed to 300ppm crude oil showing fibrinous exudates and necrosis of primary lamellae, oedema and degeneration of secondary lamellae. H&E stain x 400.

Plate 5: Photomicrograph of the histopathological section of Gill section of fish exposed to 600ppm crude oil showing hyperaemic primary and secondary lamella, oedema and degeneration of secondary lamellae. H&E stain x 400.

Fish are targets for environmental contaminants. Some fish populations are highly exposed to chemicals, especially in urban, industrial or coastal sites [3]. These fish are studied because of concern for population-level effects [22, 23] or as sentinels for the health of the environment. In addition, they may be subjects of research to investigate the mechanisms involved in physiological or evolutionary adaptation to chemical exposure [24]. Fish are also widely and increasingly used as animal models in toxicological studies, with the ultimate goal of extrapolating the results to inform questions concerning the potential human health effects of chemical exposure. Changes in the gills of fish exposed to Lead and Crude oil fall within the general responses of fish organs to environmental pollutants [3]. It was observed by [25] that fish gills are the prime target organ of all pollutants due to their extensive surface in contact with crude oil spills especially in the oil producing states. The fish even though were exposed to these pollutants yet were alive till the end of the exposure period. This means that these pollutants affect the internal metabolism of the fish since they are non-degradable, bioaccumulate in the tissues of the fish [7] getting into the human food chain. Humans are at risk of inherent toxicity upon consumption of such contaminated fish[4]. The fish also faces the dangers of impaired growth and reproduction, subsequent reduction in fish population and possible extinction [26], an indication that gill morphology and morphometrics are important biomarkers providing a rapid method for detection of the effects of pollutants.

Morphological changes in the gills recorded in this study are hyperemia, oedema, necrosis, degeneration and cellular infiltration. These have been reported in *Clarias gariepinus* after exposure to kerosene oil [27] in *Astyanax* spp.,after a brief exposure to water soluble fraction of crude oil [15] in *Clarias gariepinus* under prolonged exposure to plant extracts [28, 29]. In other studies, exposure of the fish samples to toxicants for 96 hours have produced irreversible changes in the gills [25]. Changes in the gills were thought to be adaptations by the fish to cope with the challenge of the toxicants. Oedema recorded in the gills was due to failure of the sodium pump occasioned by the toxicant leading to accumulation of Na^+ and the diffusion of K^+ outside [30]. It was also reported by [31] that degenerative changes occurred in the gills of *Gasterosteus aculeatus* exposed to cadmium. Vascular changes in the gills of exposed fish could be attempts by the fish to supply more blood to the gills to increase oxygen uptake and supply to internal organs [32]. According to [33], gills of fish are multipurpose organs that in addition to providing for aquatic gaseous exchange play dominant roles in osmotic and ionic regulations, acid-base regulations and excretion of nitrogenous wastes. Despite the fact that all fishes have functional kidneys, the gill epithelium is the site for many processes mediated by the renal epithelia in terrestrial vertebra.Therefore impairment of the gill functions by the overall effect of the pathological changes in the gill of exposed fish will have grave consequences.

The effect of lead nitrate and crude oil on the pH, temperature and dissolved oxygen (DO) of control and treated aquaria are shown in Table 1.

Table 1: Water Quality Parameters of Exposure Aquaria

Parameter	Day	Control Aquarium	Treated Aquaria			
			20mg/Pb$(NO_3)_2$	35mg/Pb $(NO_3)_2$	300ppm Crude oil	600ppm Crude oil
pH	1	7. 55 ± 0.03	7.45 ± 0.03	7.50 ± 0.01	7.55 ± 0.04	7.60 ± 0.01
	2	9.75 ± 0.03	9.65 ± 0.04	9.70 ± 0.01	9.60 ± 0.01	9.60 ± 0.01
	4	9.8 ± 0.01	9.8 ± 0.01	9.8 ± 0.07	9.75 ± 0.04	9.90 ± 0.01
Temperature (^{0}C)	1	28.0 ± 0.01	28.0 ± 0.01	27.5 ± 0.01	28.0 ± 0.04	28.0 ± 0.01
	2	26.0 ± 0.01	26.0 ± 0.01	25.5 ± 0.04	26.0 ± 0.04	26.0 ± 0.01
	4	25.0 ± 0.01	25.0 ± 0.04	25.0 ± 0.02	25.0 ± 0.01	25.0 ± 0.00
Dissolved Oxygen	1	7. 9 ± 0.01	7.8 ± 0.04	7.8 ± 0.04	7.8 ± 0.04	7.9 ± 0.01
	2	7.5 ± 0.01	7.3 ± 0.01	7.0 ± 0.04	4.85 ± 0.04	4.05 ± 0.04
	4	4.50 ± 0.01	3.75 ± 0.04	3.10 ± 0.071	3.90 ± 0.07	3.35 ± 0.02

There were no significant differences between the pH and temperature of both the control and treated aquaria containing fish samples. The values of the DO in the exposure aquaria indicated that the dissolved oxygen levels in the control and treatment groups were variable with a defined trend relative to the concentration of the pollutants. The higher the pollutant concentration, the less DO becomes. The DO levels in aquaria of control group were significantly higher ($P<0.05$) than those of the treatment groups. Among the treatment groups, aquaria exposed to crude oil (300ppm and 600ppm) had a significant

decrease ($P<0.05$)in DO from the 2nd day, while the lead nitrate treated aquaria experienced significant decrease ($P<0.05$)on the 4th day.

CONCLUSION

This study sufficiently indicates that lead nitrate and crude oil have toxic effects on the gills of exposed fish. This inherently implies that inland and marine waters are susceptible to pollutant from lead based industries effluents as well as crude oil spills especially in the oil producing states. The fish even though were exposed to these pollutants yet were alive till the end of the exposure period. This means that these pollutants affect the internal metabolism of the fish since they are non-degradable, accumulate in the tissues of the fish getting into the human food chain. Humans are at risk of inherent toxicity upon consumption of such contaminated fish. The fish also faces the dangers of impaired growth and reproduction, subsequent reduction in fish population and possible extinction.It is therefore recommended that environmental protection laws be enforced so as to prevent water bodies from pollution.

DISCLOSURE OF INTEREST

All authors declare that they have no conflicts to report.

ANIMAL STUDIES

All institutional and national guidelines for the care, use and euthanasia of live *Clarias gariepinus* fishes before they were dissected and the gills eviscerated for histological examination in the laboratory were followed.

REFERENCES

1. Hiltibran, R.C. (1971). Effect of Cadmium, Zinc, manganese and calcium in oxygen and phosphate metabolism of blue gill liver mitochondria *J.Wat. Pollut. Control Fed.* 43(5). 818-823.
2. Hoar, W.S. and Randall, D.J. (1984). *Fish Physiology.* Vol. X. *Gils.* Part *A. Anatomy*, Gas Transfer, and *Acid-Base Regulation.* Academic Press, Orlando, FL.
3. Nsofor, C.I., Ikpeze, O.O., Ngenegbo, U.C., Ikeogu, C.F., and Okonkwo, J.C. (2014a). Histopathological Alterations in the Liver and Kidney of the Fish *Chrysichthys nigrodigitatus* due to heavy metals in Niger River. *Journal of Natural Sciences Research* Volume 4, No.12, 2224-3186.
4. Nsofor, C.I. and Ikpeze, O.O. (2014). Potential hazards of consuming *Clarias gariepinus* fish contaminated with heavy metals of River Niger. *Global Journal of Biology, Agriculture & Health Sciences*, 3(2): 69-74.
5. Pitter, P. (1999): Hydrochemie (Hydrochemistry) *Vydavatelstvi* VSCHT, Praha.
6. Todd A.C., Wetmur J.G., Moline J.M., Godbold J.H., Lewin S.M., Landrigen P.I. (1996). Unveiling the chronic toxicity of Lead. An essential priority for environmental health, *Environmental Health Perspect*. 104 (Suppli): 141-146.
7. Nsofor, C.I., Igwilo, I.O., Ikpeze, O.O., Ikeogu, C.F., Umeoguagu, F.O and Okonkwo, C.J (2014b). Bioaccumulation of Heavy Metals in Shellfish *Macrobrachium rosenbergi* in Niger River at Onitsha. *International Journal of Agriculture and Biosciences,* 3(1): 38-40.
8. Hu, H. (2001). Poorly Controlled Hypertension in a painter with chronic lead toxicity. Environ. Health Perspect. 109:95-99.
9. Imevbore, A.M.A and Adeyemi, S.A. (1981): Environmental monitoring in relation to oil pollution. Pp 135-142 In: Proc of the conf on the petroleum Ind. And the Nigerian environment. NNPC/FMV and H, PTI Warri Nigeria.
10. Rivers State Environmental Protection Bureau, (1992): Ecological distribution of oil spills pollution paper. Rivers State Environmental Protection Bureau, Port Harcourt Nigeria. Pp. 15.
11. Barron, M.G., Carls, MG, Short, J.W. and Rice, S.D. (2003). Photo-enhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope Crude oil to Pacific herring eggs and larvae. *Environ. Toxicol. Chem*. 22:650-660.
12. Couillard C.M., Lee, K. Legare B. and King, T.L. (2005). Effect of dispersant on the composition of the water soluble-accommodated fraction of crude oil and its toxicity to larval marine fish. *Environ. Toxicol. Chem*., 24: 1496-14504.
13. Lui, B., R.P.D., Romaire, Elaune, R.D. and Lindau, C.W. (2006) Field Investigation on the toxicity of Alaska North Slope Crude oil and Dispersed ANSC Crude to Gulf Killifish, Eastern Oyster and white Shrimp. *Chem*., 62: 520-526.
14. Bennett, D., Schmidt, H. Meier, W. Burkhardt-Holm, P. and Wahli, T. (1999). Histopathological of fish: A proposal to assess pollution. *J. Fish Dis*., 22:25-34.
15. Akaishi, F.M., De Assis, H.C., Jaakobi, S.C., EirasStofella, D.R., St. Jean, S.D., Couteany, S.C., Lima, E.F., Wagener, A.L., Scofield, A.L., Ribeiro, C.A. (2004). Morphological and neurotoxicological findings in tropical freshwater fish *(Astyanax sp.)* after waterborne and acute exposure to water soluble fraction of crude oil. *Arch. Environ. Contam. Toxicol.* 46:244-253.
16. Roberts, R. J. (1978). Pathological and Systematic Pathology of Teleost in: Fish Pathology (Edl. R.J.O.), pp 55-59 Bailliere Tindal, London, U.K.

17. Hughes, G.M. and Perry, S.F. (1976): Morphometric study of trout gills. A Light Microscopic Method suitable for the evaluation of pollutant action. *J. exp. Biol.* 63:447-460.
18. Swartz, R.C. (1972): biochemical Criteria of Environmental Changes in the Chesa Peake Bay. *Chesapeake Sci.*, 13:517-541.
19. Jadhav B.V. and Waykar B. (2005). Influence of L-Ascorbic Acid on the Cypermethrin induced Histopathological Changes in the Gill of Fresh Water Bivalves *Perreysia cylindrical.* In: Fisheries and Fish Toxicology, pp 180-191. APH Publishing Corporation New Delhi, India.
20. Drury, R.A.B. & Wallington, E.A. (1976). *Carletons Histological Techniques.* 4th ed. Oxford Univ. Press, Ely House London. W.I. 88-100.
21. Nwuba L.A and Ikpeze, O.O. (2009). Helminth Parasites of the African Catfish (Pisces: Clariidae) from the Polluted Water Environment of the River Niger at Onitsha. *International Journal of Bioscience*, 4(2):30-33
22. Cook, P.M., Robbins J.A., Endicott. D.D, Lodge, K.B., Guiney, P.D. (2003). Effects of Aryl hydrocarbon receptor-mediated early life stage toxicity on lake trout populations in Late Ontario during the 20th century. *Environ. Science. Technol*., 37:3864-3877.
23. Nacci, D.E., Gleason. T.R., Gutjahr-Gobell, R., Huber, M and Munns, Jr., W.R. (2002). Effects of chronic stress on wild life populations; a population modeling approach and case study. In coastal and Estuarine Risk Assessment, Newman, M.C., Roberts, M.H. and Hale R.C., Eds., Lewis Publishers, Boca Raton, FL Pp 247-272.
24. Wirgin, I. and Waldman, J.R. (2004). Resistance to Contaminants in North American Fish Populations. *Mut at: Res*., 552: 73-100.
25. Fernandez, M.N. and Mazon, A.F. (2003). 'Environmental Pollution and Fish Gill Morphology. In: Val, A.L. Kapoor B.G. (Eds.), *Fish Adaptations*. Science Publishers, Enfield, USA. pp:203-231.
26. Ikeogu, F.C., Nsofor, C.I and Ikpeze, O.O (2010). *A review of risk factors for fish diseases in aquatic environments.* In: Ikpeze, O.O (editor) Proceedings of the 6th National Conference of the Society for Occupational Safety and Environmental Health. 199-204, November 3-6h, 2010. Princess Alexandria Auditorium, University of Nigeria, Nsukka, Enugu State Nigeria. ISBN: 978-8415-50-3
27. Gabriel, U.U., Amakiri, E.U., and Ezeri, G.N.O. (2007). Haematology and Gill Pathology of *Clarias gariepinus* exposed to Refined Petroleum oil, Kerosene under Laboratory Conditions. *Journal of Animal and veterinary Advances* 6(3), 461-465
28. Onusiriuka, B.C., and Ufodike, E.B.C. (2000). Effects of sublethal concentrations of Akee apple, *Bligha sapida and sausage plant, Kiegella africana* on tissue chemistry of the African catfish, *Clarias gariepinus. J. Aquat. Sci.*, 15: 47-49.
29. Fafioye, O.O., Adebisi, A.A. and Fagade, S.O. (2004). Toxicity of *Parkia biglossa and Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *Afr. J. Biotech*., 3:627-630.
30. Mitchell, R.N. and Cotran, R.S. (2004). Cell Injury Adaptation and Death. In: Kumar, V. Cotran, R. and Robbins, S.L. (Eds). Robbins basic pathology. Saunders New Delhi, India, Pp: 3-32.
31. Oronsaye J.A. (1989). Histological Changes in the Kidneys and Gills of the Stickleback, *Gasterosteus aculeatus,* exposed to Dissolved Cadmium in hard water. *Ecotoxicology and Environmental Safety* 17:279-290.
32. Guilio Richard, T. and Hinton, David E. (2008). The toxicology of Fishes. 1st edition CRC Press, Taylor and Francis Group. Florida, 3-14.
33. Evans, D.H., Piemarini, P.M. and Choe, K.P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of nitrogenous waste.