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# Liver and Kidney Histopathology: Biomakers of No. 1 Fuel Toxicosis in African Catfish, *Clarias gariepinus*

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**Abstract:** African catfish, *C. gariepinus* (mean total length, 31.49±5.45cm, sem; total length, 18.50±2.42g, sem) were exposed to grade levels of light fuel oil-No.1 fuel (0.75, 150 and 300 μL $L^{-1}$) in triplicates for 14 days The histology of the kidneys from the normal showed uniform morphology of the nuclei and areas with mild to moderate steatosis. Exposed fish showed an increased glomerular cellularity, degeneration of kidney tubules with infiltrate of few neutrophils. The inerstitia were infiltrated by inflammatory cells (neutrophils and and lymphocytes) which was concentration-dependent. There was extensive necrosis with the majority of the neoplastic tubules in the neophroblastoma stage at the highest concentration. The tubular cells were also hypertrophic and the lumina contained amorphous eosinophilic materials. The sections of the livers from the control fish had normal tubules, haematopoietic tissues and portal triad that contained dilated portal vein. There were some areas showing mild to moderate steatosis. In exposed fish, the there was degeneration of cords of hepatocytes and severe necrosis of hepatocytes, pycknosis and karyolysis of hepatocellular nuclei as well as hyalination of hepatocytes with narrowing of the liver sinusoids channels. The central veins were congested with nucleated erythrocytes, centrolobular necrosis with areas of ballooning degenerative vacuolation and steatosis particularly in the higher concentrations. There were focal areas of infiltration of the liver by inflammatory cells. Results from this study indicates that the pathology of the liver and kidney of *C. gariepinus* could be a good biomarker for the assessment of light fuel toxicosis

**Key words:** Liver, kidney, histopathology, fuel toxicosis, hecrosis, hepatocytes

## INTRODUCTION

Pollution from industrial and oil-related activities is common in many parts of the world. In Nigeria many industrial and urban centres are contaminated with a variety of pollutants that are potentially harmful to aquatic organisms. Contamination from crude oil and petroleum products are more rampant in the Niger Delta with highest concentration of crude exploration, exploitation, transporting and refining activities. According to Dublin-Green *et al.* (1998) there were 5334 reported cases of oil spills between 1976-1990 releasing about 2.8 million barrels of oil into the land swamps, estuaries and coastal waters of Nigeria. The trend continues unabated.

Water Soluble Fractions (WSF) of crude oil were reported to be toxic to various aquatic organisms such as-*Menidia beryllina* (Al-Yakoob *et al*., 1996) *M. beryllina* and *Palaemonetes pugio* (Gundersen *et al*., 1996) crude oil to pink salmon embryo (Brannon *et al*., 2006) and Gulf killifish (Liu *et al*., 2006). Remedial agents such as dispersants alone or in combination with crude oils were shown to be toxic to Pacific salmon, *Fundulus heteroclitus* hatchlings (Couillard *et al.* 2005) Pacific herring eggs and larvae (Barron *et al*., 2003) and Australian bass, *Macqueria novemaculeata* (Cohen and Nugegoda, 2000). Exposure of fish to crude, WSFs and light fuel oils produced varying degrees of pathological changes in the gill, liver and kidney of exposed organisms (Prasad 1991; Brannon, *et al*., 2006; Cohen and Nugegoda 2000; Barron *et al*., 2003; Couillard *et al*., 2005). The polychaetes, *Neanthes arenaceodentata* were shown to be resistant to WSFs of No. 2 fuel (Rossi and Anderson, 1976, 1978). There are no reports on the effects of crude or refined oil on the histopathological changes in the liver and kidney of *Clarias gariepinus*, an important aquaculture species in many parts of the world. The present investigation was conducted to provide information on the histological changes in the liver and kidney of *C. gariepinus* under exposure to light fuel oil, No.1 fuel as biomarkers of refined petroleum oil toxicosis.

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## MATERIALS AND METHODS

Juveniles *C. gariepinus* (mean total length, 31.49±5.45cm, sem; total length, 18.50±2.42cm, sem) were acclimated in six circular aquaria in 20*l* water for seven days. The fish were fed a 25% crude protein diet at 1% biomass half at 800 and 1600 h, respectively. The low boling point No. 2 fuel was obtained from a gas station. Water-fuel dispersion of the various concentrations of No.1 fuel (75, 150 and 300 uL $L^{-1}$) was prepared by adding determined amounts of the fuel to water determined amount of borehole water (dissolved oxygen, 4.43±1.4 mg $L^{-1}$; pH, 6.78±1.24; alkalinity, 28.32±3.04) and mixed vigorously. The control had no fuel oil. Each aquarium was stocked with three fish. Water fuel dispersion in the various test aquaria and the bore water in the control were renewed daily during the 14 day experimental period. At the end of the period, samples of kidneys and liver were obtained from both exposed and control fish, preserved in 10% formol saline, processed for histological studies (stained with eosin and haematoxylin) using standard methods. Permanent sections were read under the microscope.

## RESULTS

Kidneys from the control fish had glomeruli with cellular glomerular tuft. The interstitia appeared slightly infiltrated by neutrophils (Fig. 1a). Fish exposed to 75 uL $L^{-1}$ toxicant showed an increased glomerular cellularity and degeneration of kidney tubules with infiltrate of few neutrophils (Fig. 1b). The inerstitia were infiltrated by neutrophils and occasionally lymphocytes. The vessels were essentially unremarkable. Sections from fish exposed to 150 uL $L^{-1}$ 0 toxicant showed increased glomerular tuft and the inerstitia was also infiltrated by neutrophils. There was mild to moderate necrosis of the glomeruli (Fig. 1c). In fish exposed to 300 uL $L^{-1}$ fuel oil; the kidney there had marked celluarity of tubules with infiltrations by lymphocytes and neutrophils. There was also extensive necrosis with the majority of the neoplastic tubules in the neophroblastoma stage (Fig. 1d). The interstitia were heavily infiltrated by inflammatory cells. The tubular cells were hypertrophic and the lumina contained amorphous eosinophilic materials.

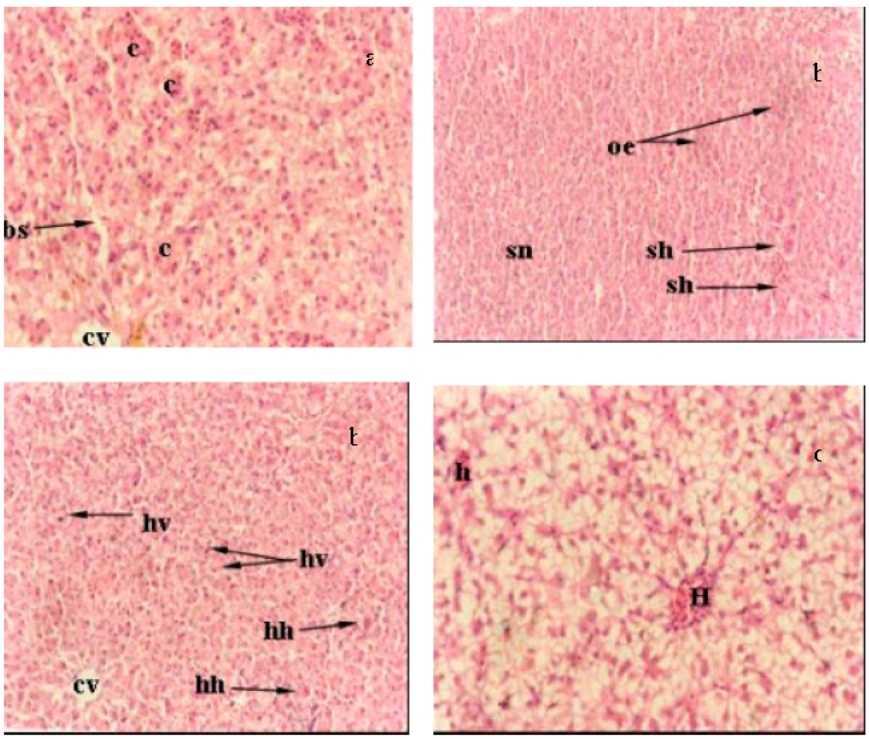


Fig. 1: Cross sections of liver from *Clarias gariepinus* exposed to various levels of kerosine for two weeks. (a) Control, (b) 75 µL $L^{-1}$, (c) 150 µL $L^{-1}$ and (d) 300 µL $L^{-1}$. A (Control)- hepatocytes in cords (c), blood sinus (bs) and central vein (cv). Note the uniform morphology of the nuclei and areas with mild to moderate steatosis. B- Degeneration of cords of hepatocytes and severe necrosis of hepatocytes (sn), pycknosis and karyolysis of nuclei and as well as hyalination of hepatocytes (sh). Note narrowing of sinusoid channels. C-Hepatocelluar vacuolation (hv) and hypertrophy (hh). D-Hepatocellular steatosis, hyperaaemia of the central vein (H) and sinusoid (h).Note the rounded vacuoles and peripherally displaced nuclei. H and E 200x

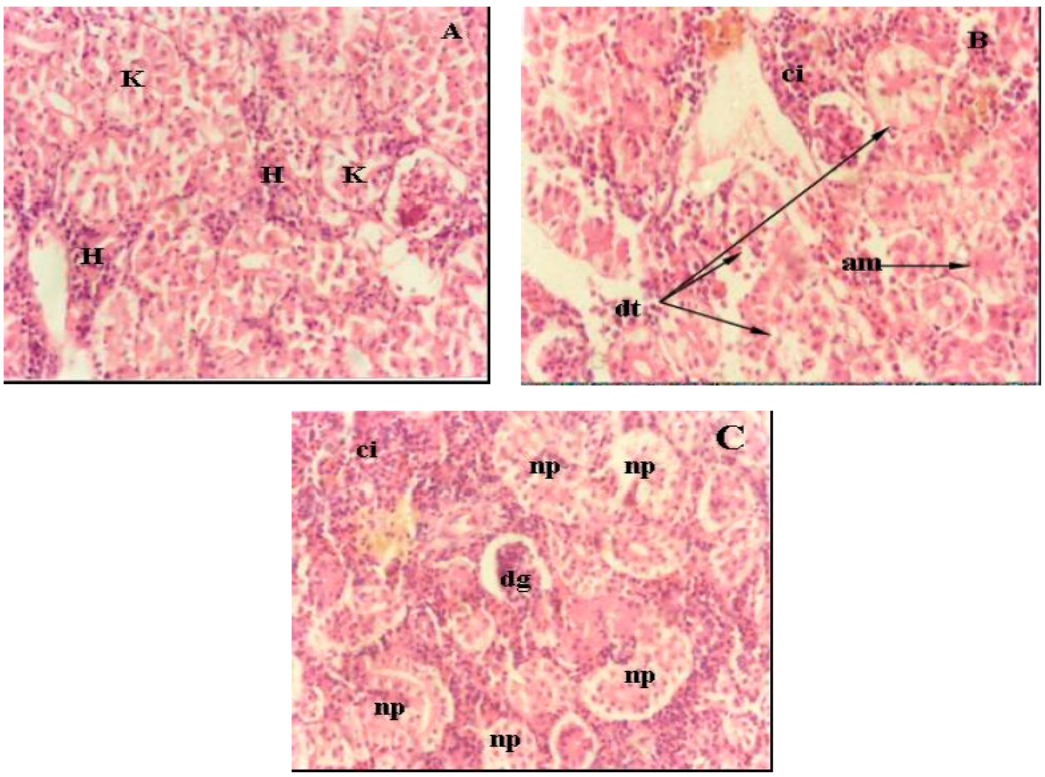


Fig. 2: Sections of kidney from *Clarias gariepinus* exposed to various levels of kerosene for two weeks. (a) Control, (b) 75 μL $L^{-1}$ and (c) 300 μL $L^{-1}$. Normal sections from the kidney of *Clarias gariepinus* (A). H= haematopoetic tissue, K=kidney tubule. (b)- degenerating kidney tubule (dg), cell infiltration, erythrocytes (ci), amorphous mateials in kidney tubule (am). C- neoplastic kidney tubules (np). Note that majority of the tubules were in the advanced stage of neoplasia (nephroblastoma). H and E 200x

The sections of the livers from the control fish had normal tubules, haematopoietic tissues and portal triad that contained dilated portal vein. The hepatocytes were arranged in plates separated by narrow or collapsed sinusoidal channels. The histology of the livers showed uniform morphology of the nuclei and areas with mild to moderate steatosis (Fig. 2a). In the lowest concentration, there was degeneration of cords of hepatocytes and severe necrosis of hepatocytes, pycknosis and karyolysis of hepatocellular nuclei as well as hepatocellular hyalination with narrowing of the liver sinusoids channels (Fig. 2b). The central veins were congested with nucleated erythrocytes. Additional disruptive changes such as areas of centrolobular necrosis with areas of ballooning degenerative vacuolation were intense in fish exposed to 150 uL $L^{-1}$ of the toxcicant. There was also congestion of the central vein and focal areas of infiltration of the liver by neutrophils and lymphocytes. Sections from fish exposed to 300 mg $L^{-1}$ had similar but more intense pathological changes such as necrosis of hepatocytes compared to those in 150 uL $L^{-1}$. There was hepatocellular steatosis, characterized by the rounded vacuoles and peripherally displaced nuclei and hyperaaemia of the central vein and sinusoid (Fig. 2c). The central veins were congested with inflammatory cells. There were also areas of infiltration of the liver by neutrophils and lymphocytes.

## DISCUSSION

Vacuolation of hepatocytes with pycknotic (condensed) nuclei in the liver was most likely due to deposition of glycogen and lipids (Myers *et al*., 1987) as a result of hepatoxicity induced by the presence of fuel oil and/ or reduced food intake (Khan and Kiceniuk, 1984). Ferguson (1989) observed that a common morphologic response of fish liver to toxicity is the loss of hepatic glycogen and/or lipid. This condition according to Wolfe and Wolfe (2005) may have occurred by direct intoxication or it may have occurred secondary to deceased body condition caused by inanition, stress or concurrent

disease. Furthermore, the authors noted paradoxically that toxic exposure may also result in the accumulation of fats as recorded in this study. Similar observations were made in *C. gariepinus* exposed to plant extracts (Fafioye *et al*., 2004) and lead (Olojo *et al*., 2005) *Onchorhynchus gorbuscha* exposed to water soluble/accommodated fractions of crude oil (Brand *et al*., 2001), *O. mykiss* exposed to water accommodated trace petroleum residues (Rudolph *et al*., 2001) and demersal rockfish exposed to crude oil (Marty *et al*., 2003).

Fatty degeneration of the liver (steatosis) recorded in the liver of exposed fish may be suggestive of metabolic disorders and it is commonly associated with dietary deficiency in response to xenobiotic (Myers *et al*., 1987). These changes are normally reported in diseased organisms or those exposed to toxicants [Khan and Kiceniuk, 1988; Hawkins *et al*., 1988; Ogbulie and Okpkwasili, 1999). It may have resulted from disturbances in any of the steps in the sequence of the events from fatty acid entry to lipoprotein exit (Mitchell and Cotran, 2004). Petroleum hydrocarbon has been shown to disrupt lipid metabolism leading to accumulation in lobsters (Capuzzo and Lancaster, 1982; Capuzzo *et al*., 1982). The degree of fatty change in exposed *C. gariepinus* especially at 300 μL $L^{-1}$ far exceeded that in the control implicating the oil as being responsible for the change. Lipid or glycogen vacuolation suggests the accumulation of triglycerides usually within the hepatocytes and may have been responsible for the hepatocyte enlargement/ hyalination (Wolfe and Wolfe, 2005). Steatosis has been correlated with neoplasms, however, its role in the progression of lesions towards neoplasm formation in fish is not well understood (McCain *et al*., 1982).

Inflammatory cell infiltration response accompanied by severe necrotic change, pycknotic and interstiti nuclei, represent the necrogenic action of the fuel on the liver and will greatly impair liver functions. Similar changes have been reported in the liver of *Astyanax* sp. Exposed to WSFs of crude oil (Akaishi *et al*., 2004) *Perca fluviatilis* and gold fish exposed to oil sand process affected water (Nero *et al*., 2006). Centrolobular necrotic change was the main change in *C. gariepinus* exposed to fuel oil and agrees with the observation of Popp (1991) who suggested that the distribution of the interstitial system in the liver resulting in a higher concentration of the toxicant in the centrolobular region account for the occurrence and frequency of centrolobular toxicity.

The changes recorded in the kidney section from the exposed fish such as increased glomerular cellluarity, interstitial being infiltrated with blood cells, distortion and disarray of the glomeruli that were concentration-dependent were similar to those caused by petroleum in English sole (Mc Cairn *et al*., 1978) and rats (Dede and Kagbo, 2001) and *C. gariepinus* exposed to plant extracts (Onusiriuka and Ufodike 2000, Fafioye *et al*., 2004) for various periods. Peripheral blood and cephalic kidney of turbot, *Scophthalmus maximus* and Atlantic cod, *Gadus morua* had micrornuclei and severe nuclei abnormality such as nuclear buds, binuleated and fragmented-apoptotic cells) induced by crude oil and nonylphenol (Barsiene *et al*., 2006). exposure. Such changes and neoplasia of kidney tubules particularly at the highest concentration recorded in this study may greatly impair the filtration functions of the kidney with grave consequences for the exposed fish. However, the onset development and extent of these changes may be toxicant, concentration and exposure time dependent.

Results from this study strongly suggest that exposure to sublethal concentrations of No.1 fuel can cause remarkable changes in the kidney and liver of exposed *C. gariepinus*. Hence, the pathology of these organs could serve as important biomarkers of No.1 fuel toxicosis. Asides, disruptive changes in the main organs involved in xenobiotic metabolism could have serious consequences on the overall p hysiology of the exposed fish.

## REFERENCES

Al-Ajmi, D., 1996. Subacute toxicity of the water-soluble fractions of Kuwait crude oil and partially combusted crude oil on *Menidia beryllina* and *Palaemonetes pugio*. Environ. Contam. Toxicol., 31: 1-8.

Al-Yakoob, S.N., D. Gundersen and L. Curtis, 1996. Effects of the water-soluble fraction of partially combusted crude oil from Kuwait's oil fires (from Desert Storm) on survival and growth of the marine fish *Menidia beryllina*. Ecotoxicol. Environ. Saf., 35: 142-9.

Akaishi, F.M., De H.C. Assis, S.C. Jaakobi, D.R. Eiras-Stofella, S.D. St. Jean, S.C. Couteany, E.F. Lima, A.L. Wagener, A.L. Scofield and C.A. Ribeiro, 2004. Morpholoical and neurotoxiclogical 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.

Barsiene, J., V. Dedonvte, A. Rybakovas, L. Andreikenaite and O.K. Anderson, 2006. Investigation of micronuclei and other nuclear abnormalities in periopheral blood and kidney of marine fish treated with crude oil. Aquat. Toxicol., 78: 99-104.

Barron, M.G., M.G. Carls, J.W. Short and S.D. Rice, 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. Environ. Toxicol. Chem., 22: 650-60.

Brand, D.G., R. Fink, W. Bengeyfield, I.K. Birtwel and C.D. McAllister, 2001. Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stress-related visceral lesions after 10-day exposure to sublethal concentrations of the waster-soluble fraction of North Slope crude oil. Toxicol. Path., 29: 574-584.

Brannon, E.L., K.M. Collins, J.S. Brown, J.M. Neff, K.R.Parker and W.A. Stebblefield, 2006. Toxicity of weathered Exxon Valdez crude oil to pink salmon embryos. Environ. Contam. Chem., 25: 962-97.

Capuzzo, J.M. and B.A. Lancaster, 1982. Physiological Effects of Petroleum Hydrocarbons on Larval Lobsters: Hydrocarbons Accumulation and Interference with Lipid Metabolism. In: W.B. Vernberg, A. Calabrese, F.P.Thunberg and F.J. Vernberg (Eds.), Physiological Mechanism of Marine Pollutant Toxicity. Academic Press, New York, pp: 477-501.

Capuzzo, J.M., B.A. Lancater and G. Sasaki, 1982. Physiological effects of petroleum hydrocarbons on lipid metabolism and energetics of larval development and metamorphosis the American lobster, *Homarus amerianus*. Mar. Environ. Res., 14: 201-228.

Cohen, A.M. and D. Nugegoda, 2000. Toxicity of three oil spill remediation techniques to the Australian bass *Macquaria novemaculeata*. Ecotoxicol. Environ. Saf., 47: 178-85.

Dede, E.B. and H.D. Kagbo, 2001. Investigations of acute toxicological effects of diesel in rat (*Rattus rattus*) using histopathological methods. J. Applied Sci. Environ. Manag., 5: 83-84.

Couillard, C.M., K. Lee, B. Legare and T.L. King, 2005. Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. Environ. Toxicol. Chem., 24: 1496-504.

Dublin-Green,W.F., J.N. Nwankwo, D.O. Irreechukwu, 1998. Effect, regulation and management of HSE issues in the petroleum industry in Nigeria. SPE Internatioinal Conf. Health, Safety and Environment in Oil and gas exploration and production. Caracas, Venezuela, pp: SPE 40/26.

Fafioye, O.O., A.A. Adebisi and S.O. Fagade, 2004. Toxicity of *Parkia biglossa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. Af. J. Biotechnol., 3: 627-630.

Gundersen, D.T., S.W. Kristanto, L.R. Curtis, S.N. Al-Yakoob, M.M. Metwally and D. Al-Ajmi, 1996. Subacute toxicity of the water-soluble fractions of Kuwait crude oil and partially combusted crude oil on *Menidia beryllina* and *Palaemonetes pugio*. Environ. Contam. Toxicol., 31: 1-8.

Ferguson, H.W., 1989. *Syste*mic pathology of fish: a text and atlas of comparative tissue responses in diseases of teleosts. Iowa State Univ. Press Ames.

Hawkins, W.E., R.M. Walkewrr, R.M. Overstreet, T.F. Lyte and J.S. Lyte, 1988. Dose related carcinogenic effects of water-borne benzo(a)pyreneon livers of two small fish species. Ecotoxol. Environ.l Saf., 116: 219-231.

Khan, R.A. and J.W. Kiceniuk, 1984. Histopathological effects of crude oil on Atlantic cod following chronic exposure. Can. J. Zool., 62: 2038-2043.

Khan, R.A. and J.W. Kiceniuk, 1988. Effect of petroleum hydrocarbons on monogeneid parasitizing Atlantic cod, *Gadus morhua*. Bull. Environ. Contam. Toxicol., 41: 91-100.

Liu, B., R.P. Romaire, R.D. Delaune and C.W. Lindau, 2006. Field investigation on the toxicity of Alaska North Slope crude oil (ANSC) and dispersed ANSC crude to Gulf killifish, Eastern oyster and white shrimp. Chemosph, 62: 520-6.

Marty, G.D., A. Hoffmann, M.S. Okihiro, K. Hepler and D. Hanes, 2003. Retrospective analysis: Bile hydrocarbons and histopathology of demersal rockfish in Prince William Sound, Alaska, after the Exxon spill. Mar. Environ. Res., 56: 569-84.

McCain, B.B., M.S. Myers, U. Varanasi, D.W. Brown, L.D. Rhodes, E.D. Gronlund, D.G. Elliot, W.A. Palsson, H.O. Hodgins and D.C. Malins, 1982. Pathology of two species of flat fish from urban estuary of Puget Sound. Interagency Energy-Environment Resaech and Development Programme Report, EPA-600/7-001. Environmental Protection Agency, Washington, DC.

McCain, B.B., H.O. Hodgins., E.D. Gronlund, J.W. Hawks, D.W. Brown, M.S. Myers and J.H. Vandermeulen, 1978. Bioavailability of crude oiled sediments to English sole (*Parophrys vetulus*) and pathological consequences. J. Fisher. Res. Bd Can., 35: 657-664.

Mitchell, R.N. and R.S. Cotran, 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.

Myers, M.S., L.D. Rhodes and B.B. McCain, 1987. Pathologic anatomy and pattern of occurrence of hepatic neoplasm, putative preneoplastic lesions and other idiopathic hepatic conditions in English sole (*Parohrys vetulus* ) from Puget Sound Washington. J. Nat. Can. Instit., 78: 333-363.

Nero, V., A. Farwell, A. Lister, G. Van der Kraak, L.E. Lee, T. Van Meer, M.D. MacKinnon and D.G. Dixon, 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Ecotoxicol. Environ. Saf., 63: 365-77.

Ogbulie, J.N. and G.C. Okpokwasili, 1999. Haematological and histological responses of *Clarias gariepinus* and *Heterobranchus bidorsalis* to some bactaerial diseases in Rivers State, Nigeria. J. Nat. Sci. Found. Sri Lanka, 27: 1-16.

Olojo, E.A.A., K.B. Olurin, G. Mbaka and A.D. Oluwemimo, 2005. Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. Af. J. Biotech., 4: 117-122.

Onusiriuka, B.C. and E.B.C. Ufodike, 2000. Effects of sublethal concentrations of Akee apple, *Bligha sapida* and sausage plant, *Kigella africana* on tissue chemistry of the African catfish, *Clarias gariepinus*. J. Aquat. Sci., 15: 47-49.

Prasad, M.S., 1991. SEM study on the effects of crude oil on the gills and air breathing organs of climbing perch, *Anabas testudineus*. Bull. Environ. Contam. Toxicol., 47: 882-889.

Popp, J.A., 1991. Hepatobiliary system. *In:* Handbook of toxicological pathology. Haschek, V. and C.G. Rousseaux Eds. Academic Press Inc. Philadelphia, USA, pp: 279-314.

Rossi, S.S. and J.W. Anderson, 1978. Petroleum hydrocarbon resistance in the marine worm *Neanthes arenaceodentata* (polychaeta: Annelida), induced by chronic exposure to fuel oil. Bull. Environ. Contam. Toxicol., 20: 513-21.

Rossi, S.S. and J.W. Anderson, 1976. Toxicity of water-soluble fractions of no. 2 fuel oil and South Louisiana cr.ude oil to selected stages in the life history of the polychaete, *Neanthes arenaceodentata*. Bull. Environ. Contam. Toxicol., 16: 18-24.

Rudolph, A., R. Yanez and L. Troncoso, 2001. Effects of exposure of *Oncorhynchus mykiss* to the water-accommodated fraction of petroleum hydrocarbons. Bull. Environ. Contam. Toxicol., 66: 400-6.

Wolfe, J.C. and J.W. Wolfe, 2005. A breif review of nonneoplastic hepatic toxicity in fish. Toxicol. Pathol., 33: 75-85.