Journal of Animal and Veterinary Advances 6 (3): 461-465, 2007

Haematology and Gill Pathology of *Clarias Gariepinus* Exposed to Refined Petroleum Oil, Kerosene under Laboratory Conditions

[1]U.U. Gabriel, [1]E.U. Amakiriand and [2]G.N.O. Ezeri
[1]Department of Fisheries, Rivers State University of Science and Technology,
P.M.B. 5080, Port Harcourt, Nigeria
[2]Department of Aquaculture and Fisheries Management,
University of Agriculture, Abeokuta, Nigeria

Abstract: Juveniles of the African catfish, *C. gariepinus* (mean total length, 31.49±5.45cm, sem; total length, 18.50±2.42 g, sem) were exposed to grade levels of refined petroleum product- kerosene (0, 75, 150 and 300 ppm) in triplicates for two under laboratory conditions.The values of all the parameters in the control except MCHC, MCH, neutrophils, monocytes and thrombocytes were higher than those in the treatment. Hb, Ht, MCV and WBC values in the treatment declined with increase in the concentration of the toxicant. But the reverse was the case with MCHC, MCH, neutrophils and thrombocytes. Significant differences between the haematological parameters of the control and the treatment levels ($p>0.05$) occurred in the Hb, Ht and RBC. But in the MCH, MCV, WBC, neutrophils, monocytes and thrombocytes differences ($p<0.05$) were recorded between the control and some of the treatment levels, particularly the highest concentration (300 ppm). Secondary lamellae of gills of exposed fish were hypertrophic, necrotic and suffered different levels of curving and blunting and fusion. There was atrophy and dystrophy of secondary lamellae. Most of the primary and secondary lamellae were distended with oedematous fluid. Gills from exposed fish showed increased vascular congestion with infiltration of the submucosa by inflammatory cells with increasing concentration of the toxicant.

Key words: Haematology, gills, *Clarias gariepinus*, refined, kerosene

INTRODUCTION

Pollution from crude and refined oil is commonplace world over 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 (Imevbore and Adeyemi, 1981). Spill incidences of various scales involving different kinds of oils are reportedly more rampant and endemic in the coastal areas because this is the site of most oil refining and terminal operations. According to the Rivers State Environmental Protection Bureau (1992) most of the spills occur in the coastal areas and swamps of the Niger Delta.

Exposure of aquatic organisms to crude and refined oils, water soluble- and water accommodated fractions of crude oil have been shown to impact on various aspects of fish physiology and sometimes leading to large scale mortality (Dambo, 1999; Barron *et al*., 2003; Couillard *et al*., 2005; Liu *et al*., 2006). Haematological and histopathological changes in fish exposed to pollutants have been proposed and used as sensitive biomarkers for assessing the effects of several environmental contaminants, including petroleum products (Heath, 1990; Bennett *et al*., 1990). Reports on blood disorder and orgn pathology in fish under exposure to crude and refined oil or their components are scanty. Prasad *et al*. (1987) observed marked changes in the blood of *Heteropneutes fossilis* exposed to crude oil. Dede and Kagbo (2002) reported similar changes in rats, *Rattus rattus* exposed to No. 2 fuel. Investigations by several workers have revealed histopathological changes in various organs of fish (ovaries, gill and liver) exposed to hydrocarbons (Stott *et al*., 1981; Akaishi *et al*., 2004).

Despite the large number of spills of various scales occurring in the country particularly in the Niger Delta region, very little is known of the haematological and histopathological changes that exposed fish population may suffer under exposure to crude or refined oil (kerosene). This study was undertaken to assess the haematological and histopathological responses of *C. gariepinus* to sublethal concentrations of kerosene under laboratory conditions.

Corresponding Author: U.U. Gabriel, Department of Fisheries, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria

MATERIALS AND METHODS

Juvenile *C. gariepinus* (mean total length, 31.49±5.45 cm, sem; total length, 18.50±2.42 cm, sem) were acclimated in six circular aquariawith daily renewal for a week. They were fed a 25% crude protein diet at 1% biomass half at 800hours and 1600hours, respectively. Three fish were exposed to the kerosene levels (75, 150 and 300 ppm) and a control (0 mg^{-1}) and each was in triplicate. The test concentrations were prepared by adding determined amount of kerosene to the aquaria. This was stirred vigorously for 3 min before three fish was randomly placed in each of the tanks. Fresh kerosene-water dispersions were prepared daily. The fish were fed as in the acclimation period. The exposure lasted for two weeks. Water temperature, pH and dissolved oxygen, DO of the exposure aquaria were monitored.

At the end of the exposure period, blood samples were collected from the fish by cardiac puncture and analysed for Haemoglobin (Hb), Haema tocrit (Ht), Red Blood Cells (RBC), White Blood Count (WBC), differential counts using the methods of (Blaxhall and Daisley, 1973). Thrombocytes and red blood cell indices (MCHC, MCH and MCV) were determined according to Brown (1980). Gill, liver and kidney samples were obtained from exposed fish and control, preserved in 10% formol saline and processed for histological examination (using standard histological techniques. Sections of organs were cut at 5μm and stained with haematoxylin and eosin. Permanent sections were read under light microscope. Haematological data were analysed with ANOVA using SAS (1990) and differences among means were separated by Duncan Multiple Range Test at 0.05% probability.

RESULTS

The fish in the toxicant were active but not feeding. The values of the DO, pH and temperature in the exposure tanks indicated that the dissolved oxygen levels in the treatment and control were variable without a defined trend relative to the concentration of the toxicant; however, the DO levels in the control were significantly higher than those in the treatment ($p<0.05$, Table 1). Among the treatment levels, those exposed to 300 mg^{-1} had the highest DO concentration. pH values in the control was higher than those from the treated group ($p<0.05$).

The values of all the blood parameters in the control except MCHC, MCH, neutrophils, monocytes and thrombocytes were higher than those in the treatment. Hb, Ht, MCV and WBC values in the treatment (Table 2) declined with increase in the concentration of the toxicant. But the reverse was the case with MCHC, MCH, neutrophils and thrombocytes. Significant differences between the haematological parameters of the control and the treatment levels ($p< 0.05$) occurred in the Hb, Ht and RBC. But in the MCH, MCV, WBC, neutrophils, monocytes and thrombocytes differences ($p<0.05$) were recorded between the control and some of the treatment levels, particularly the highest concentration (300 ppm, Table 2).

The gill sections from the control were normal with core of fibromuscules and cartilages surrounded by columnar epithelium forming papillary projections on their surface and were richly vascularised (Fig. 1a). Secondary lamellae of gills of exposed fish were hypertrophic, necrotic with different levels of curving and blunting. Some of the secondary lamellae were completely fused, atrophied or dystrophied (Fig. 1b and c). Most of the primary and secondary lamellae were distended with oedematous fluid (Fig. 1b and d). Gills from exposed fish showed increased vascular congestion with infiltration of the submucosa by inflammatory cells with increasing concentration of the toxicant. The degrees of the pathological changes in the gills were directly concentration-dependent.

Table 1: Water quality parameters of exposure aquaria

Conc. of kerosene ($mg L^{-1}$)	Dissolved oxygen, DO				
	Day 2	Day 5	Day 8	Day 11	Day 14
Control	4.60±0.20^{a}	4.60±0.00^{a}	4.50±0.00^{a}	4.30±0.10^{a}	4.20±0.00^{a}
75	1.40±0.10^{e}	1.30±0.10^{d}	1.70±0.10^{e}	1.80±0.10^{e}	2.00±0.10^{e}
150	1.70±0.10^{bc}	1.88±0.10^{c}	1.90±0.10^{c}	2.20±0.00^{bc}	1.90±0.00^{c}
300	2.4±0.10^{b}	2.30±0.20^{b}	2.60±0.10^{b}	2.30±0.10^{b}	2.50±0.10^{b}
pH					
Control	6.90±0.00^{a}	6.80±0.00^{a}	6.80±0.10^{a}	6.70±0.10^{a}	6.70±0.10^{a}
75	6.10±0.10^{b}	6.10±0.00^{b}	6.10±0.00^{b}	6.10±0.00^{b}	6.10±0.00^{b}
150	6.10±0.00^{b}	6.10±0.00^{b}	6.10±0.00^{b}	6.10±0.00^{b}	6.00±0.00^{c}
300	6.30±0.10^{b}	6.20±0.10^{b}	610±0.10^{b}	6.10±0.00^{b}	6.10±0.00^{b}
Temperature (°C)					
Control	28.0±3.03	28.2±3.03	28.0±3.03	28.4±3.03	28.5±3.03
75	28.0±3.03	28.5±3.03	28.0±3.03	28.0±3.03	28.3±3.03
150	27.0±3.03	28.0±3.03	28.6±3.03	28.2±3.03	28.2±2.03
300	27.0±2.93	28.1±3.03	28.1±.3.03	28.5±3.03	28.0±3.03

Table 2: Haematological responses (mean ±sem) of *C. gariepinus* after exposure to refined oil (kerosene) after 14 days. Mean in the same column with similar superscipts are not significantly different ($p<0.05$)

Conc. of kerosene (mg L^{-1})	Haemoglobin (g/dl)	Haematocrit (l/l)	Red blood cells ($\times10^{12}$/l)	Red blood indices		
				MCHC (gdl^{-1})	MCH (pg)	MCV (fl)
Control	7.1±0.30^{a}	0.21±0.01^{a}	2.20±0.10^{a}	34.0±1.23^{a}	31.0±1.40^{b}	94.1±1.20^{a}
75	5.5±0.10^{b}	0.16±0.01^{b}	1.70±0.10^{b}	34.5±1.14^{a}	32.0±0.90^{b}	94.1±0.23^{a}
150	4.6±0.10^{c}	0.12±0.00^{c}	1.30±0.00^{c}	35.0±2.92^{a}	35.0±0.50^{ab}	92.3±0.00^{ab}
300	4.2±0.00^{c}	0.10±0.00^{c}	1.1±0.00^{c}	42.3±0.33^{a}	38.5±0.30^{a}	90.9±0.00^{b}

Table 2: Contd. Haematological responses (mean ±sem) of *C. gariepinus* after exposure to refined oil (kerosene) for 14 days

Conc. of kerosene (mL 1^{-1})	WBC ($\times10^{9}$ L^{-1})	Differential counts			Thrombocytes ($\times10^{9}$/l)
		Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	
0 (Control)	24.0±1.0^{b}	92.0±3.00^{a}	3.0±1.00^{b}	5.00±2.00^{c}	113.0±6.00^{c}
75	37.0±5.00^{a}	85.0±7.00^{a}	4.0±0.00^{b}	19.0±3.00^{a}	142.0±10.00^{bc}
150	25.0±1.00^{b}	91.0±1.00^{a}	6.0±1.00^{ab}	3.0±1.50^{c}	197.0±13.00^{b}
300	21.0±1.00^{b}	90.0±1.00^{a}	10.0±1.00^{a}	10.± 0.50^{b}	274.0±18.00^{a}

Fig. 1: Sections of gills of *Clarias gariepinus* exposed to various levels of kerosine for two weeks (a) Control, (b) 70ppm, (c) 150ppm and (d) 300ppm. nsl-normal secondary lamella, asl- atrophied secondary almella fsl-fused secondary, de-desquamated epithelia from primary lamella, opl-oedomatous primary lamella. H and E. 200x

DISCUSSION

Haematological parameters are very sensitive to stress. Decrease in the values of Hb, RBC, Ht, MCV with increase in the concentration of the refined oil recorded in the exposed fish were similar were also reported in *C. gariepinus* and *Oreochromis niloticus* exposed to crude oil (Omoregie, 1998; Gabriel *et al*., 2001). However, Prasad *et al*. (1987) recorded a decrease in the values of all the parameters but a significant increase in the Ht value of the catfish, *H. fossilis* exposed to crude oil. The decrease in the values of these parameters could be attributed to haemolysis resulting in haemodilution, a mechanism for diluting the concentration of the pollutant in the circulatory system (Smith *et al*., 1979). Erythropaenia recorded in the exposed fish may also be accounted for by swelling of the erythrocytes (Annune and Ahuma, 1998), damages to haematopoietic tissues in the kidneys and aggregation of cells at the gills thereby causing a decrease in the number of circulating cells of stressed fish (Singh and Singh, 1982).

Exposure to various stressors elicits changes in the WBC (Wedemeyer and Yasutake, 1977). Leukopaenia and/or leukocytosis are thus a normal reaction to stressors or irritants such as kerosene. Significant leukopaenia was recorded in *C. gariepinus* exposed to the 150 and 300 mg^{-1} kerosene. Leukopaenia has been reported in *H. fossilis* exposed to crude oil (Prasad *et al*., 1987) and *C. gariepinus* exposed to copper (van Vuren *et al*., 1994), whereas leukocytosis was reported in *C. gariepinus* infected with bacteria, *Pseudomons fluorescens* (Ezeri, 2001) malachite green (Musa and Omoregie, 1999) and copper and lead (Annune and Ahuma, 1998). Changes in the values of WBC in these studies as well as ours were concentration-dependent. This may be due to the level as well as duration of exposure to the toxicant. The different levels of the toxicant may have exerted varying degree of stress on the defence mechanism of the exposed fish and hence the production of different amounts of WBC (Ellis, 1977). Besides, toxicants differ in potency and mode of actions and also fishes respond differently to different toxicants (Rice *et al*., 1977). Pollutants and other stressors as recorded in this and several other studies cause changes in the subpopulations of leukocytes (Ellis, 1977; Musa and Omoregie, 1999). *C. gariepinus* exposed to kerosene suffered neutrophilia. Erythrocytes, thrombocytes and neutrophils were reported the most sensitive to starvation in *Channa punctatus* (Mahajan and Dheer, 1983) and

heavy metal (lead) poisoning (Rai and Qayyan, 1984). And in this study there was a significant dose-dependent thrombocytosis. Erythropaenia associated with hypochromasia, increase in ESR, leukocyosis with increase in large lymphocytes, thrombocytosis and hypercoagulablity of blood was observed in *H. fossilis* exposed to mercury and zinc (Banerjee, 1998). The conditions accompanying this state are polycthermia vera and haemolytic anaemia (Seiverd, 1964). According to the author, polycthermia vera is a disease of unknown cause characterised by an increased production of red blood cells above normal values. In haemolytic anaemia the red cells break down or haemolysis at an early stage.

The changes in the gills of fish exposed to kerosene fall within the general responses of fish organs to environmental pollutants. Fernandes and Mazon, (2003) observed that fish gills are the prime target organ of all pollutants due to their extensive surface in contact with the external medium and the reduced distance between the external and internal medium. They also noted that gill morphology and morphometric are important biomarkers providing a rapid method for detection of the effects of pollutants. The general morphological changes in the gills recorded in this study have been reported in *Astyanax* sp. after 96 h brief exposure to water soluble fraction of crude oil (Akaishi *et al*., 2004) and *Clarias gariepinus* under brief or prolonged exposure to plant extracts (Onusiriuka and Ufodike, 2000; Fafioye *et al*., 2004). In some of the studies brief exposure to toxicants for about 96 h have produced irreversible changes in the gills (Ceiqueira *et al*., 2001; Fernandes and Mazon, 2003).

The changes in the gills were adaptations by the fish to cope with challenge of the toxicant. For example atrophy or dystrophy, curving, clubbing and fusion of the secondary lamellae were attempts by the fish to reduce available surface area to the kerosene. But this may result in the reduction of available surface for respiration and ionic exchange, consequently resulting in an internal hypoxic and toxic environment. 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 (Mitchell and Cortran, 2000).Vascular changes in gills of exposed fish could be attempts by the fish to supply more blood to the gills to increase oxygen uptake and supply to the internal organs. According to Evans *et al*. (2005) the gill of fish is a multi-purpose organ that, in addition to providing for aquatic gaseous exchange, plays a dominant role in osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous wastes. Thus, despite the fact that all fish groups have functional kidneys, the gill epithelium is the site many processes that are mediated by the renal epithelia in terrestrial vertebrates. Hence, impairment of the gill functions by the overall effect of the pathological changes in the gill of exposed will have grave consequences for the fish with respect to the normal functions of the gills.

CONCLUSION

This study revealed that exposure to refined oil at sublethal levels can produce significant changes in the physiology of *C. gariepinus* as manifested by changes in the haematological parameters and disruptive changes in the gills, a vital organ for respiration. Persistent exposure through pollution by kerosene may lead in the mortality of *C. gariepinus* due to due to disruption of internal physiology.

REFERENCES

Annune, P.A. and F.T.A. Ahuma, 1998. Haematological changes in mudfish exposed to sublethal concentrations of copper and lead. J. Aquat. Sci., 13: 33-36.

Akaisha, F.M., H.C De Assis, S.C. Jaakobi, D.R. Eiras-Stofella, S.D. St. Jean, S.C. Couteany, E.F. Lima, A.L. Wagener, A.L. Scofield, 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.

Barron, M.G., M.G. Carls, J.W. Short and S.D. Rice, 2003. Photoenhanced toxicity of aqueous phase and chemically dispersd weathered Alska North Slope crude oil to Pacific hering eggs and and larvae. Environ. Toxicol. Chem., 22: 650-660.

Banerjee, V., 1998. Influence o zinc and mercury on blood parameters of the fish, *Heteropneutes fossilis*. Environ. Ecol., 16: 79-84

Bennett, D., H. Schmidt, W. Meier, P. Burkhardt-Holm and T. Wahli, 1999. Histopathological of fish: A proposal to assess pollution. J. Fish Dis., 22: 25-34.

Ceiqueira, C.C. and M.N. Fernandes, 2001. Gill tissue recovery after copper exposure and blood parameter responses of in the tropical fish, *Prochilodus scrofa*. Ecotox. Environ. Saf., 52: 83-91.

Couillard, C.M., K. lee, B. Legare and T.L. King, 2005. Effect of dispersnt 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.

Dambo, W.B., 1992. Tolerance of the periwinkles *Pachymelaniaa aurita* (muller) and *Tympanotanus fuscatus* (Linne) to refined oils. Environ. Pollut., 79: 293-296.

Dede, E.B. and H.D. Kagbo, 2002. A study in the acute toxicological effects of commercial diesel in Nigerian rats, *Rattus rattus* using haematological parameters. J. Applied. Sci. Environ. Mgt., 6: 84-86.

Ellis, A.E., 1977. The leucocytes of fish: a review. J. Fish Biol., 11: 453-491.

Evans, D.H., P.M. Piemarini and K.P. Choe, 2005. the multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of gnitrogenous waste.

Ezeri, G.N.O., 2001. Haematological responses of *Clarias gariepinus* to bacteria infection and treatment with antibiotics and prophylactic treatment with antibiotics. J. Aquat. Sci., 16: 22-24

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

Fernandes, M. N. and A. F. Mazon, 2003. 'Environmental Pollution and Fish Gill Morphology. In: Val, A.L. Kapoor B.G. (Eds.), *Fish Adaptations*. Science Publishers, Enfield, USA., pp: 203-231.

Gabriel, U.U., J.K. Alagoa and M.E. Allison, 2001. Effects of dispersed crude oil water dispersion on the haemaglobin and packed cell volume of *Clarias gariepinus*. J. Aquat. Sci. Environ. Manage., 5: 9-11.

Heath, A.G., 1990. Water pollution and fish physiology. CRC Press Boca Raton, Florida, pp: 245 .

Imevbore, A.M.A.and S.A. Adeyemi, 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/FMW and H, PTi Warri, Nigeria.

Liu, B., R.P.D. Romaire, R.D. Elaune and C.W. Lindau, 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.

Mahajan, C.L. and T.R. Dheer, 1983: Haematological and haematopoietic responses to starvation in an air breathing fish, Channa punctatus. J. Fish. Biol., 22: 111- 123.

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.

Musa, S.O. and E. Omoregie, 1999. Haematological changes in *Clarias gariepnus* exposed to malachite green. J. Aquat. Sci., 14: 37-42.

Omoregie, E., 1998. Changes in the haematology of Nile tilapia, *Oreochromis niloticus* under the effects of crude oil. Acta Hydrobiol., 40: 287-292.

Onusiriuka, B.C., 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., M. Prasad and D. Singh, 1987. Some haematological effects of crude oil on fresh water fish, *Heteropneustes fossilis*. Acta Hdrochem. Hydrobiol., 15: 199-204.

Rai, R. and M.N. Qayyan, 1984. Haematological responses in freshwater fish to experimental lead poisoning. J. Experiment. Biol., pp: 553-56 .

Rice, S.D., J.W. Short and J.F. Karinen, 1977. Comparative Oil Toxicity and Animal Comparative Sensitivity. In: A. Wolfe (Ed.). Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems. Proc. Symp. Seattle, Washington. Pergamon Press N.Y., pp: 78-94.

Rivers State Environmental Protection Bureau, 1992. Ecological distribution of oil spills. Pollution Paper Rivers State Environmental Protection Bureau, Port Harcourt, Nigeria, pp: 15.

Seiverd, CE 1964. Haematology for medical pathologists. Lea and Feibger, Philadelphia, pp: 946.

Smith, G.L., J. Hattingh and A.P. Burger, 1979. Haematological assemenrt of anaestesia, MS 222 in natural and neutral forming three freshwater fish species: interspecific differences. J. Fish Biol., 15: 633-643.

Singh and Singh, 1982. Effects of copper and zinc sulphate on the blood parameters of *Mystus vittatus*. Matsya, 8: 1-6.

Stott, G.G., N.H. McArthur, R. Tarpley, V. Jacobs, R.F. Sis, 1981. Histopathological surveys of ovaries from petroleum production and control sites in the Gulf of Mexico. J. Fish Biol., 18: 264-269.

Van Vuren , J.H.J., M. van der Merwe and H.H. du Preez, 1994. The effects of copper on the blood chemistry of *Clarias gariepinus* (Clariidae). Ecotox. Environ. Saf., 29: 187-199.

Wedemeyer, G.A. and W.T. Yasutake, 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. USFWS Tech. Rep., 89: 1-18.