Journal of Research in Biology

An International Scientific Research Journal

Original Research

Hepatic enzyme markers and proteins in serum and some selected tissues in *Clarias gariepinus* from swamp around Kokori-Erhoike oil field, Nigeria

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ABSTRACT:

This study determines changes in some biochemical parameters in serum and tissues of *Clarias gariepinus* obtained from fish natural habitat in the oil exploration environs of Kokori-Erhoike in Delta State, Nigeria. Sampling sites include Ethiope River (Eku axis, reference Site A); Erhoike swamp (Site B) and Erhoike fish pond (Site C). However, Sites B and C are located in the oil exploration region of Erhoike. *Clarias gariepinus* (n=8) were collected from each site and used for the study. Levels of total proteins, albumin, haemoglobin as well as the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were determined in serum, gill, liver, brain and muscle tissues. Results showed that total protein concentrations were comparable ($p>0.05$) in serum. Albumin concentrations of fish from Site B and C were lower ($p<0.05$) as compared with that of site A in gill, muscle and brain tissues. Haemoglobin concentration was significantly lower ($p<0.05$) in fish from Site A as compared with that of Sites B and C. Results also indicated that total protein and albumin concentrations were significantly ($p<0.05$) higher in gill, muscle and brain tissues of *Clarias gariepinus* from Site A as compared with fish tissues from Sites B and C. Hepatic enzymes (ALT and AST) and ALP activities were elevated ($p<0.05$) in serum, gill, brain and liver of fish from Sites B and C as compared with that of Site A. The observed biochemical changes in fish from Sites B and C could have resulted from contaminants arising from the oil exploration activities in Site B and the presence of organic/inorganic contaminants in Site C due to the presence of fish feeds. These biochemical alterations show that the fish were under stress in their natural habitat. These biomarkers could be employed in the environmental monitoring of crude oil pollution as well as early warning signs of the adverse effects of environmental pollution.

Keywords:

Fish, Kokori-Erhoike, *Clarias gariepinus*, Albumin, Alkaline phosphatase, Haemoglobin.

Article Citation:
Osioma E, Akanji MA and Arise RO.
Hepatic enzyme markers and proteins in serum and some selected tissues in *Clarias gariepinus* from swamp around Kokori-Erhoike oil field, Nigeria.
Journal of Research in Biology (2013) 3(4): 984-992

Dates:
Received: 23 Mar 2013 **Accepted:** 23 May 2013 **Published:** 13 June 2013

INTRODUCTION

Over the years, the extent of oil exploration activities and its related environmental effects has been on the increase, (Tolulope, 2004). Some quantities of petroleum and its products may be released into the environment during oil exploration activities due to operational, accidental, transportation or other means. Apart from contaminating the flesh of commercially valuable fish, crude oil compromise fish hatcheries in coastal waters (Leighton, 1991) and its products are the most relevant to aquatic ecotoxicology (Pacheco and Santos, 2001). In Nigeria, crude oil was discovered at Oloibiri in 1959 (Akpofure *et al*., 2000) and exploration activities has been carried out in Kokori- Erhoike environment for over 30 years (Emoyan, 2009).

The cause-effect relationship and result of xenobiotic pollution in an ecosystem can be assessed through the analysis of biochemical alterations on organisms inhabiting that environment. These biochemical alterations may be sensitive and specific as early indicators of aquatic pollution (Norris *et al*., 2000; Strinac and Braunbeck, 2000)

Protein plays a vital role in the physiology of living organisms and its metabolism according to Adams *et al*., (1990) provides information on the general energy mobilization of an animal and show relationship with effects of contaminants in these organisms. The concentration of plasma albumin is a useful index of the state of protein repletion and it makes the major contribution to plasma sulphydryl groups which can function as a chain breaking antioxidant (Halliwell, 1988). Haemoglobin contained in the red blood cells which serve as the oxygen carrier in blood has been employed in assessing the health of fish and monitoring stress response of several environmental contaminants including petroleum hydrocarbons (Soivio and Oikari., 1976; Gabriel *et al*., 2007).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) catalyze the transfer of α-amino group from α-amino acid to α-keto acid. AST and ALT are biological responses of severe hepatic injury and their bioassay can serve as a diagnostic tool for estimating necrosis of the liver cells. (Cappo *et al*., 2002). The determination of ALT and AST activities has been applied in fish research to indicate bacteria, viral and parasitic infection, intoxications and water pollution (Bucher and Hofer, 1990). Alkaline phosphatase comprises group of enzymes which is responsible for hydrolyzing phosphoric ester bonds present in organic compounds at an alkaline pH (Akcakaya *et al*., 2007). The enzyme (ALP) has been reported to be a marker enzyme for the plasma membrane and endoplasmic recticulum (Akanji *et al*., 1993).

Fish species are excellent subjects for the study of various effects of contaminants (El-Shehami *et al*., 2007) and African catfish (*Clarias gariepinus*) has been used in fundamental research and toxicological studies (Nguyen and Janssen, 2002)

A significant body of research has investigated the effects of crude oil (or its derivatives) and refinery effluents on fish health. The investigators include: Yarbrough *et al*., 1976; Kuehn *et al*., 1995; Sunmonu and Oloyede, 2006; Wegwu and Omeodu, 2010; Mahmoud *et al*., 2011 and Nwaogu *et al*., 2011. However, there is dearth of information on the effect of crude oil exploration activities on African catfish (*Clarias gariepinus*) obtained from swamps (fish natural habitat) around Kokori- Erhoike oil field located in Delta State, Nigeria. This information deficiency prompted this study. Therefore, the aim of this research is to determine changes of some biochemical parameters in serum and tissues (gill, liver, brain and muscle) of *Clarias gariepinus* obtained from swamps around Kokori - Erhoike petroleum flow station in Delta State, Nigeria.

MATERIALS AND METHODS

Sampling sites:

This study was carried out in Ethiope East Local Government Area of Delta State, Nigeria. Experimental areas have been previously described by Aries *et al*., 2013 and are represented in Figure 1 below.

Site A (reference site) is the Eku axis of the Ethiope River, Delta State. There is no presence of oil facilities/operations or any industry located along the Ethiope River from its source, Umuaja, about 22km to the Eku axis. The upper axis of the Ethiope River has been reported to be relatively unpolluted (Ikomi *et al*., 2005; Agbaire and Obi, 2009; Aries *et al*., 2013). This qualifies the Eku axis of Ethiope River as a reference site for this study. Site B is the swampy environment of Kokori-Erhoike petroleum flow station where oil exploration activities have been on for more than 35 years. This area has a number of oil wells and flow stations. The aquatic ecosystem in the area is constituted by non-tidal freshwater swampy forest characteristics of those found within the freshwater survey zone of the Niger Delta. Site C is a natural fish pond located within Kokori-Erhoike environment. The main uses of water in the catchments include domestic, recreational (e.g. swimming) and fishing. Their major occupation includes farming (cassava, yam, Okro etc.), fishing and petty trading on food stuff.

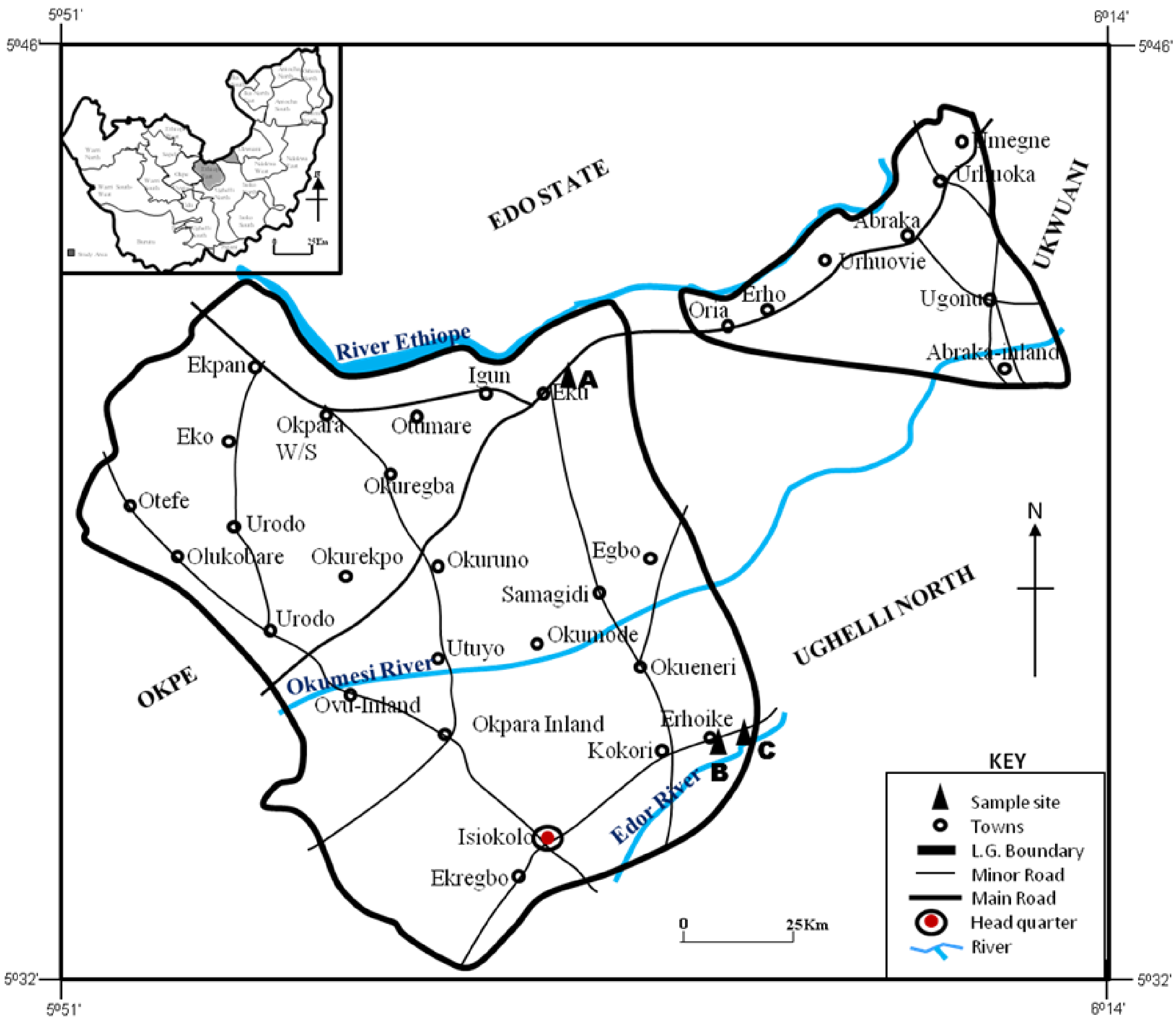

Fig 1. Map of Ethiope East showing the location sampling sites
Source: Ministry of lands, Surveys and urban Development, Asaba. (2008)

Fish

Eight African catfish (*Clarias gariepinus*) were collected from each site in October, 2011. At site A (Eku River) and site B (Erhoike swamp), fish were caught with the help of professional local fishermen and at site C (Erhoike Fish pond) fish net was used to catch the fish.

Preparation of Serum

About 1.5ml blood was taken by caudal arterial puncture from each fish into a sterilized plain tube. Blood was allowed to clot for about 5min dislodged and centrifuged at x15,000g for 15min to obtain the serum, which was stored frozen at – 20°C until analyzed.

Preparation of Tissue Homogenate

The fish were dissected and the gill, liver, brain and muscle tissues were quickly removed. The tissues (gill, liver, brain and muscles) were washed in cold saline (0.9% NaCl) solution several times and then 1g of wet tissue was homogenized in 9 ml of the physiological solution (normal saline). The resulting homogenate was centrifuged at 5000g for 20min. The supernatant was decanted and used for further biochemical analysis.

Biochemical Investigations

The concentrations of total protein and albumin were determined in serum and tissues (gill, liver, muscle and brain) employing the methods of Doumas *et al*., 1981 and Doumas *et al*., 1971 respectively. Haemoglobin level was estimated by the method of Tietz (1976), while the method of Roy (1970) was used to determine the activities of alkaline phosphatase. Alanine aminotransferase and aspartate aminotransferase were analyzed using the method of Reitman and Frankel (1957). All assays were carried out with the aid of commercially available kits supplied by TECO Diagnostics, Anahem, USA and Randox Laboratories, Ardmore, United Kingdom.

Statistics

Analysis of Variance (ANOVA) was used to analyse data obtained from the various biochemical investigations. Group means were compared by the Duncan's Multiple Range Test (DMRT) at 5% probability level. All statistical analysis was performed using SPSS version 16.

RESULTS

Results (Table 1) show that gill, muscle and brain total protein concentrations were significantly higher ($p<0.05$) in fish from Site A as compared with that of Site B and C. Total protein concentration in serum and liver of *Clarias gariepinus* from all sites were comparable at $p>0.05$. The data also indicated significant reduction ($p<0.05$) in gill and muscle albumin concentration of *Clarias gariepinus* from Sites B and C as compared with that of Site A. Levels of serum and liver albumin were comparable in fish from all sites. Results in Table 1 also revealed that fish from Sites B and C has elevated ($p<0.05$) haemoglobin concentration compared with that of Site A.

Table 1: Levels of total protein, albumin and haemoglobin concentrations in serum and tissues (gill, liver, muscle and brain) of *Clarias gariepinus* from swamps around Kokori-Erhoike Petroleum Flow Station in Delta State, Nigeria

	SAMPLING SITES		
	A	B	C
	Total protein concentration (g/dl)		
Serum (n=8)	9.31±0.53^a	9.30±0.31^a	9.29±0.33^a
Gill (n=8)	2.82±0.05^a	2.46±0.24^b	2.43±0.03^b
Liver (n=8)	2.81±0.37^a	2.91±0.15^a	2.98±0.40^a
Muscle (n=8)	5.28±0.32^a	4.29±0.10^b	4.08±0.40^b
Brain (n=8)	4.17±0.17^a	3.72±0.13^b	3.96±0.12^c
	Albumin concentration (g/dl)		
Serum (n=8)	7.25±0.32^a	7.01±0.01^a	6.96±0.13^a
Gill (n=8)	2.48±0.36^a	2.03±0.16^b	2.08±0.24^b
Liver (n=8)	2.52±0.43^a	2.51±0.26^a	2.69±0.18^a
Muscle (n=8)	4.09±0.17^a	2.09±0.42^b	2.55±0.12^b
Brain (n=8)	3.78±0.22^a	2.25±0.11^b	3.68±0.10^a
	Haemoglobin concentration (g/dl)		
Blood (n=8)	22.24±1.57^a	27.03±1.65^b	27.29±1.65^b

Values are given as Mean ± SD. Means not sharing a common superscript letter on a given row differ significantly at $p<0.05$. A= Ethiope River (Eku axis); B= Erhoike swamp; C= Erhoike fish pond.

Increased ($p<0.05$) activities of alanine aminotransferase in serum and liver of *Clarias gariepinus* from Sites B and C were observed as compared with that of Site A (Table 2). Similar trend was observed for the activity of liver aspartate aminotransferase. The table also showed that the activity of aspartate aminotransferase in serum of fish from all sites (A, B and C) were comparable ($p>0.05$), although, fish from Site A had relatively lower aspartate aminotransferase activity. Results (Table 2) also showed that the activity of alkaline phosphatase in (serum, liver, gill and brain) of *Clarias gariepinus* from Sites (B and C) were significantly ($p<0.05$) higher as compared with that of Site A.

Table 2: Changes in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in serum and tissues (liver, gill and brain) of *Clarias gariepinus* collected from swamps around Kokori-Erhoike Petroleum Flow Station in Delta State, Nigeria

	SAMPLING SITES		
	A (n=8)	B (n=8)	C (n=8)
	Alanine aminotransferase (IU/L)		
Serum	43.86±2.79[a]	48.28±0.34[b]	49.19±0.65[b]
Liver	25.10±0.80[a]	45.90±1.63[b]	42.38±1.82[c]
	Aspartate aminotransferase (IU/L)		
Serum	53.75±8.84[a]	54.34±2.88[a]	54.25±3.15[a]
Liver	52.31±0.79[a]	69.70±1.63[b]	65.94±1.82[c]
	Alkaline phosphatase (IU/L)		
Serum	32.86±3.14[a]	46.22±3.05[b]	45.76±3.49[b]
Liver	43.01±1.48[a]	52.67±1.79[b]	53.83±2.91[b]
Gill	29.73±3.73[a]	36.79±1.39[b]	32.74±5.36[a]
Brain	52.94±2.36[a]	57.34±1.21[b]	60.37±2.25[c]

Values are expressed as Mean± SD. Means not sharing a common superscript letter on a given row differ significantly at $p<0.05$. A= Ethiope River (Eku axis); B= Erhoike swamp; C= Erhoike fish pond.

DISCUSSION

Biochemical markers of pollution are considered indicators employed in fish toxicity tests and for field monitoring of aquatic contamination. They established contact of the sample with definite groups of chemical compounds and clarify their metabolic fate. Biochemical investigations allow cause-effect relationship to be established at an early stage of pollution and these sensitive and predictive diagnostic tools (Biomarkers) for assessing animal exposure and toxic effects of chemical contaminants are needed as aquatic environmental contamination assessment indicators.

The total protein concentration in serum, gill, liver, muscle and brain of *Clarias gariepinus* from Eku River is higher than the values obtained from fish samples in Erhoike swamp and Erhoike fish pond. The differences were significant ($p<0.05$) in the gill, brain and muscle tissues.

Proteins play a vital role in the physiology of living organisms and provide information on the general energy mobilization of an animal and show relationship with the effect of contamination in these organisms (Adams *et al.*, 1990). The over 30 years of petroleum exploration activities in Erhoike vicinity could have contaminated the aquatic environment and petroleum hydrocarbon can act as a mediator in free radical generation in fish (Achuba and Osakwe, 2003). During stress conditions, fishes need more energy to detoxify the toxicants and to overcome stress, thus carbohydrate reserve is depleted to meet energy demand (Nelson and Cox, 2005; Sudhanshu and Ajay, 2009). Since fish have a very little amount of carbohydrate, the next alternative source of energy is protein to meet the increased energy demand occasioned by a pollutant.

The decrease in total protein level observed in the gill, brain and muscle tissue could be to meet the higher energy demands for metabolic purposes due to the presence of petroleum hydrocarbon in Erhoike environment and could also be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress.

Albumin is the most soluble and most electrically mobile of all the major serum protein components and it is synthesized entirely by the hepatic parenchymal cells. Lower albumin concentration was observed in the serum, gill, liver, muscle and brain tissues of *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond compared with corresponding albumin level of *Clarias gariepinus* from Eku River (control site). The utilization of proteins as an alternative source of energy by fish in stress condition could have accounted for the reduced albumin level in fish from Erhoike swamp and Erhoike fish pond. Albumin has also been regarded as an antioxidant molecule. It reacts with and neutralizes peroxyl radicals (Stocker and Frei, 1991) and it is considered as a sacrificial molecule that prevents damage when it acts as an antioxidant because albumin is destroyed in the process (Halliwell, 1988). Therefore, the observed reduction of albumin concentration in African Catfish from the oil exploration areas may be linked to its participation as an antioxidant molecule to quench free radical reactions in other to mitigate the impact of oxidative stress or its utilization as a source of energy by the fish in stress condition.

This study showed that *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond have higher levels ($p<0.05$) of haemoglobin as compared with haemoglobin concentration of *Clarias gariepinus* from the control site (Eku River). Elevated levels of haemoglobin observed in African catfish from Erhoike swamp and fish pond could be as a result of stress induced by the presence of crude oil and other contaminants (as in the case of the fish pond) that leads to environmental hypoxia as a result of chronic exposure to the contaminants and anaerobic condition which lead to increase haemoglobin concentration as a compensatory mechanism for increased oxygen demand. This result corroborates with the findings of Mdegela *et al*., (2010) who reported significant elevation of haemoglobin concentration in fish from Mzumbe sewage water. Zaki *et al*., (2010) also reported significant increase in haemoglobin levels in *Tilapia zilli* exposed to acute lethal concentration dose of lead (Pb).

Alanine aminotransferase and aspartate aminotransferase (ALT and AST) are enzymes directly associated with the conversion of amino acids to keto acids. Apart from being considered to be important in assessing the state of the liver and some other organs Verma *et al*., (1981), transamination of the same represents one of the main pathways for synthesis and deamination of amino acid, thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organisms in various adaptive situations. ALT and AST activities are direct indicators of intense hepatic damage, thus their bioassay can assist as a diagnostic tool for determining necrosis of the liver cells (Whitehead *et al*., 1999; Cappo *et al*., 2002). Ugwu *et al*. (2008) concluded that AST enzyme activity in *Heterobranchus bidorsalis* adults could be used as biomarker for monitoring crude oil pollution in Nigeria.

Compared with the control (i.e. *Clarias gariepinus* from Ethiope River, Eku axis) the activities of ALT and AST in serum and liver of *Clarias gariepinus* from Erhioke swamp and Erhoike fish pond were higher. Such increase of ALT and AST may be partly due to hepatic damage resulting from petroleum pollution (in case of Erhoike swamp) or organic/inorganic contaminants (present in Erhoike fish pond, Arise *et al*., 2013) –induced oxidative insults on the hepatocytes. In addition, increased protein catabolism might be responsible for the elevation of these transaminases. These results agree with the findings of Ayalogu *et al*., (2001); Orisakwe *et al*., (2005).

In this study, serum, liver, gill and brain alkaline phosphatase activity were measured in *Clarias gariepinus* from the three sampling sites. Marked increase in ALP activity was recorded in the serum, liver, gill and brain tissues of *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond as compared with ALP activity of *Clarias gariepinus* from the control site (Eku River). ALP together with ALT and AST provide an indication of the degree of inflammation as well as possible causes of hepatocellular damage as well as distortion of the plasma membrane and endoplasmic recticulum. Results of the research agree with the findings of Yarbrough *et al*., (1976) and Ayalogu *et al*., (2001).

CONCLUSION

The study demonstrates that the level of contamination is enough to cause changes in the protein, albumin and haemoglobin level with attendant effect on the integrity of hepatocytes as evidence in the elevated activities as seen in liver marker enzymes and alkaline phosphatase activity of *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond. The above biochemical changes showed that the fish were under stress in their natural habitat (Erhoike swamp) thus; these markers could be employed in the environmental monitoring of crude oil pollutant and their associated metabolic changes as early warning signs of adverse effects of environmental pollution.

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