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# ASSESSMENT OF SOME BIOCHEMICAL INDICES IN FISHES FROM TWO DIFFERENT PARTS OF RIVERS STATE, NIGERIA

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## ABSTRACT

*Oreochromis niloticus* (tilapia) and *Chrysichthys nigrodigitatus* (Catfish) sampled from Alakiri river in Rivers State, Nigeria, were examined for some biochemical indices and blood serum enzymes activities to assess the effects of environmental stress on fish health. The results were compared with those of Omuhuechi river (baseline) in Rivers State, Nigeria. The activity of Aspartate – aminotransferase (AST), Alanine-aminotransferase (ALT) and Alkaline phosphatase (ALP) in Alakiri fish samples varied significantly ($P \leq 0.05$) from those obtained from the base-line zone. Although, Albumin, Total Cholesterol and High Density Lipoprotein (HDL) Cholesterol levels in *O. niloticus* did not differ significantly, total protein and glucose levels showed marked variations in samples from the two rivers. Such variation were also evident in the levels of total protein, glucose, albumin and HDL - Cholesterol in *C. nigrodigitatus*. The high levels of the biochemical indices in fishes (tilapia and catfish) collected from Alakiri rivers suggests that constant discharge of industrial effluents into the aquatic environment has an adverse effect on the health of aquatic fauna.

**KEY WORDS:** Assessment, Biochemical Indices, *Oreochromis Niloticus, Chrysicthys Nigrodigitatus.*

## INTRODUCTION

Clinical tests are available in human medicine to determine the general state of health, evaluate the physiological effect of environmental toxicants, and to diagnose specific diseases. Some of the methods have been adapted for use with fish blood (Blaxhall, 1972). The need for establishing normal biochemical indices in fish with a view to aiding in the diagnosis of diseases (Hesser, 1960) and in connection with pollution and its effect (Mawdesley – Thomas, 1971) has been stressed.

Pollution of the aquatic environment as a direct consequence of increase in urbanization, activities of oil exploring and exploiting industries, among others, in Nigeria, has been the subject of concern, in recent times (Horsfall et al, 1998). For instance, the discharge of water containing ammonia from a fertilizer company into Okrika river, in Rivers State, Nigeria, resulted in massive killing of fish and greatly affected the aquatic life (Horsfall and Spiff, 1999). Alakiri aquatic environment is of great economic importance to Nigeria as several oil fields are located within the environment. Wegwu (1999) reported high levels of Mercury, Lead, Cadmium, Copper, Chromium and Iron in the surface water, sediment and muscles of fishes from the river and attributed it to the increased industrial activities in the area arising from oil exploration and exploitation.

Industrial activities in Omuhuechi (base – line) river are absent and its environment is still in its natural, virgin state. This work attempts to ascertain the health of *O. niloticus and C. - nigrodigitatus* exposed to industrial pollution by determining the levels of some biochemical indices and enzymes activities often employed in pathophysiological investigations.

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## MATERIALS AND METHODS

*O. niloticus* (tilapia) and *C. nigrodigitatus* (catfish) were caught from Alakiri and Omuhuechi rivers, and transported to the laboratory. The fishes were introduced into holding tanks containing water from the rivers. The temperature of the water was maintained at 28 ± 2°C and the fishes were fed with commercial fish diet purchased from Aqua culture, Aluu for 24 hours. This was to enable the fishes from the two rivers adapt to the holding tank environment prior to collection of blood. Ten (10) fish for each of the respective species, were selected randomly from the tanks. The fish were carefully netted, avoiding stress as much as possible and immediately anaesthetized in a trough containing aerated water and 150mg/l Lignocaine (Rotex medica – GMBH). When inactivated, the fish were weighed, using the Fuji weighing balance and 3mls of blood was drawn in disposable, plastic syringes by cardiac puncture. The needle was removed, and the blood gently expressed into a bottle containing anticoagulant (fluoride – oxalate) and into a dry, anti-coagulant – free plastic container, and mixed. Plasma separation was performed by centrifugation using MSE-Minor 35 model of centrifuge. The weight of the fish fell within the ranges: 150g – 200g (for tilapia) and 230g to 350g (for catfish).

Total protein was determined using the Biuret method (Garry and Williams, 1977) while the estimation of serum albumin was performed by the Bromocresol green (BCG) binding method (Cheesbrough, 1987). The Glucose - Oxidase - Peroxidase method, described by Baker and Silverton (1985) for the estimation of Glucose was adopted. Aspartate - aminotransferase (ALT) (E.C. 2.6.1.1), Alanine - aminotransferase (ALT) (E.C. 2.6.1.2) and cholesterol estimations were performed using commercial test kits (Randox). Alkaline phosphatase (E.C. 3.1.3.1) and high density Lipoprotein (HDL) cholesterol were respectively determined by the phenolphthalein monophosphate method and dextran sulphate -mg (11) method (Quimica Clinica applicada-test kits). All determinations were carried out simultaneously with the same blood sample. The spectrophotometric technique (cam spec, m201) was used in the estimation of parameters. The enzyme activities were expressed in international units (I.U). Also, the t- test was used in the statistical analysis of the results.

## RESULTS

Table 1 illustrates the average plasma total protein, albumin, total cholestrol, high glucose values in *O. niloticus* caught from the Alakiri and Omuhuechi rivers. Broad ranges and high co-efficient of variations were recorded for all the biochemical indices in Alakiri and Omuhuechi fish plasma. There was no evidence of the influence of weight on the values of the biochemical indices obtained in this study. Also, statistical differences ($P \leq 0.05$) in the means of plasma albumin, total cholesterol and HDL - cholesterol in *O. niloticu.* from the two rivers were insignificant. However, significant differences in their means existed in the plasma total protein and glucose levels. As shown in Table 2, with the exception of total cholesterol, marked statistical differences ($P \leq 0.05$) existed in

TABLE 1: LEVELS OF SOME BIOCHEMICAL INDICES IN *OREOCHROMIS NILOTICUS* FROM ALAKIRI AND OMUHUECHI WATERS

| Parameter | Fish weight (g) | No. of fish | Range | Mean | STD | C.V (%) |
|---|---|---|---|---|---|---|
| Total protein (g/l) | 160-220 (150-200) | 10 (10) | 34.00-84.00 (23.00-55.00) | 48.70[a] (33.70)[b] | 15.46 (9.26) | 31.75 (27.48) |
| Albumin (g/l) | 160-220 (150-200) | 10 (10) | 10.00-28.00 (7.00-40.00) | 21.70[a] (15.20)[a] | 6.88 (6.89) | 31.71 (45.33) |
| Total Cholesterol (mmol/L) | 160-220 (150-200) | 10 (10) | 3.30-25.00 (3.10-5.70) | 8.46[a] (4.06)[a] | 7.29 (0.85) | 86.17 (20.94) |
| HDL-Cholesterol (mmol/L) | 160-220 (150-200) | 10 (10) | 2.20.7.60 (2.00-3.80) | 4.85[a] (2.85)[a] | 3.63 (0.67) | 74.85 (23.51) |
| Glucose (mmol/L) | 160-220 (150-200) | 10 (10) | 3.90-46.20 (2.00-6.60) | 17.68[a] (33.70)[b] | 12.49 (1.61) | 70.64 (47.77) |

* Values in parenthesis, are those of the unpolluted samples from Omuhuechi river.

* Means with different superscripts are significantly different at the 95% confidence limit

TABLE 2: LEVELS OF SOME BIOCHEMICAL INDICES IN *CHRYSICHTHYS NIGRODIGITATUS* FROM ALAKIRI AND OMUHUECHI WATERS

| Parameter | Fish weight (g) | No. of fish | Range | Mean | STD | C.V (%) |
|---|---|---|---|---|---|---|
| Total protein (g/l) | 230-350 (250-310) | 10 (10) | 30.00-48.00 (19.00-32.00) | 38.60[a] (25.90)[b] | 5.58 (5.53) | 14.46 (21.35) |
| Albumin (g/l) | 230-350 (250-310) | 10 (10) | 8.00-15.00 (6.00-11.00) | 12.00[a] (8.10)[b] | 2.45 (2.20) | 20.42 (24.94) |
| Total Cholesterol (mmol/L) | 230-350 (250-310) | 10 (10) | 4.60-6.10 (4.10-6.00) | 7.50[a] (5.04)[a] | 0.49 (0.72) | 6.53 (14.29) |
| HDL-Cholesterol (mmol/L) | 230-350 (250-310) | 10 (10) | 3.10-4.10 (2.20-4.40) | 4.74[a] (2.88)[a] | 2.42 (0.71) | 51.05 (31.14) |
| Glucose (mmol/L) | 230-350 (250-310) | 10 (10) | 3.80-34.60 (2.50-6.70) | 15.81[a] (4.48)[b] | 12.06 (1.38) | 76.28 (30.80) |

* Means with the same superscripts are not different at the P≤0.05 level.
* Values in parenthesis are those of the un-polluted sample from Omuhuechi river.

TABLE 3: LEVELS OF BLOOD SERUM ENZYMES IN *OREOCHROMIS NILOTICUS* FROM ALAKIRI AND OMUHUECHI WATERS

| Parameter | Fish weight (g) | No. of fish | Range | Mean | STD | C.V (%) |
|---|---|---|---|---|---|---|
| AST (U/L) | 160-220 (150-200) | 10 (10) | 25.00-98.50 (5.50-39.20) | 43.29[a] (16.77)[b] | 25.27 (10.24) | 58.37 (61.06) |
| ALT (U/L) | 160-220 (150-200) | 10 (10) | 20.40-132.10 (3.40-39.00) | 51.99[a] (16.83)[b] | 33.99 (11.97) | 65.38 (71.12) |
| ALP (U/L) | 160-220 (150-200) | 10 (10) | 17.00-222.00 (5.50-127.00) | 71.49[a] (24.12)[b] | 60.40 (36.53) | 84.49 (151.45) |

* Values in parenthesis are those of the unpolluted samples from Omuhuechi river.
* Means with different superscripts are significantly different at the P≤0.05 level.

TABLE 4: LEVELS OF BLOOD SERUM ENZYMES IN *CHRYSICHTHYS NIGRODIGITATUS* FROM ALAKIRI AND OMUHUECHI WATERS

| Parameter | Fish weight (g) | No. of fish | Range | Mean | STD | C.V (%) |
|---|---|---|---|---|---|---|
| AST (U/L) | 230-350 (250-310) | 10 (10) | 11.80-55.00 (3.80-30.80) | 25.06[a] (13.66)[b] | 13.28 (9.85) | 52.99 (72.11) |
| ALT (U/L) | 230-350 (250-310) | 10 (10) | 10.40-58.90 (2.30-33.30) | 22.21[a] (9.47)[b] | 13.87 (8.82) | 62.45 (93.14) |
| ALP (U/L) | 230-350 (250-310) | 10 (10) | 11.50-132.3 (3.50-38.50) | 39.78[a] (13.88)[b] | 35.89 (10.03) | 90.22 (72.26) |

* Values in parenthesis are those of the unpolluted samples.
* Means with different superscripts are significantly different at the P≤0.05 level.

the levels of plasma protein and HDL - Cholesterol in *O. nigrodigitatus* - collected from the two rivers. Apart from plasma protein and total cholesterol in *O. nigrodigitatus* obtained from Alakiri river, other parameters gave co-efficient of variations above 20%.

Results of the activities of blood serum enzymes in *O. niloticus* and *C. nigrodigitatus* caught from Alakiri and Omuhuechi rivers, are shown in Tables 3 and 4. Broad ranges and higher percentages of coefficient of variations were recorded for serum AST, ALT, and ALP activities in the two species of fish from the rivers. Also, statistical differences ($P \leq 0.05$) existed in the activities of these enzymes in the fishes from the two rivers, with those from Omuhuechi river consistently showing lower values.

## DISCUSSION

The wide range in values recorded for the biochemical indices and enzymes activities in the fishes from the study area may be attributed to various factors which significantly alter biochemical parameters. These factors would include diet sources and availability, strain, age, sex, level of exposure to toxic substances, season and sexual maturity (McCarthy et al, 1973). Barnhart (1969) compared levels of haematological parameters such as haemoglobin levels, packed cell volume,

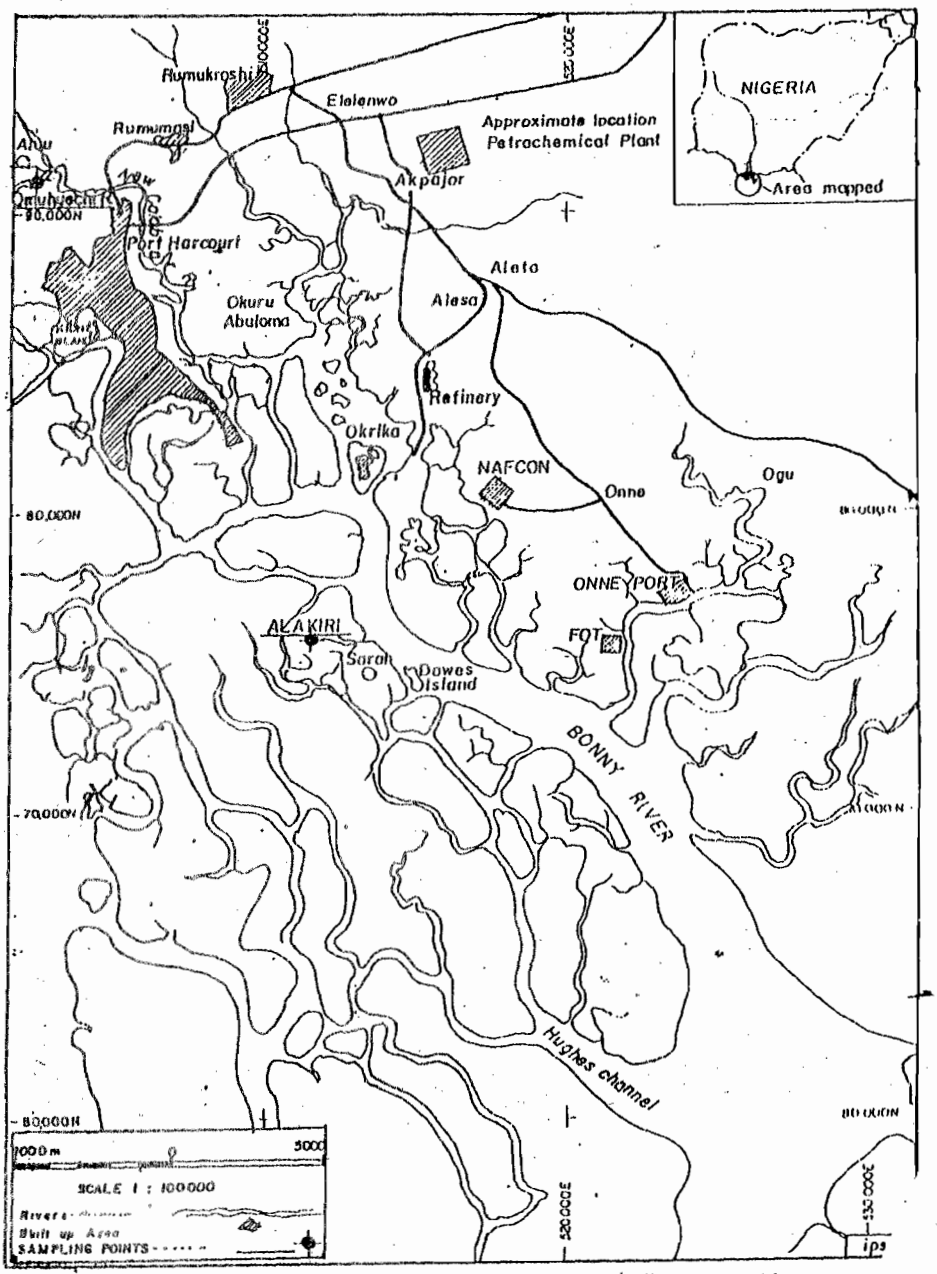


*Fig1.* PORT HARCOURT AND ITS ENVIRONS SHOWING THE SAMPLING POINTS

erythrocyte sedimentation rate and full blood count in rainbow trouts collected from a control pond and the wild. The control pond samples was defined within a narrow range while the wild type gave extremely wide range. Plasma total protein levels, which is biochemically accepted as an indicator of nutritional status (McCarthy el al; 1973) showed significant difference in *O. niloticus* serum from Alakiri and Omuhuechi rivers. Similar variation existed in the mean protein values of *C. nigrodititatus* samples from the two locations. A comparison of plasma total protein in this study with those obtained by McCarthy et al. (1973) on rainbow trout (40 - 68 g/l) indicated higher values in *O. niloticus* sampled from Alakiri river (34.0 - 84.0g/l), Table 1. Serum albumin levels in *C. nigrodigitatus* obtained from Alakiri river varied significantly ($P \leq 0.05$) from those of Omuhuechi river. Such variations were not observed in the serum albumin of *O. niloticus* caught from the two rivers. Garry and William (1977) showed that too low plasma total protein in fish indicate infectious disease, kidney damage and nutritional imbalance and too high value indicate haemo-concentration and impaired water balance. Also, too low plasma cholesterol in

fish is indicative of impaired lipid metabolism while too high values is a sign of fish that is under chronic stress and dietary lipid imbalance. As shown in Tables 1 and 2, there was no significant difference in the levels of total cholesterol in *O. niloticus* and *C. nigrodigitatus* from the two locations. Although, HDL – cholesterol levels in *O. niloticus* and *C. nigrodigitatus* showed very close ranges, marked statistical variation was evident in the HDL - Cholesterol levels for *C. nigrodigitatus* caught from Alakiri and Omuhuechi rivers.

Low glucose levels indicate inanition while too high level is indicative of acute or chronic stress in fish (Garry and William, 1977). As is evidenced in Tables 1 and 2, concentration of plasma glucose in the two species of fish from the polluted (Alakiri) and baseline (Omuhuechi) rivers showed significant variations. The disparity in the means recorded for plasma glucose level indicate that the fishes in Alakiri river are under severe stress. Heat and Pritchard (1965) demonstrated hyperglycaemia in bluegill, *Lepomis macrochirius,* subjected to short term hypoxia and correlated the increase in blood glucose with a decrease in liver glycogen. The selection of serum enzyme assays for the diagnosis of hepatic disease has been based on sufficient experience in correlating the serum values with other measures of hepatic function and disease to assure adequate sensitivity and specificity. These considerations have led to the wide-spread adoption of ALP, AST and ALT for the diagnosis of hepatic and related diseases (John, 1979). To ascertain if decisive disturbances of the equilibrium in the internal environment of fishes in Alakiri river existed, plasma ALT, AST and ALT activities were investigated. As shown in Tables 3 and 4, the extremely high values of ALT in both species of fish from Alakiri rivers as opposed to the control (Omuhuechi) and the significant levels of AST and ALP in Alakiri samples suggest the existence of disturbances of the equilibrium in the internal environment of fishes in Alakiri river. Racicot et al (1975) reported high levels of these enzymes in *Salmo gairdneri rich* exposed to $CCL_4$. They concluded that fish liver was more easily attacked by the chemical. Generally the high values of biochemical indices and increased enzymes activities recorded for the fish caught from Alakiri river, as opposed to those of Omuhuechi river with lower values, would indicate that constant discharge of wastes into the rivers has detrimental effects on the health of fish.

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