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Superoxide Dismutase and Lipid Peroxidation Levels in Fish from the Ethiope River in Southern Nigeria

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The River Ethiope is a major source of domestic water supply to many communities in Delta State, Nigeria. The river has very high fisheries potentials (Odum 1995), but there is very little information on its level of pollution except on its physicochemical properties (Ikomi 1998) and higher level of faecal materials when compared with acceptable standards (Akpomadaye 1995).

Determination of the changes in enzyme activities is one of the used methods of monitoring environmental pollution. Enzyme changes in numerous aquatic organisms such as microsomal mixed function oxidase from marine species (Pohl 1974; Porte 1991); Cytochrome P450 – dependent monooxygenase in crab (Lee 1981; Ishizuka et al 1996); and superoxide dismutase activity in fishes (Roche and Peres 1991; Roche and Boge 1996) have all been used as bioindicators of water pollution. The direct relationship between superoxide dismutase activity and lipid perioxidation has been established (Isamah et al 2000).

Water becomes polluted when the introduction of materials directly or indirectly by man impairs any or all of its uses (Luigi 1983). Several researchers have advocated the use of fish and other invertebrate organisms as bioindicators of water quality because they produce evidence of relatively stable concentrations compared with chemical analysis of water itself (Yamazaki et al 1996). This paper reports the investigation on superoxide dismutase and lipid perioxidation levels in fish from Ethiope River in Southern Nigeria and to evaluate them as index of water pollution gradient along the course of the river.

MATERIALS AND METHODS

The study area is River Ethiope (fig 1.). One of the tributaries of River Benin. It is located in the midwestern region of southern Nigeria. The river took it source at Umuaja (5^0 55^1N, 6^0 16^1E) and flows westwards for about 100km where it discharges into river Benin at Sapele.

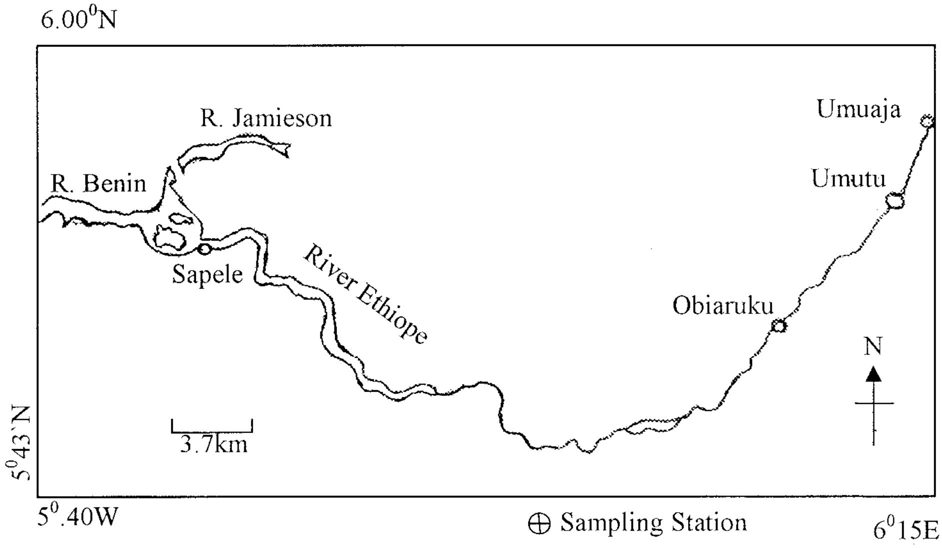

Figure 1. Location of the River Ethiope in Nigeria showing the study area

Two species of fish, Tilapia mariae and Malapterurus electricus were obtained from the source, middle and near the mouth of the River Ethiope between August and November, 2000. The Fishes, Tilapia mariae were netted by local fisherman while malapterurus electricus were harvested using locally designed fishing gear. They were transported immediately in ice to the laboratory and stored in a deep freezer at 4^0C until they were dissected to extract organs of interest (Kidney and liver). They were sorted and duly identified by the Department of Zoology, Delta State University, Abraka, Nigeria.

A total of 30 male and female samples of matured size matched Tilapia mariae (average wet length 14.3cm) and 30 male and female samples of Malapterurus electricus (average wet length 15.8cm) were collected from each site. All the reagents used were the best available and were of analytical grade.

Of the isolated organs 0.5g were washed with ice-cold water and homogenized with $10cm^3$ of ice-cold 0.05M phosphate buffer saline, pH 7.0 using MSE blender immersed in ice. Frozen fishes were partially thawed before this treatment. The extract obtained was clarified by centrifugation for 20 minutes at 7000g at 4^0C. The supernatant (S_1) obtained was used for the determination of lipid peroxidation as described by Hunter et al (1963) and modified by Gutteridge and Winkins (1982).

An aliquot of (S_1) supernatant was precipitated on ice with 0.30 volume of chloroform/method (3.5v/v), stirred on ice for 20 minutes and centrifuged at 7000g at 4^0C. The supernatant (S_2) obtained was used for the assay of SOD activity, which was based on its ability to inhibit the oxidation of epinephrine by superoxide anion (Aksnes and Njaa, 1981). One unit of SOD activity is the amount of the enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrone at 480m per minute (Misra and Fridovich 1972). The enzyme activities were assayed with an SP 1800 UV/VIS spectrophotometer.

The mean and the standard error of mean (SEM) were subjected to analysis of variance using the procedure outlined by Parker (1973).

RESULT AND DISCUSSION

Water pollution and other forms of environmental stress can have a number of harmful effects (Salvato 1976). Such effects include lipid peroxidation (Dianzani 1987), which has a number of detrimental effects on biological membranes (Robins and Cotran 1994). These effects are counteracted by the presence of antioxidant enzymes (Harris 1992). The probable most important enzyme, is however, superioxide dismutase (Fridovich 1986). The relationship between the biological behaviour of aquatic organisms, enzyme activities and levels of pollution has been established (Livingstone 1991; Winston and DiGuiulio 1991; Livingstone et al 1993).

The levels of lipid peroxidation and superoxide dismutase activity were higher in the liver compared to the Kidney of the two fish species (fig. 2 and 3). This could be attributed to the fact that the liver is the principal site of matabolism of xenobiotics and generation of superoxide anion, and therefore, becomes the more susceptible organ of free radical induced tissue damage (Shaheen and El-fattah 1995)

Comparing the ratios, activity of SOD in the liver to liver and the kidney to kidney of the two fish species living in the different ecological domains, it is obvious that Tilapia mariae has lesser SOD activity in its liver and kidney as against the high level in Malapterurus electricus (fig. 2). Izokun-Etiobhio (1990) established that environment has a role to play in the antioxidant status of organisms. Malapterurus electricus and Tilapia mariae inhabit the lentic and lotic parts of the river respectively. In the downstream part of a river, close to the shore, pollutants dilute further, dissolved oxygen is higher and organisms tolerant of such conditions, such as Catfish, Electric fish, and Protozoa inhabit the area (Smith 1992). Increased oxygen level enhances lipid peroxidation (Smith 1981), with concomitant increase in SOD activity, an inducible enzyme that protects the living cells from dangerous effect of oxygen toxicity (Canada and Calabrese 1989).

The higher level of SOD in Malapterurus electricus may, therefore, be hinged on intrinsic biological factor of the fish (Landner 1986); oxygen

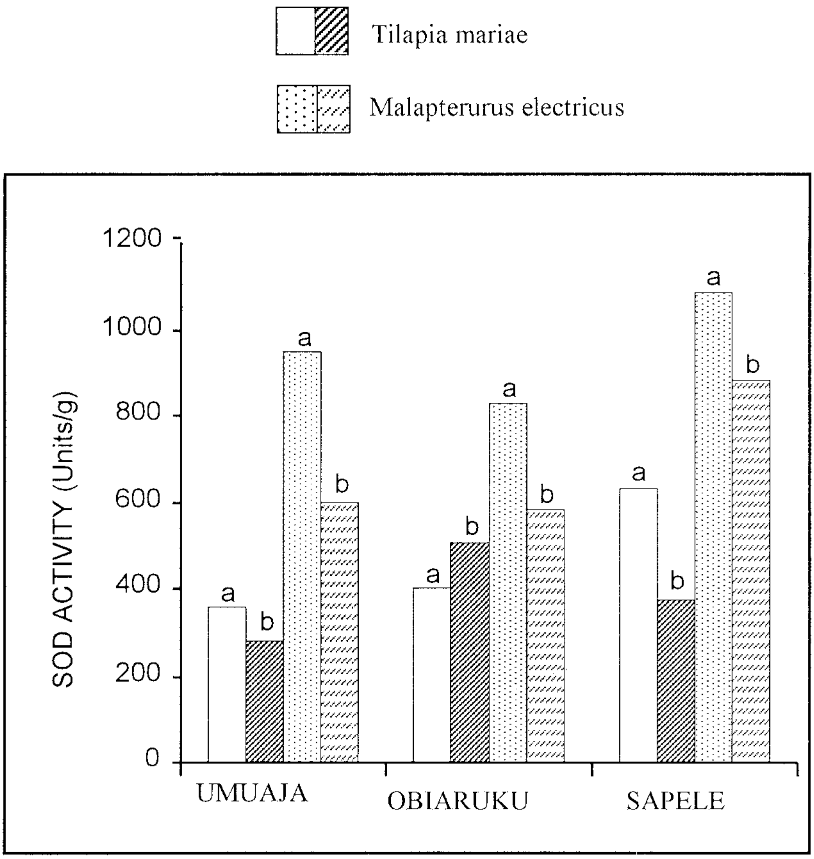

Figure 2. Superoxide dismutase (SOD) activity in lever and kidney of two fish species from three sites along the course of River Ethiope. a = liver b = kidney. *P<0.05 Sapele vs Umuaja/Obiaruku. Umuaja vs Obiaruku (not significant at P<0.05)

levels (Smith 1992) and high levels of pollutants in its environment (Roth and Hornung 1977; Ezemonye 1992). Generally the changes in SOD activities corroborate the changes in levels of lipid peroxidation. These results (fig. 2 and 3) confirm the possible implication of SOD activity in the general defence against environmental disturbances.

This study shows that there was a significant increase (P<0.05) in free radical generation in the two fish species from the lower course of the river as reflected by the increase in SOD activity and increased level of lipid perioxidation when compared with results from the middle and upper course of the river. This observation is an indication that Ethiope River is more polluted in Sapele zone. This may be due to industrialization, sitting of saw mills close to the rivers, effluents from petrochemical and rubber processing industries that are drained into the river, and because pollution

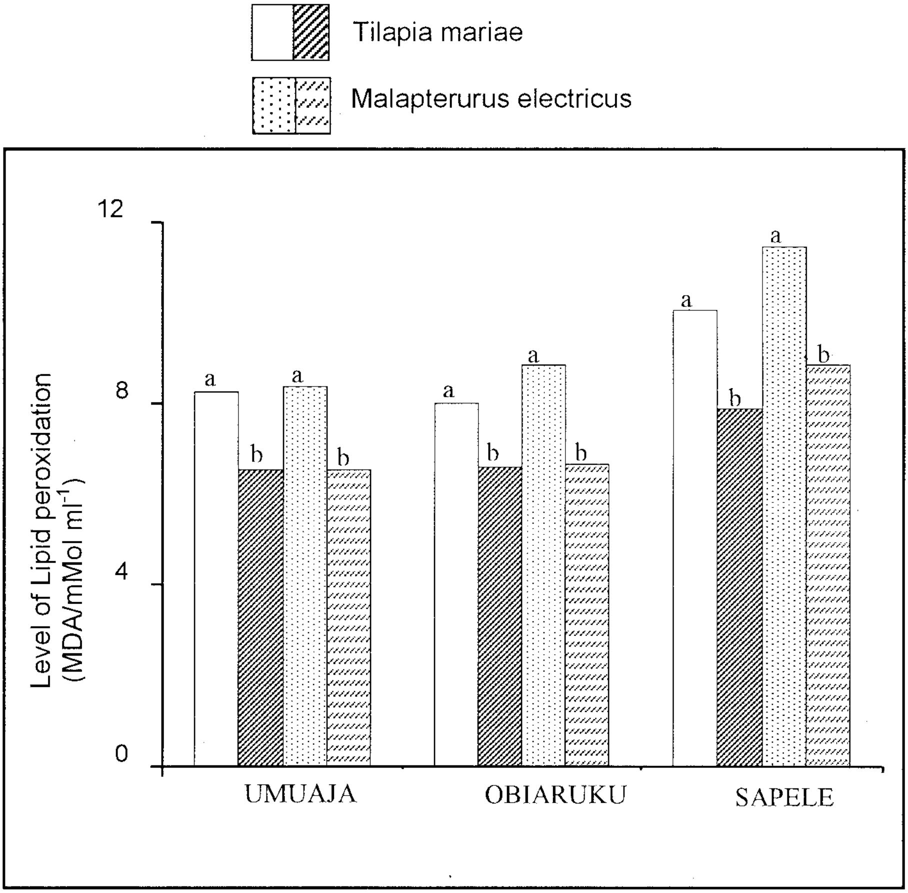

Figure 3. Level of lipid peroxidation in the liver and kidney of two fish species from three sites along the course of River Ethiope a = liver, b= kidney. *P<0.05 Sapele vs Umuaja/Obiaruku. Umuaja vs Obiaruku (not significant at P<0.05)

tends to increase downstream (Miller 1985). Thus, inhabitants of the communities close to the lower course may be exposed to toxic agents through drinking water and consumption of fishes from the river.

Recently, Akporido et al (2000) established a slight increase in the level of acidity of surface and underground water in Sapele and its environs. To check further increase in the level of pollution in the region, a constant environment auditing of the area should be sustained. This serves to preserve the water in Ethiope River and the aquatic lives therein.

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