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Comparative Evaluation of the Levels of Some Antioxidant Enzymes and Lipid Peroxidation in Different Fish Species in Two Rivers in the Western Niger Delta

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The Warri River is highly polluted (Egborge 1994). This is due to the presence of an oil refinery/petrochemical complex and the enormous oil exploration activities in Warri and its environs. Also effluents from the Government owned iron and steel manufacturing company at Ovwian-Aladja that drain into the Warri River and faecal matter are other sources of pollution (Egborge and Benka-Coker 1986). Conversely, Akpomedaye (1995) maintained that faecal pollution is the main source of pollution in Ethiope River when compared to acceptable standards.

Environmental stresses, such as air pollutants (Ranieri *et al.* 1996) and heavy metal toxicity (Weckx and Clijster 1996) cause oxidative stress. One of the consequences of increased oxidative stress is the enhancement of lipid peroxidation (Gupta *et al.* 1991).

Environmental disturbances can be considered as potential sources of stress and changes in enzyme levels are one of the markers of stress (Donaldson 1981). Roche and Boge (1996), using fish placed in synthetic resin tanks, revealed that low but general increase of total superoxide dismutase (SOD) activity is a marker of stress. We hereby report a preliminary field study of the changes in the activities of some antioxidant enzymes and the level of lipid peroxidation as an index of pollution using fish from Warri and Ethiope Rivers.

MATERIALS AND METHODS

Four sampling zones covering the entire length of Warri River were used. The fish species (five from each zone) were pooled together and five randomly selected from this pool and used for this study. A similar protocol was also repeated for the Ethiope River.

The three species of fish used in this study were *Tilapia mariae, Malapterurus electricus and Thysia ansorgii.* These fish are commonly known as tilapia, electric catfish and thysia respectively. They were netted

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by local fishermen and duly identified by the Department of Zoology, Delta State University, Abraka. With all three species of fish, the brain and heart were removed immediately following capture and store separately in polythene bag on dry ice. The samples were maintained frozen at -23°C in the laboratory until used which generally was within one week of capture. The chemical used were the best available and were of the analytical grade.

The stored samples were allowed to thaw slowly on ice prior to use and all subsequent procedures were carried out at this temperature. The thawed organs were blotted free of an excess blood, weighed and washed several times with ice-cold, 0.9% NaCl solution. The tissues (brain or heart) were sliced into approximately 0.5 segments and homogenized in a potter-Elvehjem homogenizer. The homogenisation was performed in 5 volume of ice-cold 0.05M phosphate buffer pH 7.4 and the extract was centrifuged for 20 min at 7,000g at 4°C. The supernatant (S_1) used for the determination of the amount of lipid peroxidation as described by Gutteridge and Wilkins (1982). Values for TBARS are reported as malondialdehyde (MDA) and quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed as MDA $nMolml^{-1}$. Catalase was measured in the same fraction (S_1) after addition of 1 % (V/V) ethanol and incubation in thee cold (0 - 4°C) for 15 minutes (Aksnes and Njaa 1981). Catalase activity was assayed essentially as described by Kaplan and Groves (1972); each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per min and is expressed as $Kmin^{-1}$, where K is the rate constant for a first-order reaction kinetics. As aliquot of the (S_1) supernatant was precipitated on ice with 0.3 vol of chloroform/ethanol (3.5 V/V), stirred on ice for 15 minutes and centrifuge for 20 minutes at 7,000g at 4°C (Aksnes and Njaa 1981). The supernatant (S_2) was used for the assay of SOD activity which is based on its ability to inhibit the oxidation of epinephrine by superoxide anion (Misra and Fridovich 1972). One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome during 1.0 minute. Student t-test was used for statistical analysis and for comparison between the fish species in Ethiope and Warri Rivers.

RESULTS AND DISCUSSION

The organs used for this study were the heart and the brain. The brain is highly vulnerable to attack by oxygen free radical and it does not have the abundance of processes to neutralize these molecular renegades (Reiter 1995) while the heart in an aerobic organism has one of the highest oxygen consumption rates in the body and several sources of free radical production have been identified in the heart (Ji *et al* 1991).

The level of lipid peroxidation was significantly higher ($p<0.55$) in the species of fish from Warri River compared to those from Ethiope River apart from the brain of *M. electricus* (Table 1). A preponderance of iron which followed pulses of industrial activity with a percentage increase of 1180.12 % for iron after a period of our years have been reported in Warri River (Atuma and Egborge 1986). An iron concentration of between 2.248 - 3.420 (mgL^{-1}) and 0.642 - 2.481 (mgL^{-1}) for *Tilapia mariae* from Ekpan and Ethiope Rivers respectively have been reported (Foluke 1994). Ekpan River is a segment in the Zone III of the Warri River. The level of iron in the fish species from the Warri River (Foluke 1994) puts them in jeopardy of damage by reactive oxygen species through the Fenton reaction, a modified Haber-Weiss reaction, which utilizes the redox cycling ability of iron to increase the level of lipid peroxidation. Toxic carbonyl compounds are common products of the complex processes of lipid decomposition by radicals (Reiter 1995). This result clearly indicates that the high level of iron in the fish species from Warri River might impose stress on these fish due to enhanced level of lipid peroxidation. The level of lipid peroxidation in brain homogenates from *M. electricus* from both rivers were not significantly different. This may be correlated with the ecology of *M. electricus* since it inhabits a more restricted domain in the swamp and only come to the water surface to obtain oxygen at intervals.

Table 1: A comparative level of lipid peroxidation (MDA $nmolml^{-1}$) in the fish species from Ethiope and Warri Rivers.

	Fish species	Fish Organ	Ethiope	Warri
i	*M. electricus*	Brain	4.87 ± 0.22	4.12 ± 0.40
			n = 5	n = 5
		Heart	3.03 ± 1.52	**8.29 ± 1.47
			n = 5	n = 5
ii	*T. mariae*	Brain	4.16 ± 1.17	**7.85 ± 1.72
			n = 5	n = 5
		Heart	3.14 ± 0.98	**8.29 ± 0.47
			n = 5	n = 5
iii	*T. ansorgii*	Brain	3.37 ± 1.69	**5.83 ± 1.01
			n = 5	n = 5
		Heart	4.08 ± 1.66	**12.13 ± 1.83
			n = 5	n = 5

Results are expressed as mean ± standard error of mean (S.E.M)
**Statistically significant ($p<0.05$) using the Students t-test.
n = number of fish species.

The activity of SOD from the three fish species from Warri River was significantly higher ($p<0.05$) compared to the corresponding species from Ethiope River, apart from the heart homogenates from *M. electricus* and *T.*

mariae (Table 2). This increase in SOD activity is to prevent the natural oxidation of epinephrine, the concentration of which is generally increased in stressed fish. This result therefore demonstrates that total SOD activity is potent indicator of chemical stress since its activity is generally higher in organisms containing levels of chemical toxicants (Roche and Boge 1996). This further confirms the possible implications of SOD in the general mechanism of cell defence against environmental disturbance. Although the activity of SOD in the brain homogenates of *T. ansorgii* from both rivers showed a very little variation (Table 2), it was however significantly different ($P<0.05$). The low level of standard deviation, is responsible for this observation. The lack of statistical variation in the activity of SOD in *M. electricus* and *T. mariae* is difficult to reconcile (Table 2). It is probably due to similar evolutionary adaptation to the specific conditions of aquatic environment or to established low level of SOD activity in the heart (Doroshow *et al.* 1980).

Table 2: Level of superoxide dismutase in the fish species from the two rivers (Units/g fresh weight).

	Fish species	Fish Organ	Ethiope	Warri
i	*M. electricus*	Brain	255.36 ± 0.74 n = 5	**250.21 ± 0.09 n = 5
		Heart	245.83 ± 2.02 n = 5	252.04 ± 0.21 n = 5
ii	*T. mariae*	Brain	255.48 ± 0.52 n = 5	**250.75 ± 0.51 n = 5
		Heart	251.55 ± 0.31 n = 5	251.06 ± 0.06 n = 5
iii	*T. ansorgii*	Brain	248.33 ± 0.72 n = 5	**253.96 ± 0.16 n = 5
		Heart	251.67 ± 1.66 n = 5	**256.33 ± 0.02 n = 5

Results are expressed as mean ± standard error of mean (S.E.M)
**Statistically significant ($p<0.05$) using the Students t-test.
n = number of fish species.

There was no statistical variation in the activity of catalase in the two organs examined in the fish species from both rivers. The lack of statistical variation in this enzyme suggests that unidentified stress factors such as storage, season or nutritional state could be in operation (Roche and Boge, 1996). Aksnes and Njaa (1981) reported that SOD and glutathione peroxidase are the two most important antioxidant enzymes in fish species, while Doroshow *et al.* (1980) maintained that catalase does not represent the major route of hydrogen peroxide detoxification in the heart.

Table 3: Level of catalase in the fish species from the two rivers ($Kmin^{-1}$).

	Fish species	Fish Organ	Ethiope	Warri
i	*M. electricus*	Brain	3.92 ± 0.01 n = 5	3.85 ± 0.05 n = 5
		Heart	3.78 ± 0.12 n = 5	3.91 ± 0.03 n = 5
ii	*T. mariae*	Brain	3.91 ± 0.12 n = 5	3.87 ± 0.01 n = 5
		Heart	3.92 ± 0.03 n = 5	3.89 ± 0.02 n = 5
iii	*T. ansorgii*	Brain	3.79 ± 0.09 n = 5	3.90 ± 0.01 n = 5
		Heart	3.92 ± 0.04 n = 5	3.91 ± 0.01 n = 5

Results are expressed as mean ± standard error of mean (S.E.M)
n = number of fish species.

Our result is in total agreement with the observation made by Roche and Boge (1996) that an increase in total SOD activity to a lesser extent, is an indicator of stress as a result of chemical intoxication due to pollution. Also from our data, we have presented a preliminary field report that an elevated level of lipid peroxidation could be a more superior indicator of stress due to pollution compared to total SOD activity because lipid peroxidation showed greater statistical variation than total SOD activity when the fish species from both rivers were compared using the brain and heart homogenates (Tables 1 and 2).

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