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Comparative Assessment of Some Biochemical Indices in Catfish from Rivers, Swamp and Commercial Fish Ponds in Oil and Non-Oil Polluted Areas in Rivers and Anambra States

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Abstract

This study was carried out to comparatively assess some biochemical indices in eighteen catfish harvested from rivers, swamp and commercial fish ponds in oil polluted and non-oil polluted areas. Three fishes were collected from each of the following sites; Akaraolu swamps in Ahoada East LGA (AR), a commercial fish pond within the area (AP), The New Calabar River in Ikwerre LGA (BR), a fish pond within the area (BP), Omambala River, Anambra East LGA (CR), a fish pond in Awka, Anambra state (CP), six groups in all. Analysis was done following standard procedures for the biochemical assays carried out. On analysis, the ALP and ALT activities (33.93±2.37 and 44.33±2.96 respectively) from AR were significantly ($p<0.05$) different from BR (17.00±0.58 and 35.00±5.69 respectively) and CR (22.00±2.31 and 30.67±2.60 respectively). The AST activity from AR (68.67±0.88) was not significantly different from that of BR (69.00±1.73) but was significantly different from that of CR (70.33±4.33).Also, site AP (65.53±0.37, 73.00±2.08 and 50.00±1.15 respectively) were significantly higher than CP (42.00±2.89, 64.67±4.33 and 44.67±5.46 respectively) and BP (26.00±1.15 and 50.20±1.15 respectively). However, the AST activity of catfish from AP (73.00±2.08) was not significantly different from that in BP (73.67±1.86). The creatinine, urea, Na^+ and K^+ concentrations observed from AR and AP were significantly higher in comparison to other sites. There was a significantly increased GSH, Catalase, and MDA, and a lower SOD concentration for AR and AP in comparison to other sites. The results show a variation in biochemical indices in fishes from oil polluted sites when compared to non-oil polluted sites.

Keywords: Catfish; Biochemical Indices; Pollution; River

Introduction

In recent years, the pollution of aquatic ecosystems due to man-made activities increased the need for studies to identify the impact of pollutants on the organisms inhabiting the area. Fish species were recently suggested as environmental biomarkers. Also, fishes are considered as early warning for reduction in environmental quality, and also specific measures of the existence of harmful, cancer causing and mutagenic compounds in biological materials [1]. The exploration of petroleum products has rendered agricultural lands less productive and the creeks and the aquatic lives have become more or less dead [2]. Liver and gills as the fish's main organs for metabolism and respiration are targeted by contaminants for accumulation as reported by many authors concerning damage to the structure of organs and tissues related to the exposure of fish to petroleum derivatives [3]. It has been discovered that pollution occurs frequently in the gills, liver and kidneys, because pollutants usually target these organs. When measuring the impact of heavy metals contamination, biomarkers are more efficient than bioindicators because they deal with the chemical and the physiologic changes on the organism level and make an assessment of contamination based on a direct measure of change in the organism [4]. Biomarkers in marine fish such as glutathione (GSH) and metallothionein are often used to evaluate heavy metal contamination.

An adequate knowledge of biochemical parameters of catfish in areas popularly known to be polluted is important for assessing and managing their populations, as well as assessing the health of man who depend on the aquatic organisms for food. Blood parameters used to assess the presence of fish diseases can be used to judge the health status of a population. Any deviations from established values can be used to assess the impacts of stresses such as environmental pollution and its effects. Aquatic organisms; fishes inclusive, relate to a great degree with their environment and depend on it for their survival. Hence it is expected that they will be affected by changes in it [5,6]. The aim of this work is to carry out a comparative assessment of some biochemical indices in catfish from rivers, swamp and commercial fish ponds in oil polluted and non-oil polluted areas in Rivers and Anambra States.

Materials and Methods

Collection of fish sample

The eighteen (18) African catfish (*C.gariepinus*) used in this study were collected from Akaraolu swamps, Akaraolu village in Ahoada East LGA, Rivers state (AR) (The village land and swamps are known to be within the area heavily polluted by crude oil due to explorations taking place by AGIP), the New Calabar River within Ikwerre LGA, Rivers state (BR), Omambala River in Aguleri, Anambara East LGA, a distributary of the River Niger (CR), a commercial fish pond within Ahoada-East LGA in Rivers state (AP), a commercial fish pond established close to one of the distributaries of the New Calabar River in Omuihuechi village in Ikwerre LGA, Rivers state (BP), a commercial fish pond in Awka, Anambara state (CP). Three samples were collected from each of the above named sites and kept in large containers holding water from the sample sites to keep the fishes in their original habitat so as not to alter parameters under study. On arrival, the fish samples were sacrificed, and blood collected for biochemical analysis.

Blood sample collection

This was done with the aid of a 2ml syringe from the caudal artery. The needle ran deep through a middle line, just at the rear anal fin in a dorsal-cranial direction striking the vertebrae. By drawing the needle in a slow motion backward, blood sucked into the syringe. The collected blood was put in a lithium heparin bottle and later spun in a centrifuge at 3000rpm for about 10minutes to separate plasma for biochemical analysis.

Determination of aspartate aminotransferase (AST) activity in plasma

Method: The colorimetric method was used for the determination of AST in plasma. Randox kits were used in accordance with Reitman and Frankel methods (1957) [7].

Principle: Oxaloacetate reacts with hydrazine to form 2,4-dinitro phenylhydrazine (a colored compound).

α-ketoglutarate + L-aspartate $\underset{}{\overset{\text{AST}}{\rightleftharpoons}}$ L- glutamate + oxaloacetate

Procedure

Three test tubes were collected and labelled T_1, T_2 and T_3 respectively for the blank, sample and standard respectively. One meal (1 ml) of distilled water, sample and standard were added to T_1 and T_2T_3 test tubes respectively. Then, 0.5ml of the reagent buffer added to respective test tubes, were thoroughly mixed and incubated for 30mins at 37˚C. Thereafter, 0.5ml of the colored reagent 2 (2,4-dinitrophenylhydrazine) was added to three respective test tubes and allowed to stand for 20 minutes at 25˚C. Afterwards, 5.0ml of sodium

hydroxide (NaOH) was added into all the test tubes, and their absorbance read against the blank at a wavelength of 546nm in a spectrophotometer.

Determination of alanine aminotransferase (ALT) activity

Method: The activity of ALT outside of the cell was estimated by Colorimetric method. The Randoxkits for manual evaluation as proposed by Reitman and Frankel (1957) was used [7].

Determination of alkaline phosphatase (ALP) activity in plasma

Method: Colorimetric method described by Reitman and Frankel, (1957) [7] was used in the *in vitro* determination of alkaline phosphatase in the plasma.

Determination of sodium ion (Na^+)

The concentration of Na^+ was determined using the Fogh-Anderson *et al.* (1984) [8] method.

Materials and reagents:

i. Acid reagent and color reagent

ii. Plasma

Principle: Sodium reacts with ferocyanide or acid reagent to produce a chromosphere whose absorbance is inversely proportional to sodium ion level in the sample.

Determination of plasma potassium ions (K^+) concentration

Method: Colorimetric method was utilized here in agreement to the descriptions of Reitman and Frankel, (1957) [7].

Principle: The quantity of potassium was estimated by using potassium reagent in a specially prepared mixture to form a colloidal suspension, the turbidity formed varied directly with the potassium ion concentration in the range of 2-7 mmol/L.

Determination of Urea concentration

Principle: With the help of Urease ammonia is formed when urea in serum is hydrolyzed. The ammonia is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \rightarrow 2NH_3 + CO_2.$$

NH_3 + hypochlorite + phenol → Indophenol (blue compound)

Determination of plasma creatinine concentration

The description of Reitman and Frankel, (1957) [7] colorimetric method was used.

Assay of superoxide dismutase (SOD) activity

This was determined according to Misra and Fridovich (1989 [9].

Principle

The adrenaline auto-oxidation inhibition was determined by measuring the concentration of the product adrenochrome, at 520 nm. The amount of enzyme that produced 50 % inhibition is defined as one unit of the enzyme activity.

Assay of catalase activity

This was measured using Beers and Sizermethod (1952) [10].

Principle

This method is based on the splitting of hydrogen peroxide by catalase in sample preparations into oxygen and water. The concentration of the residual hydrogen peroxide can be considered with a spectrophotometer at 420 nm. One unit of catalase activity equals the amount of protein that converts micro mole H_2O_2/min.

Assay of malondialdehyde (MDA) concentration

The method used for the analysis of malondialdehyde was that of Hunter, et al. (1963) [11] as modified by Gutteridge and Wilkins (1982) [12].

Principle

Lipid peroxidation was determined by measuring the formation of thiobabituric acid reaction substance. Under acidic condition, MDA produced from the peroxidation of fatty acid members react with the chromogenic reagent, 2-thioberbituric acid to yield a pink complex. Concentration of the resultant MDA-thiobabituric acid complex was read at 532 nm.

Determination of reduced glutathione (GSH)

The concentration of reduced glutathione was determined according to the method of Sedlak and Lindsay (1968) [13].

Principle

The reduced form of glutathione is in most cases composed of the majority of cellular non-protein sulfhydryl groups. Hence the development of a comparatively yellow stable colour when 5,5-dithiobis-2-nitrobenzoic acid (Ellman's reagent) is mixed with sulfhydryl compounds.

Statistical Analysis of Data

All data for biochemical analysis were analyzed for statistical differences and in rat treatment groups, by means of one-way ANOVA and post hoc LSD, on SPSS 20. In all, $p<0.05$ was considered significant. Data are presented as mean±S.D (standard deviation)

Results

The results for the ALT, AST, ALP activities, creatinine, urea, Na^+, K^+ concentrations, as well as Superoxide dismutase, Malondialdehyde, Glutathione, and catalase concentrations are given in table 1, 2, and 3 below.

The ALP and ALT activities (33.93±2.37, 68.67±0.88 and 44.33±2.96 respectively) from AR were significantly ($p<0.05$) different from the plasma ALP, AST and ALT activities (17.00±0.58 and 35.00±5.69 respectively) observed in catfish obtained from BR and CR (22.00±2.31 and 30.67±2.60). The AST activity from AR (68.67±0.88) was not significantly different from that of BR (69.00±1.73) but was significantly different from that of CR (70.33±4.33).

The ALP, AST and ALT activities (65.53±0.37, 73.00±2.08 and 50.00±1.15 respectively) from AP were significantly ($p<0.05$) different from the plasma ALP, AST and ALT activities (42.00±2.89, 64.67±4.33 and 44.67±5.46 respectively) observed in catfish obtained from CP. The ALP and ALT activities from AP (65.53±0.37 and 50.00±1.15 respectively) were significantly different ($P<0.05$) from those of BP (26.00±1.15 and 50.20±1.15 respectively). However, the AST activity of catfish from AP (73.00±2.08) was not significantly different from that in BP (73.67±1.86) as shown in Table 1.

Parameters	AR	BR	CR	AP	BP	CP
ALP (Iμ/L)	33.93±2.37a	17.00±0.58b	22.00±2.31b	65.53±0.37c	26.00±1.15d	42.00±2.89d
AST(Iμ/L)	68.67±0.88a	69.00±1.73a	70.33±4.33a	73.00±2.08c	73.67±1.86c	64.67±4.33d
ALT (Iμ/L)	44.33±2.96a	35.00±5.69b	30.67±2.60b	50.00±1.15c	50. 20±1.15c	44.67±5.46c

Table 1: Liver enzyme activities of catfish harvested from oil polluted swamps, non-oil polluted rivers and commercial fish ponds within oil polluted and non-oil polluted areas in Rivers and Anambra states.

Values are means ± Standard Deviation (S.D). Values with different superscript are statistical significant at ($P < 0.05$). BR and CR were compared to AR while BP and CP were compared to AP. Superscript (a,b) compares BR and CR to AR while Superscript (c,d) compares BP and CP to AP.

Description of variables: ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase.AR: Catfish from Akaraolu swamps Ahoada East LGA, oil polluted area, BR: The New Calabar River, Ikwerre LGA, non- oil polluted area, CR: Omambala River, Anambra East LGA, a non-oil polluted area. AP: commercial fish pond, Ahoada East LGA, oil polluted area, BP: commercial fish pond in IKwerre LGA, non-oil polluted area, CP: commercial fish pond in Awka, Anambra state, non-oil polluted area.

The creatinine and urea concentrations (49.13 ± 1.13 and 4.23 ± 0.28 respectively) from AR were significantly different ($P<0.05$) from those of BR (42.10 ± 2.89 and 2.53 ± 0.23 respectively). The plasma Na^+ and K^+ (134.67 ± 1.20 and 4.43 ± 0.22 respectively) from AR were not significantly different from the Na^+ and K^+ (134.67 ± 0.33 and 4.20 ± 0.25 respectively) from BR. While the creatinine and urea concentrations (49.13 ± 1.13 and 4.23 ± 0.28 respectively) from AR were significantly different from the creatinine and urea concentrations (46.97 ± 2.47 and 3.33 ± 0.24 respectively) from CR. The Na^+ and K^+ concentrations (134.07 ± 1.20 and 4.43 ± 0.22 respectively) from AR were not significantly different from the Na^+ and K^+ (135.67 ± 0.67 and 4.23 ± 0.28 respectively) collected from CR as shown in Table 2.

Also, the creatinine and urea concentrations (48.77 ± 1.24 and 4.67 ± 0.07 respectively) of catfish collected from AP were significantly different from the creatinine and urea concentrations (49.50 ± 0.75 and 2.00 ± 0.10 respectively) of catfish from CP. The Na^+ concentration (135.00 ± 0.58) of catfish collected from AP was not significantly different from (135.00 ± 0.58)Na^+ concentration collected from CP , while the K^+ concentration (4.20 ± 0.21) from AP was significantly different ($P<0.05$) from (2.13 ± 0.12) concentration from CP. The creatinine and urea concentrations (49.50 ± 0.75 and 4.67 ± 0.07 respectively) of catfish from AP were significantly different ($P<0.05$) from the creatinine and urea concentrations (47.70 ± 2.36 and 3.37 ± 0.47 respectively) of catfish from CP. The plasma Na^+ (135.00 ± 0.58) of catfish from AP was not significantly different

from (135.33 ± 1.20)of Na^+ concentration collected from BP, while the K^+ concentration (4.93 ± 0.21) from AP was significantly different from CP (3.13 ± 0.97) as shown in Table 2.

Parameters	AR	BR	CR	AP	BP	CP
Creatinine (mmol/L)	49.13±1.13[a]	42.10±2.89[b]	46.97±2.47[b]	49.50±0.75[c]	47.70±2.36[d]	48.77±1.24[d]
Urea (mmol/L)	4.23±0.28[a]	2.53±0.23[b]	3.33±0.24[b]	4.67±0.07[c]	3.37±0.47[d]	2.00±0.10[d]
Na^+ (mmol/L)	134.67±1.20[a]	134.67±0.33[a]	135.67±0.67[a]	135.00±1.15[c]	135.33±1.20[c]	135.00±0.58[c]
K^+ (mmol/L)	4.43±0.22[a]	4.20±0.25[a]	4.23±0.28[a]	4.20 ± 0.21[c]	3.13±0.97[d]	2.13±0.12[d]

Table 2: Kidney biomarkers concentrations of catfish harvested from oil polluted swamps, non-oil polluted rivers and commercial fish ponds within oil polluted areas and non-oil polluted areas in Rivers and Anambra states.

Values are means ± Standard Deviation (S.D). Values with different superscript are statistical significant at ($P < 0.05$). BR and CR were compared to AR while BP and CP were compared to AP. Superscript (a,b) compares BR and CR to AR while Superscript (c,d) compares BP and CP to AP.

Description of variables: AR: Catfish from Akaraolu swamps Ahoada East LGA, oil polluted area, BR: The New Calabar River, Ikwerre LGA, non- oil polluted area, CR: Omambala River Anambra East LGA, a non-oil polluted area. AP: commercial fish pond, Ahoada East LGA, oil polluted area, BP: commercial fish pond in IKwerre LGA, non-oil polluted area, CP: commercial fish pond in Awka, Anambra state, non-oil polluted area.

The concentrations of GSH, MDA, and Catalase (6.48 ± 0.31, 0.28±0.03, 21.77±0.39 respectively) from AR were significantly ($p<0.05$) different from the plasma GSH and MDA concentrations (5.69 ± 0.13, 0.23±0.01, and 8.79±0.38 respectively) from BR as well as those from CR (5.36±0.25, 0.11±0.01, and 7.35±0.39). The SOD activities (0.19±0.02) from AR was significantly lower than that of BR (0.24±0.01) and CR (0.27±0.02) as shown in Table 3.

Also the GSH, MDA, and Catalase concentrations of catfishes from AP (6.17±0.12, 0.31±0.02, 13.26±0.22 respectively) were significantly higher than those from BP (5.56±0.22, 0.21±0.02, 6.65±0.53 respectively), while the SOD activity in AP (0.17±0.02) was significantly lower than those from BP (0.26±0.02). The GSH, MDA and Catalase activities for catfishes from AP (6.17±0.12, 0.31±0.02 and 13.26±0.22 respectively) were significantly higher than those from CP (5.35±0.12, 0.12±0.01 and 8.39±0.10 respectively). The SOD activity from AP (0.17±0.02) was significantly lower than that from CP (0.31±0.03) as shown in Table 3.

Parameters	AR	BR	CR	AP	BP	CP
GSH (µg/ml)	6.48±0.31[a]	5.69±0.13[b]	5.36±0.25[b]	6.17±0.12[c]	5.56±0.22[d]	5.35±0.12[d]
MDA (mmol/mg)	0.28±0.03[a]	0.23±0.01[b]	0.11±0.01[b]	0.31±0.02[c]	0.21±0.02[d]	0.12±0.01[d]
Catalase (unit/mg)	21.77±0.39[a]	8.79±0.38[b]	7.35±0.39[b]	13.26±0.22[c]	6.65±0.053[d]	8.39±0.10[d]
SOD (unit/mg)	0.19±0.02[a]	0.24±0.01[a]	0.27±0.02[b]	0.17±0.02[c]	0.26±0.02[d]	0.31±0.03[d]

Table 3: Non-enzymatic (Glutathione and Malondialdehyde) and Enzymatic (Superoxide dismutase and Catalase) markers of oxidative stress in catfish harvested from oil polluted swamps, non-oil polluted rivers and commercial fish ponds within oil polluted and non-oil polluted areas in Rivers and Anambra states.

Values are means ± Standard Deviation (S.D). Values with different superscript are statistical significant at ($P < 0.05$). BR and CR were compared to AR while BP and CP were compared to AP. Superscript (a,b) compares BR and CR to AR while Superscript (c,d) compares BP and CP to AP.

Description of variables: GSH: Glutathione, MDA: Malondialdehyde, SOD: Superoxide dismutase. AR: Catfish from Akaraolu swamps Ahoada East LGA, oil polluted area, BR: The New Calabar River, Ikwerre LGA, non- oil polluted area, CR: Omambala River Anambra East LGA, a non-oil polluted area. AP: commercial fish pond, Ahoada East LGA, oil polluted area, BP: commercial fish pond in IKwerre LGA, non-oil polluted area, CP: commercial fish pond in Awka, Anambra state, non-oil polluted area.

Discussion

The liver enzymes ALP, AST and ALT are used for monitoring liver injury and serve as indicators of hepatocellular (ALT activity), and hepatobiliary (ALP

activity) injury in preclinical studies through leakage from altered membrane permeability [14]. From the results, the plasma ALP, AST and ALT activities observed in catfish obtained from AR and AP were significantly ($p<0.05$) different from the plasma ALP, AST, and ALT activities obtained from BR, CR, CP, and BP as shown Table 1. The significantly increased plasma ALP, AST and ALT activities observed in catfish from AR and AP in comparison to the plasma ALP, AST and ALT activities of catfish from BR, CR, CP, and BP are suggestive that oil pollution in the rivers from AR and AP caused damage to the hepatocytes of the catfish due to bioaccumulation. These results agree with the report of Tchounwou, *et al.* [15] which also showed that toxicants do induce organ damage still in low amount. Creatinine is produced relatively in a constant rate depending on the absolute amount of muscle mass [16]. Urea is an endogenous product of protein and amino acid catabolism. It is formed from ammonia, which is a deamination product of amino acids. Na^+ and K^+ are intracellular and extracellular ions that play vital roles in formation of nerve impulses [17]. Weight decline results in creatinine decline, while a rise in creatinine concentration is associated with mortality [18]. In this study, the creatinine, urea, Na^+ and K^+ concentrations observed in river from AR and AP were significantly higher than the creatinine, urea, Na^+, K^+ concentrations respectively of catfish obtained from BR, BP, CR and CP as shown in Table 2. The increased creatinine, urea, and electrolyte concentrations of catfish from AR and AP in comparison to those from BR, BP, CR and CP as shown in Table 2 is suggestive that oil pollution in the waters from AR and AP resulted in impairment of the glomerular filtration rate leading to renal damage. These results are in agreement with the publication of [17], on the assessment of some pesticides and heavy metals in water and fish of *Oreochromisaureus* from aquatic drainage and Nile canals and their impact on some biochemical parameters.

Glutathione is the master antioxidant that detoxifies free radicals and play immunological role in the body [19]. Malondialdehyde (MDA) is the final product of lipid peroxidation [20]. These two are used as biomarkers of oxidative stress. Damage to the liver by pollution results in increased MDA concentration [21]. In this study, the increase significant plasma level of GSH and MDA in plasma of catfish from AR and AP observed is suggestive of decreased immunological status due to free radical generated from oxidative stress. Superoxide dismutases (SODs) are enzymes that function to catalytically convert O_2^- to oxygen (O_2) and hydrogen peroxide [22]. In polluted areas, it has been discovered that GSH activity is usually elevated [23]. In this study, an increased GSH, MDA, and Catalase activities were observed in catfish from AR and AP when compared to those of catfish from BR, BP, CR and CP as shown in Table 3. The increased activities of these oxidative stress markers noticed is indicative that oxidative stress was in the fish. While a decreased activity of SOD recorded from all sites is indicative of a decrease in the conversion of reactive O_2^- to O_2. This supports the finding by Farombi, et al. [24] in the African catfish (*C.gariepinus*) from the Ogun River located close to major industries in the South Western part of Nigeria, GSH concentration was higher by 81%, 83%, and 53% in the liver, kidney and heart, respectively, compared to that from the reference site.

Conclusion

The fishes from crude oil polluted sites showed increased Liver enzyme and kidney biomarker activities, as well as increased oxidative stress markers activity, indicating the presence of oxidative stress in comparison to the non-crude oil polluted sites. Reason could be that catfish from the crude oil polluted sites may have been exposed to the accumulation of pollutants over a longer period of time in comparison to the non-crude oil polluted areas. Histological studies on the liver and kidney of these cat fish is recommended for those from the rivers, swamp and commercial fish ponds in oil and non-oil polluted areas of Rivers and Anambra states of Nigeria.

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