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Superoxide Dismutase, Alanine Transaminase and Catalase Activity of Clarias Gariepinus Cultivated in Crude Oil Contaminated Sediment

Ijeoma Jacinta Nwokolo
Department of Environmental Management and Toxicology,
Federal University of Petroleum Resources, PMB 1221, Effurun, Delta State, Nigeria.

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Abstract

This study investigated the effect of crude oil from an oil exploration company in Delta State on the blood, gills, kidney, heart and liver of Juvenile African Catfish (Clarias gariepinus). Twenty-Five Juvenile African Catfish were separated into 5 groups (5 per treatment) and used for the study. The juveniles were exposed to five varying concentrations of crude oil (0%, 0.1%, 0.3%, 0.5%, and 1%) for a period of 9days. At the end of the test period, biochemical activities were carried out on the blood, kidney, heart, and liver of the juveniles. The results of biochemical parameters revealed a significant difference ($p < 0.05$) between the control and exposed groups, indicating metabolic damage and a degenerative process. Alanine transaminase (ALT) increased in the serum, gills, kidney, heart, and liver of all exposed groups when compared to the control group serum and tissues. Superoxide dismutase (SOD) activity of kidney decreased across all exposed groups when compared to the control group. Catalase (CAT) activity increased across the serum of all contaminated groups when compared with the control group. All the juveniles held in the control stock showed no degradation. The severity of damage to the gills, heart, kidney and liver depends on the concentration of the pollutants and the period of exposure. Conclusively, this study has revealed that exposure of juvenile Clarias gariepinus to even low concentrations of crude oil may induce biochemical changes in the blood, kidney, heart, liver and gill of the fish.

Keywords: *Crude oil; African Catfish; Alanine Transaminase; Catalase; Superoxide Dismutase.*

Corresponding Author: **Ijeoma Jacinta Nwokolo**

Background to the Study

Effects of petroleum (mainly hydrocarbon) exploration in the Niger Delta, such as air pollution and oil spills is not only regional but global in scale (Kamalu and Nwokocha, 2011.).These contaminants are swept into lakes, ponds, and rivers thus polluting the aquatic environment which are prone to decline in quality by petroleum and its by-products (Sylvia, 2019).When animals come into touch with these hydrocarbons while grazing, they are exposed to a major health risk, which could result in a high rate of animal fatality (Oshienemen *et al.*, 2018). Nigerians benefit from catfish production as it consumes less space, time, and money, and has a greater feed conservation rate, according to (Amponsah *et al.*, 2021). Contaminants accumulate more in organisms than the environment, thus, Fish has been used as bio-indicators to detect the amount of pollutants in the environment (Dauod *et al.*, 2020). Biochemical changes in fishes exposed to contaminants have been proposed and used as biomarkers for pollutants such as petroleum products (Eseigbe *et al.*, 2013).

Alanine Transaminase (also known as Alanine Amino-transferase) is found in plasma and other bodily tissues, but it is most commonly found in the liver. When the liver is wounded, it spills into the blood, causing the enzyme levels in the blood to rise, indicating liver injury (Upadhayay, 2016). ALT is commonly measured as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health (Wang *et al*, 2013).

Catalase is a sensitive enzyme whose activity is influenced by a variety of circumstances, including superoxide radical overproduction (Anika *et al.*, 2019). Catalase (CAT) reacts with H2O2 to form water and oxygen molecule (Lu *et al.*, 2018). CAT is a part of the antioxidant defense system (Pallavi *et al.*, 2012). Superoxide dismutase (SOD) is the enzyme that catalyzes the dismutation of the superoxide anion to O2 and H2O2. SOD is a family of metalloenzymes that serve an important antioxidant role in aerobic organisms and are the first line of defense against superoxide radical toxicity (Zorov *et al.*, 2014).

The liver is frequently used as an environmental biomarker due to its capacity for detoxification and storage of harmful components (Stori *et al.*, 2014). The kidneys are one of the body organs responsible for excretion and water balance management in fish (Byron, 2014). The heart pumps blood (Graham and Dickson, 2004). The gills can absorb oxygen directly from the air (Byron, 2014).

It is well known that multiple studies on the exposure of African Catfish (Clarias gariepinus) to contaminants have been conducted. So far, no study has been done on the impact of crude oil produced by this specific oil business; Warri refining and Petrochemical Company (WRPC) on the blood, liver, gills, kidney, and heart of juvenile African Catfish. The company is located close to a water body and hence it becomes necessary to investigate its effects.

Materials and Methods

Collection and Acclamatization of Specimens

A local fish farm in Delta State (AB farms, Agbarho) sold us 25 healthy Clariasgariepinus juveniles at 6 weeks old with an average weight of 0.2 kg. They were carried in an open gallon

half-filled with fresh tap water. Each bioreactor had a capacity of 25 liters and contained 5 kg of soil and 20 liters of water within Department of Petroleum Resources (DPR) limits.

Ethical Clarification

All animals were kept in regular lab settings with free access to balanced pellet diet and water. The experiment methodology was authorized by the Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria ethics committee (FUPRE/ECC2019/SC/EMT001) and adhered to all ethical standards regarding the use of animals in research.

Clarias Gariepinus for Experiment

There were five groups assigned to Clarias gariepinus;
Group A: Clarias gariepinus grown in soil with no traces of crude oil
Group B: Clarias gariepinus grown on soil with 0.1% crude oil contamination
Group C: Clarias gariepinus grown on soil with 0.3% crude oil contamination
Group D: Clarias gariepinus grown on soil with 0.5% crude oil contamination
Group E: Clarias gariepinus grown on soil with 1.0% crude oil contamination

Serum and Tissue Homogenate

The fish were slaughtered, and their tissues (liver, kidney, gills, and heart) were taken and placed in a beaker containing an ice cold 0.25M sucrose solution. The blood was taken through heart puncture. Following that, each blood sample was spun at 3,500rpm for around 15 minutes using chilled centrifuge RC650s, and the serum recovered was stored at -8°C until needed. The separated tissues were weighed, and a part of each tissue was taken out, diced into extremely small bits, and homogenized in an ice-cold dish with a pre-cooled pestle and mortar. The tissue homogenates were diluted one-to-thirty times with 0.25M sucrose solution. The diluted homogenates were kept at -8°C temperature until analysis.

Biochemical Assays

Following the procedure described in the Randox assay kits, the concentration of alanine transaminase (ALT), was determined in the serum and organs. Determination of ALT activity is based on monitoring the concentrations of pyruvate hydrazone formed with 2,4 dinitrophenyl hydrazine.

The Misra and Fridovich (1972), technique was used to measure the SOD activity in the tissues of the experimental animals. A straightforward test for superoxide dismutase is based on its capacity to prevent the autoxidation of epinephrine at pH 10.2. Epinephrine is converted to adrenochrome via the xanthine oxidase process, and the yield of adrenochrome produced per O_2^- added rises with rising pH and epinephrine concentration. These findings led to the hypothesis that at least two separate mechanisms contribute to the autoxidation of epinephrine, only one of which is a free radical chain reaction involving superoxide radical (O_2^-), which is why SOD may suppress it.

Following Sinha (1971), approach, the catalase activity of the tissue homogenate produced from the experimental animals was assessed. When heated in the presence of H2O2,

dichromate in acetic acid is converted to chromic acetate, with perchloric acid forming as an unstable intermediate. On a spectrophotometer, the absorbance value of the created chromic acetate is measured between 570 and 610 nm. Dichromate does not have an absorbance in this range, hence its inclusion in the test mixture has no effect on the colorimetric detection of chromic acetate.

Statistical Analysis

All data were evaluated using Analysis of Variance (ANOVA) using the Steel and Torrie (1960) technique. Duncan's Multiple Range Test was used to evaluate if there was a significant difference between the treatment means at 5% confidence level (Duncan, 1955).

Results

The results obtained from the Alanine transaminase (ALT) activity of the blood samples and tissues of the juveniles exposed to crude oil and control is presented in Table 2. From the results, it is observed that there is an increase in ALT activities of serum and tissues of exposed groups from control. The serum and tissues of all exposed groups showed about the same range of activity. In the present study, biochemical activity such as Catalase, Superoxide Dismutase were analyzed in Serum and tissues of the juveniles exposed to crude oil and control. The biomarkers of oxidative stress superoxide (SOD) and Catalase (CAT) analyzed are presented in Table 1 and 3 respectively. From the table 1, it is observed that there is a significant difference in the activities of SOD in all the groups exposed to crude oil compared to the control. From table 3, it is observed that there is a significant difference in the activity of catalase in the serum of the juvenile exposed to crude oil when compared to control group. The tissue of the exposed group has about the same catalase activity when compared the control, with the exception of group 0.3% crude oil contaminated tissues which shows a significant difference in the catalase activity when compared to the control group.

Table 1: Superoxide Dismutase (Sod) Activity (Nmol/Min/Mg Protein) of Clarias Gariepinus Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	SERUM	HEART	LIVER	KIDNEY	GILLS
0%	203 ± 4.0^{a}	129 ± 0.5^{a}	112 ± 1.7^{a}	128 ± 0.7^{a}	120 ± 0.1^{a}
0.1%	242 ± 7.2^{b}	137 ± 0.9^{b}	124 ± 3.2^{b}	99 ± 1.1^{b}	120 ± 0.6^{a}
0.3%	134 ± 1.9^{c}	120 ± 1.5^{c}	107 ± 0.6^{c}	107 ± 0.5^{c}	105 ± 0.8^{b}
0.5%	160 ± 2.4^{d}	155 ± 0.6^{d}	140 ± 1.5^{d}	110 ± 0.5^{d}	146 ± 2.7^{c}
1%	183 ± 1.7^{e}	118 ± 0.2^{e}	112 ± 0.3^{a}	113 ± 0.3^{e}	114 ± 0.2^{d}

The results are the averages of five determinations ± standard error of the mean (SEM). The values in the same column with different superscripts differ considerably ($p < 0.05$)

Table 2: Alanine Transaminase Properties (Nmol/Min/Mg Protein) of Clarias Gariepinus Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	SERUM	HEART	LIVER	KIDNEY	GILLS
0%	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
0.1%	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
0.3%	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
0.5%	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
1%	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}

The results are the averages of five determinations ± standard error of the mean (SEM). The values in the same column with different superscripts differ considerably ($p < 0.05$)

Table 3: Catalase Activity (Nmol/Min/Mg Protein) of Clarias Gariepinus Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	SERUM	HEART	LIVER	KIDNEY	GILLS
0%	0.2 ± 0.008^{a}	0.4 ± 0.002^{a}	0.4 ± 0.001^{a}	0.4 ± 0.001^{a}	0.4 ± 0.003^{a}
0.1%	0.4 ± 0.005^{b}	0.4 ± 0.001^{a}	0.4 ± 0.001^{a}	0.4 ± 0.001^{a}	0.4 ± 0.0004^{a}
0.3%	0.1 ± 0.001^{c}	0.2 ± 0.001^{b}	0.1 ± 0.0004^{b}	0.2 ± 0.0004^{b}	0.2 ± 0.001^{b}
0.5%	0.3 ± 0.001^{d}	0.4 ± 0.002^{a}	0.3 ± 0.003^{c}	0.4 ± 0.004^{a}	0.4 ± 0.003^{a}
1%	0.4 ± 0.01^{b}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.004^{a}

Engineering, Science and Technology July, 2022The results are the averages of five determinations ± standard error of the mean (SEM). The values in the same column with different superscripts differ considerably ($p < 0.05$)

Discussion

It was suggested that enzymes such as ALT may be used as sensitive biomarkers in ecotoxicology thanks to providing early warning of potentially hazardous changes in contaminated aquatic organisms (De La Torre et al. 2000; Levesque et al. 2002). From the results obtained, the blood and tissue samples collected from the juvenile exposed to crude oil showed an increase in ALT level in the exposed group.

Alanine transaminases have a substantial role in protein and amino acid metabolism and may release into the plasma upon tissue damage and dysfunction. The present study agrees with (oner et al., 2018) who proved that copper caused substantial increases in serum AST and ALT activities in Heteropneustes fossilis with increases in exposure time. They attributed the increase to tissue damage and impairment of fish metabolism. Zikic et al., (2001) proved that plasma ALT activities were increased in Cadmium-exposed fish Carassius auratus gibelio. The scientists speculated that metal-induced damage to the liver, kidney, heart, and other organs could result in the release of this transaminase into the circulation.

The results of this study correspond with those of Edris (2017), who found an increase in ALT in goldfish exposed to nickel in a sub-chronic test. However, the findings of present study disagreed with the reports of Okechukwu et al (2007) who used sub-lethal doses of toxicants and reported significant decrease in ALT activities which may have resulted from the type of toxic compound in the solution used.

In present study, SOD activity was increased in some tissues and serum of juveniles exposed to crude oil, suggesting that crude oil triggers SOD activity. SOD provides a great deal of contributions in defence against the toxic effects of ROS, in turn might scavenge O−2 to protect cells against lesions and strike the equilibrium between oxidant and antioxidant. SOD and CAT as oxygen-free radical (OFR) enzymatic scavengers which enable the system to cope with the adverse effects of OFRs (Pallavi et al., 2012). Free radicals play a fundamental role in the toxicity of environmental chemicals. Some chemicals may lead to oxidative stress, resulting in generation of free radicals and antioxidants changes or the OFR scavenging enzyme system (Sharifi-Rad et al., 2020). The findings in this study agrees with other research, SOD activities in the Boleophthalmus pectinirostris liver were substantially triggered after the exposure to 30 mg/L benzo(a)pyrene for 7 days (Feng et al. 2000). These research results illustrated that as a sensitive marker, SOD may be used to indicate the biochemical response on organisms. The increased SOD activity denotes a higher intracellular H2O2 formation. Antioxidant enzymes such as GPx, SOD and CAT are activated to counteract the negative effect of the ROS (Parvez and Raisuddin 2005). In this work a decline in SOD activity in 0.3% and 1% crude oil exposed groups may show a reduced ability to protect cells against superoxide radicals. This study agrees with Ozmen et al. (2004) who suggested the depression in SOD activity may result in cellular injury by superoxide radical.

To protect the biological system against ROS, CAT is an inducible cytosolic enzyme characterized with important functions. Exposure to environmental contamination involving many complicated processes can be evaluated by antioxidant enzyme activity as well as by lipid peroxidation measures. Nonetheless, varied CAT responses in fish exposed to various concentrations of pollutants in laboratory trials have been studied. In the present study, CAT levels increased in some serum and tissues of exposed group which agrees with (Srinivasan et al. 2007) who reported an increase in CAT activity with laboratory exposures of channel catfish to polluted harbour sediments. Decrease in CAT activity in 0.3% contaminated group in the present study agrees with Barney et al., 1996 who noted a decline in CAT activity attributed to an increase in SOD and O_2 (Superoxide anion) production by pollutants. Furthermore, study on antioxidant enzyme activities of fish (Barbus m. petenyi Heck.) (Velcova-jordanoska, 2008) pointed out that CAT activity was inhibited upon exposure to pollution.

Conclusion

Oil and gas operations usually contaminate the Niger Delta and even global environment. There is need to minimize level of exposure. The level of crude oil in aquatic environment should not exceed 0.1% based on the biochemical changes observed in the juveniles when exposed to crude oil from WRPC. There is also a need to tighten treatment standards. The

government needs to provide more stringent standards that must be met in the treatment of crude oil discharges in terms of local legislation and international best practice.

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