

DIESEL EXPOSURE OF *TYMPANOTONUS FUSCATUS* AND ITS EFFECTS ON ENZYME ACTIVITY

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ABSTRACT

The effect of mild concentrations of diesel (10.40,15.60,21.00 and 26.00 mg/l) on some biochemical parameters such as Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP), in the tissue of periwinkle (*tympanotonus fuscatus*) were examined using a renewal static bioassay for six days. The activities of the enzymes were measured on 120 specimens of periwinkle of size between 4.5-5.5cm lengths. The result of AST activity in the muscle showed either an increased or decreased activity against the control. The activity of ALT showed significant ($p=0.05$) decrease in all the test concentrations. ALT also showed significant difference ($p=0.05$) which were either higher or lower than the control value. In the viscera, activity of AST significantly increased ($p=0.05$) than that of the control. ALT activity was significantly increased ($p=0.05$) above the control except at 10.40ml/L where a significant decrease ($p=0.05$) was observed. Significant increase ($p=0.05$) was observed in the activity of ALP above the control value except at 10.40ml/L. The result of the tissue enzyme activities indicated alteration in the biochemistry of *tympanotonus fuscatus* and therefore could be used as a biomarker of aquatic pollution and toxicities.

KEYWORDS: Diesel, *Tympanotonus fuscatus*, Enzyme, Toxicity, pollution

INTRODUCTION

Due to the introduction of substances of diverse characteristics arising from man's activities, the aquatic environment (ecosystem) is continuously subjected to changes in quality (Oluah, 2001). The effects on an ecosystem as a result of xenobiotic contamination can be estimated through the analysis of biochemical changes in organisms inhabiting such habitat (Norris *et al.*, 2000; Brewer *et al.*, 2001). These changes are often expressed using key enzymes that are involved in biotransformation system (Ozmen *et al.*, 2005). Crude oil and refined oil pollution is obvious world over and is more common in oil dependent economy. Contamination of water body by petroleum products has been reported to produce many

changes in fish and other aquatic organism when under chronic or acute exposure (Osuji and Mbata, 2004; Jee and Kang 2005; Gabriel *et al.*, 2007; Nwamba, 2009). Crude oil and refined products vary considerable in toxicity and the sensitivity of fish and other aquatic organisms to these products vary according to the product and the species (Nwamba, 2009). Crude oil and its fractions when spilled have both physical and chemical effects on aquatic animals. The physical effects are caused by oil coating the organism or the immediate environment thereby causing suffocation, loss of buoyancy and asphyxiation (Brockson and Bailey,1973; Roger, 1975; Osuji and Mbata, 2004). Chemical changes are caused by organisms physiological or biochemical response to pollutant exposure, which may be changes in haematology (Gabriel *et al.*, 2007),

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enzyme activity (Dange and Masurekar, 1981; Jee and Kang, 2005; Ugwu, *et al.*, 2008) and nitrogen content (Nwamba, 2009).

Despite high reportage of oil spills and illegal oil activities in the Nigerian coastal water, there is scarcity of report or documentation on the effect of crude oil or its fractions on tissue biochemistry of an important brackish species *Tympanotonus fuscatus*, despite the efforts towards heavy metals accumulation and total hydrocarbon content and mortality of this species in post spill incidence (Daka *et al.*, 2006; Ideriah *et al.*, 2006; Egonmwan, 2007; Renner *et al.*, 2008; Benson and Essien, 2009; Ewa-Oboho and Otogo, 2009;). This study investigates changes in the enzymes of the periwinkle (*Tympanotonus fuscatus*) exposed to diesel a refined product of crude oil.

MATERIALS AND METHODS

Periwinkles (*Tympanotonus fuscatus*) of size between 4.5cm to 5.5cm handpicked from the Eagle cement area of the New Calabar River were collected and transported in plastic buckets to the laboratory of the Department of Chemistry Ignatius Ajuru University of Education Rumuolumeni Port Harcourt. One hundred and forty (140) periwinkles were acclimated to laboratory conditions in plastic tanks of 5 litre capacity for seven days. Two hundred and fifty grams (250g) of sieved sediment collected from same points as the periwinkles was used as sediment base in the aquaria / tanks. Completely randomized design was used for the experiment. The experiment was divided into five treatment levels with three replicates. The test media diesel was prepared for the following concentration (10.40, 15.60, 21.00 and 26.00 ml/L) and a control (0.00ml/L). Twelve of the test animals were introduced into the prepared toxicants and allowed for three days before the aquaria were washed and the contents renewed for the next three days. On the sixth day the shells were broken with a rod and the tissues separated from the shell. The tissues were separated into the edible part (muscle) and the non edible part (viscera). Less than one gram (0.5g) of the tissues were macerated (homogenized) and mixed with 5ml physiological saline (8.4-9% saline solution) for enzyme assay. The saline tissue mixtures were centrifuged at 3,000rpm for ten minutes and the supernatant decanted into plain bottles.

The transaminases were analyzed based on the method of Reitman and Frankel (1957), while Alkaline phosphatase (ALP) was analysed using the method of Bessey *et al.* (1946). The obtained data were statistically analysed using the analysis of variance (ANOVA) and difference were separated using Duncan's Multiple Range Test (DMRT) (Zar,1984)

RESULTS

In the muscle of *Tympanotonus fuscatus*, there were fluctuations in the activities of aspartate transaminase (AST) in the various concentrations used. The highest activity was recorded at 21.00ml/L being 260.00 ± 0.00 IU/L, followed by that of 15.6ml/L (157.50 ± 31.81 IU/L). However, lower values to that of the control (137.50 ± 60.10 IU/L) were recorded at 10.4ml/L (115.00 ± 0.00 IU/L) and 26.00ml/L (125.00 ± 14.14 IU/L). Alanine transaminase (ALT) declined in activity in the muscle which was concentration dependent. The observed values were 227.50 ± 45.96 IU/L (control), 202.50 ± 81.32 IU/L (10.40ml/L) and 195.00 ± 0.00 IU/L (15.60ml/L), 135.00 ± 14.14 IU/L (21.00ml/L) and 92.50 ± 45.96 IU/L (26.00ml/L). The activity of alkaline phosphatase (ALP) in the muscle showed no definite pattern. The value of 147.50 ± 17.68 IU/L was recorded in the control and that 26.00ml/L test concentration. A decline was observed at 10.04ml/L and 15.6ml/L which were 145.00 ± 0.00 IU/L and 130.00 ± 0.00 IU/L respectively. The only increase was at 21.00ml/L being 155.00 ± 7.07 IU/L (Table1).

In the viscera, AST activity was raised above the control value which was 57.50 ± 31.82 IU/L. The recorded values in the test concentration were 277.50 ± 24.75 IU/L, 150.00 ± 77.78 IU/L, 135.00 ± 28.28 IU/L and 115.00 ± 91.92 IU/L at 15.6ml/L, 26.00ml/L, 10.40ml/L and 2.00ml/L respectively. ALT activity was highest at 15.60ml/L (125.00 ± 0.00 IU/L) followed by that at 26.00ml/L (52.5 ± 45.96 IU/L) then at 21.00ml/L (50.00 ± 14.14 IU/L). However, the same value was observed at 10.40ml/L and the control which was 40.00 ± 28.28 IU/L. ALP also showed increased in activity above the control value which was 215.00 ± 49.49 IU/L except at 10.40ml/L which was 142.50 ± 38.89 IU/L. The higher values were 310.00 ± 19.49 IU/L, 287.50 ± 116.67 IU/L and 217.50 ± 10.61 IU/L at 15.60ml/L, 26.00ml/L and 217.50 ± 10.61 IU/L respectively, (Table 2).

TABLE 1: AST, ALT and ALP in the muscle of *Tympanotonus fuscatus* exposed to different concentration of diesel for six days.

CONC. OF DIESEL (MI/L)	AST (IU/L)	% of Control	ALT (IU/L)	% of Control	ALP (IU/L)	% of Control
0.00	137.50 ^b	100	227.50 ^a	100	147.50 ^a	100
10.40	115.00 ^c	83.64	202.50 ^a	89.07	145.00 ^a	98.30
15.60	157.50 ^b	114.55	195.00 ^{ab}	85.91	130.00 ^a	88.14
21.00	260.00 ^a	189.09	135.00 ^{ab}	59.34	155.00 ^a	105.08
26.00	125.00 ^{bc}	90.90	92.50 ^b	40.66	147.50 ^a	100

Means with the same superscript in the same column are not significantly different (P=0.05).

TABLE 2: AST, ALT and ALP in the viscera of *Tympanotonus fuscatus* exposed to different concentrations of diesel for six days.

CONC. OF DIESEL (MI/L)	AST (IU/L)	% of Control	ALT (IU/L)	% of Control	ALP (IU/L)	% of Control
0.00	57.50 ^c	100	40.00 ^{bc}	100	215.00 ^b	100
10.40	135.00 ^d	234.78	40.00 ^{bc}	100	142.50 ^{bc}	66.28
15.60	277.50 ^a	482.61	125.00 ^a	312.50	310.00 ^a	144.19
21.00	115.00 ^d	200.00	50.00 ^b	125.00	217.50 ^b	101.16
26.00	150.00 ^d	260.87	52.50 ^b	131.25	287.50 ^a	133.72

Means with the same superscript in the same column are not significantly different (P=0.05)

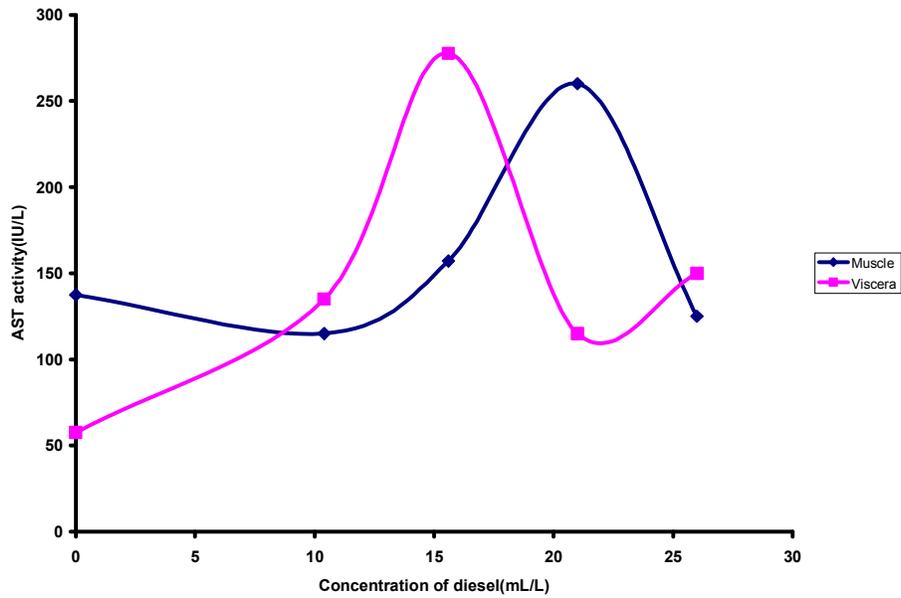


Figure 1: Comparative activity of AST in the muscle and viscera of *Tympantonus fuscatus* exposed to diesel for six days.

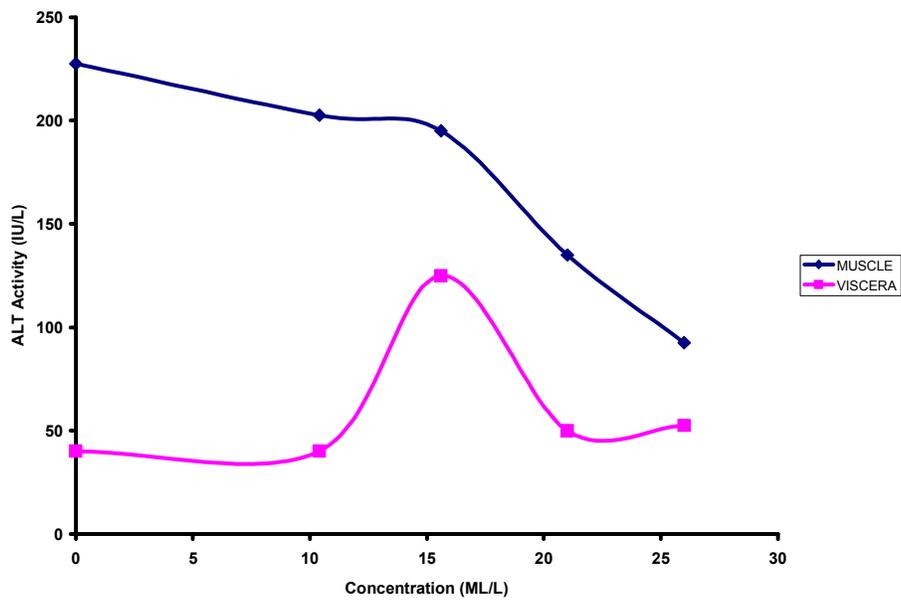


Figure 2: Comparative activity of ALT in the muscle and viscera of *Tympantonus fuscatus* exposed to diesel for six days.

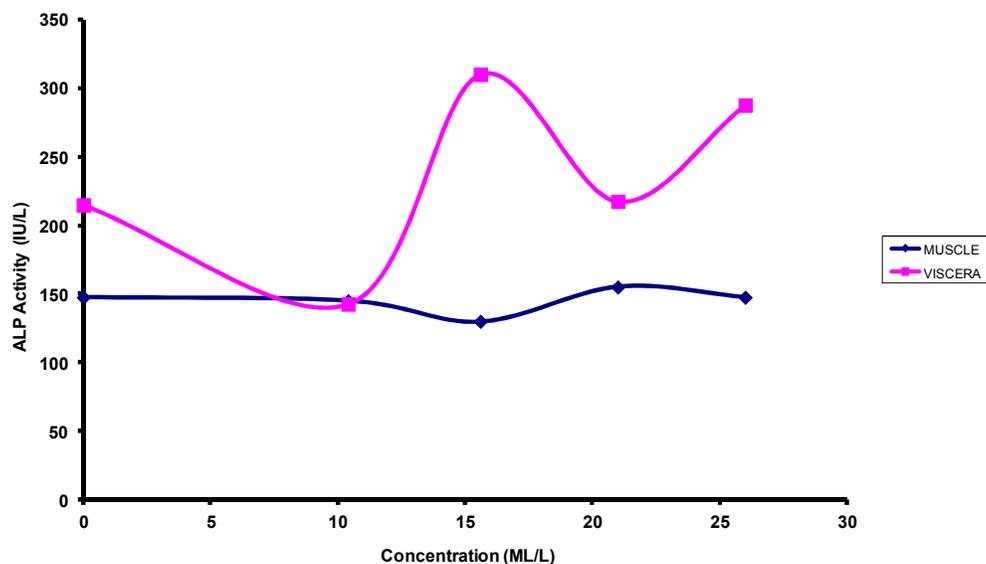


Figure 3: Comparative activity of ALP in the muscle and viscera of *Tympanotonus fuscatus* exposed to diesel for six days.

DISCUSSION

Enzymes are fragile substances which are denatured or inactivated under unsuitable conditions. These unsuitable conditions always results from man's interference or natural changes in the environment. The changes (increase/decrease) in enzyme activity in this study has been reported in similar studies by different authors working with various environmental toxicants (Humtsoe *et al.*, 2001; Greenway and Storey, 2001; Sreekala and Zutshi, 2010). The metabolic pathway in the tissues of the periwinkle was affected by the diesel due to the alteration of cellular enzymatic activities. These alterations in AST, ALT and ALP indicates disturbance in the structure and integrity of cell organelles such as endoplasmic reticulum and membrane transport (Roy, 2002; Karatas and Kalay, 2002). Roy, (2002) reported that variation of enzyme activities is due to either increased or decreased permeability of the cell as well as the direct effect of the toxicant, which in this case is diesel. Another possible cause of such observed variation could be due to increased concentration of the diesel in the tissues thereby altering the normal organ biochemistry of the organism. Decrease in AST

and ALT in the tissues infers that there is injury in the tissues of the periwinkle and that these enzymes may have leaked into the surrounding environment. Decrease in the activities of enzyme in the organ/tissue implies that the structural integrity and permeability of the tissues is in crisis (Gabriel *et al.*, 2010). On the other hand increase in enzyme activity indicates effective transamination and utilization of amino acid (Gabriel *et al.*, 2010). However the synthesis of amino acids and its precursors seem to be through the aspartate pathway, a reaction which tends to tilt to a form of anaerobic respiration of the organisms (Tiwari and Singh, 2004), which will eventually lead to death of the organism after long exposure. Increased activities of AST and ALT enhances the biosynthetic pathway for protein synthesis thereby increasing the rate of biochemical control of ATP production (Greenway and Storey, 2001).

Alkaline phosphatase (ALP) is an enzyme present in almost all the tissues of organism. It is a hydrolytic enzyme that is majorly concerned with the process of transphosphorylation (transfer of phosphate group) and have an important role in the general energetics of an organism (Sreekala and Zutshi, 2010). According to Srivastava *et al.* (1995), ALP

along with acid phosphatase (ACP) is associated with transport of phospholipids, phosphoproteins, nucleotides and carbohydrates and also active in the synthesis of proteins.

The decrease in the activities of ALP in the tissue of the periwinkle is similar to those observed by Parthasarathi and Karappasamy, (1998) in the muscle, intestine and liver of *C. punctatus* exposed to fenvalerate, and Sreekala and Zutshi, (2010) in *Labeo rohita* sampled from polluted lake. Reduction in the enzyme (ALP) activity increases the rate of degradation of protein and its synthesis under anoxic condition (Greenway and Storey, 2001) and this can underlie the reduction in activities of several other equilibrium enzymes in tissues such as AST and ALT in the muscle of the periwinkle. The decrease in ALP suggested the uncoupling of phosphorylation resulting from toxicity. It has been observed that alkaline phosphatase splits various phosphate esters at an alkaline pH and mediates membrane transport and therefore the decrease may result in altered transport and inhibitory effect on cell growth and proliferation (Goldfischer *et al.*, 1998). Accordingly Parthasarathi and Karappasamy, (1998) observed that ALP is capable of inactivating phosphorylase enzyme, thereby promoting glycogen synthesis. Therefore its variation in the tissue can cause alteration in glycogen content.

The inhibition of ALP may also result from severe acidosis (Shaikila, *et al.*, 1993), which may be a mechanism for adaptive changes for the periwinkle to meet the energy demand by anaerobic breakdown of glycogen. Accordingly the authors posited that inhibition in activity could also be due to interaction of toxicant with co-factors and regulators (Ramesh *et al.*, 1994; Shaikila *et al.*, 1993). The decreased activities of these enzymes indicate disturbance in the structure and integrity of cell organelles, such as endoplasmic reticulum and membrane transport system (Nchumbeni *et al.*, 2007)

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