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Petrol Induced Alteration in Enzymes and Electrolytes in Tympanotonus fuscatus after Exposure

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Abstract: *Tympanotonus fuscatus collected from the New Calabar River were exposed to different concentrations of petrol (10.40, 15.60, 21.00 and 26.00ml/L) and a control for six days to determine the activities of enzymes, aspartate transaminase(AST), alanine transaminase(ALT) and alkaline phosphatase(ALP) and electrolytes namely sodium(Na^+), potassium(K^+) and chloride(Cl^-) in the tissues of the organism. There was a marked increase in the activities of all the enzymes in the viscera of Tympanotonus fuscatus with ALP being the most prominent enzyme. ALP was highest at 26.00ml/L(390,00 ± 70.71IU/L) which was 2X and 3X that of AST and ALT respectively at 10.40ml/L. In the muscle, elevation of activity was observed in AST only while ALT and ALP declined in all the exposure concentrations with AST being the most prominent enzyme. The levels of the electrolyte (Na^+, K^+ and Cl^-) all declined in value in comparison to the control in the viscera of Tympanotonus fuscatus. The most prominent ion was sodium followed by chloride and finally potassium. In the muscle, decrease in levels was also observed in all the ions except at 15.60ml/L and 26.00ml/L for sodium and ions respectively. The increase or the decrease observed in either the enzymes or the electrolytes did not follow any pattern.*

Key words: *Pollution, Tympanotonus fuscatus, petrol, enzymes, electrolytes, environment*

I. Introduction

One of the environmental consequences of crude oil or petroleum exploration and exploitation is oil pollution and this has been found to cause serious aquatoxicological effects that are deleterious to aquatic life (Agbogidi et al.,2005). The domestic and industrial use of its crude or refined form has rapidly increased in recent times. Diesel, kerosene and petrol are the most commonly used fraction of crude oil (EHC 20,1992). Unsaturated hydrocarbons at different concentrations are found to be entrained in these fractions thereby causing variable toxicities to aquatic organisms (Kato et al., 1996).

During oil spills, a number of physical and chemical properties such as evaporation, dispersion, emulsification, sedimentation, biodegradation and photo-oxidation are altered which help in the distribution and partitioning of petroleum components into the water, organisms and sediments (Clark, 1992). Hydrocarbons are quantitatively the most important component or constituent of petroleum and arise from both natural and anthropogenic sources (Law and Biscaga, 1994). However, among the anthropogenic sources, point source contamination by run-offs, refineries and other coastal effluents are in substantial amount and are important in causing chronic pollution in the vicinity of estuaries, creeks, harbour and coastal settlements (Abu-Hilal and Khordagui, 1994). Petroleum hydrocarbons are among the most common contaminants bound to estuaries and sediments (Medeiros et al.,2005; benson et al., 2008). These substances, mostly of organic origin may be deleterious to aquatic plants and animals and may cause damage to wildlife and fish (Atlanta, 1994).

Tympanotonus fuscatus is a group of molluscan shellfish with either smooth or rough spiral shells. They are found in the inter tidal zones at low tide in several parts of the world (Doerffer, 1992). It is a dominant species in West African coastal lagoon and also a high source of animal protein. Periwinkles crawl about under water but usually remain passive when left uncovered by the tide. They feed on microscopic algae, detritus matter and diatoms and have been found to be rich in protein and carbohydrate (Egonmwan, 1980). It is a delicacy and competes favourable with tilapia catfish in marketability in the Niger Delta area of Nigeria.

This study is carried out to investigate the effect of petroleum product, petrol (gasoline) on the tissue enzymes and electrolytes of Tympanotonus fuscatus after mild exposure.

II. Materials And Methods

Periwinkles (Tympanotonus fuscatus) of size between 4.5 - 5.5cm were handpicked at the Eagle cement area of the New Calabar River near the Ignatius Ajuru University of Education Rumuolumeni, Port Harcourt. They were transported in plastic buckets to the Chemistry Department Laboratory of the University. Two hundred apparently healthy periwinkles were acclimated to laboratory conditions in plastic tanks of six litre capacity. The tanks were half filled with brackish water and sediments collected from same source. The

acclimation was done for seven days. The substrate was prepared by air drying the sediment and then macerated in a mortar and sieved in 2mm mesh.

250g of finely prepared sediment were put into each of the plastic tanks to serve as the substrate base. Completely randomized design (CRD) was used for the experiment. The experiment was divided into five treatment levels with two replicates. The test media were prepared in the following concentrations: 10.40ml/L, 15.6ml/L, 21.00ml/L, 26.00ml/L and a control (0.00ml/L) of diesel. Twelve of the test animals were introduced into each of the toxicant media. The content of the aquaria were washed thoroughly on the forth day and was renewed with fresh concentrations which lasted to the sixth day.

On the sixth day the periwinkles were removed from the toxicant and the shells were broken with a small rod and the tissues separated from the shell. The tissues were divided into the edible part (muscle) and the non edible part (viscera).

0.5g of the tissues were macerated or homogenized and mixed with 5ml de-ionized water for electrolyte analysis and another 0.5g of the tissues were homogenized and mixed with 5ml of physiological saline for enzyme analysis. They were centrifuged at rate of 3000rpm for ten minutes and the supernatant poured into 5ml plain bottles. The samples were immediately transferred to the laboratory for analysis. The electrolytes were analysed by the method of Schales and Schales (1941), while the enzymes were analysed by the method of Reitman and Frankel (1957).

The result obtained were subjected to analysis of variance (ANOVA) using one way classification to test whether differences existed between the means. Where differences existed, Duncan's multiple range test was used to separate the means (Zar, 1984).

III. Results

In the vicera of tympanotonus fuscatus, aspartate transaminase (AST) activities were higher than the control value (57.50 ± 31.82). The percentage increase in AST activity were 282.60% (10.40ml/L), 213.04% (26.00ml/L), 195.65%(21.00ml/L) and 134.78 (15.60ml/L). Alanine transaminase (ALT) activity also were all higher than the control value of 40.00 ± 28.28 IU/L. The highest value obtained at 10.04ml/L was 218.75% rise in activity. This was followed by 212.50%, 162.50% and 156.25% rise at 15.60ml/L, 26.00ml/L and 21.00ml/L respectively. Alkaline phosphatase (ALP) activity in the treatment groups were higher in value than that of the control (215.00 ± 49.49IU/L) except at 10.40ml/L where inhibition of 1.17% was recorded. The higher values were 81%, 80% and 27% rise at 26.00ml/L, 15.40ml/L and 21.00ml/L respectively (Table 1). In the muscle, elevated activity of AST was observed in all the exposure concentrations above the control value of 137.50 ± 60.10 IU/L. the rise in activity were 160.0%, 147.27%, 114% and 69% at 10.40ml/L, 26.00ml/L, 15.60ml/L and 21.00ml/L respectively. However, ALT activity declined in all the exposure concentrations as against the control value (227.50 ± 45.96IU/L) except at 15.60ml/L which was equal in to the control value. The inhibitions were 9.89%(10.40ml/L), 24.18%(26.00ml/L) and 53.85% at 21.00ml/L. ALP activity declined in all the exposure concentrations which were 10.17%(21.00ml/L), 23.73%(26.00ml/L), 30.57%(15.60ml/L) and 33.90% at 10.40ml/L as against the control value of 147.50IU/L (Table 2).

In the viscea of Tympanotonus fusatus, the levels of sodium ion (Na^+) depreciated in all the exposure concentrations. The de crease in levels were 4.76%(26.00ml/l), 16.67%(21.00ml/L), 52.38%(10.40ml/L) and 60.00% at 15.60ml/L as against the control value of 105.00 ± 7.07meq/L. potassium ion (K^+) also depreciated in value in all the exposure concentrations. However , the depreciations were not concentration dependent. The percentage decline in levels obtained were 51.25%(26.00ml/L) 47.97%(21.00ml/L), 46.34%(10.40ml/L) and 45.53%(15.60ml/L). chloride ion (Cl^-) declined in levels in all concentrations except at 15.60ml/L which was 21.21% above the control being 82.50 ± 88.38meq/L. the decreased levels were 51.52% (26.00ml/L), 27.88%(21.00ml/L) and 21.82% at 10.40ml/L (Table3). In the muscle Na^+, value depreciated in all the test concentrations except at 15.60ml/L which was 33.33% above the control value of 67.50 ± 24.75meq/L. Decline of 33.33% were observed at 21.00ml/L and 26.00ml/L concentrations while that observed at 10.40ml/L was 25.93% lower in value. K^+ ion decreased in levels as well in all the concentrations, the highest being 52.70%(15.60ml/L), which was followed by 48.65%(10.40ml/L), then 14.86%(26.00ml/L) and 12.57%(21.00ml/L) as against the control value of 18.50 ± 1.41meq/L. Cl^- ion declined in all the exposure concentrations except at 26.00ml/L which was 98.95% above the control value of 47.50 ± 38.89meq/L. decline of 68.42%, 58.42% and 27.37% were observed 10.40ml/L, 15.60ml/L and 21ml/L respectively.

Table 1. AST, ALT and ALP activities in the viscera of Tympanotonus fuscatus exposed to petrol for six days.

Concentration of petrol(ml/L)	AST(IU/L)	% of control	ALT(IU/L)	% of control	ALP(IU/L)	% of control
0.00	57.50 ± 31.82^c	100	40.00 ± 28.28^c	100	215.00 ± 49.49^b	100
10.40	220.00 ± 56.57^a	382.61	127.50 ± 60.10^a	318.75	212.50 ± 10.61^b	98.83

15.60	135.00 ± 28.28^{bc}	234.78	125.00 ± 68.01^{a}	312.50	387.50± 215.67^{a}	180.23
21.00	170.00 ± 49.50^{b}	295.65	102.50 ± 28.50^{b}	256.25	375.00 ± 28.28^{a}	127.90
26.00	180.00 ± 313.04^{b}	313.04	105.00 ± 56.57^{b}	262.50	390.00 ± 70.71^{a}	181.39

Means with the same alphabeth in the same column are not significantly different (P>0.05).

Table 2. AST, ALT and ALP activities in the muscle of *Tympanotonus fuscatus* exposed to petrol for six days.

Concentration of petrol(ml/L)	AST(IU/L)	% of control	ALT(IU/L)	% of control	ALP(IU/L)	% of control
0.00	137.50 ± 60.10^{c}	100	227.50 ±45.96^{a}	100	147.50 ± 24.75^{a}	100
10.40	357.50 ± 31.82^{a}	260.00	205.00 ±14.140^{ab}	90.11	97.50 ± 10.61^{bc}	66.10
15.60	295.00 ± 0.00^{ab}	214.55	227.50 ± 81.32^{a}	100	102.50 ± 38.89^{b}	69.49
21.00	232.50 ± 38.89^{ab}	169.09	105.00 ± 28.28^{bc}	46.15	102.50 ± 60.10^{b}	89.83
26.00	340.00 ± 148.49^{a}	247.27	172.50 ± 123.74^{b}	75.82	112.50 ± 10.61^{ab}	76.27

Means with the same alphabeth in the same column are not significantly different (P>0.05).

Table 3. Sodium, potassium and chloride ion in the muscle of *Tympanotonus fuscatus* exposed to petrol for six days. .

Concentration of petrol(ml/L)	Na^+ (meq/L)	% of control	K^+ (meq/L)	% of control	Cl (m^-eq/L)	% of control
0.00	105.00 ± 7.07a	100	30.75 ±12.37^{a}	100	82.50 ± 88.38^{b}	100
10.40	50.00 ± 0.00^{c}	47.62	16.25 ± 1.06^{b}	53.66	64.50 ± 21.29^{b}	78.18
15.60	42.50 ± 3.54^{d}	40.00	16.75 ± 8.86^{b}	54.47	100.00 ± 0.00^{a}	121.21
21.00	87.50 ± 53.03^{b}	83.33	16.00 ± 4.95^{b}	52.03	59.50 ± 14.85^{c}	72.12
26.00	100.00 ± 70.71^{a}	95.24	15.00 ± 6.36^{b}	48.78	40.00 ± 42.43	48.48

Means with the same alphabet in the same column are not significantly different (P>0.05)

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Table 4.Sodium, potassium and chloride ion in the muscle of *Tympanotonus fuscatus* exposed to petrol for six days.

Concentration of petrol(ml/L)	Na^+ (meq/L)	% of control	K^+ (meq/L)	% of control	Cl (m^-eq/L)	% of control
0.00	67.50 ± 24.75^{b}	100	18.50 ± 1.41^{a}	100	47.50 ± 38.89^{b}	100
10.40	50.00 ± 0.00^{b}	74.07	9.50 ± 6.36^{b}	51.35	15.00 ± 7.07^{d}	31.58
15.60	90.00 ± 49.50^{a}	133.33	8.75 ± 5.30 ^{b}	47.30	19.75 ± 13.79^{d}	41.58
21.00	45.00 ± 7.07^{c}	66.67	16.00 ± 4.95^{ab}	87.49	34.50 ± 7.07^{c}	72.63
26.00	45.00 ± 7.07^{c}	66.67	15.00 ± 6.36^{ab}	85.14	94.50 ± 64.34^{a}	198.95

Means with the same alphabeth in the same column are not significantly different (P>0.05).

IV. Discussion

Behaviour of animals is known as a neurotropically mediated phenomenon which is mediated by neurotransmitter substances (Sambasiva, 1999). The increase and decrease in enzyme activities observed in this study is similar to ones observed in other studies (Mousa *et al.*, 2008; Gabriel *et al*., 2011; Humtsoe *et al*., 2007). In stress mediated reactions organisms need energy to detoxify, biotransform and excrete the toxicant so that the effect of the toxicant can be minimized. This can be achieved by the use of immediate and principal source of energy which is carbohydrate (Umminger, 1977). To achieve this, AST and ALT activities may either be stepped up or down so that transamination processes would favour the organisms need. Increase in AST and ALT in the muscle of the periwinkle infers active of transamination (the interplay between carbohydrate and protein synthesis during energy demand) in the muscle of the periwinklr, (Gabriel et al., 2011). The increase in the transaminases is to gain energy in order to cope with the stressed condition which resulted from higher demand carbohydrate and its precursors to keep both the glycolytic pathway and TCA cycles at sustained levels to cope with the energy required during stress (Tiwari and Singh, 20004).

Elevation of the transaminases is an indication of stress augmentation resulting from toxicants which in this case is the petrol. Increase in the transaminases is in line with increasing energy demands to fulfill the organisms need through amino acids (Tiwari and Singh, 2004). However, in the viscera, their was observed increase in AST and decrease in ALT after exposure to petrol. This response may be a counter reaction to Maintain the integrity of the organ and to offer protection to the structural integrity of the cellular membrane (Pari and Amali, 2005). It may also suggest that their were no damage to the parenchymatous tissue and the permeability and integrity of the cell membrane were intact. The increase and decrease observed in the organs is also an indication of concentration dependent enzymatic responses of the enzymes in the target tissue/organ of the experimental animal under petrol toxicity (Gabriel *et al*., 2011). Decrease in these enzymes can also be attributed to inhibition of enzyme synthesis (Mousa et al.,2004; Shalaby et al., 2007) as a result of petrol toxicity. There was a marked increase in ALP activity in the muscle with a corresponding decrease in the viscera of the periwinkle in this study. ALP and acid phosphatase (ACP) are important biomarkers because of their presence in all tissues of organism and are involved in adaptive cellular response to pollutants (Lohner *et al*., 2001). ALP plays the major role in the phosphate metabolism and without enzyme the external membrane may be damaged by toxicants (Durriue and Tran-Minh, 2002). Increase in ALP may have resulted possibly from phosphate ingestion by the periwinkle. ALP is a hydrolytic enzyme that is responsible with transphosphorylation processes and plays an important role in the general energetics of an organism such as the conversion of NADP to NAD (Morton, 1995; Sreekala and Zutshi 2010) and are associated with metabolic transport of phospholipids, phosphoproteins, nucleotides and carbohydrates and with synthesis of proteins (Srivastava et al., 1995). ALP splits various phosphate esters at an alkaline pH and mediates membrane transport. Decrease in ALP activity can result in altered transport and inhibitory effect on cell growth and multiplication (Goldfischer *et al*., 1964). ALP promotes glycogen synthesis by inactivating phosphorylase enzymes (Parthasarathi and Karuppasamy, 1998), therefore its inhibition can cause reduced glycogen content. Reduction in ALP activity can also result from severe acidosis (Shaikila *et al*., 1993)and this in turn could be adaptive for the fish to meet the energy required by the anaerobic breakdown of glycogen. Inhibition of ALP could also be from the interaction of the toxicant with co-factors and regulators (Sarabadhikary and Sur, 1992; Ramesh *et al*., 1994). The decrease in this enzyme indicates a disturbance in the structure and integrity of cell organelles such as endoplasmic reticulum and membrane transport system (Nchumbeni et al., 2007) of the organism which in this case is the periwinkle.

As the primary link between environmental change and physiological response, the neuro-endocrine system is a critical part of osmoregulation. The capacity to regulate ions in the face of changing environmental conditions due to the presence of toxicants is an obvious necessity for organisms that live in water (McCormick, 2001). There was observed decrease in the electrolytes (Na^+, K^+ and Cl^-) in all the tissues tested.

The electrolytes are distributed in solution throughout all the body fluids. The cations are the ions of sodium, potassium, calcium and magnesium with the predominating ones being sodium and potassium. The major anions are the chlorides and hydrogen carbonates. Maintenance of constant internal ion concentration is essential for the active regulation of water influx and ion efflux in aquatic organisms (Karthikeyan *et al*., 2006). Studies have shown that both organic and inorganic pollutants impair ionic balance in various biological systems (Oksama and Kristoffersson, 1980; Gabriel *et al*., 20009; Uboh *et al*., 2012). Na^+ and K^+ ion are essential for the activity of many enzymes and have been implicated in the transport of ATP which participates in several metabolic processes such as Na^+ and K^+ ATPase which are located the cell membrane (Rajanna et al ., 1981). Decrease in these ions is an indication of stress mediated injury in the tissues (Olwole, 2001; Gabriel *et al* 2009). Decrease in Na^+ indicates a change in permeability properties of the muscle and viscera of the periwinkle (Karthikeyan *et al*., 2006). Decrease in these (Na^+, K^+ and Cl^-) implies that the rate at which these ions are lost to the environment is greater than their absorption by the organs from the environment.

Na^+ and Cl^- are used as index of osmoregulation because they react similarly in organisms under situations of strong circulating levels and are useful in stress measurement. The decrease in Na^+ and Cl^- contents may be due to increase in the permeability of the organs or inhibition of active transport, aconsequence of petrol toxicosis. According to Pic (1998) and Ando (1981) Cl^- permeability is increased in stressful animals resulting in an enhanced efflux rate and an appriopriate decrease of Cl^- ion concentration. Cl^- decrease in the periwinkle might also have resulted from reduced activity of carbonic anhydrate or sterodogenesis. The decrease in K^+ ion concentration have been associated with stress (Karthikeyan *et al*., 2006). K^+ is essential for the activity of nerves and is present in nerve fibres which are related to carbohydrate metabolism (Shaanmugan, 1993). However, reduction in K^+ ion can be attributed to increase in extracellular space.

The result of this study reveals that petrol is toxic to periwinkle and causes underlying injury in its tissues, therefore its use should be effectively managed and where there is spill adequate clean-up exercise should be carried out immediately.

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