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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (4): 545-549, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.545.549

Comparative Effects of Petrol and Diesel on Enzyme Activity in *Tympanotonus fuscatus* after Sublethal Exposure

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Abstract: Pollution of the aquatic environment by petroleum and its products is common the world over. This study is aimed at examining sublethal effects of petrol and diesel on enzymes in *Tympanotonus fuscatus* namely: Aspartate Transaminase (AST) (E.C. 2.6.1.1), Alanine Transaminase (ALT) (E.C. 2.6.2.2) and Alkaline Phosphatase (ALP) (E.C. 3.1.3.1) activity after exposure. The periwinkles were exposed to 10.40, 15.60, 21.00, 26.00 ml L^{-1} and a control. The organs were removed on the sixth day and were prepared for enzymatic analysis. Enzyme activities were compared to the control value and between the toxicants. The effects of the toxicants on AST activity in the muscle and viscera were significantly different ($p>0.05$) from the control value (137.50±15.10 IU L^{-1}). AST activity were raised more in petrol concentrations than the diesel concentrations in the muscle. The reverse was the case in the viscera at 15.60 ml L^{-1} (227.50±24.75 IU L^{-1}). ALT activity in the muscle were not significant ($p>0.05$) between the toxicant media. In the viscera, significant differences ($p>0.05$) were observed in some of the concentrations with petrol showing higher activity. ALP activity in the muscle were not significant ($p>0.05$) in both media, but were more elicited in the diesel concentrations. In the viscera, the activities of ALP were more pronounced in the petrol concentrations and were significant ($p>0.05$) at the higher diesel concentrations. The exposure of *Tympanotonus fuscatus* to petrol and diesel concentrations caused changes in the enzymatic activities in the organism with those of petrol more pronounced than those of the diesel.

Key words: Enzyme, petrol, diesel, sublethal exposure, *Tympanotonus fuscatus*

INTRODUCTION

Petroleum or crude oil is the major source of hydrocarbons in the world all over. The various components of the crude oil are collected as distillate over wide range of temperatures during fractional distillation. These distillates are transported to different parts of the country (Nigeria) for different uses or purposes. Nigeria, which has a wide range of pipeline network and deports for the transportation and distribution of crude oil and its fractions (Renner *et al*., 2008) has lost a lot due to vandalisation and bunkering activities. Apart from vandalisation, most of these pipelines are old and poorly maintained which results in oil spillages and pollution of the aquatic environment (Brume, 2004) resulting in stress of aquatic organism.

Crude oil and its refined products which are found to contain polycyclic aromatic hydrocarbons and other toxic substances are constantly discharged into the aquatic environment through different anthropogenic activities (Afolabi *et al*., 1985). Although, the components of petroleum share similar physical and chemical properties, yet the toxicological properties of the components can be quite different (Anderson *et al*., 1974; Chukwu and Okhumale, 2009) based on specific individual physical and chemical properties of the component under investigation. It is also established that in the event of oil spill, that man, animals, vegetation, soil and the entire environment are affected through the injection of toxic substances into the environment (Ngodigha *et al*., 1999; Amakiri *et al*., 2009). Crude oil and its fraction are found to cause mortality in organisms (Moles and Norcoss, 1998; Renner *et al*., 2008) and changes in species composition, low abundance, loss of species and tainting (Widdows *et al*., 1982).

To effectively manage the environment, the effect of crude oil and its components on aquatic species should not only be examined in the macro level but also in the micro level where examination of their effects should be on the internal changes within the organism. Such internal

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changes may be alteration in the biochemistry and physiology of the organism, which may not be immediately pronounced but can be noticed after long time of exposure in the event of chronic or sublethal concentrations.

This study therefore was conducted to examine the effects of petrol and diesel on the enzyme activity of a very important commercial brackish gastropod, periwinkle (*T. fuscatus*).

MATERIALS AND METHODS

Source of test animals/acclimation of animals: The test animals (periwinkle) of length 4.5-5.5 cm were handpicked from the Eagle Cement area of the New Calabar River near the Ignatius Ajuru University of Education Rumuolumeni Port Harcourt, Rivers State, Nigeria at low tide and were transported in a plastic container to the Chemistry Department Laboratory of the University. Four hundred periwinkles were acclimated to laboratory conditions for four days in plastic tanks of dimension 30×30×10 cm half filled with brackish fetched from same source.

Preparation of substrate: Sediments were collected from same source. The sediments were air dried to constant weight and macerated in a mortar with pestle and sieved with 2 mm mesh to separate stones. Two hundred and fifty grams of the finely prepared sediments were measured into each of the plastic tanks to serve as substrate.

Experimental design: The Completely Randomized Design (CRD) was used for the experiment. The experiment was divided into four treatment levels and a control with three replicates.

Preparation of toxicant/exposure of periwinkle to the toxicant: The toxicants (petrol and diesel) were prepared in the following concentrations (10.40, 15.60, 21.00 and 26.00 ml L^{-1}) and a control. These concentrations were chosen based on the 96 hr LC_{50} (104.68 ml L^{-1}) observed by Renner *et al.* (2008) on periwinkle exposed to petrol. Ten periwinkles were exposed to each of the replicates and allowed in the solution for three days before fresh solution or toxicant concentrations were prepared and left of the next three days, all totaling six days of exposure.

Collection and preparation of samples: On the sixth day, the periwinkles were brought out of the toxicant media and the shells were broken with a small steel rod to separate the tissues from the shell. The tissue was then separated into the muscle and the viscera. About 0.5 g of each of the organs were macerated or homogenized in a ceramic mortar. The homogenized organs were mixed with physiological saline (normal saline solution) and centrifuged at 3000 rpm for ten min. The supernatant was collected with a dropper or teat pipette and transferred into labeled 5 mL plain bottles for enzyme analysis.

Sample analysis: The samples were analysed for Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP). The colorimetric end point technique by Reitman and Frankel (1957) was used to determine the activities of AST and ALT in the organs. ALP activity was analysed by the method of Bessey *et al.* (1946), which take into consideration the effect of colour change of a buffered phenolphthalein substrate.

Statistical analysis: The data obtained were subjected to Analysis of Variance (ANOVA) to determine if there is any significant difference between the exposures. Where differences existed, Duncan's multiple range test (DMRT) was used to compare the means (Zar, 1984).

RESULTS

The activity of AST in the muscle in petrol concentrations increased significantly ($p>0.05$) when compared to the control value which was 137.50±15.10 IU L^{-1}. The increase were higher in the lower concentrations, 10.40 and 15.60 ml L^{-1} which were 357.50±31.82 and 295.00±0.00, respectively than the higher concentration 21.00 and 26.00 ml L^{-1} which were 232.00±38.89 and 240.00±48.49 IU L^{-1}. In the test concentrations of diesel, the activity of AST were either higher or lower than the value of the control. The higher values were observed in the middle concentrations, 15.60 and 21.00 ml L^{-1} which were 157.00±16.82 and 260.00±0.00. In the viscera, the activity of the enzyme were significantly ($p>0.05$) higher than that of the control value (57.50±3.82) in both the petrol and the diesel test concentrations. The highest activity was recorded in the diesel concentration at 15.60 ml L^{-1} (227.50±24.75) which was followed by the value observed for petrol at 10.40 ml L^{-1} (220.00±18.57) as compared to the value of 157.50±3.82 for the control (Table 1).

The activity of ALT in the muscle in petrol concentrations decreased significantly ($p>0.05$) below the control value (227.50±15.32 IU L^{-1}) except at 15.60 ml L^{-1} where the same value as that of the control was observed. In diesel concentrations, there was a significant ($p>0.05$) dose response decrease in the activity of ALT. The least activity observed was 92.50±15.32 IU L^{-1} at 26.00 ml L^{-1}.

Table 1: Aspartate transaminase, AST (IU L^{-1}) in the muscle and viscera of *Tympanotonus fuscatus* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant	Muscle AST (IU L^{-1})		Viscera AST (IU L^{-1})	
(ml L^{-1})	Petrol	Diesel	Petrol	Diesel
0.00	137.50±15.10		57.50±3.82	
10.40	357.50±31.82^{a}	115.00±0.000^{b}	220.00±18.57^{a}	135.00±9.90^{b}
15.60	295.00±0.00^{a}	157.00±16.82^{b}	135.00±9.90^{b}	227.50±24.75^{a}
21.00	232.00±38.89^{a}	260.00±0.000^{a}	170.00±16.50^{a}	115.00±22.00^{a}
26.00	240.00±48.49^{a}	125.00±14.14$_{b}$	180.00±12.66^{a}	150.00±17.28^{a}

Values with the same superscript in the same in the same row are not significantly different (p>0.05)

Table 2: Alanine transaminase, ALT (IU L^{-1}) in the muscle and viscera of *Tympanotonus fuscatus* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant	Muscle AST (IU L^{-1})		Viscera AST (IU L^{-1})	
(ml L^{-1})	Petrol	Diesel	Petrol	Diesel
0.00	227.50±15.32		40.00±5.82	
10.40	205.00±14.14^{a}	202.50±8.32^{a}	127.50±20.32^{a}	40.00±9.90^{b}
15.60	227.50±21.32^{a}	195.00±0.00^{a}	125.00±22.00^{a}	125.50±0.00^{a}
21.00	105.00±28.28^{a}	135.00±14.14^{a}	102.50±9.50^{a}	50.00±7.70^{b}
26.00	172.50±10.74^{a}	92.50±15.32^{a}	105.00±18.85^{a}	52.50±8.32^{b}

Values with the same superscript in the same in the same row are not significantly different (p>0.05)

Table 3: Alkaline phosphatase, ALP (IU L^{-1}) in the muscle and viscera of *Tympanotonus fuscatus* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant	Muscle AST (IU L^{-1})		Viscera AST (IU L^{-1})	
(ml L^{-1})	Petrol	Diesel	Petrol	Diesel
0.00	147.50±8.75		215.00±16.49	
10.40	97.50±10.61^{b}	145.00±0.00^{a}	212.00±10.61^{a}	142.50±13.45^{a}
15.60	102.50±12.20^{b}	130.00±0.00^{a}	387.50±43.67^{a}	310.00±19.49^{a}
21.00	132.50±20.00^{a}	155.00±7.07^{a}	375.00±9.90^{a}	217.50±10.61^{b}
26.00	112.50±10.61^{a}	147.50±17.68^{a}	390.00±35.35^{a}	287.50±38.67^{b}

Values with the same superscript in the same in the same row are not significantly different (p>0.05)

In the viscera, the activity of ALT were significantly (p>0.05) raised above the control value (40.00±5.82 IU L^{-1}) in both the petrol and the diesel test concentrations. However, ALT activity observed at 10.40 ml L^{-1} was 127.60±20.32 IU L^{-1} in the petrol test medium, while the values observed at 15.60 ml L^{-1} for both petrol and diesel which were 125.50±22.00 and 125.50±0.00 IU L^{-1}, respectively (Table 2).

The activity of ALP in the muscle in petrol concentrations decreased significantly (p>0.05) below the control value (147.50±8.75 IU L^{-1}). The most noticeable decrease was observed at 10.40 ml L^{-1} which was 97.50±10.61 IU L^{-1}. In the diesel concentrations, the activity of ALP were all below the control value except at 21.00 ml L^{-1} which was equal to the control value. In the viscera, the activity of ALP in both toxicant concentrations were significantly (p>0.05) higher than the value of the control (215.00±16.49 IU L^{-1}) except at 10.40 ml L^{-1} where decrease in activity was observed in both toxicant media (Table 3).

DISCUSSION

The behaviour of organism is a neurotropically controlled phenomena which is mediated by neurotransmitter substances which alters the internal biochemistry of organisms (Sambasiva Rao, 1999). Enzymes are fragile substances which are denatured or deactivated in unsuitable conditions. These unsuitable conditions mainly results from man's interference with the natural environment. The changes observed in the activities of the enzymes in this study is similar to those observed in other studies with toxicants (Greenway and Storey, 2001; Humtsoe *et al*., 2007; Mousa *et al*., 2008; Sreekala and Zutshi, 2010). In stress induced reactions organisms need energy to detoxify, biotransform and excrete the toxicant inorder to reduce the effect of the toxicant. This can be achieved by the use of the immediate and principal source of energy which is carbohydrate (Umminger, 1977).

Generally, there was an increase in AST activities in the muscle and the viscera of the periwinkle in both petrol and diesel toxicant media while decrease in ALT activity was only in the muscle in both toxicant media but increased in the viscera in both media. In all cases, petrol was found to have induced or elicited more enzymatic activity than the diesel. In the event of environmental assault, AST and ALT may either be stepped up or down so that transamination process can favour the organism. The associated increase in the activities of AST and ALT in the organs in the toxicant media infers active/effective transamination (Gabriel, *et al*., 2011). During stress conditions the transaminases are raised to gain more energy so as to nullify the effect of higher demand of carbohydrate and its precursors to maintain the glycolytic pathway and TCA cycles at sustained levels (Tiwari and Singh, 2004) so that the organism can cope with the new environmental conditions.

Increase in the transferase indicates stress augmentation resulting from the toxicants which in this case is petrol and diesel. Therefore the observed increase in these enzymes was to fulfill the organisms need through amino acid pool (Tiwari and Singh, 2004). Changes in these enzymes results from alteration in enzymatic activities which was more in the petrol than the diesel. Enzyme changes depicts disturbance in the structure and integrity of cell organelles (Roy, 2002; Karatas and Kalay, 2002). According to Roy (2002), variation in enzyme activity is due to either increased or decreased permeability of cell as well as the toxicant. The higher values of the activities of the enzymes in the organs in petrol medium to that of diesel showed that petrol may have induced the sites responsible for

enzymatic reaction than the diesel and that it may have penetrated more into the tissues than the diesel to have caused more interaction with the tissue biochemistry of the organism.

However, decrease was observed in the ALT activity in the muscle in both petrol and diesel media. ALT is more indicative of cell injury than AST (Gabriel *et al*., 2011) and this implies that there was tissue damage. Since AST activity did not decline in any of the tissues, it follows that the synthesis/production of the amino acids and its precursors are generated through the aspartate pathway (Tiwari and Singh, 2004), a reaction which shifts towards anaerobic respiration of the organism which can lead to death after long exposure. The biochemical control of ATP production can be enhanced by increased AST and ALT activity which promotes the production pathway for protein synthesis (Greenway and Storey, 2001).

Alkaline phoshatase (ALP) is present in all tissues of organism. It is a hydrolytic enzyme that is mainly concerned with phosphate group transfer and plays an important role in the general energetics of an orgamism (Sreekala and Zutshi, 2010). ALP along with acid phosphatase (ACP) is associated with the metabolic transport of carbohydrates, nucleotides, phosphoproteins and phospholipids and also active in protein synthesis (Srivastava *et al*., 1995).

There were marked increase in ALP activity in the viscera of the periwinkle with a corresponding decrease in the muscle of the organism in both petrol and diesel media. In the viscera, petrol was found to have elicited more ALP activity than the diesel while the reverse was the case in the muscle. Increase in ALP in the viscera may have resulted from phosphate absorption and ingestion (Durrieu and Tran-Minh, 2002). Its increase plays the role of general energetics of the orgamism by converting high energy compounds such as NADP to NAD (Sreekala and Zutshi, 2010). Increase in ALP also promotes the synthesis of glycogen by deactivating phosphorylase enzymes (Parthasarthi and Karuppasamy, 1998), therefore its variation can cause changes in glycogen content which may not be healthy for the organism.

However, decrease in ALP activity was also observed in the muscle in the two toxicant media (petrol and diesel). Decrease in the activity of ALP increases the rate of degradation of protein synthesis in anoxic conditions (Greenway and Storey, 2001) which can cause reduction in the activities of several other enzymes such as AST and ALT. Decrease in ALT suggests uncoupling of phoshorylation which results from toxicity on the organism. According to Goldfischer *et al*. (1964), ALP splits various phosphate esters at alkaline pH and mediates membrane transport and therefore its decrease production can cause altered transport and inhibitory effect on cell growth and multiplication. Another possible cause of reduction in ALP can be due to acidosis (Shaikila *et al*., 1993) which may be a mechanistic pathway for adaptive changes by the periwinkle to meet the required energy during anaerobic breakdown of glycogen. Inhibition of this enzyme (ALP) can also be from the interaction of the toxicants with co-factors and regulators (Shaikila *et al*., 1993; Ramesh *et al*., 1994).

Generally, all the enzymes followed the same pattern of activity in both petrol and diesel media. Also the alteration in the activities of AST, ALT and ALP in the organs/tissues of the periwinkle in the toxicant media (petrol and diesel) is an indication of disturbance in the structure and integrity of cell organelles, endoplasmic reticulum and membrane transport system (Nchumbeni *et al*., 2007) of the organism. Again, it was observed that petrol induced more activity in all the enzymes than the diesel which is an indication that petrol was more toxic to the orgamism than diesel. Since petrol is more volatile than diesel, it may have been easier for the diesel to penetrate into the tissues of the organism (periwinkle) to cause biochemical alterations than the diesel.

CONCLUSION

Petrol and diesel caused changes in the enzymatic responses of *T. fuscatus*. This implies that they are toxic to *T. fuscatus* and the environment in general even at very low concentrations. Generally, the effects produced by toxicants were more pronounced in petrol than diesel thus signifying that petrol is more toxic to *T. fuscatus* than diesel. If the presence of these substances in the environment goes unchecked it can result in death of many aquatic organisms after long exposure.

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