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Comparative Effects of Kerosene and Diesel on Ion Regulatory Characteristics in *Tympanotonus fuscatus* after Subchronic Exposure

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Authors' contributions

This work is a collaborative effort of all the authors. The experiment was designed by author EOS, who equally drafted the manuscript, which was read and corrected by author DOAN. Author EES executed the experiment in the laboratory. All the authors finally read and approved the final manuscript as presented.

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ABSTRACT

This study was done to examine the comparative effect of different concentrations of kerosene and diesel on the ion regulatory characteristics in *Tympanotonus fuscatus* after exposure. *Tympanotonus fuscatus* were exposed to different concentrations of kerosene and diesel (10.40, 15.60, 21.00 and 26.00 ml/L) and a control to examine their effect on sodium, potassium and chloride ions in the muscle and viscera for six days. In the muscle, kerosene generally increased the levels of sodium, potassium and chloride ions in the lower concentrations (10.40 and 15.60 ml/ L) above the control values. In the higher concentrations (21.00 and 26.00 ml/L), these parameters were observed to be lower than the control value. Whereas the levels of sodium, potassium and chloride ions in the viscera increased above the control value in all the exposure concentrations

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except potassium 15.60 ml/L and chloride at 21.00 and 26.00 ml/L concentrations. In the viscera, sodium ion levels were lower than the control value in both kerosene and diesel media except at 21.00 ml/L concentration. Potassium ion levels in kerosene increased above the control values except at 26.00 ml/L, while lower levels of potassium ions were recorded in all the exposure concentrations. Chloride ions were lower than the control value in all the exposure concentrations of kerosene and diesel. The results of this study showed that both kerosene and diesel altered the ion regulatory and osmolality of *Tympanotonus fuscatus* and kerosene being more effective. The toxicants seem to be parameter and tissue specific in their mode of action.

Keywords: Electrolytes; contaminant; crude oil; kerosene; diesel; Tympanotonus fuscatus.

1. INTRODUCTION

Crude oil is found thousands of metres beneath the earth's crust and is only brought to the surface through drilling activities. It is a naturally occurring complex mixture of hydrocarbon gases and liquids [1]. Crude oil and its products are common contaminants in the aquatic environment through offshore and onshore oil production [2]. Despite the rapid progress in monitoring and assessing the impacts of oil on the coastal environment with the use of biological organism, pollution cannot be reduced completely to zero. This is due to the risk of tanker spills, blowouts, pipeline leaks and other forms of accidental discharges during transport, which will carry the spill beyond the production area [3].

The general environment and wetland ecosystem are extremely subjected to destructive effects of pollution. This is as a result of exploration and exploitation of crude oil. The contamination of aquatic environment petroleum and petroleum products coupled with other environmental factors constitute stress to aquatic organisms [4]. Exploration activities impart negatively on environment. Effects of petroleum on organism can also arise from permissible (allowable) discharges of operation wastes which may be in the form of drilling mud, cuttings and produce water.

In Nigeria, pipeline leakages and tanker accidents is the major cause of oil pollution. Over the years these pipes undergo corrosion and leakage due to inadequate surveillance and are subject to wear and tear and other dangers [5]. Apart from death of organisms, crude oil constituents are incorporated in the water column [6] and are taken up by marine organisms and thereby become toxins in their system. This toxicity can be acute (causing rapid death) or causing serious disturbance to the basic biochemical functions of the organism. Toxicity can also be delayed or chronic, in which case the survival capacity of the organism is diminished, thus resulting in reduced growth rate, reproduction rate and resistance to stress or biological attack.

This study was carried out to investigate the comparative effects of two fractions of crude oil (kerosene and diesel) on some electrolytes in an important commercial gastropod, *Tympanotonus fuscatus* after subchronic exposure.

2. MATERIALS AND METHODS

2.1 Source and Acclimation of *Tympanotonus fuscatus*

The test animals, *Tympanotonus fuscatus* (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 (400) periwinkles were acclimated to laboratory conditions for four days in plastic tanks of dimension 30 cm × 30 cm × 10 cm half filled with brackish water fetched from same source.

2.2 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 (250 g) of the finely prepared sediments were measured into each of the plastic tanks to serve as substrate.

2.3 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.

2.4 Preparation of Toxicant/Exposure of Periwinkle to the Toxicant

The toxicants (kerosene and diesel) were prepared in the following concentrations (10.40, 15.60, 21.00 and 26.00 ml/L) and a control. These concentrations were chosen based on the 96 hr LC_{50} (111.14 ml/L) observed by the author on periwinkle exposed to kerosene in a trial test. Ten periwinkles were exposed to each of the replicates and allowed in the solution for three days before fresh solutions or toxicant concentrations were prepared and left for the next three days, all totaling six days of exposure.

2.5 Collection and Preparation of Samples

On the sixth day, the *T. fuscatus* 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 de-ionized water and centrifuged at 3000 rpm for ten minutes. The supernatant was collected with a dropper or teat pipette and transferred into labeled 5 ml plain bottles for electrolytes analysis.

2.6 Sample Analysis

The samples were analysed for sodium ion (Na^+), potassium ion (K^+) and chloride ion (Cl^-). The electrolytes were analysed according to the method described by [7], which used the automatic analyzer and optimal test by means of flame photometry. This method is based on colorimetric end point techniques.

2.7 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 or separate the means [8].

3. RESULTS

In the muscle, Na^+ increased in content at the two lower concentrations (10.40 and 15.60 ml/L) and decreased at the higher concentrations of kerosene when compared to the control value of 67.50±6.19 Meq/L. In the diesel concentrations, the response of Na^+ was higher in all the concentration solutions. The response of the ion (Na^+) in the muscle, were more in the diesel concentrations. In the viscera, sodium ion (Na^+) in kerosene concentrations were, all lower than that of the control value (105.00±5.16 Meq/L) except at 21.00 ml/L (327.50±0.00 Meq/L). Similar pattern of (Na^+) levels were also exhibited in the diesel concentrations with the highest level of (Na^+) observed at 21.00 ml/L (122.50±3.14 Meq/L) as against the control value of 105.00±5.16 Meq/L (Table 1).

In the muscle, K^+ levels appreciated in the lower concentrations (10.40 and 15.60 ml/L) which was 25.00±1.41 and 25.00±2.12 Meq/L respectively as against the control value of 18.50±1.41 Meq/L. In the higher concentrations, lower levels were observed. In the diesel concentrations, K^+ only depreciated at 15.60 ml/L which was 17.75±3.53 Meq/L as against the control value of 18.50±1.41 Meq/L. The highest level of K^+ in diesel concentration was observed at 21.00 ml/L which was 23.75±3.86 Meq/L. In the viscera, K^+ levels increased in all kerosene concentrations as against the control value of 30.75±3.09 Meq/L except at 26.00 ml/L which was 26.00±1.42 Meq/L. There was a complete decrease in K^+ levels in the viscera in all the diesel concentrations with the most decrease observed at 15.60 ml/L, which was 17.75±1.96 Meq/L (Table 2).

In the muscle, increase in Cl^- level was observed only at 15.60 ml/L concentration of kerosene which was 112.50±5.25 Meq/L as against the control value of 47.50±4.86 Meq/L. The same value as that of the control was observed at 10.40ml/L, while at 21.00 and 26.00 ml/L, the observed value was 20.00±0.00 Meq/L. In the diesel concentrations, the value of 20.00±0.00 Meq/L was observed at the lower concentrations (10.40 and 15.60 ml/L), while the value of 65.00±3.35 Meq/L was observed at the higher concentrations (21.00 and 26.00 ml/L) as against the control value of 47.50±4.86 Meq/L. In the viscera, the levels of Cl^- were far lower than that of the control value (82.50±8.84 Meq/L) in both the kerosene and the diesel media. The lowest level was observed in the kerosene solution at 26.00 ml/L (26.00±1.42 Meq/L) which was followed by the value observed at the same concentration for the diesel which was 30.00±3.54 Meq/L as against the control value of 82.50±8.84 Meq/L (Table 3).

Table 1. Sodium ion (Na^+) in the muscle and viscera of *T. fuscatus* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant (ml/L)	Muscle Na (Meq/L)		Viscera Na (Meq/L)	
	Kerosene	Diesel	Kerosene	Diesel
0.00	**67.50±6.19**		**105.00±5.16**	
10.40	142.50±7.96[a]	127.50±2.65[a]	100.00±8.84[a]	50.00±0.00[b]
15.60	107.50±8.84[a]	102.50±8.84[a]	76.00±0.00[ab]	95.00±7.07[a]
21.00	50.00±0.00[b]	202.50±7.96[a]	327.50±0.00[a]	122.50±3.14[b]
26.00	50.00±0.00[b]	265.50±5.31[a]	95.00±7.07[a]	50.00±0.00[b]

Figures with the same superscript in the same in the same row are not significantly different (P>0.05)

Table 2. Potassium ion (K^+) in the muscle and viscera of *T. fuscatus* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant (ml/L)	Muscle Na (Meq/L)		Viscera Na (Meq/L)	
	Kerosene	Diesel	Kerosene	Diesel
0.00	**18.50±1.41**		**30.75±3.09**	
10.40	25.00±1.41[a]	18.75±1.80[b]	31.50±2.12[a]	19.25±1.68[b]
15.60	25.00±2.12[a]	17.75±3.53[b]	36.00±4.24[a]	17.75±1.96[b]
21.00	17.00±2.12[a]	23.75±3.86[a]	34.00±1.46[a]	29.50±2.12[a]
26.00	15.00±2.83[b]	20.25±3.35[a]	26.00±1.42[a]	27.00±1.08[a]

Figures with the same superscript in the same in the same row are not significantly different (P>0.05)

Table 3. Chloride ion (Cl^-) in the muscle and viscera of *T.* after exposure to petrol and diesel for six days (Mean±SD)

Conc. of toxicant (ml/L)	Muscle Na (Meq/L)		Viscera Na (Meq/L)	
	Kerosene	Diesel	Kerosene	Diesel
0.00	**47.50±4.86**		**82.50±8.84**	
10.40	47.50±2.65[a]	20.00±0.00[b]	31.50±2.12[b]	47.50±2.65[a]
15.60	112.50±5.25[a]	20.00±0.00[b]	36.00±4.24[b]	65.00±1.96[a]
21.00	20.00±0.00[b]	65.00±3.53[a]	34.00±1.46[a]	40.00±0.00[a]
26.00	20.00±0.00[b]	65.00±3.35[a]	26.00±1.42[a]	30.00±3.54[a]

Figures with the same superscript in the same in the same row are not significantly different (P>0.05)

4. DISCUSSION

Biochemical responses of organisms to altered environmental conditions, is an adaptive mechanism to detoxify chemical effects on the organism and to maintain equilibrium in the internal environment of the organism. Changes or disruption in organism on contact with xenobiotics takes place in cell components of the organism [9].

The regulation of ions in organisms is very important because of their role they play [10,11]. Electrolytes regulates acido-basic balance, neuromuscular excitability, osmotic pressure, enzymatic reactions, retention of membrane permeability, buffer and energy exchange [12-14]. A diversion from any of these factors will culminate in a change in the ion composition [13] and therefore will constitute toxicity to the organism.

In this study, there were irregularities in the responses of sodium, potassium and chloride ions to the toxicants (kerosene and diesel). The levels of sodium, potassium and chloride ions were either increased or decreased in the toxicants media, except chloride ions in the viscera which was lower than the control value in both media. Changes in the levels of these ions in the toxicants media corroborates an earlier work [13] and it arose from ionic imbalance which was caused by increase in the activity of the muscle tissues of the organism which consequently altered ion fluxes or flow across the muscle membrane of the periwinkle. In toxicant media, osmoregulation is mostly concerned with the maintenance of proper water and salt balance in various tissues of animals, which help to regulate the ion content of the organism [15,16] which was absent in the periwinkle.

The changes observed in the ions (sodium, potassium and chloride) ie osmo/ionregulatory alterations induced by the toxicants (kerosene and diesel) were found to be associated with increased permeability and inhibition of active ion intake which in the long run caused a decrease in the number of active chloride similar to observations made by [17] in Oreochromis niloticus exposed to copper. Reports have shown that toxicants inhibit Na^+/ K^+- ATPase, a protein located in the basolateral region of the gills of fish which is responsible in ionic regulation in teleost [10,18-20], thus the toxicants (kerosene and diesel) may have acted on organ in the periwinkle that performs similar functions.

The responses of the electrolytes in the periwinkle (*T. fuscatus*) were different from those of the diesel. The lower concentrations of kerosene showed higher than control values in all the ions examined, while in the higher test concentrations, the ion values were lower than those of the control value, whereas the reverse was the case generally in the diesel concentrations. Difference in toxicity arose from the nature of the toxicants and the responses of the organism to these organisms [21,22]. The difference in the mode of response can also be attributed to the nature and chemical and physical characteristics and composition of kerosene and diesel and the tolerance levels of the periwinkle to the toxicants [23,24]. According to [25], the properties of a substance plays active roles in the absorption into the organs of living organisms and the mod of action of the metabolites which results in toxicity model exhibited on the organism.

Generally the lower fractions of crude oil (petroleum) are more toxic or potent due to easier penetrability or permeation into the cells of the animals to effect action [26] and the volatility of the products which makes for easier penetration into the various organs/ tissues of animals.

5. CONCLUSION

Kerosene and diesel were both toxic to *T. fuscatus.* The mechanisms of interaction with the ions in both concentrations were also different. The ions showed more response in kerosene solution than that of the diesel. The response pattern showed that kerosene is more toxic to this organism than diesel. However, active roles should be played by government and relevant agencies to check spill incidences and willful disposal of these hydrocarbons in the aquatic environment and take adequate measures on offenders and remediation processes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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