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Effect of Toluene on the Organosomatic Indices and Electrolytes in the Liver and Muscle of Adult *Clarias Gariepinus*

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Abstract

The study investigated the effect of toluene on organosomatic indices and electrolytes in the liver and muscle of adult Clarias gariepinus. Eighteen adult Clarias gariepinus with mean weight and length of 97.00g and 14.02cm, respectively were acclimatized to laboratory conditions for 7days and then exposed to varying sublethal concentration (3.33, 6.66, 9.99 ppm) of toluene for 14 days. Then after, the organosomatic indices and electrolytes in liver and muscle were determined following standard processes. Results of the organosomatic indices (Cardiosomatic, Renatosomatic, Spleenosomatic and Hepathosomatic) showed no significant variations ($p>0.05$) across the various concentrations. The concentration of sodium, potassium and chloride were in the range of 3.00 – 5.55 mmol/L, 12.27 – 17.66 mmol/L and 1.02 – 1.65 mmol/L, respectively (for liver), and 0.30 – 5.15 mmol/L, 12.88 – 24.41 mmol/L and 0.70 – 1.30 mmol/L, respectively (for muscle). Analysis of variance showed a significant variation ($p<0.05$) across the various concentrations, though not in a dose depended pattern. The fluctuations in the electrolytes is an indication of stress which therefore suggest that toluene is toxic to fish at certain concentration. Hence, there is the need for caution to be exercised when using substances containing toluene close to surface water resources.

Keywords: Electrolytes; *Clarias gariepinus*; Organosomatic indices; Toluene; Toxicants.

1. Introduction

Environmental degradation is on the increases in many parts of the world. In developing nation like Nigeria, authors have attributed population growth, industrialization, urbanization, unsustainable agricultural practices to be a major factor leading degradation processes of the various ecosystem [1-5]. The degradation of the ecosystem is majorly due to human activities and to lesser extent natural effects [6]. The environmental contaminants affect all the major ecosystem including soil, water and air.

Most human activities take place in soil. Through agents of water erosion, the soil contaminants could end up the in the aquatic ecosystem. In many parts of the coastal region of Niger Delta particularly Bayelsa state, many waste stream resulting from household end up in the aquatic ecosystem through direct discharge and indirectly through runoff of wastes deposited close to the aquatic ecosystem. Sewage are deposited in the surface water resources through the use of pier toilet system [7], wastes emanating from slaughter house and market activities are discharged into the aquatic ecosystem in many coastal regions in Bayelsa state [8, 9]. In addition, crude oil exploration and production is carried out in onshore and offshore in Nigeria. Crude oil could alter the characteristics of the receiving environment (soil and water). In Nigeria crude oil enter the environment through well blow out, corrosion and rupture of pipeline, sabotage and illegal bunkering activities.

Toluene (an aromatic alkylbenzene hydrocarbon compound) is a distillate product of petroleum, and it is flammable. According to Inyang, *et al.* [10], toluene has high vapour pressure and fairly soluble in water. The authors further reported that toluene is produced during the processing of crude oil products such as gasoline. Studies have indicated that gasoline contain some fractions of toluene [11]. Toluene has several industrial applications including paints, lacquers, glues, and some solvents [11, 12]. Toluene have been widely reported to induce Neurobehavioural effects on exposure [11, 12].

Due to the fact that toluene sources are readily available in our environment, hence, there is need to assess their toxicity level on potential organisms they could affect. In aquatic ecosystem, fish have been widely used to assess the effects of contaminants on the water. Some of the notable changes commonly observed include organosomatic indices, metabolites, enzymes, electrolytes, haematological indices, behavioural response and acute toxicity. Therefore, this study aimed in assessing the effect of toluene on the organosomatic indices and electrolytes in liver and muscle of adult *Clarias gariepinus.*

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2. Materials and Methods

2.1. Source of Fish, Experimental Location and Acclimatization

Eighteen healthy African sharptooth catfish *Clarias gariepinus* were purchased from a private fish farm at Okaka, Yenagoa metropolis, Bayelsa State, Nigeria. The experiment was carried out in the Department of Biological Sciences, Niger Delta University. The mean weight and length of the fish were 97.00g and 14.02cm. The fish were acclimatized for 7 days in a circular aquarium. During the process the fish was fed with normal fish diet and the aquarium water renewed daily.

2.2. Bioassay

The Completely Randomized Design experimental was adopted in this study. Sublethal concentration of the toxicant was determined based on the range finding test of Inyang [13]. The concentration was prepared by pipetting 0.10mls, 0.20mls, and 0.30mls of the original concentration of the toluene (1000g/L) and making it up with 30L borehole water. This translates to 3.33ppm, 6.66ppm and 9.99ppm. The fish were grouped into four treatments including control in triplicates. The desired concentration was prepared following this approach previously applied by Inyang, *et al.* [10], Inyang, *et al.* [14], Inyang, *et al.* [15].

N1 V2 = N2V2

N1 = Manufacturer concentration (1000g/l)

N2 = Concentration of test solution desired.

V1 = Volume of the original solution added.

V2 = Volume of the test solution (30 litre).

During the one-week bioassay the aquarium water was renewed daily. Some of the physicochemical characteristics of the aquarium water used were carried following American Public Health Association (APHA) [16], method, and the resultant values ranged from 26.00 – 26.13 ºC (Temperature), 6.23 – 6.37 (pH) 6.17 – 7.21 mg/l (dissolved oxygen), 99.50 – 136.12 µS/cm (conductivity), 0.20 – 0.50 NTU (Turbidity) and 12.25 – 14.88 mg/l (alkalinity).

2.3. Determination of Organosomatic Indices and Electrolytes Level

The fish was dissected at the end of the experimental period (14 days), and the heart, kidney, liver and spleen were obtained and weighed using electrical balance. From the values the organosomatic indices was calculated following the method previously described by Inyang, *et al.* [15]. About 0.5g of liver and muscle of the fish were macerated and deionized water was added for stabilization. The samples were centrifuged at the rate of 3000rpm for 10 minutes [17]. Then the supernatant was analyzed for electrolytes (sodium, potassium and chloride) following the methods previously described by 18. Logaswamy, *et al.* [18], and American Public Health Association (APHA) [16].

2.4. Statistical Analysis

SPSS software version 20 was used for the statistical analysis. The data were expressed as Mean ± standard deviation. One-way analysis of variance was carried out at p = 0.05, and multiple comparison was carried using Duncan statistics.

3. Results and Discussion

The Organosomatic indices of *Clarias gariepinus* exposed to toluene for one week is presented in Table 1. Among the four indices assessed (Cardiosomatic, Renatosomatic, Spleenosomatic and Hepathosomatic) there is no significant difference at p>0.05 with an increase in the concentration of the toxicants. The trend reported in this study has some similarity with the work of Inyang, *et al.* [15], that reported no significant variation in organosomatic indices i.e. Renatosomatic, Spleenosomatic and Hepathosomatic, and significant variation in Cardiosomatic index of *Heterobranchus bidorsalis* exposed to varying concentration of Rhonasate 360SL for 14 days. Though no significant variations were recorded, but apparent differences exist though not in a dose dependent pattern. Insignificant variation suggests no major alteration in the Organosomatic indices of *Clarias gariepinus* exposed to toluene for 14 days. Though with increased exposure a major alteration that could lead to significant difference in the Organosomatic indices at varying concentration is possible owing to the toxicity of toluene. Inyang, *et al.* [10], have reported that toluene can cause an alteration in blood platelets, packed cell volume, haemoglobin, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and metabolic enzymes (such as acid phosphatase, alkaline phosphatase and alanine amino transferase) in adult *Clarias gariepinus.*

Table-1. Organosomatic indices of *Clarias gariepinus* exposed to toluene for 14 days

Concentrations, ppm	Kidney	Liver	Spleen	Heart
0.00	1.65±0.3a	3.80±0.6a	0.25±0.1a	0.35±0.1a
3.33	1.65±0.2a	5.80±0.3a	0.35±0.1a	0.45±0.2a
6.66	1.85±0.2a	3.70±0.1a	0.35±0.1a	0.50±0.0a
9.99	1.30±0.1a	2.90±0.6a	0.35±0.1a	0.50±0.1a

Data is expressed as mean± standard deviation; Different letters along the column indicates significant variations at p<0.05 according to Duncan statistics

The activities of Electrolytes in liver and muscles of *Clarias gariepinus* exposed to toluene for one week is presented in Tables 2 and 3, respectively. In the liver the concentration of sodium was highest 5.55mmol/L at 9.99ppm and least (3.00mmol/L) at 6.66ppm. Basically there was no significant variations ($p>0.05$) among the various concentrations except for 9.99ppm (Table 2). While in the muscle sodium were highest 5.15mmol/L at 3.33ppm and least (0.30mmol/L) at 9.99ppm. There were significant variations ($p>0.05$) among the various concentration except for 0.00ppm and 6.66ppm that did not differ significantly (Table 3).

In the liver the concentration of potassium was highest 17.66mmol/L at 6.66ppm and least (12.27mmol/L) at 9.99ppm. There were no significant variations ($p>0.05$) among the various concentrations except at 3.33 and 6.66ppm that was not significantly different (Table 2). While in the muscle, the potassium were highest (24.41mmol/L) at 3.33ppm and least (12.88 mmol/L) at 9.99ppm. There were significant variations ($p>0.05$) exist. Furthermore, multiple comparison shown not significant variations between 0.00 and 9.99ppm and 3.33 and 6.66 ppm (Table 3).

The chloride concentration in the liver ranged from 1.02 to 1.65mmol/, being not significant different among the various concentrations except for 0.00ppm (Table 2). In the muscle the chloride concentration ranged from 0.70 to 1.30ppm, being significantly different at $p<0.05$. Multiple comparison showed that the variation occurred between 0.33 and 9.99ppm.

Table-2. Activities of Electrolytes in liver of *Clarias gariepinus* exposed to toluene 14 days

Concentrations, ppm	Sodium, mmol/L	Potassium, mmol/L	Chloride, mmol/L
0.00	3.45±0.45a	14.64±1.43b	1.65±0.05b
3.33	3.75±0.75a	16.79±1.01c	1.13±0.13a
6.66	3.00±1.00a	17.66±0.94c	1.18±0.18a
9.99	5.55±0.55b	12.27±0.90a	1.02±0.25a

Data is expressed as mean± standard deviation; Different letters along the column indicates significant variations at $p<0.05$ according to Duncan statistics

Table-3. Activities of Electrolytes in muscle of *Clarias gariepinus* exposed to toluene for 14 days

Concentrations, ppm	Sodium, mmol/L	Potassium, mmol/L	Chloride, mmol/L
0.00	3.03±0.28b	13.14±0.86a	0.90±0.30ab
3.33	5.15±0.15c	24.41±3.85b	1.30±0.20b
6.66	3.41±0.41b	22.67±2.17b	1.20±0.21ab
9.99	0.30±0.20a	12.88±1.96a	0.70±0.21a

Data is expressed as mean± standard deviation; Different letters along the column indicates significant variations at $p<0.05$ according to Duncan statistics

The electrolytes were not in dose depended pattern. Electrolytes are among the major blood cells in fish. Some electrolytes such as potassium and sodium are positively charged while chloride is a negatively charged ion. These ions help in the balance of pH and acid base. Inyang, *et al.* [14], reported that these electrolytes help in the passage of fluids between and within cells. The authors further reported that these electrolytes are very crucial in regulating the functioning of neuro-muscular, endocrine and excretory system of fishes. In fish, electrolytes are also useful in osmoregulation processes in fish. Hence in this study the significant variations in the in electrolytes suggest reduction in osmotic processes which may lead to collapse in the circulatory system. The fluctuation in values of the electrolytes suggests that the toluene is toxic to fish causing alteration in metabolic processes in the fish. An apparent increase in the electrolytes values may distort some physiological and metabolic processes in the fish, since electrolytes such as Sodium and Potassium are vital in many enzymatic processes essential for the activity of many enzymes and also in the transport of ATP which participates in several metabolic processes.

4. Conclusion

This study assessed the effect of toluene on organosomatic indices and electrolytes in liver and muscle of adult *Clarias gariepinus.* The study found that after 7 days the 0 – 9.99ppm of toluene did not cause a significant alteration in the organosomatic indices, but cause a significant fluctuation in the electrolytes (potassium, sodium and chloride) in the muscle and liver of the fish. An alteration in electrolytes could alter the role of this ions in fish causing stress and even dead at prolong period of time. As such, caution should be exercised in the use of substances containing toluene close to aquatic ecosystem.

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