Research Article
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Evaluation of the effect of toluene (produced water component) on some blood cells and enzymes of *Clarias gariepinus*

Abstract

The aim of the study was to unveil the toxic nature of toluene on some haematological parameters and metabolic enzymes of *Clarias gariepinus* (a common Niger Delta wetland fish). Thirty two adult *Clarias gariepinus* (mean length 23.20±0.06cm) and mean weight (180.0.00±0.09g) were acclimated to laboratory conditions for 7 days and then exposed to varying sublethal concentration of toluene (viz: 3.33, 6.66, 9.99mgl^{-1}) in a semi-static bioassays for 14 days. Blood cells (white blood cell (WBC), pack cell volume (PVC), haemoglobin (HB) etc were determined in the plasma, while metabolic enzymes (acid phosphatase (ACP), alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined in the kidney and liver of the probe organism. WBC and Hb values were statistically significant, akin to mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). A progressive decrease in values within the experimental group were observed in all the blood cells mentioned above. Enzymes in the kidney decreases down the experimental group akin to liver enzymes except ALT and ALP at 33.3mgl^{-1}. Enzymes and blood cells tested are more useful biomarkers of sublethal effect of toluene on aquatic organisms. The trend of parameters tested in this research indicates that toluene could be toxic at high concentration. Adequate care should be taken to avoid produced water components moving into the aquatic environment.

Keywords: toluene, *clarias gariepinus,* blood cells, enzymes

Volume 4 Issue 6 - 2018

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Received: November 19, 2018 | **Published:** December 28, 2018

Introduction

Most aquatic ecosystems are defiled on a daily basis as a result of industrial and anthropogenic releases. In the Niger Delta ecosystem, there are a number of industries such as petroleum refinery, petrochemical industries, liquefied natural gas industry, fertilizer plant, aluminium smelting plant and a lot more sited either on the bank or close to banks of the estuaries.[1] These industries discharge their partially treated and untreated effluent into the estuary.[2] The world consumed over 62 quadrillion BTU (British thermal unit) of petroleum and natural gas based energy resources in 2012,[3] and demand continue to rise as the global population grows and per capital gross domestic product (GDP) and energy consumption increase.[4] Crude oil and natural gas produced from offshore oilfields usually are extracted from subsea geological formations as part of an oil-gas-water emulsion.[5] The volume of water associated with oil is typically low during early stages of production, but may increase to several times the volume of oil produced towards the end of a well's lifespan.[5] According to Fakhru l-Razi et al.,[6] once this water has been separated from the oil and gas, it is known as produced water and becomes a waste material that is most commonly either discharged to sea or injected into the hydrocarbon-bearing formation to enhance oil production or into another subsea formation for disposal.

Fakhru'l-Razi et al.,[6] summarize the components of produce water to include crude oil, which is a mixture of mono aromatic hydrocarbons (MAH), polycyclic aromatic hydrocarbon (PAH), dissolved formation minerals, including heavy metals and radioactive materials; production chemicals, which are typically synthetic additives, solids such as formation solids, corrosion and scale materials, bacteria, waxes and asphaltenes; and dissolved gasses. A typical example of MAH includes benzene, toluene, ethylbenzene and xylene (all are low molecular weight mono aromatic hydrocarbons). Majority of these MAH are moderately soluble in water. Toluene is a colourless in nature, possessing high vapour pressure and low to moderate water solubility. It is a mono-substituted benzene derivative consisting of a methyl group attached to the phenyl group. Toluene is produced during the processing of gasoline and other fuels from crude oil. Toluene has numerous commercial and industrial applications which is a solvent in paints, contact cements and model airplane glue.[7]

Problems of toxic substances in the environment

Lethal and sublethal concentration of poisons are known to have toxic effects on fish behaviour, haematology, enzymes, metabolites, histopathology, growth, reproduction, feeding, respiration and general physiological processes of exposed organisms.[1,2,8–19] In recent years, haematological variables were used more when clinical diagnosis of such physiology was applied to determine the effects of external stresses and toxic substances as a result of the close association between the circulatory system and the external environment.[20] They also suggested that haematology biochemical changes, growth and oxygen consumption of fish be used in determining the toxicity of pollutants. Biochemical indicators of environmental contamination such as enzymes and metabolites may be sensitive and early warning indicators of short or long term detrimental effect of toxicants. Information on produced water effect on African catfish is scarce, again, some chemical constituent of produced water are rarely included when considering the ecotoxicological effect of produce water on the

aquatic environment, hence this present research is aimed at assessing the toxicity of toluene on haematological and biochemical parameters of *Clarias gariepinus,* a common Niger wetland fish.

Material and Methods

Fish sources and acclimatization

Fish samples (adult *Clarias gariepinus*) for this study were obtained from a private fish farm at Okaka, Yenagoa, Bayelsa State, Nigeria. They were transported to wet laboratory of the Department of Biological sciences, Niger Delta University, Bayelsa State, where the assay were conducted from January to March, 2018. Thirty two adult *Clarias gariepinus* (mean weight, 180.00±0.09g and mean length 23.20±0.06cm) were acclimatized individually in a rectangular aquaria for seven days during which they were fed once a day (9.00-11.00hr) with 35% crude protein diet at 1% biomass. Sublethal concentrations of toluene (1000g/l) for the assay (3.33mgl^{-1}, 6.66mgl^{-1} and 9.00mgl^{-1}) were determined based on the range finding test.[21] These were prepared by transferring 0.1, 0.2 and 0.3mls, respectively of the original concentration of the toxicant and making it up to 30 litres with borehole water in the test aquaria. 30litres of the diluent (borehole water) was used as control. Fishes were introduced individually into each aquarium. The exposure period lasted for 14 days during which the exposure media were renewed every forty-eight hours. The physiochemical characterization of the water used for fish bioassay was carried out using standard methods APHA,[22] and the results obtained ranged from 26.01–26.12°C (Temperature), 6.22-6.32 (pH), 12.29-12.31mg^{-1} (alkalinity), 135.01-136.91µs/cm (conductivity), 6.60-6.92mg^{-1} (dissolve oxygen) and 0.45 -0.50NTU (turbidity)

Blood cells and enzyme determination

Blood samples for haematological analysis were collected from the probe organism behind the anal fin with 23G size needle and syringe. All samples were preserved in EDTA bottles. After blood collection fish were sacrificed for the collection of kidney and liver for enzyme analysis. Approximately 0.5g of each organ was grounded with clean pestle and mortar. Physiological saline was used for preservation and stabilization. All samples for enzyme analysis were preserved in physiological saline. Samples were centrifuged for 15 minutes and the supernatant were carefully removed and stored in plain bottles at -20ºC for final analysis Blood cells analyses were conducted based on standard haematological procedures by Blaxhall and Daisley.[23] While activities of metabolic enzymes viz: AST, were analyzed using the colorimetric method of Reitman and Frankel,[24] ALP was assayed by using Kind and King[25] method and ACP assay were determined according to Bessey et al.[26]

Data analysis

The data were subjected to analysis of variance (ANOVA) to test if the exposure of the probe organism to toluene produced any significant difference in the haematological and enzyme parameters where differences existed, Ducan's multiple range test (DMRT) was used to compare difference between means.

Results and Discussion

Enzymes kidney ACP values decreases down the group in a dose dependent pattern. The control group recorded the value (57.50±0.02µ/l) while the least value (37.50±0.00µ/l) was recorded at the highest concentration of the toxicant (Table 1). Quite unlike the kidney ACP, liver ACP values fluctuate down the experimental group (Table 1). The highest value was recorded at 3.33mgl^{-1} while the least value (38.50±0.10µ/l) was recorded at 6.66mgl-1. Kidney ALT values decreases down the group except at the highest concentration 9.99mgl^{-1}) values obtained here were statistically significant ($p<0.05$). The least value was recorded at 6.66mgl^{-1} (3.00±0.00µ/l) compared to the control group that had 7.50±0.01µ/l. Liver ALT pattern was akin to Liver ACP. Values fluctuate down the experimental group. The highest value was obtained at 3.33µ/l (11.00±0.03µ/l). Kidney ALP values were statistically significant ($p<0.05$). This suggests the effect of the toxicant on *Clarias gariepinus* kidney. Liver ALP was not akin to liver ALT and ACP patterns. Values decreases down the experimental group. The highest values were recorded at 3.33mgl^{-1}, while the least value was obtained at the highest concentration.

Table 1 ACP, ALT and ALP in the kidney and liver of *Clarias gariepinus* (Adults) exposed to chronic levels of toluene for 14 days

Conc. Of Toluene (mgl^{-1})	organ	ACP (µ/l)	ALT (µ/l)	ALP (µ/l)
0.00	Kidney	57.50±0.02^{a}	7.50±0.01^{a}	51.50±0.09^{a}
	Liver	60.50±0.03^{ab}	6.00±0.01^{b}	13.00±0.03^{ab}
3.33	Kidney	50.00±0.01^{ab}	5.50±0.02^{b}	41.00±0.01^{ab}
	Liver	66.50±0.21^{a}	11.00±0.03^{a}	15.50±0.01^{a}
6.66	Kidney	38.00±0.03^{b}	3.00±0.00^{c}	36.00±0.04^{b}
	Liver	38.50±0.10^{c}	3.50±0.01^{c}	11.00±0.01^{ab}
9.99	Kidney	37.50±0.00^{b}	3.50±0.00^{c}	34.50±0.01^{b}
	Liver	56.50±0.01^{ab}	4.50±0.00^{c}	7.00±0.00^{b}

Means within column with different superscript are significantly different ($p<0.05$)

The trends in this study suggest the effect of the toxicant on the fishes. Typically disruption of the integrity of biochemical and physiological processes in fish have been monitored by determining changes in the activities of enzymes in plasma/serum and functional organs (gill, brain, liver, muscle, kidney) of the fish or organism in question.[27–29] According to Begum,[30] Tripathi and Verma,[31] these enzymes include alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and acid phosphatase (ACP). The authors added that they are of great interest to researchers as they are used to determine disease condition in affected organism.

Activities of these enzymes generally decreased as the concentration of the toxicant increases (in a dose dependent pattern). Similar results was also unveiled by Inyang,[32] Luscova[33] when they exposed diazinon

Citation: Inyang IR, Puanoni AR, Izah SC. Evaluation of the effect of toluene (produced water component) on some blood cells and enzymes of *Clarias gariepinus*. *MOJ Toxicol.* 2018;4(6):440–444. DOI: 10.15406/mojt.2018.04.00142

to *Clarias gariepinus* and *Cyprinus Carpio* respectively. Decreased levels of ALP activities in the liver and kidney of *Clarias gariepinus* depicts that liver tissue of the exposed fish may not have been impaired. This view was also opined by Ovuru and Mgbere.[34] ALP activity reflect a change in endoplasmic reticulum mass, it is also known to occur in the cell membrane and may be involved in metabolic transport,[35] thus a decrease may denote a decrease in metabolic transport (Begum). According Ovuru and Mgbere,[34] profound decrease in ALP activity after exposure will eventually result in a shift in biosynthesis and energy metabolism pathway of the exposed organism. Additionally, Sastry and Sharma[36] reported decreased activities of alkaline and acid phosphatases in the brain of *Channa punctatus* following the effects of diazinon. The authors unveiled that ALP and ACP activities were inhibited after 96hrs and then resumes its normal values. According to Aly and El-Gendy,[37] ACP is known to be localized in the lysosomes and surrounded by a lipoprotein membrane. The authors further added that decrease in ACP values (akin to values recorded in this research) may be related to leakage of the enzymes into extracellular compartments or tissue damage.

Acid phosphatase is hydrolytic lysosome enzymes and is released by the lysosome for the hydrolysis of foreign materials.[38] The author further added that acid phosphatase is an enzyme of lysosomes origin which hydrolysis the phosphorous ester in acidic medium, moreover helps in autolysis of the cell after its death. Decrease in values of acid phosphatase may signify a serious problem in the probe organism lysosomes. According to Jawale[39] and Sherekar and Kulkarni,[40] increase or decrease in the lysosome enzyme activity depend upon the concentrations of the pesticide.

The Red blood cell (RBC) values were not statistically significant ($p>0.05$) except at 3.33mgl^{-1}. A slight elevation of values (2.69±0.01) was recorded at 3.33mgl^{-1}. The white blood cells (WBC) values profoundly showed a marked distinct within the experimental group. Elevated values were recorded at 3.33mg^{-1} and 6.66mg^{-1} values slightly decreases down the experimental group. There was no significant difference between the control and the last concentration (9.99mg^{-1}). Pack cell volume (PCV) pattern was akin to WBC while haemoglobin (Hb) values decreases down the experimental group in a dose dependent pattern. Mean cell haemoglobin concentration (MCHC) values were not statistically significant except at the highest concentration while mean cell volume (MCV) and mean cell haemoglobin (MCH) values fluctuate down the experimental group (Table 2). Typically the evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal pathological process and toxicological impacts.[41] According to Wendelaar-Bonga,[42] haematological alterations are usually the first detectable and quantifiable responses to environmental change. Haemoglobin values recorded in this research were profoundly significant as values decreases down the experimental group in a dose dependent pattern. Decreased haemoglobin have been reported after exposure of fish to chemical insults.[43,44] Typically, haemoglobin combines reversibly with oxygen to form oxyhaemoglobin in areas of high oxygen concentration and releases the oxygen in regions of low oxygen concentration.[45] Haemoglobin decreases as observed in this study may have serious physiological aberrations in the probe organism. The RBC at 3.33gm^{-1} and 6.66mgl^{-1} showed a slight elevation in values. Similar report was also unveiled by Pereira et al.,[46] when they exposed *Prochilodus lineatus* to clomazone, an organophosphate insecticide. This elevation according to Pereira et al.,[46] and Health[47] is as a result of acute stress, when the adrenergic stimulus triggers splenic contraction, releasing large quantities of red cells into the blood stream.

Table 2 Blood cells of *Clarias gariepinus* (Adults) exposed to chronic levels of toluene for 14 days

Conc. Of Toluene (mgl^{-1})	RBC (10^6mm^{-3})	WBC (10^6mm^{-3})	PCV(%)	Hb (gl^{-1})	Lymph ($\times10^9$l^{-1})	Platelets ($\times10^9$l^{-1})	MCV (µ/3)	MCH (pg)	MCHC (%)
0.00	1.68±0.00^{ab}	191.35±0.35^{ab}	18.05±0.11^{c}	12.90±0.05^{a}	92.00±1.01^{a}	62.00±0.90^{c}	145.50±0.18^{ab}	40.60±0.02^{a}	28.00±0.20^{a}
3.33	2.69±0.01^{a}	207.65±2.50^{a}	39.00±0.10^{a}	11.65±0.01^{a}	85.50±0.90^{ab}	88.50±0.08^{b}	145.00±2.10^{ab}	43.25±0.01^{a}	29.85±0.08^{a}
6.66	1.89±0.01^{ab}	205.10±1.90^{a}	28.50±0.00^{b}	7.95±0.00^{b}	83.50±1.00^{ab}	128.00±0.02^{a}	153.10±3.15^{a}	43.45±0.02^{a}	28.35±0.00^{a}
9.99	2.04±0.00^{a}	186.45±2.33^{ab}	18.50±0.03^{c}	3.55±0.00^{c}	83.00±0.65^{ab}	80.00±0.13^{bc}	115.30±0.71^{b}	22.49±0.02^{b}	19.30±0.01^{b}

Means within column with different superscript are significantly different ($p<0.05$). RBC, Red blood cells; WBC, White blood cells; PCV, Pack cell volume; Hb, Haemoglobin; Lymph, Lymphocyte; MCV, Mean cell volume; MCH, mean cell haemoglobin; MCHC, Mean cell haemoglobin concentration

The white blood cells (WBC) also known as leucocytes functions in organism against foreign bodies. There is a general believe that as organisms are exposed to toxicants, the WBC will increase in the blood stream in order to fight intruders. In this present research, values of WBC increased at 3.33mgl^{-1} and 6.66mgl^{-1}(The first and second concentration of the toxicant respectively) and suddenly decreases at the last concentration indicating that as the concentration of the toxicant (toluene) increases the more severe effect of the toxicant on the WBC. The WBC decrease also unveiled profoundly the effect of toluene on primitive haematopoitic cells responsible for production of the WBC in the probe organism.

Haematological aberrations were also recorded in the following parameters MCV, MCH and MCHC. A slight increase in values at 3.33mgl^{-1} and 6.66mgl^{-1} were recorded in MCV, MCH and MCHC, while decrease in values characterized the parameters at the last concentration of toluene. The elevation according to Svobodora et al.,[48] is an evidence of macrocytic anaemia. This findings was also reported by Inyang,[49] according to the author, a decrease in values of some experimental group is probably an indication of red cell swelling and/or decrease in haemoglobin synthesis. This seems to be the case in this present research.[50]

Conclusion

In conclusion, the profound changes in haematological and enzyme indices of *Clarias gariepinus* compared to control value can seriously affect the probe organism physiology. Additionally, various indices used in this study can be used as bio indicator of exposure of toluene to *Clarias gariepinus,* one of the most prevalent wetland fish in the Niger delta, Nigeria.

Acknowledgments

None.

Conflicts of interest

The author(s) declares that there is no conflict of interest.

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