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EVALUATION OF TOXICITY POTENTIAL OF PROCESS WATER USING FISH ACUTE TOXICITY AND MICRONUCLEUS TESTS

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ABSTRACT: In this study, the potential toxicity of process wastewater from oil and gas exploration in the Niger Delta region of Nigeria was investigated on *Clarias gariepinus* juveniles using a renewable static bioassay with continuous aeration to determine its acute toxicity. Physicochemical analysis of the wastewater showed that it characterised by a foul odour, was slightly alkaline (pH 7.72) and had high values of chemical oxygen demand (COD) and conductivity (100.20 mg/L and 2793.33 µS/cm) respectively. Lead, chromium and nickel were present at amounts above national (FEPA, NESREA) and international (USEPA) set limits for effluent discharge. The LC_{50} value obtained for the fish juveniles exposed to various concentrations of the wastewater for 96 hrs under laboratory conditions was 4.45 ml/L with lower and upper limits being 4.11 ml/L and 7.847 ml/L respectively. Toxicosis symptoms observed includes loss of balance, respiratory distress, vertical and erratic movement and death. The fishes were exposed to 2.0, 4.0, 6.0, 8.0, and 10 ml/L (v/v; wastewater/tap water) for 28 days to evaluate the genotoxic effect of the wastewater using the micronucleus test on peripheral blood erythrocytes. The process wastewater induced significant ($p<0.05$) concentration-dependent increase in micronuclei, binucleated, notched nuclei and immature erythrocytes in *C. gariepinus* which were considered as cytogenetic damage indicators. Results of this study showed that the tested wastewater was a potential cyto-genotoxic agent and could induce adverse health effects in exposed individuals.

Keywords: process water, *clarias gariepinus,* acute toxicity, micronucleus, Niger delta

INTRODUCTION:

Water is one of the most commonly used liquids for injection into the reservoirs through specific wells (injection wells) for oil production support in the refinery. This is done during so-called "secondary" recovery in order to compensate for the drop in pressure inside the reservoir after it has started production. Quality tolerances for process water vary widely with the purpose for which it is used. Process wastewater refers to any water which, during manufacturing or processing, comes into direct contact with or results from the production or use of any raw material, intermediate product, finished product, byproduct, or waste product (USEPA, 2011). With respect to the oil and gas industry, process water is defined as water that has been in intimate contact with hydrocarbons in the refinery. Water that is generated in the process units is represented by desalter effluent, sour water, tank bottom draws and spent caustic (IPIECA, 2010).

In general, process water should be clear, colourless, and free from iron, manganese, hydrogen sulfide, and organic growths (Nordell, 1951). Process water should typically have a conductivity ranging from 0.1 to 50 µS/cm. Although progress has been made over the last few years by the oil refining sector to make improvements to the way in which water is managed, the extent of compliance with standards and global best practices in their treatment and discharge still remains a challenge (Isehunwa and Onovae, 2011).

In the Niger-Delta region of Nigeria, water effluents are usually pumped through discharge pipelines to streams or the sea. Before disposal, the main regulatory agency for the management of wastewaters from the oil and gas industry (Department of Petroleum Resources) requires the constituents of wastewaters to be within the approved limits. Non-compliance with these limits is backed by sanctions (Onojake and Abanum, 2011).

Fish are extremely valuable in toxicity monitoring as they appear to posses the same biochemical pathways as mammalian species does, to deal with the toxic effects of endogenous and exogenous agents (Ahmad, 2012). The hazardous compounds they accumulate in their tissues are directly or indirectly consumed by humans, and are capable of transforming xenobiotic compounds into carcinogenic and mutagenic metabolites (Ergene,*et al.*, 2007). Besides, fish constitute an important link in food chain and their contamination by industrial wastewaters imbalance the aquatic system, therefore it is imperative to examine the toxic effects of process water on them. Often, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish due to their close association with the environment (Okomoda *et al.*, 2010).

The effect of environmental contamination on the health conditions of the African catfish, *Clarias gariepinus* has been reported (Ololade and Oginni, 2010; Okayi *et al.*, 2010; Ayuba *et al.*, 2013; Dahunsi and Oranusi, 2013). In spite of the adverse effects of wastewaters generated during oil and gas exploration activities in the Niger Delta region of Nigeria on the aquatic environment, there is dearth of information on the physicochemical parameters and acute toxicity of process water using African catfish, *C. gariepinus* as

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test organism. This study was therefore undertaken to investigate the physicochemical characteristics and effect of process water on the mortality rate and behavioural pattern of juveniles of *C. gariepinus*.

MATERIALS AND METHODS:

Collection of Samples

The process water used for this study was obtained fresh from the Nigerian Agip Oil Company (NAOC) facility at Ogboinbiri (4°50'0"N, 5°58'0"E) in Bayelsa State in March, 2012. The plastic containers were washed with distilled water and then rinsed with the effluent. The wastewater was collected at the point of discharge with a funnel into 10 litre plastic containers which were previously washed and rinsed with distilled water. They were kept in an ice chest for onward transport to the laboratory and stored in the refrigerator at 4°C and analysed within 24 h of collection for physicochemical analysis.

Physicochemical analysis

The wastewater was analyzed for a number of standard physicochemical parameters including pH, hardness, total dissolved solids, conductivity, alkalinity, chloride, nitrates, ammonia, sulphates, phosphates and 13 metals and heavy metals namely: Ca, Na, K, Mg, Fe, Cu, Zn, Al, Cr, Pb, Ni, Mn and Cd using standard analytical methods (USEPA, 1999; APHA, 2005).

Acute Toxicity

Juveniles of the African catfish, *C. gariepinus* used for this investigation measured 17±1.0 cm and had a mean weight of 12.4±0.5 g. They were purchased from a fish farm at Ekehuan Road, Benin City, Nigeria (6°15′N, 5°25′E). The fishes were acclimatized for fourteen days in glass aquaria tanks measuring 20 × 15 × 30 cm containing de-chlorinated water at room temperature of 27±1.7 °C. During the acclimation period, the fish were examined for pathogens and diseases. Water was changed at two days interval to prevent the build-up of metabolic wastes. Juveniles were fed twice daily with fish meal at 3% body weight. Feeding was stopped 24 hours prior to and during exposure period that lasted for 96 hours.

Acute toxicity test followed methods recommended by UNEP (1989). Water was put into the aquaria using a measuring cylinder and mixed up with the test effluents into the aquaria making it up two litres. The same method was applied to the duplicate, and ten fishes were stocked per aquarium. The test solution was stirred with a rod to ensure adequate mixing before the test organisms were introduced into the experimental tanks. The process water concentrations used were 2.0, 4.0, 6.0, 8.0 and 10 ml/L and a control. The fish were examined for abnormal behaviours and mortality for 12, 24, 48, 72 and 96 hours. Dead fish were removed from test solutions as soon as observed. A fish was considered dead when it was totally immobile and had no respiratory/opercula and tail movements. The 96 hour LC_{50} toxicity was determined as a probit analysis using the arithmetic method of percentage mortality (Randhawa, 2009).

Micronucleus Assay

Twenty fishes were randomly selected for the control at 27±1.7 °C. Similarly, 20 fishes each were randomly selected and exposed to process water concentrations of 2.0, 4.0, 6.0, 8.0 and 10 ml/L for a period of 28 days in renewal bioassay conditions. Natural photoperiod of 12 h light and 12 h dark were maintained during the experiment.

During the time of exposure, 5 fishes were randomly selected on day 28; and peripheral blood collected from their caudal vein using sterile syringes and needles for the micronucleus (MN) assay. A thin smear of blood was made onto clean, grease-free slides and air-dried overnight at room temperature before fixing in absolute methanol for 20 min and subsequently stained with May-Grunwald and 5% Giemsa (Singh *et al.*, 2005). Three slides were prepared per fish per concentration and control. The frequencies of micronuclei and other nuclei lesions were expressed per 1000 cells per fish. The nuclear abnormalities (binucleated cells, immature erythrocytes) were scored along with MN as biomarkers of cytogenotoxicity in accordance with Carrasco *et al.* (1990) and Cavas and Ergene-Gozukara (2003, 2005).

Results obtained for the lower and upper confidence limits of the LC_{50} were subjected to regression statistical analysis with Duncan's multiple range test in one way ANOVA, using SPSS version 16.0 for windows at $p<0.05$ level of significance to compare the various concentrations of process water and the control.

RESULTS AND DISCUSSION:

Table 1 shows the result of the physicochemical analysis of process water. The wastewater had a foul odour with a slightly alkaline pH of 7.72. The wastewater was characterised by relatively high values of chemical oxygen demand (100.20±2.01 mg/l), conductivity (2793.33±20.46 µS/cm), phosphates (90.67±4.5 mg/l), and low dissolved oxygen (0.73±0.01 mg/l). The values of lead, chromium, and nickel (0.13, 0.17, and 0.10 mg/l) respectively were above the limits set for effluent discharge by national [Federal Environmental Protection Agency (FEPA), National Environmental Standards and Regulation Enforcement Agency (NESREA) and international [United States Environmental Protection Agency (USEPA)] regulatory bodies (FEPA, 1991; NESREA, 2009; USEPA, 2009).

Among the aquatic pollutants contaminated the fish habitats at alarming rate heavy metals are most injurious for fish life. Metals are becoming increasingly concentrated at higher trophic levels, possibly due to food-chain magnification (Martin and Knauer, 1973; Parvathi *et al.*, 2013). Bioaccumulation of heavy metals in tissues of marine organisms has been identified as an indirect measure of their abundance and availability in the marine environment, for this reason, monitoring fish tissue contamination serves an important function as an early warning indicator of sediment contamination or related water

quality problems (Murtala *et al.*, 2012). Many studies have been carried out on different fish species which revealed that both essential and non-essential metals cause toxic effects in fish through disturbing the physiological activities, biochemical processes, reproduction and growth and finally lead to their mortality (Gaber, 2007). The high levels of lead, chromium, and nickel and the probable synergistic with other metals in the process wastewater could possibly account for its toxicity. The heavy metals in the wastewater may have affected organs like the gills, liver, brain or kidney of the fish. Similar observations have been reported with agricultural and pharmaceutical effluents (Adewoye *et al.*, 2005; Agboola and Fawole, 2014). The foul smell of the produced water may have resulted from the biodegradation activities of anaerobic bacteria of organic matter in it, possibly facilitated by the low dissolved oxygen and high biochemical oxygen demand of the wastewater (Adewoye *et al.*, 2005).

Table 1
Physicochemical properties of process water

Parameter	Process water (Mean± S.E.)	FEPA (1991) Limit	NESREA (2009)Limit	USEPA (2009) Limit
pH	7.72±0.12	6-9	6-9	6.5.-8.5
Dissolved oxygen	0.73±0.01	5	-	-
BOD_5@ 20°C	34.09±0.1	50	50	250
COD	100.20±2.01	-	-	-
Total hardness	8.12±1.46	-	-	0-75
Total Dissolved Solids	0.05±0.01	2000	500	500
Conductivity	2793.33±20.46	-	-	-
Alkalinity	0.05±0.01	-	150	20
Ammonia	0.33±0.18	-	1	0.03
Sulphates	88.50±6.93	500	250	250
Nitrates	0.06±0.03	20	10	10
Phosphates	90.67±4.5	5	2	-
Potassium	123.97±12.1	-	-	-
Sodium	105.53±8.69	-	-	-
Calcium	82.37±5.55	200	-	-
Magnesium	00.00±0.00	-	-	-
Chloride	8.57±4.28	600	250	250
Iron	4.47±0.41	20	-	0.3
Lead	0.13±0.03	<1	0.05	0.02
Copper	0.20±0.12	<1	0.5	1.3
Zinc	0.00±0.00	<1	-	0.12
Cadmium	0.00±0.00	<1	0.02	0.002
Manganese	0.33±0.18	5	0.2	0.05
Aluminum	0.00±0.00	-	-	-
Chromium	0.17±0.01	0.05	0.05	0.1
Nickel	0.10±0.01		0.05	0.005

All values are expressed in mg/l except conductivity (µS/cm) and pH (no units). BOD = Biochemical Oxygen Demand, COD = Chemical Oxygen Demand, FEPA = Federal Environmental Protection Agency (1991), NESREA = National Environmental Standards and Regulations Enforcement Agency (2009), USEPA = United States Environmental Protection Agency (2009) maximum permissible limits for effluent from wastewater.

The mortality rate of *C. gariepinus* juveniles exposed varied concentrations of process water is presented in Tables 2 and 3. There was increase in mortality as the concentration of the wastewater increased. Normal behaviours were observed in the control groups; however, restlessness, erratic movement, loss of equilibrium and gasping for breath, were some abnormalities observed in fish exposed to the different concentrations of process water. The affected fish became very weak and died with increase in concentration.

The LC_{50} (the effective concentration at which fifty percent (50%) of the test organisms are killed) derived at 95 hours was 4.45 ml/L with lower and upper limits being 4.11 ml/L and 7.847 ml/L respectively. The fitness of the natural population at this concentration would be relatively impeded and the mortality rate increases with increase in concentration. The computed regression equation was found to be Y= -1.8571 + 2.9* (R = 0.65, Y = probit kill) (Fig. 1).

Table 2:
Mortality rate of *C. gariepinus* juveniles exposed varied concentrations of process water

Conc. (ml/l)	Mortality					No of mortality	Percentage mortality
	12 hours	24 hours	48 hours	72 hours	96 hours		
Control	0	0	0	0	0	0/10	0
2.0	0	0	0	0	2	2/10	20
4.0	0	0	1	1	2	4/10	40
6.0	0	0	1	2	3	6/10	60
8.0	0	0	1	3	4	8/10	80
10.0	0	0	1	4	5	10/10	100

Table 3

Percentage mortality rate of *C. gariepinus* juveniles exposed varied concentrations of process water

Conc. (ml/l)	No of deaths at 96 hours			Mortality	Percentage mortality	*Corrected % of mortality	Probit
	1	2	3				
Control	0	0	0	0/10	0	2.5	3.04
2.0	1	0	1	2/10	20	20	4.16
4.0	1	2	1	4/10	40	40	4.75
6.0	2	2	2	6/10	60	60	5.25
8.0	3	2	3	8/10	80	80	5.84
10.0	4	2	4	10/10	100	97.5	6.96

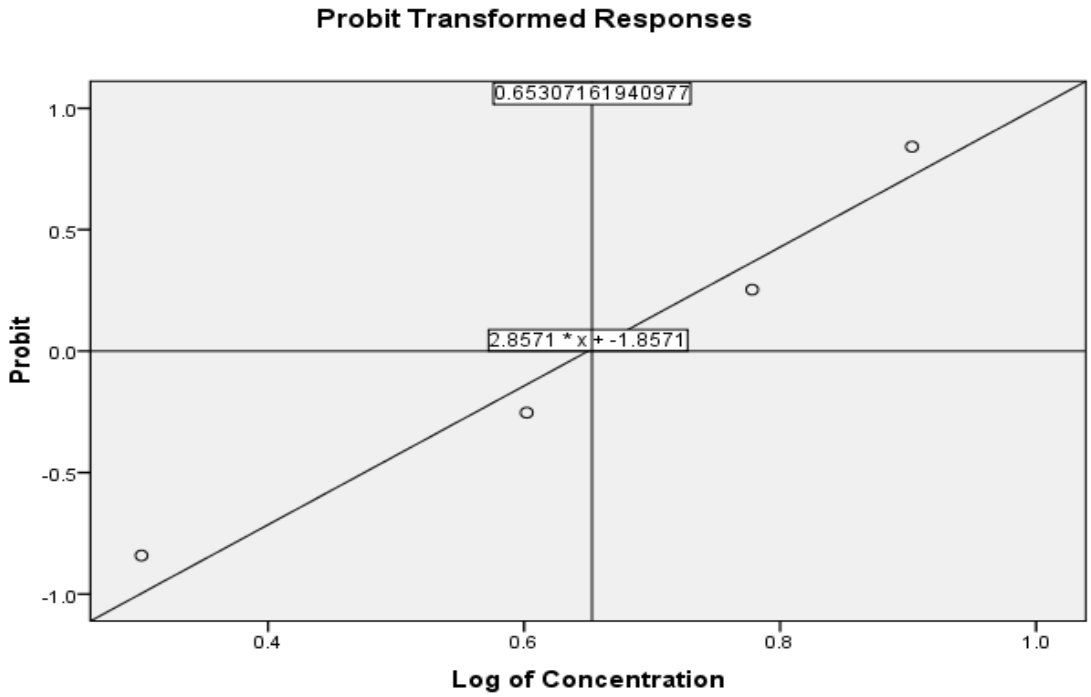

Fig. 1 Linear relationship between mean probit mortality and log concentration of *C. gariepinus* juveniles exposed to process water for 96 hours

Assessment of water quality by use of only physicochemical methods do not provide integrated information on the effects of pollutants on aquatic life because toxicity is a biological characteristic (Kazlauskiene *et al.*, 2012). In this study, respiratory abnormalities (gasping for breath prior to mortality) were observed in the test organism. These observations are in agreement with results obtained for the same test organisms exposed to rubber processing effluents (Dahunsi and Oranusi, 2013) which are indications of depleted oxygen content due to higher demand for oxygen. The disruption of the behavioural responses of the organisms has been attributed to the increase in Biochemical Oxygen Demand increases (and the decrease in oxygen content) which eventually reduces the fitness of a natural population (Adewoye *et al.*, 2005). The fish were also erratic in their swimming patterns and became motionlessness thereafter. These are indications that mortality of the exposed fish is not only due to impaired metabolism, but could in addition be due to nervous disorder (Okayi *et al.*, 2013). Results obtained from this study showed that process water was toxic to *C. gariepinus*. There was concentration-dependent increase in mortality of the test organism exposed to the wastewater. Our results are in agreement with observations made with the African fish exposed to rubber processing, agricultural, pharmaceutical effluents and synthetic resin (Adewoye *et al.*, 2005; Dahunsi and Oranusi, 2012; 2013; Agboola and Fawole, 2014). Our findings in this study are, to the best of our knowledge, the first documented report on the acute toxicity of process water on *C. gariepinus* juveniles.

Table 4 shows the result of the genotoxicity of process water by micronucleus test. Compared with the control, the frequency of micronucleus induction in erythrocytes of *C. gariepinus* increased significantly ($p<0.05$) with increase in concentration of the wastewater. There was a slightly over two-fold increase over the frequency in the control (31±2.53) at 10 ml/L effluent concentration (73.5±1.41). Similarly, there was concentration-dependent significant ($p<0.05$) increase (3.40 ± 0.51) of immature erythrocytes in the circulating blood of *C. gariepinus* exposed to process water at the same concentration.

Table 4

Mean frequencies of the micronucleus (MN), binuclei (BN), and immature induction in erythrocytes of *C. gariepinus* Juveniles exposed to sub-lethal concentrations of process water

Process water (ml/L)	Cells with MN (Mean ± S.E)	Cells with BN (Mean ± S.E)	Immature Cells (Mean ± S.E)
Control	31.0 ± 2.53	1.20 ± 0.20	1.25 ± 0.25
2.0	71.6 ± 2.25	1.40 ± 0.25	2.00 ± 0.55
4.0	77.5 ± 5.20	3.80 ± 0.49	3.00 ± 0.54
6.0	82.5 ± 3.61	3.40 ± 0.51	3.20 ± 0.87
8.0	92.5 ± 0.93	3.80 ± 0.97	2.40 ± 0.98
10.0	73.5 ± 1.41	3.00 ± 1.09	3.40 ± 0.51

Micronucleus formation and nuclear abnormalities in fish have been considered as useful indicators in evolution of genotoxic and cytotoxic effects of contaminants in aquatic organisms (Rocha *et al.*, 2009; 2010; Cavas and Ergene-Gozukara, 2005; Bariene, 2006). The induction of the micronucleus and nuclear abnormalities in the peripheral erythrocytes of *C. gariepinus* treated with process water in this study suggest that this wastewater contained clastogenic substances capable of causing DNA damage and genome instability in the test organisms (Kligerman, 1982; and Malla and Ganesh, 2009).

CONCLUSION:

Results of the physicochemical analysis, acute toxicity and micronucleus test of process wastewater have shown that it contains chemicals that are harmful to the environment and is capable of exerting genotoxic effect on living organisms. The indiscriminate discharge of wastes from oil and gas exploration activities in the Niger Delta region of Nigeria, if left unchecked, could lead to the depletion and possible extinction of natural fish population. This should be a clarion call the regulatory bodies, whose mandate it is to protect the environment, to enforce laws enacted to protect our environment.

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