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ACUTE TOXICITY OF PRODUCED WATER ON *CLARIAS GARIEPINUS* JUVENILES

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ABSTRACT: One of the greatest challenges posed by environmental risks to aquatic lives in the Niger Delta region of Nigeria results from the indiscriminate discharge of produced water from oil and gas exploration activities in the area into the environment. In this study, *Clarias gariepinus* juveniles (measuring 17±1.0 cm and mean weight 12.4±0.5 g) were exposed to different concentrations of produced water under laboratory conditions to determine the 96 h LC_{50} value. The acute toxicity of exposed juveniles was found to be 3.31 ml/L with lower and upper confidence limits of 2.14 ml/L and 3.10 ml/L respectively. Juveniles showed abnormal behaviours, including erratic swimming, restlessness, loss of balance, respiratory distress and death. The degrees of the observed abnormalities were directly proportional to the produced water concentration.

Keywords: produced water, clarias gariepinus, acute toxicity, niger delta, aquatic lives

INTRODUCTION

Produced water is mainly salty water trapped in the reservoir rock and brought up along with oil or gas during production. These waters exist under high pressures and temperatures, and usually contain oil and metals (USEPA, 2000). The Niger-Delta region is the main producer of oil and gas in Nigeria; and as such the greatest generator of produced water. Produced water discharged into the environment is yet to meet set standards for disposal as the concentration of oil/grease content, total dissolved solids, total suspended solids and some other parameters are still very high (Isehunwa and Onovae, 2011).

There are laws, guidelines and regulations for the management of produced water in Nigerian oil and gas operations; however, they do not include regulations aimed at further reducing discharges until zero discharge is achieved in the long term. The disposal guidelines and limits set by the regulatory agencies are not properly monitored and effectively enforced. Although there has been no reported environmental disaster of high magnitude associated produced water disposal in the country, much of this waste water is dumped in the environment, especially during drilling operation indiscriminatorily (Onojake and Abanum, 2012).

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 produced 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).

Health conditions of the African catfish, *Clarias gariepinus* has been reported for environmental contamination by trace metals (Olaifa et al., 2004; Ololade and Oginni, 2010), herbicide (Okayi et al., 2010), formalin (Okomoda et al., 2010; Ayuba et al., 2013), rubber processing effluent (Dahunsi and Oranusi, 2013). Previous studies undertaken for the evaluation of produced water toxicity in the Niger-Delta employed physicochemical analysis to determine the extent of compliance with standard and global best practices for disposal of the wastewater into the environment (Isehunwa and Onovae, 2011, Onojake and Abanum, 2012). There is no documented information here in Nigeria on the acute toxicity of produced water using the African catfish, *C. gariepinus*. The objective of this study was therefore aimed at investigating the effect of produced water on mortality rate and behavioural pattern of juveniles of *C. gariepinus*

MATERIALS AND METHODS

Collection of Samples

The produced 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.

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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* measuring 17±1.0 cm and mean weight 12.4±0.5 g were purchased from a fish farm at Ekehuan Road, Benin City, Nigeria (6°15′N, 5°25′E) and used for this investigation. The fish were acclimatized for fourteen days in glass aquaria tanks measuring 20 × 15 × 30 cm containing de-chlorinated water (tap water exposed to air for greater than 24 hours) 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 (produced water) 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 (*C. gariepinus)* were introduced into the experimental tanks. The produced water concentrations used were 1.5, 2.0, 3.0, 4.0, 5 ml/L and a control (de-chlorinated water). The fish were examined for abnormal behaviours and mortality for 6, 12, 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 (no response to a gentle prodding) and 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. The lower and upper confidence limits of the LC_{50} were determined as described by UNEP (1989). Results obtained 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 produced water and the control.

RESULTS AND DISCUSSION

Table 1 shows the result of the physicochemical analysis of produced water. The wastewater had an unpleasant (foul) odour and was slightly alkaline with a pH 7.71. The wastewater was characterised by relatively high values of conductivity (2793.33±20.46 µS/cm), alkalinity (274.53±2.43 mg/L), biochemical oxygen demand (61.20±0.1 mg/L) and low dissolved oxygen (2.11±0.03 mg/L). The amounts of lead, manganese, zinc and nickel (0.07, 0.37, 0.69, and 0.03 mg/L) respectively were above national (NESREA) and international (USEPA) limits for effluent discharge. These data obtained from the physicochemical analysis shows that the effluent is toxic for discharge into our immediate environment.

Table 1

Physicochemical properties of produced water

Parameter	Produced water (Mean± S.E.)	NESREA (2009) Limit	FEPA (1991) Limit	USEPA (2009) Limit
pH	7.71±0.15	6-9	6-9	6.5.-8.5
Dissolved oxygen	2.11±0.03	-	5	-
BOD_5@ 20°C	61.20 ±0.1	50	50	250
Total hardness	8.90±8.06	-	-	0-75
Total Dissolved Solids	0.03±0.02	500	2000	500
Conductivity	2793.33±20.46	-	-	-
Alkalinity	274.53±2.43	45	-	-
Ammonia	10.37±0.19	1	-	0.03
Sulphates	50.17±25.59	250	500	250
Nitrates	21.01±0.95	10	20	10
Phosphates	44.00±23.34	2	5	-
Potassium	55.57±27.81	-	-	-
Sodium	60.30±32.19	-	-	-
Calcium	48.80±14.43	-	200	-
Magnesium	3.24±3.24	-	-	-
Chloride	650.43±8.93	250	600	250
Iron	0.17±0.07	1	20	0.3
Lead	0.07±0.07	0.05	<1	0.02
Copper	0.07±0.04	0.5	<1	1.3
Zinc	0.69±0.26	-	<1	0.12
Manganese	0.37±0.19	0.2	5	0.05
Nickel	0.03±0.03	0.05	<1	0.005

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

Wastewater persistent pollutants (heavy metals) are the most dangerous as they migrate and accumulate in organisms in ecosystems (Kazlauskiene et al., 2012). Nickel, manganese and lead are known to affect the health status of living organisms (SON, 2007). Annune et al. (1994) has reported that gills tissues are most sensitive to water pollutants since gills are the primary site for osmo-regulation and respiration. They are highly vulnerable to lesions due to their immediate contact with aquatic pollutants. Some of these pollutants exert their effects on the external surface of the fish especially the gills. The high toxicity of the produced water could be attributed to some possible synergistic effect likely to be produced by the active ingredients in the wastewater. The relatively high amounts of the heavy metals in the produced water 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).

It has been argued that water pollution assessment 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, therefore investigations should be carried out under controlled laboratory conditions on test objects of different trophic level, phylogenesis and ontogenesis (leeches, daphnids and fish), taking into account the test set of functions (Kazlauskiene et al., 2012). Unlike the normal behaviours observed in the control groups, restlessness, gasping for breath, erratic movement, loss of equilibrium were some abnormalities observed in fish exposed to the wastewater. The affected fish became very weak, gasping for air and died with increase in concentration (Table 2). The respiratory abnormalities observed in the test organism in this study (gasping for breath prior to mortality) are in agreement with results obtained by Dahunsi and Oranusi (2013) for the same test organisms exposed to rubber processing effluents 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 erratic swimming and motionlessness of the fish 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 produced water was toxic to *C. gariepinus*. There was increase in mortality as the concentration of the wastewater increased (Tables 2 and 3). Similar results on concentration-dependent increase in mortality rates have been reported for the same organism exposed to agricultural effluents (Adewoye et al., 2005), rubber processing effluents (Dahunsi et al., 2013), resin effluent (Dahunsi and Oranusi, 2013) and pharmaceutical effluents (Agboola and Fawole, 2014).

Table 2.
Mortality rate of *C. gariepinus* juveniles exposed varied concentrations of produced water (n=10)

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
1.5	0	0	0	0	0	0/10	0
2.0	0	0	0	1	3	4/10	40
3.0	0	0	1	1	4	6/10	60
4.0	0	0	1	2	5	8/10	80
5.0	0	1	1	2	6	10/10	100

Table 3.
Percentage mortality rate of *C. gariepinus* juveniles exposed varied concentrations of produced water (n=10)

Conc. (ml/l)	No of deaths at 96 hours			Mortality	Percentage mortality	Probit
	1	2	3			
Control	0	0	0	0/10	0	-
1.5	0	0	0	0/10	0	3.36
2.0	1	2	1	4/10	40	4.75
3.0	2	2	2	6/10	60	5.25
4.0	3	2	3	8/10	80	5.34
5.0	4	2	4	10/10	100	6.64

The LC_{50} value derived from the toxicity revealed that *C. gariepinus* is sensitive to produced water. The LC_{50} at 95 hours was 3.31 ml/L with lower and upper confidence limits of 2.14 ml/L and 3.10 ml/L respectively. This means that at this concentration of the effluent in the aquatic environment, half of the entire natural population will become dead. At this concentration, the fitness of the natural population of an aquatic environment would be relatively impaired and as the concentration increases, the mortality rate also increases. The computed regression equation was found to be Y= -1.45 + 3.5* (R = 0.52, Y = probit kill) (Fig. 1). To the best of our knowledge, this is the first documented report on the acute toxicity of produced water on *C. gariepinus* juveniles. Varying LC_{50} values have been obtained by various investigators on exposure of the same organism to different effluents from agricultural wastes, rubber processing, resin and pharmaceutical industries (Adewoye et al., 2005; Dahunsi and Oranusi, 2012; 2013; Agboola and Fawole, 2014). The differences in these values may be due to differences in the nature of the pollutant, age of the organism and environmental conditions (Ayuba et al., 2013).

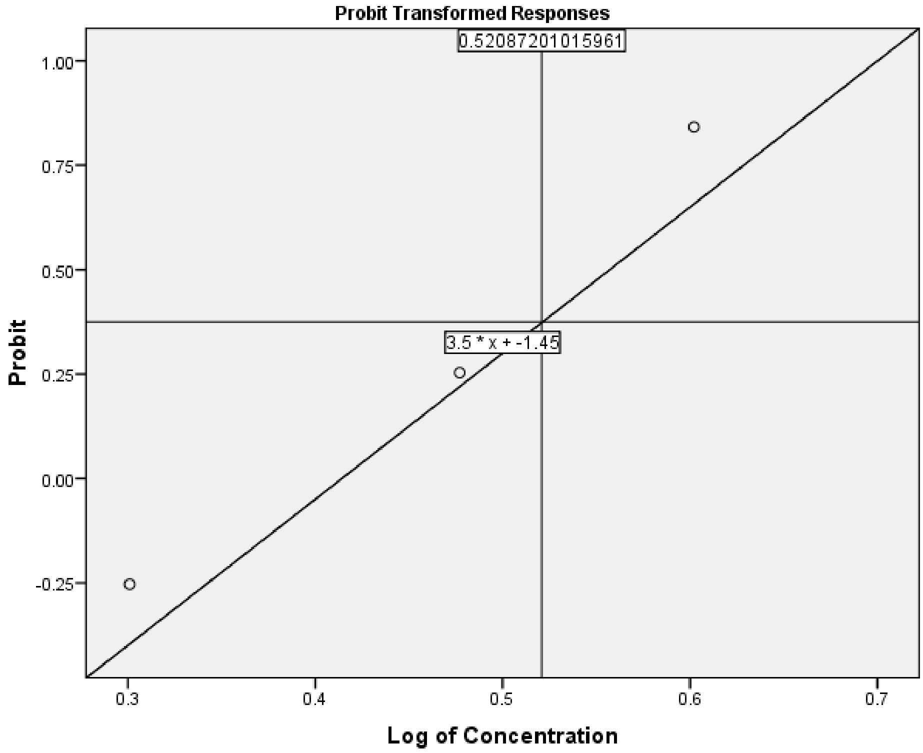

Fig. 1 Linear relationship between mean probit mortality and log concentration of *C. gariepinus* juveniles exposed to produced water for 96 hours

CONCLUSION

The present investigation has shown that produced wastewater contains substances that are harmful to a natural fish population. The effects of a pollutant on any aquatic organism could be acute, chronic, sub-chronic or delay toxicity. In this study, the symptoms of toxicity on the fish started gradually with unusual and erratic swimming behaviour in the wastewater which eventually led to death. The indiscriminate discharge of wastes from oil and gas exploration activities in the Niger Delta region of Nigeria should receive urgent attention from the regulatory bodies, whose mandate it is to protect the environment. This will reduce the deleterious effects on the environment, other living aquatic organisms and man.

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