

ESTIMATION OF ACUTE TOXICITY OF BILGE WATER TO THE AFRICAN CATFISH [*Clarias gariepinus* (BURCHELL 1822)] JUVENILES

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ABSTRACT

Clarias gariepinus (Burchell) juveniles were exposed to different concentrations (10, 20, 30, 40 and 50%) of bilge water for 96 hrs under laboratory conditions using a renewable static bioassay with continuous aeration to determine its acute toxicity. The LC_{50} of exposed juveniles was found to be 35.97 ml/L with lower and upper confidence limits of 18.98 ml/L and 30.28 ml/L, respectively. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargies and sudden death were observed in the exposed fish and these varied greatly with increase in concentration of the bilge water. The differences observed in the mortalities of *C. gariepinus* at varying concentrations were significant ($p < 0.05$).

Keywords: Bilge water, African fish, *Clarias gariepinus*, Acute toxicity

INTRODUCTION

Shipping, which has led to a phenomenal growth in world merchandise trade and has been recognized as one of the strong catalysts for socio-economic development, has consistently grown faster than output. The world seaborne trade in 2013 amounted to 9.35 billion tons of cargo (Werschkun, 2014). Cargo throughput handled at the Nigerian Ports stood at 22,324,223 million metric tonnes in the third quarter of 2014, showing an increase of 12.5 per cent over 19,849,258 metric tonnes achieved in 2013 (NPA, 2014). During the same period, a total of 1,405 oceans going vessels with a total Gross Registered Tonnage (GRT) of 38,047,705 called at all Nigerian Ports, an increase of 2.78 % over the number of vessels that called in 2013.

Associated with phenomenal growth and development there is significant increase in the amount of oil used in the shipping industry worldwide, most of which find their way into the marine environment causing pollution. Oils and fuels have a tendency to accumulate in sediments and in the marine organisms. The gateway of these harmful substances into the marine ecosystems include: bilge pumping, fueling, and improper response to spills. Oily wastewater pollution not only affects drinking water and groundwater

resources, but it endangers aquatic resources and human health; causes atmospheric pollution; affects crop production and destroys the natural landscape (Poulopoulos *et al.*, 2005).

Bilge water containing oily wastes is normally treated by regular operation of oily water separators, filtration and other related shipboard systems (Han *et al.*, 2014). However, accidental and intentional bilge water spills continually occur worldwide. Although Nigeria is a signatory to the Memoranda of Understanding on Port State Control in the West and Central African sub-region, the MOU is yet to effectively reduce, control and prevent marine pollution in the country (Igbokwe, 2001). The regulatory bodies charged with the responsibility for the management of the marine environment are still grappling with the modalities to enforce full implementation of the important international agreements and programmes, including the London Protocol to protect the marine environment (IMO/NIMASA, 2013).

Fish, like the mammalian species, possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents, hence, they are valuable in toxicity monitoring (Ahmad, 2012). Several investigations on the effect of environmental contaminants on the

African catfish, *Clarias gariepinus* have been documented (Okayi *et al.*, 2010; Okomoda *et al.*, 2010; Ayuba *et al.*, 2013; Dahunsi and Oranusi, 2013). Information on the health condition of bilge water on the African catfish, *C. gariepinus* has however not been documented. The objective of this study is therefore to investigate the effect of bilge water on mortality rate and behavioural pattern of juveniles of *C. gariepinus*.

MATERIALS AND METHODS

Samples of bilge water were collected from a seagoing vessel used to transport crude oil along the Atlantic Ocean in April 2014. The vessel which originated from Port Vila, Vanuatu, located on the island of Efate was berthed on High Sea at Lagos Port Complex located at the Apapa area of Lagos, Nigeria. The bilge water samples which were collected in washed and sterilized plastic containers, were thoroughly mixed, transported in an ice chest and was stored in the *Haier Thermocool* refrigerator (Model HR-157B) at 4 °C in the laboratory. The physicochemical characteristics of the bilge water samples were determined within 24 hours of collection using standard analytical methods (USEPA, 1999; APHA *et al.*, 2005).

Juveniles of the African catfish (measuring 17 ± 1.0 cm, 12.4 ± 0.5 g) used for this investigation were purchased from a fish farm in Benin City, Nigeria. The test organisms were transported in a sealed oxygenated polythene bag which contained freshwater from the farm. The specimens were kept in large glass aquaria tanks measuring (20×15×30 cm) containing well aerated dechlorinated borehole water at room temperature (27 ± 1.7 °C) for fourteen (14) days to acclimatize to the laboratory conditions. Water was changed at two days interval to prevent the build-up of metabolic wastes. The fish were fed twice daily with fish meal at 3% body weight.

Feeding was discontinued 24 hours prior to the commencement of the experiment which lasted for 96 hours. Before the bioassay started, ten acclimated juveniles were introduced into each tank containing different concentrations of the wastewater with two replicates. The control experiment was similarly set up in replicates with dechlorinated water.

Acute toxicity test conducted followed methods recommended by UNEP (1989). Ten fishes were stocked per aquarium in bilge water concentrations (10, 20, 30, 40, 50 ml/L) and control. The fish were observed 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 with no respiratory/opercula and tail movements. The 96 hour LC₅₀ toxicity was determined as a probit analysis using the arithmetic method of percentage mortality (Randhawa, 2009). Results obtained for the lower and upper confidence limits of the LC₅₀ were subjected to regression statistical analysis and one way ANOVA at $p < 0.05$ level of significance with SPSS version 16.0 for Windows. Significant means of the concentrations of bilge water and the control were separated using Duncan's multiple range test.

RESULTS

Table 1 shows the physicochemical characteristics of bilge water. The wastewater was slightly acidic with a pH 6.09 and a complex mixture with a variety of dissolved cations and suspended particles. Some of the parameters notably nitrates, phosphates, chlorides, iron and nickel showed deviation from national (NESREA) and international (USEPA) specifications for maximum limits allowed for effluent discharge into water bodies for all categories of industries.

Table 1: Physicochemical Properties of Bilge Water

Parameter	Bilge Water	NESREA (2009) Limit	USEPA (2009) Limit
pH	6.09	6-9	6.5.-8.5
Turbidity	12.1	-	-
Ammonia	0.04	1	0.03
Sulphates	43.00	250	250
Nitrates	57.01	10	10
Phosphates	54.04	2	-
Carbonates	188	-	-
Chloride	338.00	250	250
Iron	0.13	-	0.3
Zinc	0.04	-	0.12
Manganese	0.02	0.2	0.05
Aluminum	0.02	-	-
Nickel	0.09	0.05	0.005
Cobalt	0.01	-	-

All values are expressed in mg/L except pH (no units). 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 to varied concentrations of bilge water is presented in Table 2. Observations show the fish exposed to the wastewater were restless, erratic in their movement and were gasping for breath.

However, normal behaviour was observed in the control groups. The results show that as the concentration of the bilge water increased, the affected fish became very weak and eventually died.

Table 2: Mortality Rate of *C. gariepinus* Juveniles Exposed Varied Concentrations of Bilge Water

Conc. (ml/L)	Mortality (hours)					No of mortality	Percentage mortality	*Corrected % mortality	Probit
	12	24	48	72	96				
Control	0	0	0	0	0	0/10	0	2.5	3.04
10	0	0	0	0	0	0/10	0	2.5	3.04
20	0	0	0	0	4	4/10	40	40	4.75
30	0	0	6	0	0	6/10	60	60	5.25
40	0	8	0	0	0	8/10	80	80	5.84
50	0	10	0	0	0	10/10	100	97.5	6.96

The LC_{50} at 95 hours was 35.97 ml/L with lower and upper confidence limits of 18.98 ml/L and 30.28 ml/L respectively. At this concentration of the bilge water in the aquatic environment, half of

the entire fish population would die. The computed regression equation was found to be $Y = -3.8571 + 2.8571 * X$ ($R = 1.5283$, $Y =$ probit kill) (Fig 1).

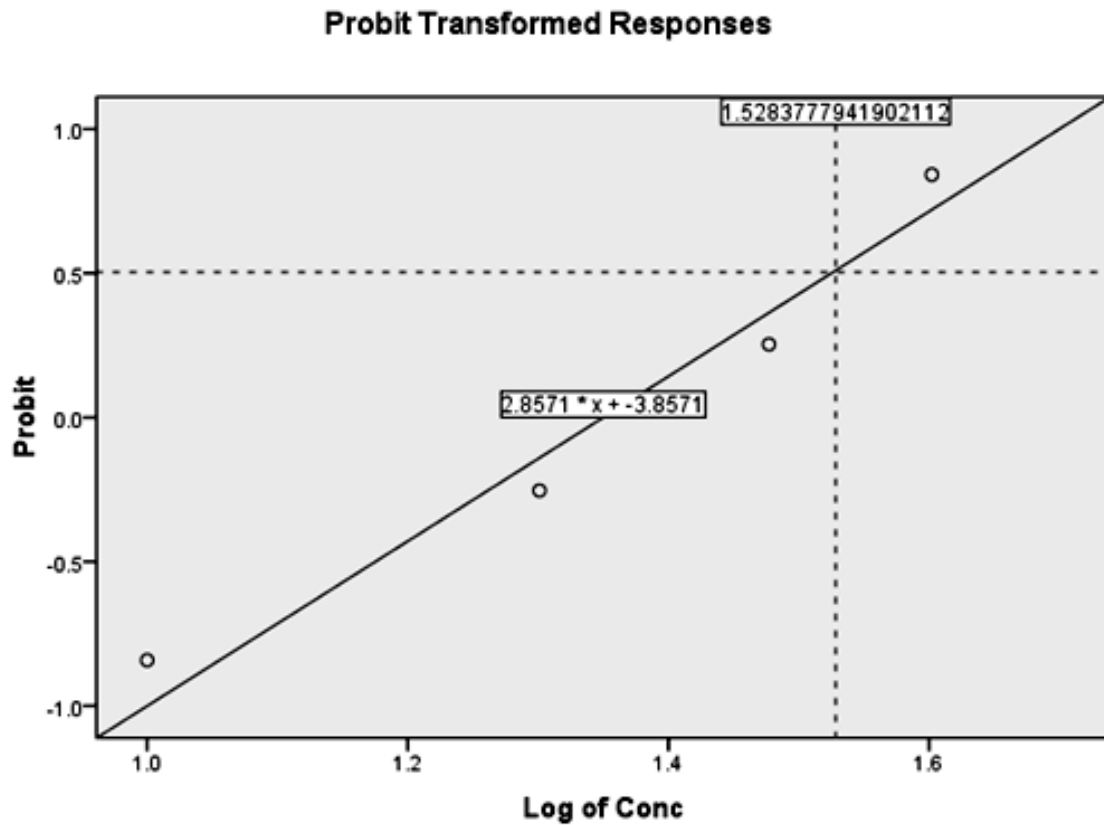


Figure 1: Linear relationship between mean probit mortality and log concentration in *C. gariepinus* juveniles exposed to bilge water for 96 hours

DISCUSSION

The physicochemical characteristics of the bilge water sample used in this study showed a number of deviations from national and international specifications for maximum limit allowed for effluent discharge into water bodies (NESREA, 2009; USEPA, 2009). The toxicity of the wastewater could be attributed to the synergistic effects of the pollutants which could affect the eco-physiology of the fish. Similar observations have been reported with agricultural and pharmaceutical effluents (Adewoye *et al.*, 2005; Agboola and Fawole, 2014). The unpleasant odour of the bilge water may have resulted from the biodegradation activities of anaerobic bacteria of organic matter, possibly facilitated by the low dissolved oxygen and high biochemical oxygen demand of the wastewater (Adewoye *et al.*, 2005).

Kazlauskienė *et al.* (2012) has argued that the assessment of water pollution using only physicochemical methods is not sufficient to provide integrated information on the effects of

pollutants on aquatic life because toxicity is a biological characteristic. The fish in this study gasped for breath prior to mortality. This observed respiratory abnormality is in agreement with results obtained by Dahunsi and Oranusi (2013) for the same test organisms exposed to rubber processing effluents. Also observed was the erratic swimming pattern and motionlessness of the fish, which 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).

Several investigators have reported similar results on the concentration-dependent increase in mortality rates for the African fish exposed to agricultural effluents (Adewoye *et al.*, 2005), resin effluent (Dahunsi and Oranusi, 2012), rubber processing effluents (Dahunsi and Oranusi, 2013) and pharmaceutical effluents (Agboola and Fawole, 2014). The degree of acute toxicity values differ with different types of pollutants and this could be attributed to differences in the nature of the pollutant, age of the organism and

environmental conditions (Ayuba *et al.*, 2013).

In conclusion, results obtained in the study has shown that bilge water induced acute toxicity in *C. gariepinus* juveniles. Precautions must therefore be taken in the manner in which bilge water is disposed into the aquatic ecosystem.

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REFERENCES

- Adewoye, S.O., Fawole, O.O., Owolabi, O.D. and Omotosho, J.S. 2005. Toxicity of cassava wastewater effluent to African catfish: *Clarias gariepinus*. *Ethiopian Journal of Science* 28(7): 189-194.
- Agboola, O.A. and Fawole, O.O. 2014. Chronic toxicity of pharmaceutical effluent to *Clarias gariepinus* (Burchell, 1822). *Covenant Journal of Physical and Life Sciences (CJPL)* 1(2): 27-42.
- Ahmad, Z. 2012. Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. *African Journal of Biotechnology* 11(34): 8578-8585.
- American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF) 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st Ed. American Public Health Association, Washington DC, 1200p.
- Ayuba, V.O., Iyakwari, S.P. and Oyeniyi, M.E. 2013. Acute toxicity of formalin on *Clarias gariepinus* juveniles. *Production, Agriculture and Technology* 9(1): 21-28.
- Dahunsi, S.O. and Oranusi, U.S. 2012. Acute toxicity of synthetic resin effluent to African catfish, *Clarias gariepinus* [Burchell, 1822]. *American Journal of Food and Nutrition* 2(2): 42-46.
- Dahunsi, S.O. and Oranusi, U.S. 2013. Haematological response of *Clarias gariepinus* to rubber processing effluent. *Annual Review and Research in Biology* 3(4): 624-635.
- Han, M., Chen, Y., He, F. and Yu, L. 2014. Treatment of oily wastewater with WPO and CWO. *Journal of Korean Chemical Society* 58(1): 68-71
- Igbokwe, M.I. 2001. Assessment of existing national legislations and regulations related to pollution prevention. A paper presented at the National Workshop on MARPOL 73/78 Ratification, Implementation and Enforcement. Organised by International Maritime Organisation in conjunction with Federal Ministry of Transport, 27-29 August at Le Meridien Eko Hotel, Victoria Island, Lagos. pp. 15.
- International Maritime Organisation/Nigeria Maritime Administration and Safety (IMO/NIMASA) 2013. Report of the National Workshop on the London Protocol in Nigeria, Lagos, 26-28 February, 2013. 42p.
- Kazlauskienė, N., Svecevičius, G., Marciulionienė, D., Montvydiene, D., Kesminas, V., Staponkus, R. and Taujanskis, A. 2012. The effect of persistent pollutants on aquatic ecosystem: A complex study. *Baltic International Symposium (BALTIC)*, IEEE/OES, Klaipėda 8-10 May, 2012 pp.1-6.
- National Environmental Standards and Regulation Enforcement Agency (NESREA) 2009. Federal Republic of Nigeria Official Gazette, National Environmental (Sanitation and Waste Control). Federal Government of Nigeria Printer, Abuja, Nigeria, FGP 112/102009/L000 (OL54). No.60 (96). pp.1057-1102.
- Nigerian Ports Authority (NPA) 2014. Nigerian Ports Authority Press Releases, 26/28 Marina, Lagos. Available online: <http://www.nigerianports.org/news.aspx?id=375> (Accessed on 11 November, 2014).
- Okayi, R.G., Annune, P.A., Tachia, M.U. and

- Oshoke, O.J. 2010, Acute toxicity of glyphosate on *Clarias gariepinus* fingerlings. *Journal of Research in Forestry, Wildlife and Environment* 2(2): 150-155.
- Okayi, R.G., Daku, V. and Mbata, F.U. 2013, Some aquatic macrophytes and water quality parameters of River Guma, Benue, Nigeria. *Nigerian Journal of Fisheries and Aquaculture* 1(1): 25 - 30
- Okomoda, J., Ayuba, V.O. and Omeji, S. 2010. Hematological changes of *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to acute toxicity of formalin. *Production, Agriculture and Technology* 6(1): 92-101
- Poulopoulos, S.G., Voutsas, E.C., Grigoropoulou, H.P. and Philippopoulos, C.J. 2005. Stripping as a pretreatment process of industrial oily wastewater. *Journal of Hazardous Materials* 117 (2-3): 135-139.
- Randhawa, M.A. 2009. Calculation of LD₅₀ values from the method of Miller and Tainter, 1944. *Journal of Ayub Medical College Abbottabad* 21(3): 184.
- United Nations Environmental Programme (UNEP) 1989. Estimation of the Lethal Toxicity of Pollutants in Marine Fish and Invertebrates. *Reference Methods for Marine Pollution Studies* No. 43, 27p.
- United States Environmental Protection Agency (USEPA) 1999. National Recommended Water Quality Criteria – Correction: EPA 822/Z – 99 -001, USEPA. Washington DC.
- United States Environmental Protection Agency (USEPA) 2009. Drinking Water Contaminants. Washington, DC, USA. (Available online : <http://water.epa.gov/drink/contaminants/index.cfm#Lis>.)
- Werschkun, B., Banerji, S., Hofer, T. 2014. Emerging risk from ballast water treatment: The run-up to the International Ballast Water Management Convention. *Journal of Chemosphere* 112: 256-66.