PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 23 (6) 1169-1174 June 2019

Acute Toxicity of Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

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ABSTRACT: This research was conducted to determine the lethal concentration (LC_{50}) of benzalkonium chloride (BAC) mixture with treated produced water capable of causing mortality of at least 50% of the Nile-tilapia-*Oreochromis niloticus*juveniles in a static 96h acute toxicity. Juveniles of mix sexes of *O. niloticus* of average length (6.80 cm) and weight (25.05g) were exposed to various concentrations (20.20, 50.50, 101.00 and 202.00 ppm.) of the toxicant with tap water used as control under laboratory condition. The LC_{50} values at 24, 48, 72 and 96hours were determined to be 96.427, 73.799, 73.799 and 69.772 ppm, respectively. Water quality check and general fish behaviour (respiratory distress, loss of balance, erratic swimming and death) were observed. Regression equation(y =-0.270 + 93.44) and correlation (R^2 = 0.751) obtained from the study depict direct positive relationship between fish mortality and BAC mixture. Hence, unregulated release of benzalkonium chloride and treated produced water into aquatic environment is capable of causing acute toxicity to juveniles of *O. niloticus*. Its disposal into the environment should therefore be discouraged.

DOI: https://dx.doi.org/10.4314/jasem.v23i6.26

Dates: Received: 17 April 2019; Revised: 19 May 2019; Accepted 22 June 2019

Keywords: Acute-toxicity, LC_{50}, Benzalkonium-chloride-mixture, Nile tilapia

Environmental pollution emanating from human industrial activities is said to have commenced in the early part of the twentieth century following industrialization. The insatiable needs of humans for industrialization and development resulted in various inventions and the manufacture of synthetic and semi-synthetic compounds that have a very wide array of application such as oil and gas, agriculture (use of pesticides and herbicides), cosmetics, pharmaceuticals and health and personal care products etc. These actions of man have engendered prosperity and well-being over time. However, the continuous exploration and exploitation of natural resources within the environment not only brought prosperity and development but also resulted in environmental degradation of various degrees. One of such chemicals that have a very wide range of application is benzalkonium chloride (BAC) which belongs to a parent group of quaternary ammonium compounds (QACs).The benzalkonium chloride (BAC) has a very wide range of application such as oil and gas (used as biocides and corrosion inhibitor), agriculture (used as pesticides and herbicides), cosmetics, pharmaceuticals and health and personal care products (USEPA 2006).The QACs are classified as cationic surfactants used as disinfectants in various industrial, medical and domestic application. These chemicals are known for their pollutant properties in wastewater and host aquatic habitat (Zhang *et al*., 2015). The toxic potentials of these compounds are dependent on their capability to interrupt membrane reliability through interaction with membrane lipids and /or trans-membrane protein, giving rise to harmful impacts on exposed organisms (Eleftheriadis *et al*., 2002; Tischer *et al*., 2012). Furthermore, some QACs have been known to trigger genotoxic impacts in mammalian and plant cells (Ferk *et al*., 2007) as well as crustaceans (Lavorgna *et al*., 2016). Also, as a result of their eco-toxicological characteristics and relative abundance in wastewaters and aquatic habitats, quaternary ammonium compounds are of great environmental apprehensions. Consequently, the anthropogenic activities of man emanating from petroleum hydrocarbon exploitation have resulted in the discharge of different levels of pollutants into both aquatic and terrestrial environment resulting in a deleterious effect on the biota. One of these chemical constituents of oil and gas exploration is the produced water generated from the formulation process.

The introduction of large volumes of generated contaminants into the natural environment has become a major apprehension to environmentalists. Studies revealed that, annually several tones of pharmaceutical

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and municipal solid wastes are generated and disposed (Gualtero, 2005). The discharge of petroleum hydrocarbon and xenobiotic compounds into the environment is now a major concern for both the public, regulatory bodies and scientific world due to their cancer causing and genetic alteration capabilities, genotoxicity and toxicity possibility (Randhawa and Kullar, 2011). This research therefore, was aimed at investigating the acute toxicity potential of benzalkonium chloride (BAC) mixture with treated produced water (TPW) to freshwater tilapia-*Oreochromis niloticus.*

MATERIALS AND METHODS

Sample Collection and Acclimatization: A total of one hundred juveniles of freshwater tilapia-*Oreochromis niloticus* were obtained from the African Regional Aquaculture Centre (ARAC), Aluu, Port Harcourt, Rivers State, Nigeria in the early hours of the day and transported to the experimental lab (aquarium) at the Department of Animal and Environmental Biology, University of Port Harcourt, Rivers State in four 50Ltr black plastic containers with an open end containing sufficient habitat water to sustain fish life. The fish of average weight (25.05g) were acclimatized for 14 days in two (2) large glass holding tanks measuring 100 x 100 x 50 cm with well aerated de-chlorinated water. During the acclimatization period, the fish were fed twice daily (8:00 am and 4pm) at3% total body weight with commercial feeds (Coppens) obtained from source. Mortality rate was below 3% during acclimatization as the water was renewed every 24 hours to eliminate hoarded faecal material and unconsumed feed. Feeding was discontinued 24 hours prior to 96hours (acute) exposure.

Experimental Treatments: Procedure followed recommendations of UNEP (1989). After acclimatization, initial rage finding test was conducted to determine appropriate concentrations for the 96 hours experiment. Four concentrations with control in triplicate were prepared as follows; 0.00 (control), 20.20, 50.50, 101.00 and 202.00 ppm. 5 juveniles of the fish were randomly collected and stocked in each of the 15 glass containers measuring 40x25x25 cm with 0.25mm diameter with opening covered by means of fastened wire gauge were used for the experiment. A static bioassay was employed to establish the toxicity of benzalkonium chloride mixture with treated produced water for *O. niloticus* juveniles.

Analytical Procedure: Following exposure, fish behaviours were monitored from the beginning and at 6 hours interval. Water quality check was done at every 24 hours by means of physicochemical parameters (Temperature, pH, Dissolved Oxygen, Salinity and Chemical Oxygen Demand) analysis according to methodology of APHA *et al.,* (1985). Fish were considered dead and isolated as soon as possible when the opercula and tail movements stopped and there was no response to a gentle prodding.

Data Analysis: The 96h $LC_{50}$was determined as a probitanalysis using the arithmetic method of percentage mortality as described by UNEP (1989). Results obtained were subjected to statistical analysis with SPSS to test for significant difference ($P < 0.05$) between the various concentrations of bezalkonium chloride mixture with treated produced water and the control.

RESULTS AND DISCUSSION

The mean and standard deviation values and mean range as compared with Federal Ministry of Environment values of the physicochemical parameters analysis are as presented in Tables 1 and 2 while the mean and percentage values are presented in Table 3.The lethal concentration (LC_{50}) values of the 96h toxicity and its corresponding plot are presented in Table 4 and Figure 1, respectively. Consequently, the corresponding plots of the physicochemical parameters (pH, Temperature, Dissolved Oxygen, Salinity and Chemical Oxygen Demand) analysis of before and during 96h exposure are presented in Figures 2, 3, 4, 5 and 6, respectively. It was observed that variable degree of fish mortality was recorded across exposure time at different concentrations. However, it observed that fish in control experiment survived the 96 h exposure duration. The 96h LC_{50} of *O. niloticus* juveniles exposed to various concentrations of benzalkonium chloride (BAC) mixture with treated produced water was69.772ppm.The regression equation of the relationship was calculated to be probit y=-0.270 + 93.44, log concentration and on R-square value R^2 = 0.751This expression i.e. the regression equation, R^2 value indicates that mortality rate of fish increased with increase in concentration of benzalkonium chloride (BAC) mixture with treated produced water.

The physicochemical study revealed that during exposure pH, Dissolved Oxygen (D.O) and salinity (figures: 3, 4 and 5) decreased with increasing concentration of the toxicant (benzalkonium chloride (BAC) mixture with treated produced water while Temperature and Chemical Oxygen Demand (figures: 2 and 6) increased with increasing concentration at the end of 96hour (acute) exposure of tilapia-*Oreochromissniloticus* to the toxicant. In a similar vein, physicochemical parameters analysis of test medium (test water) prior to exposure showed similar trend except Salinity that slightly deviated in trend with increase.

Table 1: Mean and Standard Deviation Values of Physicochemical Analysis during (96h) Exposure

Parameters	Control	20.20 ppm	50.50 ppm	101.00ppm	202.00ppm
pH	7.67± 0.56	7.52± 0.22	7.47 ±0.28	7.36±0.24	7.28± 0.18
Temp(°C)	27.75 ±0.42	27.73 ±0.36	27.8 ±0.34	27.8 ±0.41	27.83 ±0.35
DO(mg/l)	4.43 ±1.28	4.4± 1.22	4.3 ±1.04	4.03 ±1.15	3.73 ±1.89
Salinity(ppt)	0.04 ±0.01	0.05± 0.02	0.04± 0.02	0.04± 0.01	0.02 ±0.01
COD(mg/l)	2.41± 1.11	1.25± 0.58	1.54± 0.66	1.54± 0.67	1.54± 0.67

Table 2: Mean Physicochemical Parameters Range of before and during (96 hours) Exposure Compared to FME Standard

Parameters	Before Exposure	During Exposure	FME Standard
Temp. (°C)	27.0 – 27.4	27.73 – 27.83	20 – 33
pH	6.2 – 6.95	7.28 – 7.67	6 – 9
D.O (mg/l)	4.5 – 6.6	3.73– 4.43	6.8
Salinity (ppt)	0.01 - 0.23	0.02 – 0.05	
COD (mg/l)	0.96 – 5.32	1.25 – 2.41	

Table 3: Acute Toxicity Mortality Values in Mean and Percentage

Conc. (ppm)	Mortality Time (Hours)							
	24	%	48	%	72	%	96	%
0.0 (control)	0	0	0	0	0	0	0	0
202.00	5	100	5	100	5	100	5	100
101.00	4	80	5	100	5	100	5	100
50.50	0	0	1	20	1	20	1	20
20.20	0	0	0	0	0	0	0	0

The decreasing trend of some physicochemical parameters during exposure was in agreement with the reports of Warren, (1977) who states that the release of toxic substance into aquatic habitat could result in the reduction of D.O which in return could lead to asphyxiation in fish.

Table 4: The LC_{50} (ppm) Values of Acute Toxicity Bioassay

Time (Hours)	LC_{50} (ppm)
24	91.427
48	73.799
72	73.799
96	69.772

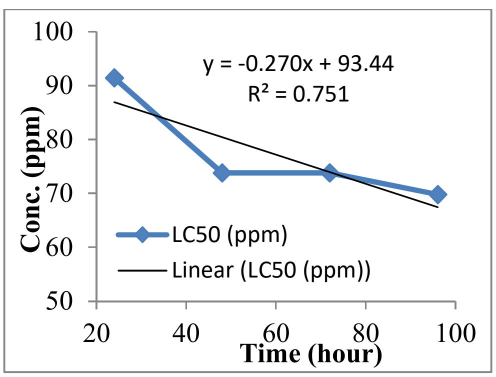

Fig 1: Plot of LC_{50} in a 96 Hours Acute Toxicity

In this study, it was observed that dead fish exhibited mucus accumulation on body surfaces and gill filaments. This observation was in accordance with the reports of Omitoyin *et al*., (2006) and Adesina, (2008) who stated that flux of toxic substance on gill epithelium is a function of rise in ventilation. Adesina, (2008) stated in his report that high harmful impacts of most toxic substances on fish is as a result of low dissolved oxygen (D.O).

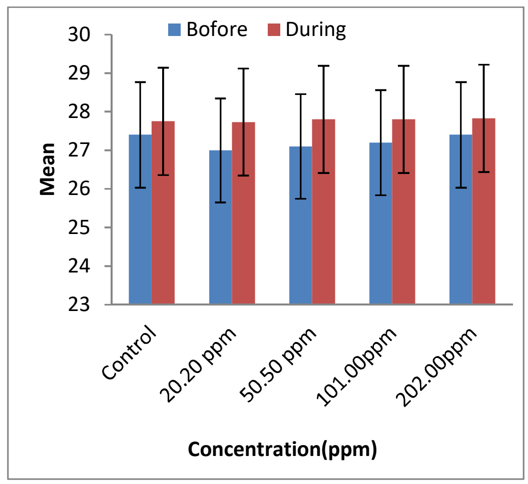

Fig. 2: Effect of Temperature on concentration before and after 96h exposure to Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

In a related report, Rahman *et al.,* (2002) reiterated that different degrees of behavioural changes (loss of balance, erratic swimming, respiratory distress and vertical movement and death) manifested by fish in the course of toxicity study is a pointer that fish mortality could be as a result of insufficient oxygen supply. According to the report Ndimele *et al*., (2010) and Lawson *et al*., (2011) temperature increase across increasing concentration could be due to increase metabolic activity and osmo-regulatory mechanisms exhibited by the test fish in an attempt to counterbalance the impact of the toxicant. However, reduction in pH with increasing concentration could result in bio-concentration of unionized ammonia which is lethal to fish continued existence.

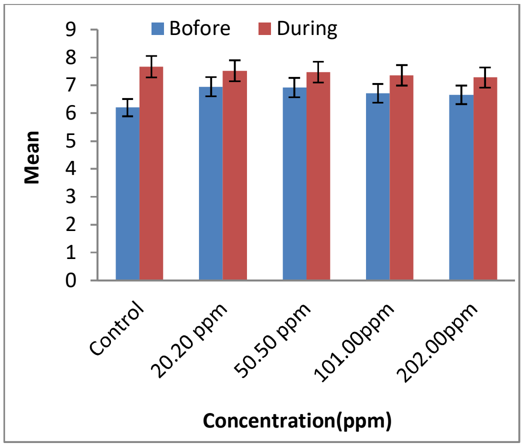

Fig. 3: Effect of pH on concentration before and after 96h exposure to Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

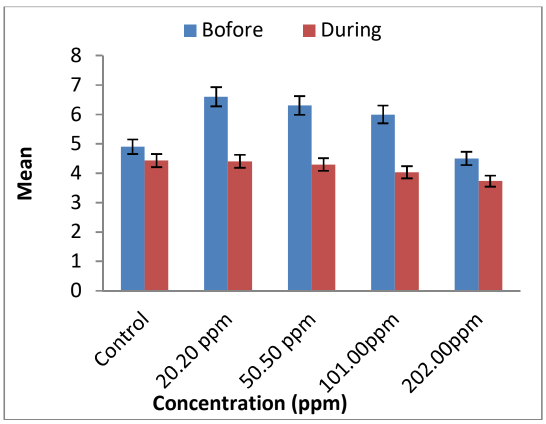

Fig. 4: Dissolved Oxygen on concentration before and after 96h exposure to Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

The 96hour LC_{50} value (Table: 4) determined from the bioassay showed that *O. niloticus* juveniles were sensitive to benzalkonium chloride mixture (with treated produced water).The regression equation obtained from the study was y =-0.270 + 93.44, R^2 = 0.751(Figure: 1) which when compared with y = 1.38 +7.84x R^2 = 0.84 as reported by Kori-Siakpere (2008) showed slight disparity. This regression disparity could be as a result of the variation in fish weight and toxicant despite the positive correlation that existed between mortality rate of *O. niloticus* juveniles and increasing concentrations of benzalkonium chloride mixture (with treated produced water). Ezekiel and Benedict (2008) reported 96h LC_{50} of aqueous extracts of pawpaw seed powder to *O. niloticus* as 4.2mg/l with 95% confidence (lower and upper) limits of 31.86 – 93.81 mg/l.

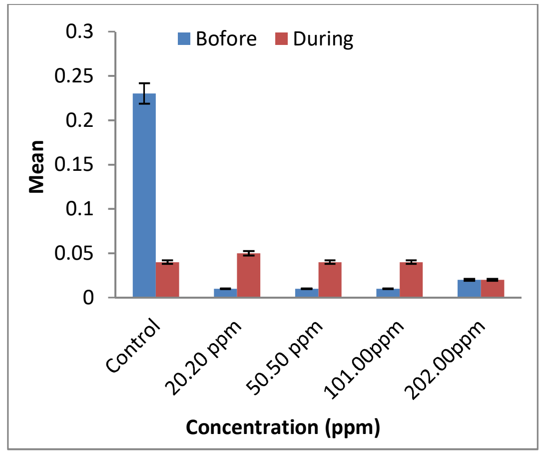

Fig. 5: Salinity on concentration before and after 96h exposure to Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

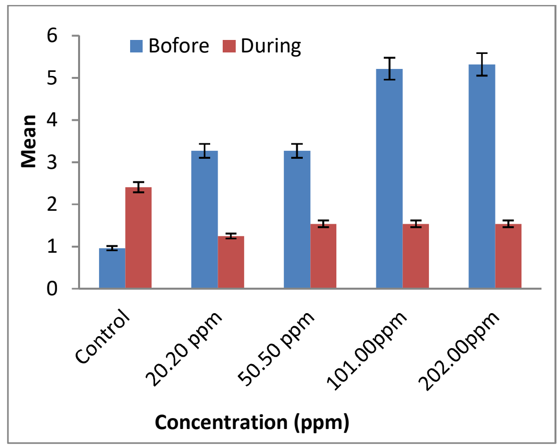

Fig 6: Chemical Oxygen Demand on concentration before and after 96h exposure to Benzalkonium Chloride Mixture with Treated Produced Water to Juveniles of Freshwater Tilapia- *Oreochromis niloticus*

Consequently, Annune *et al.,* (2002) reported 96h LC_{50} of extracts of Ringworm plant *Sennaalata* to *O. niloticus* juveniles as 13.93 mg/l.While Wade *et al.,* (2002) who reported 96h $LC_{50}$of 0.19mg/l for the *O. niloticus*exposed to cassava (*Manihot esculenta*) toxic. However, the variation in the 96hour LC_{50} between findings of this study (69.772ppm) and those of cited researchers could be as a result of variation in certain study specific factors such as toxicant, fish age and environmental conditions.

The impacts of a toxicant on any aquatic biota could be classified into one of the following classes of toxicity viz; acute, chronic, sub chronic and delay toxicity. In this study, it was revealed that abnormal behaviour exhibited by exposed fish which eventually led to fish mortality are manifestations of toxic potentials of toxicant (benzalkonium chloride mixture (with treated produced water).

Conclusion: The 96h LC_{50} obtained from the acute exposure of *O. niloticus* juveniles to benzalkonium chloride mixture with treated produced water) was 69.772ppm as compared with 2mg/l, 13.93 mg/l and 0.19mg/l cited by various researchers. Hence, unregulated release of benzalkonium chloride and treated produced water into aquatic environment should be discriminated.

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