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Acute toxicity of Tilapia (*Oreochromis niloticus* TREWAVAS) exposed to Cassava (TMS 30572) Processing Wastewater

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Abstract

Acute toxic effects of cassava processing waste water on juveniles of tilapia, *Oreochromis niloticus* (14.67±0.5 g) was investigated in a static bioassay. Six (6) treatments with control inclusive were used at different concentrations of 0.94mg/l, 2.80mg/l, 4.70mg/l, 6.60mg/l, 8.40mg/l and 0.00 in triplicate. Ten (10) fish were stocked per 20lt capacity plastic tank. Physico-chemical parameters were monitored. The 96-hrs LC_{50} is 2.82mg/l. Results obtained were subjected to statistical analysis of variance (ANOVA) method to test for the level of significance ($p < 0.05$) between control and treatments. Experimental fish exhibited skin hemorrhages, loss of scales, restlessness, uncoordinated swimming behavior, settling at the bottom of the tank and eventually, death. Mucus accumulation was observed on body surfaces and gill filaments of the dead fish. The 96-hr LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality data.

Keywords: Tilapia, *Oreochromis niloticus*, Acute toxic effects, Processing wastewater.

INTRODUCTION

Cassava is a good source of carbohydrates in the world (FAOSTAT, 2012) and is also a major staple food in the developing countries such as Nigeria, providing a basic diet for over half a billion of people. The technology of processing cassava roots involves essentially; peeling, grating, fermenting, pressing out water and sieving, these processes generate liquid and solid wastes that are hazardous to the environment (Jyothiet *al.*, 2005; Cumbanaet *al.*, 2007).

Like other roots and tubers, cassava contains anti-nutritional factors and toxins (FAOSTAT, 2010). Cassava contains cyanogens and glycosides that are hydrolyzed into hydrogen cyanide (Oti, 2002; Arimoroet *al.*, 2008) and cyanide is one of the most toxic chemicals to fish if cassava effluent is released into the nearby water bodies.

During the process of producing starch from cassava (*Manihot esculenta*) roots, large amounts of cyanoglycosides are released, which rapidly decay to CN⁻ following enzymatic hydrolysis. Depending on the varying cyanoglycoside content of the cassava varieties, the cyanide concentration in the wastewater is as high as 200mg/l (Siller and Winter 1998). Cyanides are harmful substances readily absorbed by inhalation, and in oral and dermal routes of exposure as well as in the aquatic environment (Adekunleet *al.*, 2007). This causes a stress condition in aquatic lives and fish. Adewoyeet *al.* (2008) in the study on the effect of cassava wastewater on *Clarias gariepinus* observed signs of serious stress, changes in swimming pattern and mortality of the observed fish thereby submitted that deposition of cassava wastewaters into aquatic environments may impair the natural population size while the consumption of fish from such environment is deleterious. This submission corroborated the reports of Adekunleet *al.* (2007) which stated that acute exposure to high concentrations of cassava wastewater results in impaired respiratory activities, growth retardation and death of fish.

In this study, the 96hrLC₅₀ was studied using tilapia *Oreochromis niloticus* juvenile exposed to acute concentration of cassava processing waste water for 96hrs.

MATERIALS AND METHODS

The study was carried out in the Fisheries Department laboratory Federal University of Agriculture Makurdi located on latitude 7° 46'N and longitude 8° 29'E. Makurdi has two main seasons: the wet season usually between April to September and the dry season usually between October to March. Average annual rainfall is between 1000 to 1500mm (Stock, 2006). Juveniles of tilapia *Oreochromis niloticus* (14.67±0.5g) obtained from the Departmental Fish Farm were used for this investigation. The fish were acclimatized to laboratory condition for fourteen (14) days before the exposure period. During the acclimatization period the fish was fed twice daily at 0800 and 1600 hrs at 5% of their body weight with coppers pellets. Feeding was stopped 48 hours before and during the exposure period. Freshly harvested cassava TMS 30572 (*Manihot esculenta*) tubers were obtained from cassava farmers in Agan, Makurdi. The tubers were peeled, weighed using metler Toledo (k) and washed with water out of which 3kg was removed and then immersed wholly in a 20liter capacity plastic containing 5litres of deionized water for fermentation for 3 days at ambient temperature (32°C). After fermentation, the softened cassava product is removed from the water and grinded in the feedmill. The white slurry was collected in a sack and the waste water was squeezed into a receiving plastic container as described by Adekunle *et al.* (2007). Stock solution of cassava was prepared by collecting one litre of the 100% cassava processed waste water. From this stock solution, various concentrations used in the investigations were prepared by dilution. Cassava processing waste water concentrations used for the acute test was 0.94mg/L, 2.80mg/L, 4.70mg/L, 6.60mg/L, and 8.40mg/L. These percentages were derived from the cassava processing waste water tolerance tests and dilution series previously conducted on fish species. A total of six (6) treatments control inclusive, all in triplicate was used. A total of one hundred and eighty (180) tilapia *Oreochromis niloticus* juveniles (14.67±0.5g) were randomly distributed ten fish per experimental tank. Syringe and needle was used to collect the cassava processing waste water into the treatment tanks. Water quality parameters total dissolved solids, electrical conductivity, pH, and temperature were determined using (Hanna HI 91284) multi parameter water quality checker, dissolved oxygen was checked using the method of (APHA, 1985). This was done before and after the test. Behavioral changes and mortality was observed and recorded after 0min, 30min, 1, 2, 4, 8 and subsequently every 12 hours, for the next 96hrs. The inability of fish to respond to gentle prodding was used as an index of death. Results obtained was subjected to statistical analysis of variance (ANOVA) method to test for the level of significance ($p < 0.05$) between control and treatments. The 96-hr LC₅₀ was determined as a probit analysis using the arithmetic method of percentage mortality data as described by UNEP (1989).

RESULTS

At high concentrations of 6.60mg/l and 8.40mg/l, the fish swarm in an uncoordinated manner, became very weak, gulping for air at the surface of water exhibited skin hemorrhages and settled at the bottom. Restlessness and settling at the bottom of the tank was observed in concentrations 4.70, 2.80 and 0.94mg/l. At 8.40mg/l, 96.67% mortality was recorded, at concentrations of 6.60, 4.70, 2.80 and 0.94mg/l, mortality recorded was 80.0%, 56.7%, 36.7% and 23.3% respectively, increase in concentration of the cassava waste water resulted in higher mortalities, Mucus accumulation was observed on the body surfaces and gill filaments of dead fish. In the control group no mortality was recorded throughout for 96hours. Results obtained are presented.

Table 1: Acute toxicity of cassava TMS 30572 processing waste water on *Oreochromis niloticus* juveniles for 96hours.

Conc mg/l	Log Conc	No. of Fish Stocked	Mortality @ 96 Hrs	% Mortality	Probit
0.00	0	30	0	0	0
0.94	-0.0269	30	7	23.33	4.26
2.80	0.4472	30	11	36.67	4.67
4.70	0.6721	30	17	56.67	5.18
6.60	0.8195	30	24	80	5.84
8.40	0.9243	30	29	96.67	6.88

The relationship between log Concentration and Probit Kill of *Oreochromis niloticus* Juveniles for 96 h LC₅₀ is presented in Figure 1. The LC₅₀ value obtained from the Relationship between Probit Kill and Log Concentration for 96 h LC₅₀, based on regression analysis was found to be 2.82mg/l. The regression equation for the relationship was calculated to be $\text{probit } y = 2.425x + 3.990$, log concentration and on R-square value, $R^2 = 0.786$. The expression, R^2 value indicates that, mortality rate of fish increased with increase in concentration of cassava waste water.

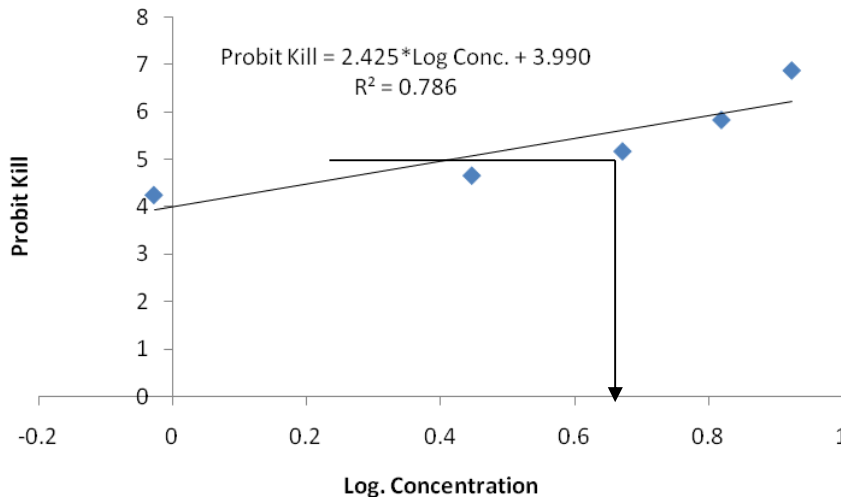


Figure 1: Linear Relationship between Log Concentrations of Cassava TMS 30572 processing waste water and Probit kill of *Oreochromis niloticus* Juveniles.

The result of Water quality parameter obtained during exposure of *O. niloticus* juveniles to acute concentrations of cassava waste water for 96hrs. Total dissolved solids, electrical

conductivity, pH, Temperature and Dissolved Oxygen varied significantly ($p < 0.05$) from the control. Dissolved oxygen varied significantly ($p < 0.05$) from 5.50mg/l to 3.03mg/l with increased concentration. Temperature varied from 26.90°C to 26.55 °C with increased concentration. pH varied slightly from 9.05 to 9.09 with increased concentration. Total dissolved solids varied significantly from 302mg/l to 347 mg/l with increased concentration. Electrical conductivity varied significantly ($p < 0.05$) from 606 μ S/cm to 689 μ S/cm with increased concentration of cassava waste water as presented

Table 2: Water Quality Parameters of Acute Concentrations of Cassava Processing Waste Water for 96 hours on tilapia *Oreochromis niloticus* Juveniles.

	Water Quality Parameters				
	TDS(mg/l)	EC (μ S/cm)	pH	T(°C)	DO(mg/l)
Concentrations of wastewater (mg/l)					
0.00 (Control)	302.00 \pm 1.00 ^a	606.00 \pm 1.00 ^a	9.05 \pm 0.02 ^{bc}	26.90 \pm 0.10 ^b	5.50 \pm 0.06 ^d
0.94	310.50 \pm 0.50 ^b	627.50 \pm 1.50 ^b	8.24 \pm 0.51 ^a	26.90 \pm 0.20 ^b	5.35 \pm 0.05 ^d
2.80	313.00 \pm 1.00 ^b	629.50 \pm 1.50 ^b	9.03 \pm 0.01 ^b	26.40 \pm 0.10 ^{ab}	5.27 \pm 0.15 ^{cd}
4.70	321.50 \pm 0.50 ^c	636.00 \pm 1.00 ^c	9.06 \pm 0.01 ^{bc}	26.60 \pm 0.10 ^b	5.18 \pm 0.08 ^c
6.60	341.00 \pm 1.00 ^d	681.50 \pm 1.50 ^d	9.12 \pm 0.01 ^c	26.10 \pm 0.10 ^a	4.07 \pm 0.03 ^b
8.40	347.50 \pm 0.50 ^e	689.00 \pm 1.00 ^e	9.09 \pm 0.01 ^{bc}	26.55 \pm 0.15 ^b	3.03 \pm 0.03 ^a

Means on the same column with different superscript are significantly different ($p < 0.05$)

TDS=Total Dissolved Solids, EC=Electrical Conductivity, T=Temperature, DO=Dissolved Oxygen

DISCUSSION

The fluctuations of the physico-chemical parameters showed no significant difference ($p < 0.05$) between the different treatments and control, the effects on this study could be negligible. However, the wide fluctuations with significant difference might have been altered by the experiment and hence produced deleterious effect such as stressful conditions of abnormal behaviors prior to death and mucus secretion on the gills of the moribund fish. In the study, significant difference ($p < 0.05$) was observed in the values of total dissolved solid (TDS), electrical conductivity (EC) and dissolved oxygen (DO) between control and various treatments. The TDS and EC increases with increasing concentrations of cassava processing waste water indicating electrolyte concentrations and organic matter content. These are in agreement with literature reports on the effects of cassava effluent on water quality which (Bengtson and Triet 1985; Wade *et al.*, 2002). While DO decreases considerably with increasing concentrations, the reduction in the value of DO is in line with the report of Gabriel *et al.*(2007) who observed same in *Clarias gariepinus* exposed to proxone under laboratory condition. This may be due to stress induced condition by the cassava processing waste water which results in agitation and abnormal behavior of the test fish and thereby reduces the DO level of the experimental water. No changes were observed in the values of pH and Temperature between control and the treatments, the result agreed with that of Nteet *et al.*(2011) who reported no significant difference ($p < 0.05$) in water quality in the experimental water on

exposure to *Sarotherodon melanocephalus* industrial effluent. The 96-hour LC₅₀ of cassava processing waste water to juveniles of *Oreochromis niloticus* is 2.82mg/l, this value is lower than the result of Adekunle *et al.*, (2007) who reported the 96 h LC₅₀ of 0.25% and 0.45% for *Oreochromis niloticus* and *Clarias gariepinus* exposed to cassava effluent the reason may be as a result of higher toxicant concentration in the cassava effluent. and that of Wade *et al.* (2002) who reported that the 96-hour LC₅₀ of 0.19mg/l-1, for the Nile tilapia *Oreochromis niloticus*, exposed to the toxicity of cassava (*Manihot esculenta*), the reason may be as a result of environmental factors, age and size of the fish. The 96-hour LC₅₀ of Atrazine administered to *Oreochromis niloticus* in water was 7.2mg/l as reported by (Fidelis *et al.*, 2012) which is higher than the present study the difference may be attributed to difference of toxicant and environment used. The test fish showed various behavioral changes at different cassava waste water concentrations. The behavioral changes observed were gulping for air at the surface of water, uncoordinated swimming, settling at the bottom of the tank, restlessness, loss of scale, skin hemorrhage and death. Mucus accumulation was observed on the body surfaces and gill filaments of dead fish. The type and duration of the behavioral changes increased with increased concentrations. No behavioral changes or death occurred in the control group at any time during the experiment. All control fish were active and swam normally.

CONCLUSION

Results of the acute toxicity of *O. niloticus* juveniles exposed to cassava processing waste water for 96 hours shows 96 hours LC₅₀ of 2.82mg/l. Mortality was recorded at all the acute concentrations and increased from 23.3% at lowest level 0.94mg/l to 96.7% at highest level 8.40mg/l. No mortality was observed in control group, the cassava waste water used appear to have deleterious toxic effects which increases with increased dosage on *O. niloticus* juvenile.

RECOMMENDATION

Due to acute effects of cassava processing waste water which causes 50% mortality of the test fish after 96hrs on exposure to 2.82mg/l, it is imperative that the local cassava processors and cassava processing industries are educated on the toxic effect of the waste water they are generating on aquatic lives around them and disposal of such toxic wastewater without proper treatment should be discouraged.

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