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Evaluation of Acute Toxicity of Cassava Effluent on the African Catfish [*Clarias gariepinus* (Burchell 1822)] and Freshwater Clam [*Egera radiata* Lam (Bivalvia, Donacidae)]

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ABSTRACT: The indiscriminate discharge of the wastewater generated during the processing of *Manihot esculenta* Crantz into the environment or public sewers remains a source of concern. In this study, the acute toxicity (96-h LC_{50}) of cassava effluent was evaluated using *Clarias gariepinus* juveniles and *Egera radiata*. The two organisms were exposed to different concentrations of the effluent using a renewable static bioassay with continuous aeration. The LC_{50} of exposed *C. gariepinus* juveniles was found to be 1.92 ml/L with lower and upper confidence limits of 1.35 ml/L and 2.19 ml/L, while that of *E. radiata* was 1.17 ml/L with lower and upper confidence limits of 0.72 ml/L and 1.29 ml/L respectively. The relationship between exposure concentrations and mortality of juvenile *C. gariepinus* indicate a dose dependent relationship. On the contrary, the relationship between toxicant exposure concentration and mortality of the clams showed a polynomial or biphasic relationship. The biphasic mortality curve exhibited by exposed clams suggests the possibility of adaptive responses occurring at higher effluent exposures. The mean 96 h LC_{50} values of the freshwater African catfish and freshwater clams exposed to the cassava mill effluent indicate that the shellfish are more sensitive to the effluent.

Keywords: Acute toxicity, Cassava effluents, *Clarias gariepinus*, *Erega radiata*

Introduction

Cassava tuber, like most other members of the family Euphorbiaceae, produces latex which must be processed to detoxify the cyanogenic glucosides in it before it becomes safe for human and animal consumption (Onwueme and Sinha, 1991). Cyanogens and glycosides are easily hydrolysed into hydrogen cyanide which is toxic to aquatic animals and pose serious threat to the environment (Abiona *et al*., 2005; Uhegbu *et al*., 2012).

The extraction of starch from cassava tubers require large amounts of water. Besides the high concentration of poisonous cyanide, the waste water also contains varying concentrations of heavy metals (Olorunfemi *et al*., 2007; 2008; 2011; Jideofor, 2015). In Nigeria, the smallholders are estimated to account for over 80% of cassava production (Knipscheer *et al*., 2007); and the processing mills are usually located on arable lands and near fresh water bodies. Studies have shown that when the effluent is indiscriminately released directly or

indirectly into streams and rivers without prior treatment (which is usually the case), it may lead to detrimental effects on plants and animals and aquatic organisms (Ubalua, 2007; Olorunfemi *et al*., 2011; Ogunyemi, 2017).
Managing water quality is particularly important to fisheries. Water pollution can affect the abundance, location, and/or size of fish. Fish are extremely valuable in toxicity monitoring. 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). Mussels have been extensively utilized in the past as biological indicator of pollution in monitoring programmes and the reason for this is that they are able to accumulate within their tissues many of the contaminants (pesticides, hydrocarbons, metals, etc.) present in sea water (Viarengol and Canesi, 1991). Clams have earned their reputation as pollution indicators of the marine environment for metal accumulation and depuration (Zorba *et al*., 1992).
The deleterious effect cassava effluents to a natural fish population and ostracods have been reported (Adewoye *et al.*, 2005; Onyedineke *et al*., 2010). There is however, no documented information here in Nigeria on the acute toxicity of cassava effluent using the clam *(E. radiata)*. The objective of this study was therefore aimed at investigating the comparative effect of cassava wastewater on mortality rates and behavioural patterns of the freshwater African catfish *(C. gariepinus)* juveniles and freshwater clam *(E. radiata)*.

Materials and methods

Sampling site and collection of cassava effluents: Fresh effluents from the processing of cassava were obtained from small-scale cassava processing mills in Uselu Quarters, Benin City (6°15' N and 5°25' E). In order to form a homogenous mixture, the effluents were scooped from ten different positions into 10 L plastic containers from moulds in the mills where grated cassava packed into sacs are kept before putting them into the manual pressing machines. The effluents obtained from the processing of the cassava roots were collected at three different times (morning, afternoon and evening) and stored at 4°C until analysed for physicochemical properties and used for the acute toxicity study.
Determination of physicochemical parameters: Cassava effluent samples, together with control (tap water of good quality) were analysed for a number of standard physicochemical properties, including total dissolved solids (TDS), sulphates, phosphates, nitrates, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) according to methods described by APHA (2005). Twelve metals namely potassium, sodium, calcium, lead, copper, cadmium, chromium, iron, zinc, silver, nickel, and manganese were analysed in the test samples according to standard analytical methods (USEPA, 1996; APHA, 2005) using an atomic absorption spectrophotometer (AAS) (PerkinElmer A Analyst 100). The metal standards were prepared to known concentrations, labelled, and kept inside plastic bottles that were pre-cleansed with concentrated nitric acid and distilled water. The absorbance of the standards, effluent samples and control was taken in triplicates. Graphs of the concentrations against the absorbance of each of the standards for the metals were plotted. Thereafter, the concentrations of the metals in the test samples were interpolated from their respective graphs.
Test organisms: Collection of test organisms followed conventional methods. Juveniles of the African catfish *(C. gariepinus)* measuring 17±1.0 cm and mean weight 12.4±0.5 g (n=100) were purchased from a commercial fish farm in Benin City, Nigeria (6°15′N, 5°25′E). The fish 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. Water was changed at two days interval to prevent the build-up of metabolic wastes. They 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.
The experimental freshwater clams (*E. radiata*) measuring body mass of 1.6 ± 0.2 g and shell length of 2.0 ± 0.2 cm (n = 100) were collected manually from a freshwater swamp, a natural habitat for clams, in Effurun, Delta State, Nigeria (5°33'0"N, 5°47'0"E) and subsequently transferred to the glass tanks with de-chlorinated water and under a 12 h light-dark cycle with constant aeration where the clams were acclimated for 2 weeks before the start of the experiment (Cid *et al*., 2015). The water was changed every 24 hours, and the clams were fed daily *ad libitum* with a suspension of minced fish pellets. The temperature and the pH of the water were monitored daily. Clams were kept under laboratory conditions for one week prior to use.
Acute Toxicity Test: Acute toxicity test followed methods recommended by UNEP (1989). For the African catfish, ten fishes were stocked per aquarium in cassava effluent concentrations (0.1, 1.0 and 2.5 ml/L) and control (de-chlorinated water). 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 (no response to a gentle prodding) and no respiratory/opercula and tail movements.
Similarly, for the bivalves, ten clams were stocked per aquarium in cassava effluent concentrations (0.1, 0.5, 1.0, 2.5 and 5 ml/L) and control (de-chlorinated water). The shellfish were examined for abnormal behaviours

and mortality for 12, 24, 48, 72 and 96 hours. Dead clams were removed from test solutions as soon as observed. A clam is considered dead, when the shell opens (gaps) to expose the animal.

Data Analysis: The 96 hour LC_{50} toxicity was determined as a probit analysis using the arithmetic method of percentage mortality (Randhawa, 2009). 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.

Results

The cassava effluent used in this study was cloudy and had an unpleasant odour. Results of the physicochemical analysis of the effluent show that the cyanide content was high (78.40±1.20 µgHCl/ml) and highly acidic with a pH value of 3.61. The amounts of nickel, chromium, cadmium, lead, copper, iron, zinc and manganese (1.02, 0.20, 0.40, 1.94, 3.08, 72.40, 25.10 and 2.80 mg/L) respectively were above international (USEPA) limits for effluent discharge (Table 1).

Table 1: Physicochemical parameters of cassava effluent

Parameter	Cassava Effluent	Tap Water	NESREA (2009) Limit	USEPA (2009) Limit
pH	3.61±0.02	7.13±0.02	-	6.5-8.5
Dissolved oxygen	6.20±1.70	1.80±0.40	-	-
Conductivity	14.60±0.60	-	-	-
COD	10.10±4.20	1.02±0.04	-	-
BOD_5@ 20°C	10.86±0.03	1.40±0.04	50	250
Total dissolved solids	262.00±6.12	2.30±0.08	-	500
Turbidity	400.32±4.60	-	-	-
Sulphates	75.42±3.10	-	250	250
Nitrates	17.40±1.00	1.20±0.01	10	10
Phosphates	22.20±1.17	-	2	-
Chloride	216.00±3.22	1.60±0.04	250	250
Potassium	28.60±2.10	10.90±1.20	-	-
Sodium	18.60±0.06	2.30±0.10	-	-
Calcium	14.80±1.00	32.50±0.20	-	-
Copper	3.08±0.08	0.10±0.01	-	0.009
Iron	72.40±1.60	0.20±0.01	-	0.3
Zinc	25.10±0.09	0.30±0.01	-	0.12
Manganese	2.80±0.04	0.01±0.01	0.2	0.05
Silver	2.30±0.06	0.00±0.00	-	-
Nickel	1.02±0.01	0.00±0.00	0.05	0.005
Chromium	0.20±0.01	0.00±0.00	-	0.005
Cadmium	0.40±0.02	0.00±0.00	-	0.002
Lead	1.94±0.20	0.00±0.00	-	0.003
Cyanide	78.40±1.20	0.00±0.00	-	-

All values are expressed in mg/l except temperature (°C), cynogenic potential [µgHCl/ml (ppm)] and pH (no unit). BOD = Biochemical Oxygen Demand, COD = Chemical Oxygen Demand, NESREA = National Environmental Standards and Regulations Enforcement Agency (2009), USEPA = United States Environmental Protection Agency (2009) maximum permissible limits for wastewater.

The mortality rate of *C. gariepinus* juveniles exposed to varied concentrations of cassava effluent is presented in Table 2. Unlike normal behaviours observed in the control groups, the fish exposed to the effluent were restless, erratic in their movement and gasping for breath. Erratic movement was ≥30% while loss of breath was ≤20%. The affected organisms became very weak and eventually died as the concentration of the effluent increased.

Table 2: Mortality rate of *C. gariepinus* juveniles exposed to varied concentrations of cassava effluents

	Duplicate W1					Duplicate W2				
	Mortality(hours)					Mortality (hours)				
Control	**12**	**24**	**48**	**72**	**96**	**12**	**24**	**48**	**72**	**96**
(%)	0	0	0	0	0	0	0	0	0	0
0.1	0	0	0	0	1	0	0	0	0	1
1.0	1	1	2	1	0	1	1	1	2	0
2.5	1	2	1	1	0	1	1	2	1	0

Table 3 shows the mortality rate of *E. radiata*. The clams exhibited degrees of restlessness, random motion, overturning (loss of balancing), sluggishness and eventual death with the shell gaping; the degrees of this behaviour increasing as the effluent concentration increased.

Table 3: Mortality rate of *E. radiata* exposed to varied concentrations of cassava effluents

	Duplicate W1					Duplicate W2				
	Mortality(hours)					Mortality(hours)				
Control	**12**	**24**	**48**	**72**	**96**	**12**	**24**	**48**	**72**	**96**
(%)	0	0	0	0	0	0	0	0	0	0
0.1	0	0	0	1	0	0	0	1	1	0
0.5	1	1	2	1	0	1	2	1	2	0
1.0	0	1	2	1	0	0	0	2	2	0
2.5	1	2	1	2	0	1	1	2	3	0
5.0	0	1	3	2	0	1	1	2	3	0

Probit analysis was used to calculate LC_{50} because probit transformation will straighten the cumulative distribution line and make it easier for data analysis. The relationship between exposure concentrations and mortality of juvenile *C. gariepinus* indicates a dose dependent relationship. This dose dependent relationship also indicates mortality will increase with increased exposure until 100% mortality is attained (Fig. 1). On the contrary, the relationship between toxicant exposure concentration and mortality of the clams, as indicated by the parabolic curve, showed a polynomial or biphasic relationship (Fig. 2). The mean LC_{50} of the fish exposed to cassava effluent was 1.92% while that of the clam was 1.17%. The dose dependent relationship also indicates mortality will increase with increased exposure until 100% mortality is attained.

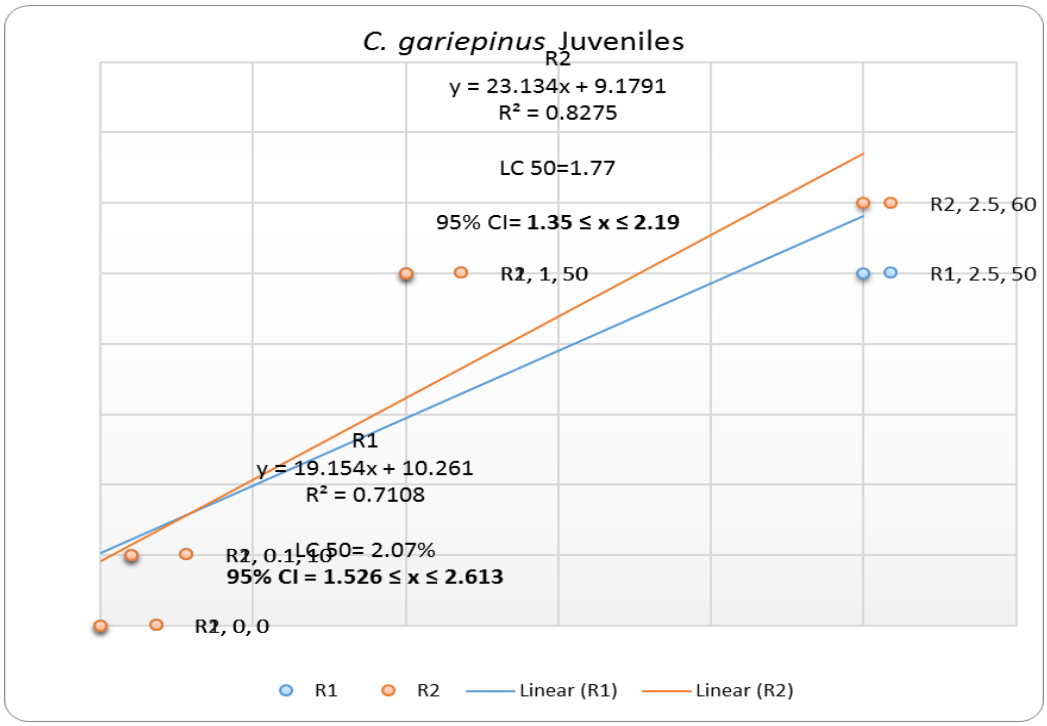

Fig. 1. Linear relationship between mean probit mortality and exposure concentration of *C. gariepinus* juveniles exposed to cassava effluent for 96 hours

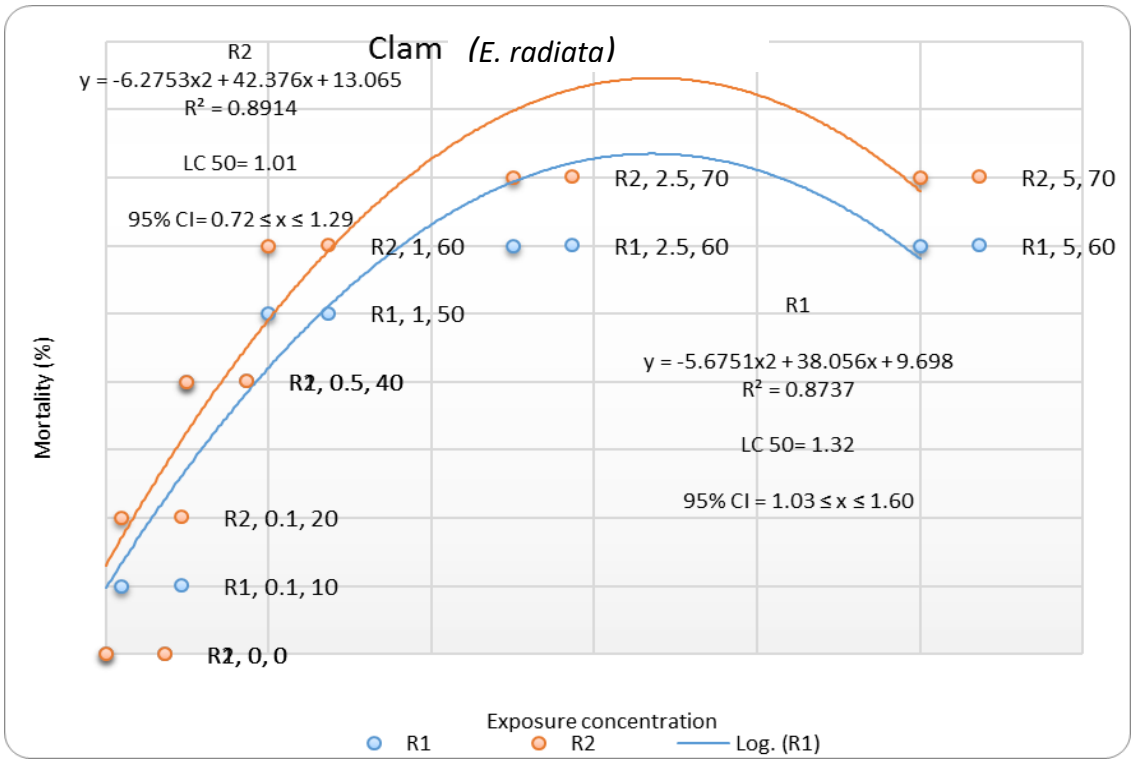

Fig. 2. Linear relationship between mean probit mortality and exposure concentration of *E. radiata* exposed to cassava effluent for 96 hours

Discussion

The physicochemical properties of the cassava effluent used in this study showed that the effluent was highly acidic and also contained high amounts of nickel, chromium, cadmium, lead, copper, iron, zinc and manganese. Acute toxicity of cassava effluents have been reported on fish (Adewoye *et al*., 2005) and benthic macroinvertebrate (Arimoro *et al*., 2008). While Adewoye and his colleagues (2005) opined that the residual effects of chromium and cadmium in the untreated cassava water was suggested to be capable of affecting organs like the gills, liver, brain, kidney and genital organs in the fish, Arimoro and his collaborators (2008) stated that the effluent caused the absence of crustaceans and mollusks in their study. Results from another investigation attributed the high values of physicochemical parameters of cassava effluent as well as the low pH to have accounted for the physiological stress, thereby causing high mortality on ostracoda (Onyedineke *et al*., 2010). Similar reports attributing toxicity of pharmaceutical effluents to the abnormalies observed in the liver tissues of fish (congestion of the central vein, vacoulation of hepatocyte, oedema, cellular infiltration and cellular necrosis) to high concentrations of heavy metals have also been reported (Agboola and Fawole, 2014). Consequently, mortality of the fish and clam observed in this study may well be as a result of the presence of dissolved hydrocyanic acids and synergic interaction of the heavy metals in the cassava effluent.

The high BOD and COD levels from this study might be attributed to the presence of high organic matter in the effluent. Low dissolved oxygen and high biochemical oxygen demand of the wastewater may have facilitated biodegradation activities of anaerobic bacteria of organic matter in the effluent. In the same vein, the disruption of the behavioural responses of the organisms could be 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).

Behavioral abnormalities (restlessness, random motion, loss of balancing, sluggishness) have been observed in fish (Dahunsi and Oranusi 2013) and ostrada (Onyedineke *et al*., 2010) exposed to rubber processing effluents. Similar effects of ballast water and bilge water on fish have also been reported (Olorunfemi *et al*., 2014; 2015). The respiratory abnormalities of fish and clams observed in this study (gasping for breath prior to mortality) are in agreement with these results. 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).

There were increases in mortalities of *E. radiata* and *C. gariepinus* as the concentration of the wastewater increased. Similar results on concentration-dependent increase in mortality rates have been reported for the same organisms exposed to rubber processing effluents (Onyedineke *et al*., 2010; Dahunsi and Oranusi, 2013).

The varying LC_{50} values obtained in these studies may be due to differences in the nature of the pollutant, age of the organism and environmental conditions (Ayuba *et al*., 2013). The biphasic mortality curve exhibited by exposed clams suggests the possibility of adaptive responses occurring at higher effluent exposures. In summary the mean LC_{50} of the fish and clam exposed to cassava effluent indicates that shellfish are more sensitive to the effluent compared to the fish.

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