BEHAVIOURAL AND BIOCHEMICAL RESPONSES OF JUVENILE CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO GRADED CONCENTRATIONS OF CASSAVA WASTE WATER

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ABSTRACT

The behavioural and serum liver enzyme responses of juvenile catfish (Clarias gariepinus) were evaluated for 72 hours. Thirty-six (36) healthy fishes with standard weight, 20 ± 1.52 g and standard length, 18.25 ± 0.50 cm were used for the experiment in non-renewable bioassay system. The test fish exhibited stressful behavioural changes such as erratic swimming, vertical swimming, gasping, and body discolouration. The 24 and 48-hours LC50 were determined to be 96.937 and 9.765 mg/ml respectively. Increased serum aspartate amino transferase and alanine transferase concentrations were recorded on the final day of the experiment (p<0.05).

Keywords: Behavioural, Biochemical, Catfish, Cassava wastewater

INTRODUCTION

Cassava (*Manihot esculenta* crantz) is primarily grown for its starch-containing tuberous roots, which are the major source of dietary energy for more than 500 million people in the tropics (Lynam, 1993). The ability of cassava to grow and produce relatively well in marginal environment under low management levels makes it an attractive crop for poor resource farmers (Bencini, 1991). Cassava is generally considered to have a high content of dietary fibre, magnesium, sodium, riboflavin, thiamine, nicotinic acid and citrate (Bradbury and Holloway, 1988). It also contains cyanide which is extremely toxic to humans and animals. The roots have high moisture content (60 – 65%), making transportation from rural areas difficult and expensive.

To forestall spoilage following harvest and also due to its bulky nature, cassava is usually traded in some processed form. Processing reduces moisture content and converts it to a more durable and stable product, which makes it more transportable (IITA, 1990; Ugwu, 1996). Although, over the years, cassava has been processed into a number of domestic and industrial products, cyanide toxicity arising from cassava is still being reported. There is therefore a need to process it to reduce the cyanide content to safe levels (Eggleston *et al.,* 1992).

Cassava effluents generated during cassava processing are usually neither treated nor properly disposed in Nigeria.

The concentration profile of the chemical component of cassava effluent is in the order of sodium > potassium > magnesium, and iron (Adejumo and Ola, 2010). The occurrence of these chemicals and cyanide in the aquatic ecosystem may be beneficial or toxic. The hydrocyanic acid content of cassava tubers is removed by either washing, exposure to air, heating or pressing with the aid of processing equipment and technology (IITA, 2005). There are two important biological wastes derived from cassava processing which are the cassava peels and the liquid squeezed out of the fermented parenchyma mash (Oboh, 2006). The polluting potential of an effluent is measured by the amount of oxygen needed to oxidize the organic matter, the chemical oxygen demand (COD) and the amount of oxygen necessary to stabilize the organic matter by microorganisms and enzymes i.e. the biochemical oxygen demand (BOD) (Adejumo and Ola, 2010).

Fish and other aquatic life are killed by cyanide concentrations in the microgram per liter range (Adeyemo, 2005). Cassava peels are normally discharged as wastes and allowed to rot in the open with a small portion used as animal feed, thus resulting in health and environmental hazards. Also, the waste water that comes with grinding and pressing is indiscriminately disposed. Cassava is also soaked in water bodies (whether lentic or lotic); such could have enormous adverse impact on aquatic life. This necessitated the present research.

MATERIALS AND METHODS

The Test Organisms: One hundred and twenty *Clarias gariepinus* juveniles with standard weight and standard length, 20 ± 1.52 g and 18.25 ± 0.50 cm, were purchased from Otuocha River, Otuocha, Anambra State, Nigeria. The fish were transported in improvised plastic containers of 25 litre capacity, which were partially cut open at about 15 cm below the handle to the Fisheries and Hydrobiology Research Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Seven catfish each were placed in 12 bowels containing freshwater. The bowels were covered mesh net of size 0.8 mm. The fishes were acclimatized at room temperature for 14 days in which the fishes were not fed in the first three days for them to digest all food materials in their stomach in which they would have acquired from the wild but were fed once a day in other day with commercial fish feed. Mortality during acclimatization was 3%. The fish were not fed for the period of the experiment.

Cassava Wastewater: Fresh cassava tubers (*Manihot utilissima*) (average total weight, 50.2 ± 2.05 kg) were bought from a farm in Eha-Alumona, Nsukka, Enugu State. Identification of the cassava was done at the taxonomic unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The tubers were first washed to remove sand and other debris. After, the tubers were reweighed to get a final weight of 41.5 kg. The tubers were then soaked in an air tight plastic container of about 175 litres, with 47 litre of water without peeling. This was to mimic the soaking pattern used in water bodies around Nigeria. Soaking lasted for five days. The waste water was removed after five days, stirred and sieved to remove debris. The resulting solution was poured into a 25 litre container and stored at 4ºC to avoid further microbial degradation. Also, the container was filled to the brim and was properly covered in order to minimize the volatilization of some constituents which might cause a reduction in the toxicity of the effluent. This is in accordance with the instructions of EPA (2002).

Lethal Toxicity Test: Forty fishes were divided into four experimental groups, one control and three treatments, each with three replicates. The treatments received 10 mg/ml, 20 mg/ml and 30 mg/ml of the waste water. The experiment was monitored for 72 hours while behavioural, morphological, and mortality responses were recorded.

Physico-Chemical Analysis: Dissolved oxygen (DO), alkalinity, PH, hardness, free CO2, biochemical oxygen demand (BOD), phosphate, hydrogen cyanide (HCN), chemical oxygen

demand (COD) were determined using the protocols described by Bhatia (2009).

Liver Enzymes Analysis: Two (2) ml of venous blood was collected from each fish and transferred to sample bottles to allow coagulation. Plasma was obtained by centrifugation for 15 minutes at 3,000 rpm and separated into plain bottles for analysis. Randox enzymatic kit (Randox Laboratories, United Kingdom) was employed for the *in vitro* determination of the activity of alanine transaminase (ALT) and aspartate transaminase (AST) in plasma, using the colorimetric method of Reitman and Frankel. Randox enzymatic kit (Randox Laboratories, UK) was also used for the *in vitro* determination of ALP activity in plasma, according to the colorimetric method of Englehardt.

Data Analysis: Data collected from the study were subjected to analysis of variance. Significant means were separated using Fisher's Least Significant Difference (F-LSD) at p = 0.05.

RESULTS

Behavioural Responses of the Test Organisms to the Toxicity of the Cassava Wastewater: During the lethal toxicity test, *Clarias gariepinus* showed marked distress (Table 1). This was recorded as erratic swimming, gasping of breath, weak movements and continuous surfacing. Distress in behavioural responses increased with increase in concentration of the waste water.

Table 1: Behavioural response of *Clarias gariepinus* exposed to graded concentrations of cassava wastewater

Concentrations (mg/ml)	Behavioural Responses Erratic swimming	Gasping
0	-	-
10	+	+
20	++	++
30	+++	++
	Vertical swimming	**Body discolouration**
0	-	-
10	+	-
20	++	+
30	+++	+++

The fish got weaker as the experiment progressed, with vertical swimming, ventral surface turned upward and finally, death. The control fish exhibited normal behavioural responses and zero mortality.

Lethal Concentration: The LC_{50} of the cassava waste water at 24 and 48 hours were 96.937 and 9.765 mg/ml, respectively (Table 2). The LC_{50} for the 24 and 48 hours were deduced from the probit plots (Figures 1 and 2).

Table 2: Mortality of *Clarias gariepinus* exposed to different concentrations of cassava wastewater

Concentration (mg/ml)	Duration (hours) 24	48	72
0	0	0	0
10	0	0	0
20	0	4	0
30	1	4	0

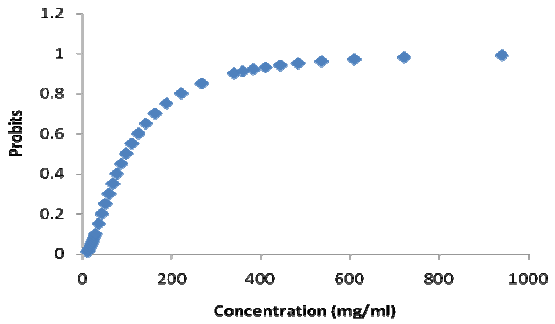

Figure 1: 24 hours LC_{50} of cassava wastewater

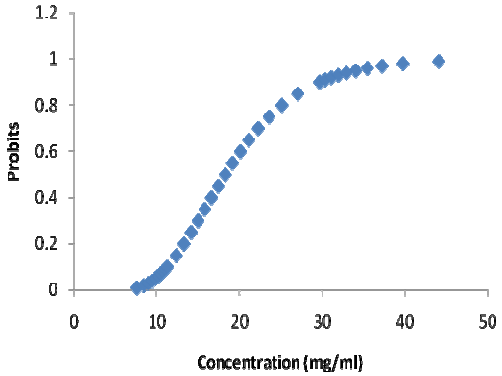

Figure 2: 48 hours LC_{50} cassava wastewater

Physicochemical Parameters of Test Cassava Wastewater: The pH, CO_2 and water hardness were significantly increased at the end of the study ($p<0.05$), whereas the biochemical oxygen demand and phosphorus content decreased significantly.

Table 3: Physicochemical parameters of the test cassava wastewater

Parameters	Initial	Final	P-value
pH	6.20 ± 0.06	6.58 ± 0.09*	0.00
HCN (mg/l)	0.09 ± 0.00	0.09 ± 0.00	0.88
Phosphorus (mg/l)	0.71 ± 0.00*	0.05 ± 0.00	0.00
BOD (mg/l)	8.23 ± 0.29*	7.12 ± 0.16	0.00
DO (mg/l)	3.70 ± 0.03	3.51 ± 0.10	0.06
CO2 (mg/lCO_3)	27.61 ± 5.04	183.33 ± 37.18*	0.01
Hardness (mg/l $CaCO_3$)	61.92 ± 6.90	97.93 ± 10.51*	0.01
Alkalinity(mg/l $CaCO_3$)	41.54 ± 4.20	107.53 ± 4.26*	0.00

Mean values with asterisk as superscript are significant (p<0.05)

Table 4: Effect of cassava wastewater on the liver enzyme concentration of *Clarias gariepinus*

Liver enzymes	Initial	Final	P-value
AST	11.50 ± 2.01a	66.58 ± 11.96b	0.00
ALT	31.58 ± 1.19a	63.75 ± 11.21b	0.01
ALP	19.00 ± 0.77	24.00 ± 4.20	0.21

There were changes in other physicochemical parameters, but such were not significant (p>0.05) (Table 3).

The Effect of Cassava Wastewater on the Liver Enzymes of *Clarias gariepinus*: The final value of the AST concentration was significantly (p<0.05) higher than the initial value of AST concentration (Table 4). Similar to the values of AST, the variation between the initial and the final ALT concentration was significant (p<0.05). Though, a variation existed in the ALP values, the final values of the ALP concentration was not significant when compared to the initial (p>0.05).

DISCUSSION

Agricultural practices and industrialization, all geared towards food production are ways of alleviating food scarcity and poverty. These practices have led to water and other forms of pollution thus, creating health hazards to living organisms including man. The annual cassava production statistics in Africa stands at about 84 million tonnes with Nigeria leading with a total production of 30 million tonnes, Tanzania 5.7 million tonnes, and Madagascar 2.4 million (Adeyemo, 2005). This upsurge in production in Nigeria has led to creation of cassava processing units where various cassava products are produced and waters are discharged into the environment and waters bodies.

Reduced distressful behavioural responses and mortality present in the lower concentrations used in the current study suggest that the test fish can tolerate low doses of the waste water. The behavioural responses of the fishes exposed to the cassava effluent may be attributed to the decreased in dissolved oxygen concentration resulting from the cassava effluent. The mortalities recorded in the course of this study were an indication of the toxicity of the waste water in the fish.

The increase in the dissolved CO_2 content on the final day of the experiment was an indication of heightened respiratory activities. This observation is supported by the fact that the biological oxygen demand level decreased significantly at the final day of the experiment. This is also an indication of elevated oxygen uptake. Water hardness and alkalinity also rose appreciably at the end of the study. Metallic cations such as Ca^{2+}, $Mg^{2+,}$ Na^{+}, K^{+} etc play vital roles in cellular functions. They regulate muscle contraction, generation of nerve impulses, and activation of enzymes during protein production and transport etc. The continuous addition of cations could result in uncoordinated muscular contractions, and when this occurs in vital organs, paralysis and even death could result. of the treatment water recorded no significant increase or decrease between the initial and final value. This suggests that fermentation may not appreciably reduce the cyanide content with time. Though, the extent of reduction may depend on the

quantity of cassava soaked in a particular volume of water. This tends to suggest that depending on organisms' susceptibility, cassava waste water may still be toxic due to its cyanide content irrespective of fermentation time.

The dissolved oxygen content did not change significantly at the end of the experiment. This is not surprising as the study took place in an open, shallow bowls in which oxygen dissolution could have been relatively stable for the duration of the experiment.

The 24 and 48 hours LC_{50} of the cassava waste water (96.939 and 9.765 mg/ml) showed that the toxicity increased maximally with fermentation time. This is also not surprising as the essence of fermentation is to get rid of toxic chemicals from the cassava that will be consumed as food. No wonder the mortality of the test fish increased to the extent that all had died by the 72^{nd} hour. Gintaras (2010) reported that the 96-hr LC_{50} values obtained from the toxicity test of Nickel on some freshwater fish; rainbow trout, three-spinned stickleback, roach, perch and dace ranged from 19.3 to 61.2 mg Ni/l.

The present study recorded significant increases in both the aspartate amino transferase alanine transferase concentrations on the final day of the study. The dose-dependent response could be why all fish in the 30 mg/ml concentration died before the completion of experiment. This could be as a result of adverse effects on some vital organs such as the liver, operculum, and gills etc. However, the alkaline phosphatase (ALP) concentration in the present study decreased when compared with the 0 mg/ml concentration. This reduction is also in line with the report of Olaniyi *et al.* (2013) in *C. gariepinus* exposed to graded levels of cassava mill effluent. They reported increase in the AST and ALT concentrations. They attributed such to hepatic cellular damage in the test fish. Also recorded was heightened leucocytosis which was associated with physiological response to maintain the health of the fish. Das *et al.* (2004) reported increase in the AST and ALT of Indian major carps exposed to nitrate toxicity and suggested that the elevation of the transferase could be as a result of the use of the α-amino groups in the tricarboxylic acid cycle of keto acids to augment energy production. Sepici-Dincel *et al.* (2009) observed that the increase in activities of AST and ALT in the muscle and liver of the common carp exposed to 10 mg/l of cyfluthrin may be due to a disturbance in the Kreb's cycle.

Iwama and Ackermen (1994) observed similar result in rainbow trout exposed to graded concentrations of MS-222 and clove oil.

The right amount of all twenty amino acids is important for protein build-up needed for growth and repair of damaged tissues (Kaslaw, 2013). However, eight of these cannot be produced by the body and therefore, must be generated from our diet. Keto acids of the Krebs cycle can be converted to amino acid by the addition of an amine group, produced by the breakdown of mother amino acid. Such transamination occur continuously in the body depending on its physiological status and, passable be is driven by the particular protein needed by the body of which the building block is in short supply.

Phosphatases remove phosphate groups from molecules. This removal of the phosphate group from a molecule within a cell makes it unable to leave the cell. For instance, the removal of the phosphate group from glucose makes it impermeable and even unable to be used for energy production during glycolysis. The response of plasma alkaline phosphatase during the experiment was mostly lower compared to the baseline but, the treatments had alkaline phosphatase concentrations similar to the control group.

Measurement of blood alkaline phosphatase is used to access the existence of biliary dysfunction. Since the mucosal cells lining the bile system of the liver are the sources of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gall bladder are responsible for maintaining the right homeostasis of this enzyme in the blood (EPA, 2002). The significant reduction in the plasma concentration of this enzyme recorded in the present study showed protection, rather than toxic impacts on the bones, the liver and associated billary system. Also, the production and secretion of bile is

central to the effective digestion and utilization of fat; the result of this study suggested that biliary function was not impaired during the study period.

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