Revue Scientifique et Technique Forêt et Environnement du Bassin du Congo
Volume 7. P. 10-17, Octobre (2016)

Article Scientifique

The effect of cassava mill effluent on histology of Clarias gariepinus juveniles

Olufayo M. O.[1] and David O.T.[1]

(1) Aquatic toxicology Research Unit, Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Po Box : 703, Akure, Nigeria / e-mail: moakinbulumo@yahoo.co.uk

DOI : http://doi.org/10.5281/zenodo.160042

Abstrat

One of the major challenge of aquatic environment particularly in agricultural areas is the discharge of toxic effluent. Most cassava varieties contain a substance called cyanide that can make the crop toxic to the aquatic ecosystem if they are inadequately processed. Untreated effluents from cassava factories and local processing sheds have effects on fish population. The study aims at addressing the problem of cassava effluent on aquatic organisms especially fish. Bio-assay test was carried out in the laboratory in order to assess the toxicity of cassava effluent on aquatic organisms such as fish. Different concentrations of cassava effluent (100, 120, 130, and 140 ml/l) were administered to the various treatments for 96 hours. The LC_{50} *of Cassava effluent to Clarias gariepinus was determined using probit analysis. Data collected were analyzed using two way analysis of variance (ANOVA) at 0.05 level of probability using SPSS (version 14.0). After 96 hours, no mortality was observed in the control (0.0 ml) and 20-100 % mortality was recorded during the 96-hour experiment. Behavioural responses were dose dependent, it increased with increase concentrations. The 96-hour Lethal Concentration (*LC_{50}*) value was 116.62 mls. Histological changes in the gill, liver, and kidney of fish treated with different concentrations of cassava mill effluent for 96h showed gill necrosis, infiltration and degeneration of cells, gill lamellae. The liver showed fibrosis, pyknosis, and vacuolation, while the kidney showed destruction of tubule, fusion of tubules, pyknosis and condensation of glomeruli content. In conclusion, the alteration observed in the gill, liver and kidney structures of fish showed that cassava mill effluent is toxic to the fish and had profound impact on behavior and respiration of C. gariepinus in both lethal and sub-lethal concentrations; therefore their use near aquatic environment should be discouraged.*

Keywords: *Cassava effluent, toxicity,* LC_{50}, *aquatic environment, Clarias gariepinus*

Résumé

L'un des principaux défis du milieu aquatique, surtout dans les zones agricoles, est le rejet d'effluents toxiques. Plusieurs variétés de manioc contiennent une substance appelée cyanure qui peut rendre la récolte toxique pour l'écosystème aquatique si elle est mal traitée. Cette étude à pour objectif, de résoudre le problème des effluents de manioc sur les organismes aquatiques et spécialement sur les poissons. Le bio-essai a été effectué au laboratoire afin d'évaluer la toxicité des effluents du manioc sur les organismes aquatiques tels que les poissons. Différentes concentrations des effluents du manioc (100, 120, 130 et 140 ml/l) ont été administrées à divers traitements pendant 96 heures. Le LC_{50} *de l'effluent du manioc à C. gariepinus à été déterminée en utilisant l'analyse des probits. Les données recueillies ont été analysées à l'aide de deux analyses de la variance (ANOVA) à 0,05 du niveau de probabilité en utilisant SPSS (version 14.0). Après 96 heures, aucune mortalité n'a été observée dans le contrôle (0,0 ml) et une mortalité comprise entre 20 et 100 % a été enregistrée au cours de l'expérience de 96 heures. Les réponses comportementales étaient dépendantes de la dose, elles se sont accrues avec une augmentation des concentrations. La valeur léthale à 96 heures de concentration (*LC_{50}*) était de 116,62 ml. Les modifications histologiques dans les branchies, le foie et les reins des poissons traités avec différents effluents de manioc des usines à 96h de concentration ont montré une nécrose des branchies, l'infiltration et la dégénérescence des cellules, des lamelles de branchies. Le foie a montré une fibrose, pyknose et vacuolisation, tandis que le rein a montré la destruction de tubule, la fusion des tubules, la pyknose et la condensation des contenus de glomérules. L'analyse des données de la présente enquête met en évidence que les effluents de manioc des usines sont toxiques et ont un impact profond sur le comportement et la respiration chez C. gariepinus dans les deux concentrations léthales et sub-léthales. Ainsi, leur utilisation proche d'un environnement aquatique devrait être découragée.*

mots clés :*Effluent de manioc, toxicité,* LC_{50}, *Environnement aquatique, Clarias gariepinus.*

1. Introduction

In the rain forest environment, cassava is one of the main crop grown for food by small-scale farmers. Apart from food, cassava is very versatile and its products such as starch, gum, glues, confectionery sweeteners, biodegradable products and drugs are used in animal feed and alcohol production (Olaniyi et al., 2013). Nigeria is the second largest consumers of cassava in sub-Sahara Africa and it is estimated that 37 % of dietary energy comes from cassava (Eruvbetine et al., 2003). Most cassava variety contains a substance called cyanide that make the crop toxic to the aquatic ecosystem if they are not well processed (Adeyemo, 2005 ; Olaniyi et al., 2013 and Eruvbetine et al., 2003). Cyanide is one of the most toxic chemical to fish and fish are more sensitive to cyanide than human (Huang et al., 2008). Increase in industrial and agricultural wastes has resulted in pollution of aquatic environment and cassava processing, especially in environments where cassava industry is highly concentrated is regarded as polluting the natural resources and this has contributed significantly to environmental damage and water deficit (Olufayo and David, 2013). Most of these aquatic pollutants have under gone some toxicity test to evaluate their effects on non-target organisms and the concentration of potential toxicants should be below that which causes mortality on the fish (Boyd, 2005). The continual use and indiscriminate disposal of cassava effluent has prompted some concern of the effects of cyanide on the early life stages of fish (Huang et al., 2008). Many reports have discussed the impact of man-made xenoestrogenic compounds on man and wildlife. According to Fatoki et al., (2010), careless handling, and accidental spillage or discharged of untreated cassava effluents into the natural water ways have harmful effects on the fish population and other forms of aquatic life and may contribute to long term effects in the environment (Adeyemo. 2005; Olaniyi et al, 2013; Olufayo and David, 2013).

Histological indices are important parameters use in the area of toxicology, fish physiological or pathological status in fishery management and disease investigation (Olufayo and David, 2013). Since discharge of untreated cassava effluents into natural water bodies are very common among cassava processors and have harmful effects on fish population and production, there is need to study fingerling and juvenile stages of fish which are more susceptive to toxicants than the adult fish because most researchers have worked on adult fish. This study therefore investigates the toxic effects of cassava mill effluents on histological features of *C. gariepinus* juveniles which is one of the most common fish species used in fish culture in Nigeria because this stage in the life history of the fish is susceptible to toxicants than adult fish.

2. Material and Methods

An investigation was carried out in the laboratory to evaluate the effects of cassava mill effluents on the histology (gills, liver and kidney) of *C.gariepinus* juveniles. The material and methods used are:

Collection and Acclimation of Experimental Organisms

The juveniles of *C. gariepinus* weighing 17±0.5g were obtained from the Federal University of Technology Teaching Farm and acclimatized to laboratory conditions for 48 hours in glass aquaria well aerated with electric aerators prior to the bio-assay.

Collection of Cassava Effluents

One hundred fifty (150) liters fresh cassava effluents were collected from some small–scale cassava processing mills in Akure, Nigeria and transported to the Limnology Laboratory of Fisheries and Aquaculture Department, Federal University of Technology Akure, Nigeria prior to the experiment.

Experimental Setup

The laboratory experiment was laid in completely randomized design (CRD) with two replicates per treatment. Five graded concentrations of cassava mill effluents were prepared from the 150 litres stock solution collected from cassava factory at varying concentrations of 100, 110, 120, 130 and 140 mls and poured into 10 litres of water in 15 litres aquarium each to give concentrations of 0.01 mg/l , 0.011 mg/l, 0.012 mg/l, 0.013 mg/l, 0.014 mg/l. Ten (10) fish with mean weight of 17±0.5g were distributed into each aquarium containing different concentrations of cassava effluents of 0.01 mg/l, 0.011 mg/l, 0.012 mg/l, 0.013 mg/l, 0.014 mg/l and a control (0.00 mg/l). The fish were starved for 24 hours prior to the experiment in order to reduce the faecal and unconsumed wastes. The bio-assay lasted for 96 hours. The behavioral changes and mortality of the experimental fish were observed and recorded.

Water Quality Parameters

The temperature, pH, conductivity and dissolved oxygen (DO) were monitored throughout the duration of the experiment using standard laboratory methods as described by American Public Health Association (APHA et al., 1989).

Histological Parameters

Fish were sampled after the 96 h experiment for histological analyses of the selected tissues (gills,

Table 1: Behavioral Changes and Mortality Rate of C gariepinus Juveniles Exposed to Different Concentrations of Cassava Mill Effluents

	24 hours						48 hours						72 hours						96 hours					
Concentration (ml)	0.0	100	110	120	130	140	0.0	100	110	120	130	140	0.0	100	110	120	130	140	0.0	100	110	120	130	140
frequent jumping	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
erratic swimming	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
convulsions	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
loss of reflex	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
hyper ventilation	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
discolouration	-	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Mortality (%)	-	-	-	10	60	90	-	-	-	10	80	100	-	-	10	20	100	100	-	20	30	60	100	100
Presence (+) of specific observation Absence (-)of specific observation																								

Table 2 : Water quality parameters obtained during exposure of C. gariepinus juveniles to cassava mill effluent for 96hrs

Parameters	Control	100ml	110ml	120ml	130ml	140ml
Temperature (0°C)	22.70	22.73	22.75	22.73	22.75	22.76
	(0.58)	(0.58)	(0.58)	(0.58)	(0.58)	(0.58)
pH	6.67	7.71	8.72	7.14	7.69	7.30
	(0.19)	(0.19)	(0.19)	(0.19)	(0.19)	(0.19)
DO_2 (mg/l)	5.81	4.14	4.14	4.31	4.51	4.81
	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)	(0.07)

Mean values with error in parentheses.

livers and kidneys). Fish were dissected and the tissues collected were fixed in 10 % formal saline, processed routinely, embedded in paraffin, sectioned at 6µm thickness. They were stained with haematoxylin and eosin (H&E) and examined using light microscopy (NIKON TE 3000). Photomicrographs were taken atx10, x20, and x40 magnification with a digital camera (Nikon 9000).

LC_{50} Determination:

The Lethal Concentration (LC_{50}) at 96h was computed and determined using the probit analysis (Boyd, 2005). Data collected were analyzed using two way analysis of variance (ANOVA) at 0.05 level of probability using SPSS (version 14.0).

3. Results

After 96 hours, no mortality was observed in the control tank (0.0 ml) while 20-100 % mortality was recorded in concentrations 100, 110, 120, 130 and 140 ml/l during the 96-hour experiment (table 1). Behavioral responses were dose dependent, it increased with increase concentrations. In higher concentrations of the test effluent, fish showed initial disturbed swimming movements and surfacing behavior indicative of avoidance response. This was followed by bleaching of the skin, sudden quick movement, unusual lethargy and fish settling at the bottom of the aquaria motionless with slow opercula movements, gulping for air. Fish at lower concentrations of cassava mill effluents (control 0.0 mls-100 mls) showed normal behavior, they did not exhibit any abnormal behavior through the entire duration of the experiment (table 1). Throughout the 96 h experiment, the water quality parameters (temperature, DO and pH) did not vary significantly (P>0.05) within the various concentrations of all the cassava effluents as well as with the control experiments (table 2). The 96-hour

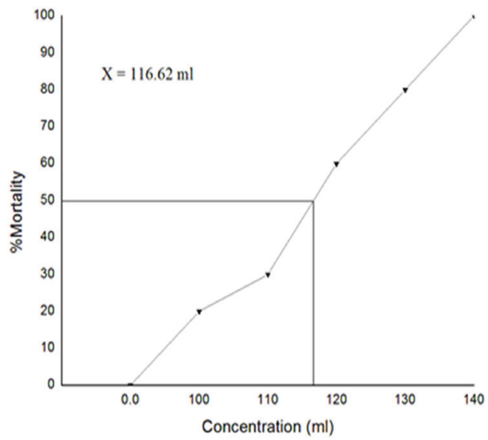

Figure 1 : 96 h LC50 of cassava mill effluent on C. gariepinus

lethal concentration (LC_{50}) value was observed at concentration 116.62 mls (figure 1).

Histological changes in the gills of *C. gariepinus* juveniles exposed to cassava mill effluents showed varying degrees of degeneration of the lamellae (plates 1-6). There was no appreciable microscopic lesion in the gill of *C. gariepinus* from the control tanks (plate 1). Slight vacuolation, congestion and degeneration of filament were observed from the gills of *C. gariepinus* exposed to lower concentration (100 ml) of cassava mill effluent (plate 2), degeneration of filament, necrosis, infiltration and degeneration of cell were observed in concentrations 110 and 120 mls respectively (plates 3 and 4) while complete degeneration of the filament, high level of degeneration of lamellae were observed in the gills of *C. gariepinus* exposed to higher concentrations (130 and 140 mls) of cassava mill effluent (plates 5 and 6). Normal situation was observed in the kidneys of fish from the control tank (plate 7). However, the kidneys of *C. gariepinus* exposed to 100 and 110 ml/l of cassava effluents showed appreciable cellular changes particularly fusion of tubules, pyknosis, tubule pyknosis as well as degeneration of the kidney cells (plates 8 and 9) while the Kidneys of *C. gariepinus* juveniles exposed to 120, 130, and 140 mls of cassava mill effluents showed karyolysis, rupture of nuclear membrane, fragmentation of nuclear chromatin, shrinkage, dense nucleus and vacuole formation (plates 10, 11 and 12). Plates13 showed the normal histo-structure of liver of *C. gariepinus* from control fish. The livers of test fish exposed to cassava mill effluents (100 and 110 mls) showed observable fibrosis and dissolution of nucleus (plates 14 and 15) while fish exposed to high concentrations of cassava effluents (120-130 mls) showed complete dissolution of nucleus loss of chromatin materials, vacoulation and finally a complete absence of the nucleus (plates 16 and 17). The livers of *C. gariepinus* exposed to 140 mls of cassava mill effluent showed total nucleus dissolution and vacuolation (plate 18).

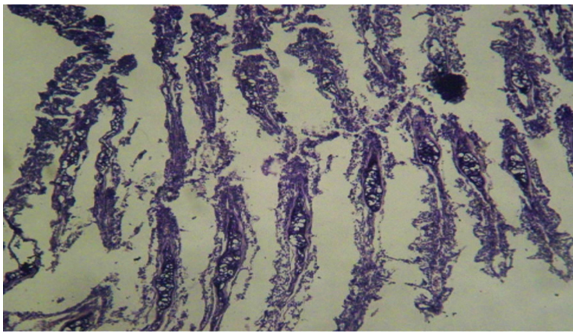

Plate 1: Gill of C. gariepinus in the control tank × 400

Plate 2: Gill of C. gariepinus exposed to 100ml of cassava mill effent showing Congestion, vacuole formations and Degeneration of filaments × 400

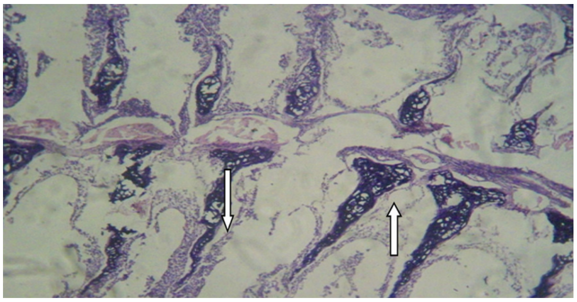

Plate 3: Gill of C. gariepinus exposed to 110ml of cassava mill effluent showing degeration of filament . × 400

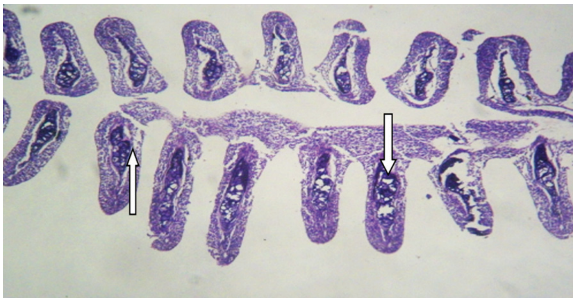

Plate 4: Gill of C. gariepinus exposed to 120ml of cassava mill effluent showing necrosis ,Infilteration of the cell and degeneration of cell × 400

Plate 5: Gill of C. gariepinus exposed to 130ml of cassava mill effluent showing complete degeneration of the filaments. × 400

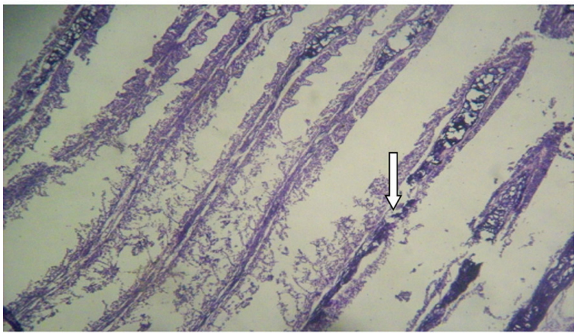

Plate 6: Gill of C. gariepinus exposed to 140ml of cassava mill effluent showing high level of degeneration with lamellae. × 400

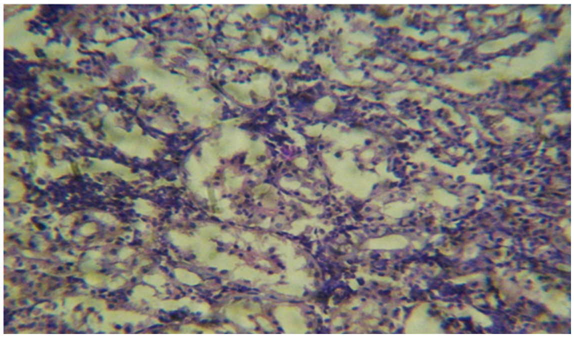

Plate 7: Showing Kidney of C. gariepinus in the control tank.× 400

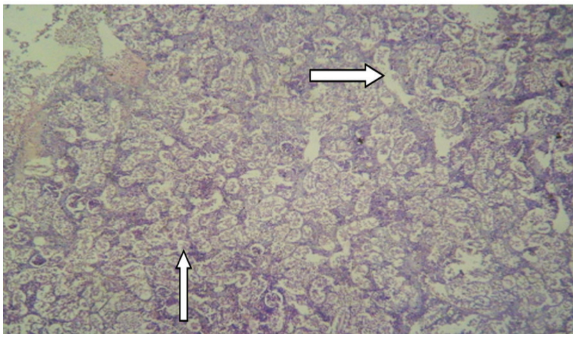

Plate 8: Kidney of C. gariepinus exposed to 100ml of effluent showing space formation and fragmentation of nuclear chromatin. × 400

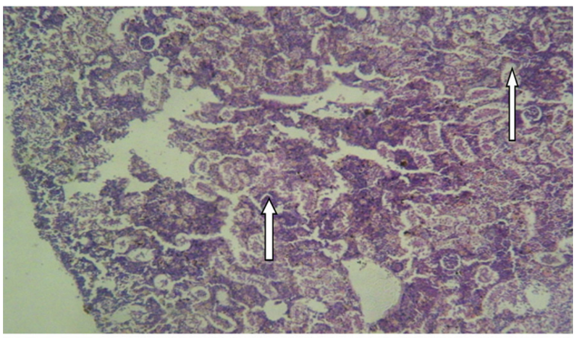

Plate 9: Kidney of C. gariepinus exposed to 110ml of effluent showing shrinkage of nucleus and vacuole formation× 400

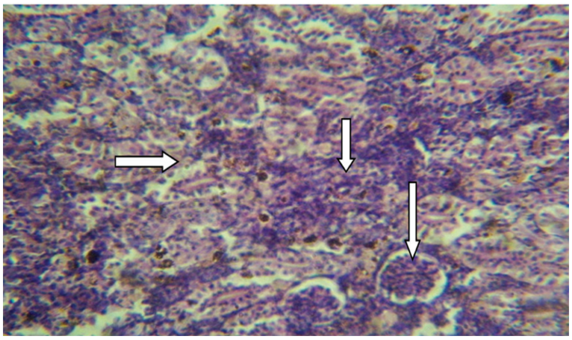

Plate 10: Kidney of C. gariepinus exposed to 120ml of effluent showing Karyolysis rupture of nuclear membrane and fragmentation of nuclear chromatin. × 400

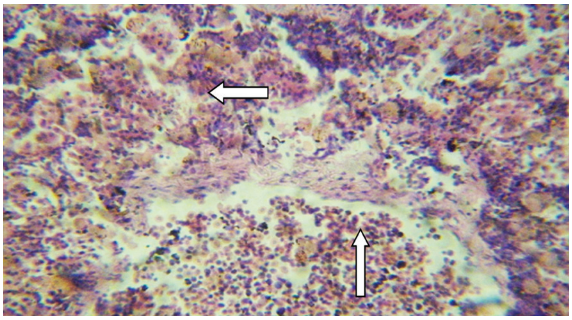

Plate 11: Kidney of C. gariepinus exposed to 130ml of cassava mill effluent showing shrinkage and dense nucleus × 400

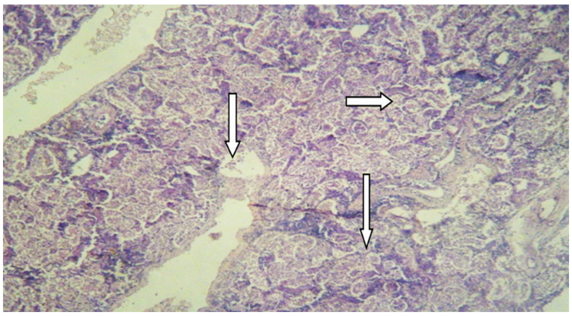

Plate 12: Kidney of C. gariepinus exposed to 140ml of effluent shows shrinkage of nucleus and vacuole formation× 400

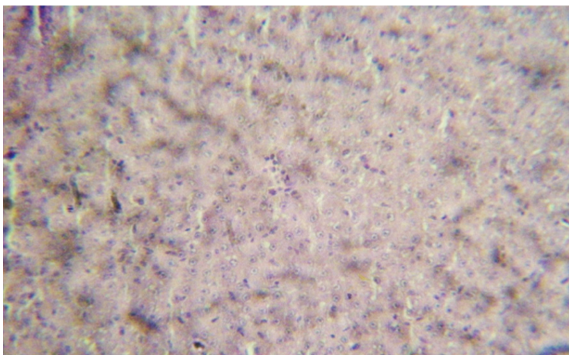

Plate 13: Liver of C. gariepinus in the control tank × 400

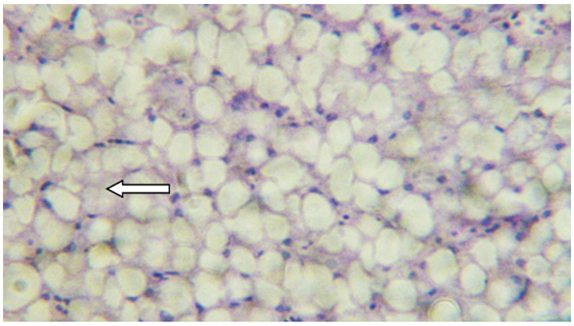

Plate 14: Liver of C. gariepinus exposed to 100ml of cassava mill effluent showing fibrosis × 400

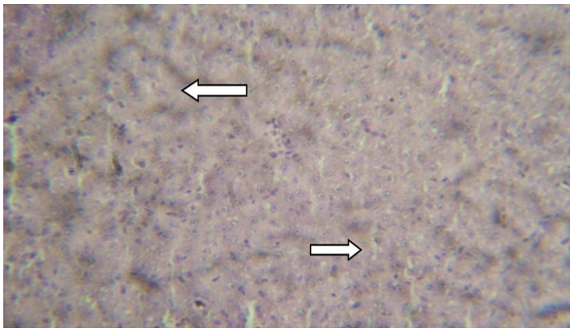

Plate 15: Liver of C. gariepinus exposed to 110ml of cassava mill effluent showing dissolution of nucleus× 400

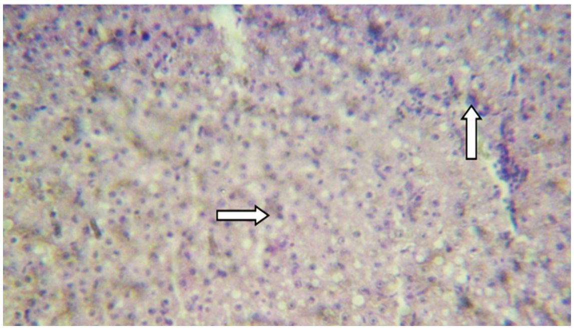

Plate 16: Liver of C. gariepinus exposed to 120ml of effluent showingcomplete dissolution of nucleus with loss of chromatin materials × 400

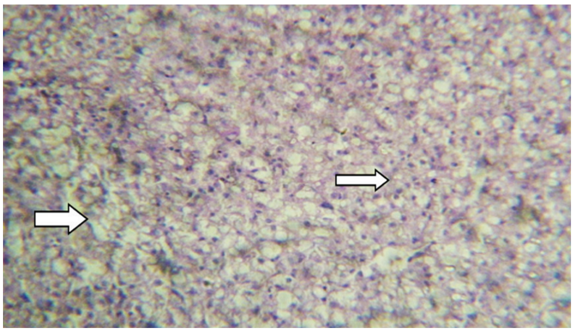

Plate 17: Liver of C. gariepinus exposed to 130ml of cassava mill effluent showing vacuolation and loss of chromatin material× 400

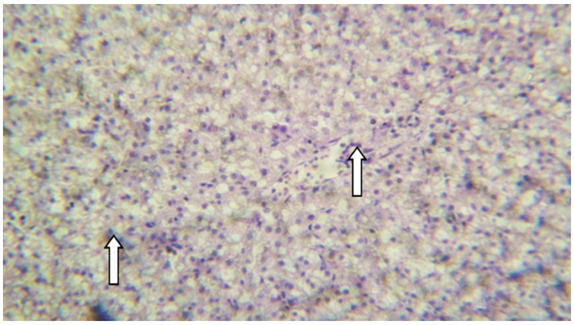

Plate 18: Liver of C. gariepinus exposed to 140ml of cassava mill effluent showing nucleus dissolution and vacuolation× 400

4. Discussion

Environmental pollution is one of the major primary causes of aquatic biodiversity depletion in Nigeria. The indiscriminate use of toxicants and the discharges of waste effluents from industry into the water ways have harmful effects on fish production and aquatic environments (Ayoola 2008; Olufayo and David 2013). In this study, the physicochemical parameters of the test media fluctuated slightly during the toxicity test. These values were normal for toxicity test which is the standard value and this agreed with Olufayo and David (2013) who reported that there was a significant negative correlation between pH and dissolved oxygen values. Since most fish breathe in water in which they live, changes in the chemical properties thereof may be reflected in the animal's respiratory activity, particularly if the environmental factors affect respiratory gas exchanges (Ayoola, 2008; Olufayo and Fagbenro, 2007; Adeyemo, 2005 and Holden, 1973) had earlier reported that the introduction of a toxicant into an aquatic system might decrease dissolved oxygen concentration, which will impair respiration leading to asphyxiation. Variation in the oxygen consumption in cassava mill effluent treated fish is probably due to impaired oxidative metabolism and pesticide induced respiratory stress. Hence, dysfunction of behavior and respiration can serve as an index of cassava mill effluent toxicity (Olufayo and David, 2013 ; Ural and Simsek, 2006).

Several abnormal behaviors such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increased opercula activities, surface to bottom movement, sudden quick movement, and resting at the bottom observed in this study were similar to the observations of Omoniyi et al., (2002) who worked on effects of tobacco leaf dust on *Clarias gariepinus*, and Oti, (2002) who observed increase in opercula movement of Catfish fingerlings exposed to cassava mill effluents. The stressful and erratic behavior of

C. gariepinus fingerlings in the experiment indicates respiratory impairment, probably due to the effect of the toxicant cassava mill effluent on the gills. The stressful behavior of respiratory impairment due to the toxic effect of cassava mill effluent on the gills was similar with the reports of Omitoyin et al., (2006) and Aguigwo (2002) who observed that pesticide impairs respiratory organs. Cassava mill effluents toxicity is shown to increase with increased concentrations. The behavioral effect observed in this study is in consonance with earlier reports of Murty et al., (1983), Omoregie and Ufodike, (1991) and GESAMP (1991). It was observed that the higher the concentration of the toxicant, the higher the mortality rate. The histological examination of the liver, gill, and kidney of the exposed fish indicated that the kidney, liver and gills were the organs most affected. In fish, gills are critical organs for their respiratory, osmo-regulatory, and excretory functions. Gills are generally considered good indicator of water quality, being models for studies of environmental impact, since they are the primary route for the entry of effluents (Omitoyin et al., 2006). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply, and demand to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress. If gills would be destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a changed permeability oxygen uptake rate would even rapidly decreased (Aguigwo 2002; Olufayo and Alade 2012).

The result of histopathological studies carried out in this work agrees with Olufayo and David (2013),Wade et al., (2002), Adeyemo (2005), and Olaniyi et al., (2013) who reported that 96 h toxicity assay of cassava effluents on Catfish and Nile tilapia, histological results indicated the gill was the primary target tissue affected by cassava mill effluents. The liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to pesticides (Aguigwo, 2002). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed in this study resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, and this is similar to the observation of Rahman et al., (2002). The inability of the fish to regenerate new liver cells might also have led to necrosis. The developmental stages of the fish under study were generally more sensitive to toxic pollutant than adults, the result agreed with the reports of Olufayo and Alade (2012). The inability of the fish to regenerate new liver cells might also have led to necrosis while the kidney showed karyolysis, destruction of tubule, fusion of tubules, pyknosis and condensation of glomeruli content. Necrosis observed in the test fish resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, and this is similar to the observation of Rahman et al., (2002). In the present study the kidney of *C. gariepinus* juveniles exposed to varying concentrations of cassava mill effluents which showed tubular destruction or fusion of the tubules, pyknosis, condensation of glomeruli content was similar to findings of Omoniyi et al., (2002) and Rahman et al., (2002). The 96-hour lethal concentration (LC_{50}) value observed at concentration 116.62 mls agrees with toxicological procedure (Olufayo and David 2013). In a 96-hr bioassay test performed on the toxic effect of Cassava mill effluent to the African Catfish - *Heteroclarias Hybrid* of *Heterobranchus bidorsalis* (Male) and *Clarias gariepinus* (Female), the LC_{50} was determined as 50.12 mgl $^{-1}$ (Adeyemo, 2005).

Conservation value

The result of this study had showed that untreated cassava mill effluents from cassava processing factories or farm lands have threatened aquatic environment and the fish species that occurred only in the sampled aquatic environment demanded urgent attention for conservation because biological diversity is becoming an important economic realities, if the environments are not well guided, the fish species around the processing areas will be totally lost and such areas will have impaired negative effects on diversity of life for improvement in their conservation and management.

5. Conclusion

It was discovered from this study that cassava mill effluents when released into the aquatic environment at high concentrations had dramatic effects on aquatic organisms and this suggested that environmental risk assessment linked with cassava mill effluents uses would be required. The analysis of data from the present investigation evidenced that cassava mill effluent is toxic and had profound impact on behavior and respiration in *C. gariepinus* in both lethal and sub-lethal concentrations. Damages of the gills indicated that the sub-lethal concentrations of effluent caused impairment in gaseous exchange efficiency of the gills and this is similar to the observation of Rahman

(2002), Omitoyin et al. (2006) and Aguigwo (2002). It can be concluded that cassava mill effluents was toxic to fish organs, *C. gariepinus* juveniles are more susceptible to pollutant, therefore the use of cassava effluents near fish ponds and water ways or aquatic environment should be discouraged generally.

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