EFFECTS OF LETHAL AND SUB-LETHAL DOSES OF CASSAVA MILL EFFLUENTS ON GILLS AND HEART OF AFRICAN CATFISH

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ABSTRACT
Most of the effluents from cassava processing units are directly or indirectly discharged into aquatic system without any prior treatment. This study examined the response and toxic effects of cassava effluent on the heart and gills of Clarias gariepinus. Eighty four African catfishes (Clarias gariepinus) were subjected to lethal and sub-lethal tests. Ten fishes were introduced to five different levels of cassava effluent concentrations - 0.002, 0.005, 0.008, 0.04 and 0.014mg/1 (lethal test) for 96 hours and 0.025, 0.030, 0.040, 0.050 and 0.060mg/1 (sub lethal) for 14 days replicated three times. It was observed that most of the test organisms that died within the 96hrs of the experiment died in the first 24 hours. The 96hrs LC50 was 0.020mg/1 and 14 days LC50 was 0.011mg/1. The alterations observed on the heart and gills tissues were presented. In the control experiment, the photomicrograph of the heart showed normal arrangement of heart tissues (Mg x 400). Heart tissues of the fish exposed to 0.002mg/1 concentration of effluent showed the partially disrupted epicardium (Mg x 400). The heart tissues of fish exposed to 0.005mg/1 showed fairly disrupted myofibrils (M) while at 0.008mg/1, the heart tissues presented a fairly disrupted myofibrils (M) with congested lumen (CL) and poorly disrupted myofibrils. A necrotic cell congestion of lumen and total disappearance of the myofibrils were expressed in 0.014mg/cc concentration. Gill section of the fish at 0.002mg/1 showed a mild level of desquamation (epithelial lifting leading to diffuse mass of gill lamella). Gill section of fish of 0.005mg/1 showed the disappearance of the gill lamella, hyaline cartilage place with haemorrhage and severe of pigmentation. At 0.008mg/1, the gill tissues showed severe diffusion of both primary and secondary lamella with disintegration of epithelial lining. White at 0.011mg/1 and 0.014mg/1, there was necrosis of gill tissues and chronic deformation of gill tissues with total disappearance of both primary and secondary lamella respectively. This study established that, exposure of C. gariepinus to very low concentrations (0.008mg/1) of cassava waste water effluent can induce various toxicological effect and histological degradation.

Key words: Histology, Cassava effluents, Lethal and sub-lethal tests, Environment.

INTRODUCTION
Cassava (Manihot esculenta) is a good source of carbohydrate, widely cultivated crop used as staple food in the world. It is highly acceptable and rated as the third largest source of carbohydrate for meal in the world, while Nigeria is the world’s largest producer. However, cassava is endemic to African countries (Wade et al., 2002). Cassava are mostly processed into a Nigerian staple food - Garri and cassava mill plants are often directly or indirectly discharged into aquatic system without any prior treatment, this has negative impact on the environment and biodiversity (Goodley, 2004) resulting into water pollution. The chemical compound found in the waste water during cassava processing is called cyanide. This results from the hydrolysis of cyanogens and glycosides present in the cassava (Oti, 2002). It is toxic to aquatic animals and poses serious threat to the environment (Abiona et al., 2005).

The toxicity of hydrogen cyanide is caused by the cyanide ion (CN-) which halts cellular respiration by inhibiting an enzyme in the mitochondria called Cytochrome C Oxidase. Cyanide ions (CN-) also interfere with iron-containing respiratory enzymes (Osuntokun, 1994). Toxic chemicals in cassava effluent cause tissue damage and histopathological degradations and generally, such degradation have been observed in the gills, liver, heart, kidney, and epidermis of these aquatic organism-catfishes (Van Dyk et al., 2005). Extraction of starch from cassava roots requires large amounts of water. After separation of starch and fibre, the remains are the residual water. Cassava waste water contains unextracted nitrogenous compounds and cyanoglycosides. When this water is released directly into streams and rivers, residual starch can cause rapid growth of bacteria, resulting in oxygen depletion and detrimental effects on aquatic life (Goodley, 2004).
Industrial effluent discharges are worldwide sources of potential pollution (Ajao, 1985). Acute toxicity tests give first-hand information on the effects of such discharges on organisms and the ecosystem as a whole and are valuable in creating awareness as to the potential harmful effects of such industrial discharges to the environment. In recent years, histopathological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment (Wendelaar Bonga, 1997). Therefore, this study investigated the level of tolerance of catfish exposed to cassava effluent and extent of mutilation caused to the heart and gills of after its exposure.

MATERIALS AND METHODS

The experiment was conducted at the fishery unit of LAUTECH Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso. The cassava effluents used for the tests was collected from a Gari processing mill at Isale-Ora, Ogbomoso Eighty four African catfishes (Clarias gariepinus) were purchased from the ministry of Agriculture, Ogbomoso. During transportation of the fish to the laboratory, proper aeration was ensured. The containers in the laboratory were washed with KNO₃ to remove any adsorbed metals and they were sun dried. The fishes were acclimatized for 14 days and were not fed for the first three (3) days, this is to ensure that all the food present in their stomach is completely digested to enable their adaption to the new feed. They were fed once daily.

The experimental set ups were designed for both lethal and sub-lethal tests. For lethal test five concentrations were used based on the results obtained in range finding test. Each of the treatments had three replicates according to Marison (1991) methods. The effluent concentrations (0.025, 0.030, 0.040, 0.050 and 0.060mg/l) were prepared before the commence ment of the experiment and ordinary water serves as control. Ten fishes were introduced into the test solutions and the control experiment. The experimental set ups were monitored for 96 hours. The treatments had three replicates according to the new feed. They were fed once daily.

Acute toxicity tests give first-hand information on the effects of such discharges on organisms and the ecosystem as a whole and are valuable in creating awareness as to the potential harmful effects of such industrial discharges to the environment. In recent years, histopathological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment (Wendelaar Bonga, 1997). Therefore, this study investigated the level of tolerance of catfish exposed to cassava effluent and extent of mutilation caused to the heart and gills of after its exposure.

RESULTS

The mortalities from the lethal and sub-lethal tests were dissected and the selected organs such as gills and hearts were removed from each experimental set up, washed in saline water, fixed with 10% formalin for 24 hours. The washed tissues were dehydrated though a series of graded alcohol, then cleared in xylene, infiltrated with paraffin in a vacuum oven at 56°C and embedded in 40% paraffin wax for 5 hours (Luna, 1968). The embedded sanytes were sectioned to 5nm thickness, mounted on slides with neutral canadal balmsa, stained with haematoxylin and eosin for 5 minutes (Anon, 1969) and examined under microscope at x 100 and x 200 magnifications in order to desorbed the normal histological structures, appearance, arrangement and their physiological conditions the photo-micrographic. Impressions of the slide for each tissue of lethal and sublethal test were taken and interpreted.

Histopathological investigations: The photomicrographs of the heart and gills tissues were presented in plates A-L, showing heart and gills tissues alterations that were observed. In the control experiment the photomicrograph of the heart showed normal arrangement of heart tissues (Plate A) (Mg x 400). Heart tissues of the fish exposed to 0.002mg/ l concentration of effluent (treatment 2) showed the partially disrupted epicardium (Plate B) (Mg x 400). The heart tissues of fish of 0.005mg/ l showed fairly disrupted myofibrils (M) (Plate C) while at 0.008mg/ l the heart...
tissues presented fairly disrupted myofibrils (M) with congested lumen (CL) and poorly disrupted myofibrils (Plate E). Necrotic cell congestion of lumen and total disappearance of the myofibrils were expressed at 0.014mg/l concentration.

The gill section showed the arrangement of the Primary (P) and Secondary (S) lamella, Hyaline cartilage (HC) prominently in the central core. All were well arranged in the gill structure of the control fish treatment (Plate G). Gill section of the fish at 0.002mg/l showed a mils level of desquamation (epithelial lifting leading to diffuse mass of gill lamella). Gill section of fish of 0.005mg/l showed the disappearance of the gill lamella, hyaline cartilage place with haemorrhage and severe of pigmentation. At 0.008mg/l, the gill tissues showed severe diffusion of both primary and secondary lamella with disintegration of epithelial lining white at 0.011mg/l.
DISCUSSION
The 96 hours LC$_{50}$ of the cassava effluent was 0.020mg/1 while the 2-week LC$_{50}$ of sublethal was recorded to be 0.011mg/1. These values fell within the lethal concentration ranges for O. niloticus and C. gariepinus respectively as reported in the previous studies by Ikpi et al., (2003) and Adewoye et al. (2005). They however submitted that such concentrations would disrupt the fitness of the natural population and this may be attributed to the toxic effect of the effluent on the gills; a trend that conformed to the observation of Adewoye et al., (2005); Ayoola (2008) and Ogundiran et
Histological observation in the heart tissues (Plates A-F) showed a typical structural organization of the parenchymatous cell in the untreated fishes (Plates A), however in this present study, the fishes in the treated effluent showed major histological abnormalities such as epicardium, cellular infiltration, congestion of lumen and cellular necrosis, conditions that impaired the population size of an aquatic environment.

The level of toxicity of any toxicant depends on its bioaccumulation, the chemistry of the compound and the reactions of the organisms to the toxicant (Ayoola, 2008). Behavioural abnormalities have been attributed to nervous impairment as a result of blockage of nervous transmission between the various effector sites, enzyme dysfunctions that may induce paralysis of respiratory muscles and or depression of respiratory centre and disturbances in energy or metabolic pathways which results in depletion of energy. No adverse behavioural changes or any mortality were which results in depletion of energy. No adverse behavioural changes or any mortality were recorded in the control experiment throughout the period of the bioassay. While in the effluent treated fishes, several abnormal behavioural response were observed and recorded such as incessant jumping and gulping of air; restlessness, frequent surface to bottom movement, sudden change of direction during movement, resting at the bottom, loss of skin colouration, loss of equilibrium and gradual onset of inactivity. The observation was similar to the observation of Omoregie et al., (1990); Okwuosa and Omoregie, (1995); Avoajah and Oti (1997); Omoniyi et al., (2002); Rahman et al., (2002).

The small changes observed in the lower lethal and sub lethal concentrations of the cassava effluent may be due to the avoidance behaviour of the test organisms to the cassava effluent, this conform with the submission of Donalson and Dye (1975) who opined that, fish exposed to low concentration of toxicant do not reach the stage of exhaustion, rather they quickly become adapted to the stressor. The stressful and erratic behaviour of the fish in this progressive architectural distortion varied concentrations and period of exposure, this is in agreement with the submission of Strivastava and Strivastava (1994) who reported that teleost accumulated lead (Pb) both directly from diet and indirectly from diet and in directed from aqueous medium through an active food chain by the surface lamella. The most generally encountered type of degenerative changes was congested, cellular infiltration and focal necrosis cellular necrosis as observed in this work probably resulted from excessive work required by the fish to get rid of the toxicants from its body during it process of detoxification. Frieberg et al., (1971) submitted that, fishes are known to possess sequestering agent (metallothianein), the bioaccumulation of these trace elements in the heart tissue reaches a proportion in which the function of the heart is impeded, this resulting in a progressive degeneration of the heart cells syntactical arrangement.

Therefore, necrosis became evident as the concentration increases and this may be due to the inability of fishes to regenerate new heart cells. It was also observed that the histopathological changes in the heart caused circulatory problems. This is evident and more pronounced as observed in the myofibrils (which is an indication congested lumen in the heart of the fish). An increase in the degree of damages done to the heart tissue of the fishes (C. gariepinus) held in 0.008mg/1, 0.01mg/1 and 0.014mg/1 cassava effluents, is generally related to important hepatic lesions such degenerative and necrotic processes, this observation was in line with submission of Chang et al., (2000) and Pacheco and Santos (2002). Furthermore, the presence of melanomacrophages in great quantity in the heart of exposed C. gariepinus is strong evidence that this organ suffered structural and circulatory disorder due to the exposure to the effluent. This is in a way signalling the fact that this environment where the effluent is discharges is grossly polluted and impaired.

Histological observations in the gills (Plates G-L) - The main sites of heavy metal uptake and accumulation of effluents are the gills and gastrointestinal tracts (Annume and Iyaniwura, 1993). Gills are the primary initial stage of various effluents and cytological changes in gill morphology in fish usually occur as a result of contaminant exposure. A negative effect on the gills serves as a stressor and may result in fish death and it also predisposes the fishes to disease.

The progressive architectural distortion at the varied periods of exposure and dosage observed in my studies support the findings of
Strivastava and Strivastava (1994). The major histological abnormalities observed in (Plate H-L) were desquamation, disappearance of gill lamella, occurrence of haemorrhage and pigmentation, disintegration of epithelial line and total occurrence of necrosis. Whereas they were all well placed in the non-treated fish with no signs of necrotic occurrence in Plate A, Johnson and Bergman (1984) found that gills lesion occurred in all organism at contaminant concentration above a threshold level. Munktittirck and Power (1990) have suggested that measurement of gill histological change is valuable since they have been linked to changes at the organism level. The lesions observed in the present study also indicate an initial defence mechanism of the fish against cassava effluent concentration and duration of exposure. Roncero et al., (1990) also reported the occurrence of oedema and epithelia hyperplasia and necrosis in the gills of tench (Tinca tinca, L.) subjected to experimental cassava waste water effluent.

Therefore, the histological changes observed in the heart and gills of the C. gariepinus in the present study indicate that the fish were responding to the direct and the additive effects of the contaminants more than other effect such as stress. Such information confirms that histopathological alterations are good biomarkers for both food and laboratory assessment, particularly in tropical areas that are naturally subjected to a multiplicity of environmental variations of depletion due to chemical contamination.

CONCLUSION

Conclusively, this study have been able to establish the fact that, exposure of C. gariepinus (adult size) to even low concentrations (0.008mg/l) of cassava waste water effluent can induce various toxicological effect and histological degradation which depended on the period of exposure and concentration of toxicant. In view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of cassava waste water effluent can induce damages to the tissue and organs, which might make all the living entities in polluted environment vulnerable to disease, and eventually leads to death.

REFERENCES