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TOXICITY OF CALCIUM HYPOCHLORITE ON FINGERLINGS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822).

Ayotunde S. Kolawole and Oyinkansola Olukunle[1]
Dept. of Aquaculture and Fisheries Management
University of Ibadan, Nigeria

Corresponding author – E-mail address: oyinkansolaolukunle@gmail.com

Abstract

The toxicity of calcium hypochlorite (containing 65% chlorine disinfectant) was investigated on fingerlings of Clarias gariepinus.Fish of mean weight of 5.5 ± 0.8g and total length of 7.8 ± 0.5cm were exposed to various concentrations (0.00, 0.011, 0.033, 0055 and 0.077 mg/l) of calcium hypochlorite resulting in residual chlorine of (0.00, 0.002, 0.006, 0.010 and 0.014 mg/l respectively) for 12, 24, 48 and 96h, adopting a static bioassay technique. The water quality, tolerance and behavioural responses, haematological and histopathological parameters of fingerlings of Clarias gariepinus were monitored and measured using standard methods. Blood samples were obtained from the caudal circulation and used for the measurement of Packed Cell Volume (PCV), Haemogolobin level (Hb), Red and white Blood cell count. Erratic swimming, excited competitive movements and gasping for air at the surface were the behavioural changes observed with increase in time of exposure and concentration of the chemical. The water quality parameters were measured during and after the experiment. Significant differences were observed. The PCV showed significant (P<0.05) decrease in values between 25% and 20% in all concentrations compared to the control value of 29% at 96h exposure. Haemoglobin levels were similarly significantly (P<0.05) lowered from the control value of 8.3g/dL to 6.7g/dL in the varying concentrations with increase in time of exposure. Deteriorating changes were observed in the liver, gill and brain of fingerlings of C. gariepinus exposed to calcium hypochlorite with increase in both concentration and time of exposure. It is therefore concluded that treated municipal water supply which usually contains residual chlorine of 0.20ppm is not suitable for aquaculture. It is recommended that the water should be exposed to light between 24 to 48 hours in order to allow for the evaporation of the residual chlorine present in it if it must be used at all. Calcium hypochlorite should not be used indiscriminately in aquaculture systems.

Keywords: Chlorine, Hypochlorite, Haematology, Histopathology, *Clarias gariepinus*.

Introduction

A number of chemicals are used in aquaculture for improving pond soil, water quality and for controlling problems of phytoplankton blooms, aquatic plant infestations, disease vectors and proliferation of wild fish (Boyd and Tucker, 1998).

Aquaculturists are usually aware of the dangers these chemicals pose to man when handled, but often overlook their potential impact on the surrounding pond environment and on the quality of aquatic food products. Only few chemicals have been ecologically tested in Nigeria for safety in spite of their environmental and ecological impact (Ogundiran *et al.*, 2007).

Water supplies for aquaculture are sometimes means for the introduction and spread of infectious diseases. A pathogen-free water source is essential for success in aquaculture. Surface waters commonly used in aquaculture come from coastal waters or rivers which may contain some fish pathogens and such open water supplies should not be used without treatment. Disinfection of the surface water is very important to avoid pathogen contamination of the culture environment.

Calcium hypochlorite is widely used as a

source of chlorine for disinfecting fish rearing tanks and also for household water treatment. The chlorine is a powerful disinfectant when used either on its own or as calcium hypochlorite. It has the major advantage of ensuring clean water right up to the tap, whereas the actions of other disinfectants such as ozone, ultraviolet light and ultrafiltration are only temporary. In addition to purifying water, chlorine helps remove tastes and odours, controls the growth of slime and algae in main pipes and storage tanks. It also removes unwanted nitrogen compounds from water. Today, much of the world's drinking water depends on chlorination.

Aquatic toxicity testing in water pollution control is very necessary to determine whether a potential toxicant is dangerous to aquatic life and, to find the relationship between toxicant concentration and its effect on aquatic animals (Onuoha, 2010). One significant objective is often to ascertain the concentration which may be permitted in receiving waters without adversely affecting the fauna or prejudicing other uses (Hunter, 1978).

The use of haematological technique in fish culture is growing in importance for toxicological research, monitoring of culture environment and fish health conditions (Onuoha, 2010). Sampath *et. al.*, (1993) noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is an outward manifestation of disease. Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari and Sarkar, 2004; Maheswaran *et al.*, 2008). Toxic chemicals cause tissue damage and histopathological degradations as the fish show haematological responses to toxicants, and generally, such degradation of histological origin occurs in the livers, gills and brain. The liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Myers *et al.*, 1998), and exhibit the effects of a variety of environmental pollutants (Hinton *et al.*, 1992).

Adeogun (2004) reported that different toxicants produce remarkable lesions on the gills of fish such as scattered gill filaments, thickened gill filament, inflammation of lamellae and necrosis at higher concentration. Fagbenro (2002) also reported that high mortality occurred in fish showing severe gill epithelia hyperplasic, separation of the gill epithelial layers from supportive tissues and necrosis.

Fishes are widely used to evaluate the health of aquatic ecosystem and physiological changes serves as biomarkers of environmental pollution (Kock *et al.*, 1966). *Clarias gariepinus* is most widely used because, it is hardy. It is able to tolerate both well and poorly oxygenated waters. It is widely cultivated in Nigerian water bodies, hence used as biological indicators of ecotoxicological studies. This paper report findings of a study done to investigate the effects of calcium hypochlorite, a commonly used disinfectant in aquaculture management; on water quality, haematological and histopathological parameters of the African catfish, *Clarias gariepinus* fingerlings.

Materials and Methods

The 96-hour static bioassay was conducted in the laboratory of the department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria to determine the effects of a disinfectant, Calcium hypochlorite on fingerlings of *Clarias gariepinus*. 250 fingerlings of *Clarias gariepinus* of mean weight 5.5 ± 0.8g and 7.8 ± 0.5cm total length were obtained from a private hatchery in Ibadan, Oyo State , Nigeria and transported to the laboratory. The disinfectant used was obtained from an agro-chemical sales and distribution centre, located in Ibadan, Oyo State, Nigeria. The fish were acclimated for 21 days in rectangular plastic aquaria prior to tests. During the acclimation, the test water was changed every three days.The tanks were monitored daily for mortality. Experimental fish were fed daily with 1.8mm 45% Crude Protein Coppens® catfish feed.

The stock solution of calcium hypochlorite was prepared by diluting 1.10mg of calcium hypochlorite in 1 litre of distilled water to give a residual chlorine level of 0.20ppm.

In order to determine the amount of calcium hypochlorite to be used to disinfect water, it is essential to determine the chlorine demand of the water. 1% chlorine solution was prepared by dissolving 1.35g of calcium hypochlorite in 100ml of distilled water. Then 2.5 ml of the chlorine solution was dispensed into 100ml volumetric flask and made up to mark with distilled water. Dispensed 200ml of the raw water sample into each of the 4 amber bottles of 250ml capacity. Added 0.5, 1.0, 1.5, and 2.0 ml of the distilled chlorine solution prepared into 200ml of the raw water dispensed in the 4 amber bottles. Mix and allow to stand for 30minutes.Determine the residual chlorine at the end of 30minutes This was done for the water to be used for the experiment, Body (1995a). At the end of the acclimatization period, the range finding test was carried out according to the method described by Odiete (1999) and mortality monitored every two (2) hours for 24 hours.

Concentrations of the disinfectant which caused death of fish within 30 minutes were omitted from the test. Twelve experimental tanks were used for the range finding test. The following concentrations in milligram per liter (mg/l) were used for the range finding test: 0.011, 0.11 and 1.1mg/l.

At the end of the range finding test, the definitive test commenced. After determining that 0.011mg/l of calcium hypochlorite recorded 33% mortality, the concentrations introduced for the definitive test which lasted for 96 hours were 0.011, 0.033, 0.055 and 0.077mg/l. The control was included containing no disinfectant with each treatment having here (3) replicates. 15 experimental tanks, each with 10 litres of water were used for the definitive test .The tanks were covered with lid made of fine polyethylene gauze screen of 1mm mesh size to prevent the fish from jumping out of the containers. In each case, 10 fingerlings of *C. gariepinus* were sorted randomly into each tank with known concentration of disinfectants. The fish were subjected to photoperiod of 12 hours light and 12 hours darkness.

Observations for the behavioural responses of fish to the toxicant such as

loss of equilibrium, respiratory function, swimming behaviour, pigmentation and death were done in each replicate. Death of the fish were confirmed when the respiratory movement of the gills stopped (opercula movement), also when the fish does not respond to stimuli. The dead fish were removed during the experiment and the mortality recorded at 3 hours interval until 96 hours.

Selected water quality parameters were measured before the introduction of the disinfectant and at the end of the experiment. The parameters measured before disinfectant was introduced included pH 7.10 ± 0.25, temperature 27.50 ± 2.50°C, dissolved oxygen 8.50 ± 1.20mg/l. Nitrite 0.10 ± 0.13mg/l and ammonia 0.30mg/l. Also, residual chlorine was measured before, during and after the disinfectant was introduced. See Table 1, 2 and 3

Table 1: Mean values of water quality parameters before the 96-hour exposure of various concentrations of calcium hypochlorite

pH	Temp(°C)	DO(mg/l)	Nitrite NO_2(mg/l)	Ammonia NH_3(mg/l)
7.10	27.50	8.50	0.10	0.30

Table 2: Values of water quality parameters during the 96-hour exposure of to various concentrations of calcium hypochlorite
Water Quality Parameters

Calcium Conc(mg/l)	pH	Temp(°C)	DO(mg/l)	Nitrite NO_2(mg/l)	Ammonia NH_3(mg/l)	Residual Chlorine
0.000	7.15	25.30	8.20	0.12	0.35	0.000
0.011	6.90	25.80	8.00	0.20	5.00	0.002
0.033	6.30	26.20	7.68	0.33	9.50	0.006
0.055	6.05	26.50	7.20	0.50	10.05	0.010
0.077	5.85	26.80	6.80	0.65	10.65	0.014

Table 3: The values of water quality parameters after the 96-hour exposure of C.gariepinus to various concentrations of calcium hypochlorite

Calcium Conc(mg/l)	pH	Temp(°C)	DO(mg/l)	Nitrite NO_2(mg/l)	Ammonia NH_3(mg/l)	Residual Chlorine
0.000	6.90	25.00	7.80	0.10	0.45	0.000
0.011	7.05	25.80	7.50	0.25	7.00	0.002
0.033	7.28	26.00	7.20	0.35	10.55	0.006
0.055	7.30	26.10	7.70	0.15	0.55	0.010
0.077	7.30	26.50	7.60	0.15	0.50	0.014

The experiment was performed using static bioassay in which the fish is kept in the same test stagnant solution throughout the test duration of 96 hours.

Blood from the selected fish was drawn from the caudal vessels with a heparinized disposable plastic syringes into heparinized microhaematocrit tubes sealed with plasticine at one end and centrifuged for 5 minutes at 3,000 rpm. The blood samples were then used for the measurement of Packed Cell volume (PCV), Haemoglobin level, Red and white Blood Cell Count. All determinations were carried out in duplicates for each sample.

The PCV (%) was measured using a haemocrit reader. Haemoglobin levels were obtained by means of Boehringer-Mannheim® commercial kits, based on colorimetric determinations. Total Erythrocyte Count (TEC) was performed with microscope Nebauer count chambers diluting the blood (20 times) Toisson's solution and total leukocytes Count (TLC) was performed with microscopic Nebauer count chambers diluting the blood (200 times) in Turk's solution. The haematological indices of Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated using the equations given by Anderson and Klontz (1965).

For the histopathological examination, fish samples were taken from each treatment and the abdominal cavities of the sacrificed fish were then opened and some organs removed. Omoregie and Wade (2002) reported organs that are mostly affected by toxicant to include liver, brain, gills and kidney among others. Samples of the liver, brain and gill of the exposed fish including the control experiment were extracted and fixed in Bouins solution as reported by Akpokodje et. *al.* (2005). The sections collected were stained with Haematoxylin and eosin.

The organs selected for histopathological examination went through series of processes. Formaldehyde was used to fix the tissues in the volume 10 times that of specimens for 24 hours. The second stage was dehydration which was done manually to remove the inherent water content of the specimen in a gradual way considering osmotic dynamics. Other processes were clearing, infiltration, embedding, blocking, sectioning and staining. Visual observation of the effect of different concentrations of calcium hypochlorite on the fish was made every three hours for 96 hours.

Results obtained for the triplicates from the experiment were combined, subjected to statistical analysis using two-way analysis of variance (ANOVA) to test differences between the various levels of concentration of calcium hypochlorite and the exposure periods.

Results and Discussion

At the 12^{th} and 21^{st} hour into the experiment, the definitive test recorded 100% mortality at both 0.055 and 0.077mg/l concentrations of the disinfectant (see Tables 1 and 2) The experimental unit containing 0.033mg/l concentration of the disinfectant recorded an average of 56.67% mortality at the end of the experiment. (Table 4).

Table 4: Rate of mortality of *C.gariepinus* fingerlings exposure to different concentrations of Calcium hypochlorite per treatment

Treatment/hr	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	Total Mortality	%Mortality
TO_1																																			
TO_2																																			
TO_3																																			
TA_1																																			
TA_2																																			
TA_3							01																											01	10
TB_1					01	02		02																										05	50
TB_2				02		03	02																											07	70
TB_3					01	01	01	02																										05	50
TC_1			03	02	02	01	02																											10	100
TC_2		02	02	01	01	03	01																											10	100
TC_3			01	03	03	01		02																										10	100
TD_1		02	03	05																														10	100
TD_2		03	01	04	02																													10	100
TD_3		02	04	02	02																													10	100

TO=Control treatment without toxicant, TA= 0.002ppm of toxicant, TB= 0.006ppm of toxicant, TC= 0.010 of toxicant, TD= 0.014ppm of toxicant

All fish in the control experiment showed no sign of stress of discomfort. On introduction of the disinfectant into other experimental units, fish showed instant response to toxicant upon contact during which the swimming pattern changed, suggesting stressful condition. Behavioural changes observed include uncoordinated erratic swimming, excited competitive movement, frequent jumping and gasping for air at the surface and swimming upside down up due to respiratory impairment and skin irritation which is an indicator of the effects of the toxicant on the nervous system as was reported by Ayoola (2008). These were followed by weak swimming, settling down at the bottom of the tanks motionless as time of exposure increases and finally death. These observations agrees with the reports of Omitoyin *et al.,* (2006) and Adesina (2008). The colour of the fish skin was changed from normal darkly pigmentation in the dorsal and lateral parts to very light pigmentation and bleaching of the skin.

Several authors had reported works on similar patterns of abnormal behavioural responses in fish exposed to toxicant (Wade et al., 2002, Rahman et al., 2002, and Ajani, 2006). However, it was observed that the fingerlings of *Clarias gariepinus* showed variation in their tolerance of same concentration of calcium hypochlorite. Fryer (1977) found out that in all toxicants, a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance.

The pH and temperature did not display significant differences before and after the introduction of the disinfectant and even after the experiment. The dissolved oxygen decreased with increase in concentration to 6.80mg/l from 8.00mg/l in the control experiment after the toxicant was introduced. This could be as a result of the chemical reaction between calcium hypochlorite and the experimental water as the disinfectant is highly soluble in water and initially increase the respiration of the fish as a result of stress-induced introduction of the disinfectant especially at high concentrations. Warren (1977) reported that the introduction of toxicant into an aquatic system might decrease the dissolved oxygen in the water thereby impairing respiration and leading to asphyxiation. The nitrite and ammonia levels had varying values with time of exposure and increase in concentration (Table 3).

In Tables 5 and 6 the PCV of the fish showed significant decrease from 27% in the control experiment to 20% after 24hr andfrom 29% to 18% after 96hr in 0.077mg/l calcium hypochlorite exposure group. Results of ANOVA showed that there were significant differences in the mean levels values of PCV with increase in the concentration levels of the toxicant. The decreased value of PCV may be due to anaemia and haemodilution or haemolysis of RBC as was reported by Fafioye (2002). The effect of sublethal concentration of 15 mg/l of malachite green on the blood composition of the fish Clarias gariepinus exposed under static bioassay also caused anaemia.
(Musa and Omoregie, 1999).

Table 5: Haematological parameters of *Clarias gariepinus* fingerlings subjected to different concentration of Residual Chlorine at 24hours exposure

Treatment Conc(ppm)	PCV (%)	Hb(g/dl)	RBC($10_{/\mu l}$	WBC(10^3 /mm^3)	Platelets	Lympho cyte(10^3/ mm^3)	Heterop hil(10^3/m m^3)	Monocyte(10^3/ mm^3)	Eosino phil(10^3 /mm^3)	MCV(fl)	MCHC (pg)	MCH
O(Control)	27	8.8	1.64	22,700	54,000	74	20	3	3	164.63	32.59	53.66
A(0.002)	25	7.0	1.88	17,200	102,000	67	25	4	2	132.99	28.00	37.23
B(0.02)	20	6.8	2.77	16,650	133,000	61	28	2	3	72.20	34.00	24.55
C(0.20)	18	5.4	1.45	18,900	106,000	69	31	4	4	52.17	30.00	37.24

PCV=Packed cell volume, **Hb**= Haemoglobin, **RBC**= Red blood cell count (erythrocyte), **WBC** = White blood cell count, **MCV** = Mean cell volume, **MCHC** = Mean cell haemoglobin count, **MCH** = Mean cell haemoglobin

Table 6: Haematological parameters of *Clarias gariepinus* fingerlings subjected to different concentration of Residual Chlorine at 96-hour exposure.

Treatment Conc.(ppm)	PCV (%)	Hb (g/dl)	RBC ($10_{/\mu l}$)	WBC (10^3/mm^3)	Platelets (10^3)	Lympho cyte(10^3 /mm^3)	Hetero phil(10^3 /mm^3)	Monocyte (10^3/mm^3)	Eosino phil(10^3 /mm^3)	MCV(fl)	MCHC (pg)	MCH
O(Control)	29	8.3	1.65	16,000	126	83	12	3	1	175.76	28.62	50.30
A(0.002)	25	7.7	1.58	15,650	83	89	8	2	0	158.23	30.80	48.73
B(0.006)	15	4.6	1.60	15,200	86	84	13	1	0	93.75	30.67	28.75
C(0.010)	23	7.3	2.71	17,750	133	62	33	3	2	84.87	31.73	26.94
D(0.014)	20	6.7	1.48	14,200	109	67	29	3	1	135.14	33.50	45.27

PCV=Packed cell volume, **Hb**= Haemoglobin, **RBC**= Red blood cell count (erythrocyte), **WBC**= White blood cell count, **MCV**= Mean cell volume, **MCHC**= Mean cell haemoglobin count, **MCH**= Mean cell haemoglobin

The variation in the PCV level as recorded in the experiment could be as a result of immunological status of the fish used as this could vary among species of the same strain, age and environmental conditions (Onuoha, 2010) (Table 4).

The Hb, RBC and MCV of *C. gariepinus* fingerlings showed congestion significant decreasing from control to the fish exposed to the highest concentration (0.077mg.l). The lymphocyte level decreased from 83 x 10^3/mm^3 in the control to 62 x 10^3/mm^3 in concentration 0.033 and 0.055mg/l respectively but increased to 89 x 10^3/mm^3 in 0.011 and 84.10^3/mm^3 in 0.033mg/l (Table 4).

Changes in haematological parameters of *C. gariepinus* due to stress caused by environmental pollutants, disease or attack by pathogens have been reported by a number of authors (Ezeri, 2001, Gabriel *et al.*, 2001). The statistically significant ($P < 0.05$) decrease in values of the haematological parameters studied is not uncommon in fish exposed to sublethal concentrations of toxicants and therapeutic agents. Omoregie *et al.*, (1994) also made similar observations when *Oreochromis niloticus* was exposed to sublethal concentrations of formalin. The general reduction of the blood parameters is an indication of anaemia caused by exposure of *C. gariepinus* to calcium hypochlorite over the period of the study, there were no significant histological changes observed in the photomicrograph of the brain in the control experiment (0.000mg/l of toxicant) hence no visible lesion observed in Plate 1.1 . However, in contrast, Plates 1.2, 1.3 and 1.4 show different stages of spongiform vacoulations in the neurophil; graduating to moderate neuronal necrosis /degeneration leading to widespread vacuolation of the neurophil and finally autolysis as shown in Plate 1.5. The trend observed is in response to increasing concentration of the toxicant respectively (Table 7).

Table 7: Summary of histopathological changes observed in the liver, brain and gill of *C.gariepinus* to various concentrations of calcium hypochlorite

Treatment	Calcium hypochlorite conc.(mg/l)	Residual chlorine concentration(ppm)	Liver	Brain	Gill
Control	0.000	0.000	There are uniformly small sized cytoplasmic vacuoles in the hepatocytes.	No visible lesion	No visible lesion
A	0.011	0.002	There are variable sized (ranging from very small to extremely large) in the hepatocytes.	No visible lesion. Except for a few foci of moderate vacuolation (spongiosis) in the neurophil.	There is slight thickening of the gill lamella
B	0.033	0.006	There are variable sized cytoplasmic vacuoles in the hepatocytes. There is also moderate congestion of the central veins	Widespread vacuolation on the neurophil.	There is moderate thickening of the primary and secondary gill lamellae: there is also moderate congestion of the gill capillaries
C	0.055	0.010	Hepatocytes contain moderately sized cytoplasmic vacuoles	Widespread vacuolation on the neurophil.	There is moderate thickening of the primary and secondary gill lamella: there is also the congestion of the gill capillaries
D	0.077	0.014	Hepatocytes contain very large cytoplasmic vacuoles	Autolysis	Poor section

The indicated spongiosis was as a result of prolonged effect of calcium hypochlorite resulting to severe brain damage. Omitoyin *et al.* (2006) reported similar findings. The gills of the fish in the control set-up showed a well arranged primary and secondary lamellae or filaments with slender branching projection lined by highly vascularized simple epithelium. The gills of the fish exposed to different concentration of calcium hypochlorite showed varying degree of damages and the intensity increased with increase in concentration. Slight and moderate thickening of lamellae, moderate congestion, necrosis, degeneration of cells and haemorrhage were all the changes observed in the fish gill.

Fagbenro (2002) reported that high mortality occurred in fish showing severe gill epithelia hyperplasia, separation of the gill epithelial layers from supportive tissues and necrosis. This is similar to reports by Adeogun (2004) that different toxicant produced remarkable lesions on the gills of the fish such as scattered gill filaments, thickened gill filament, inflammation of lamellae and necrosis in higher concentration. These effects are linked to gill function disorders which may affect their physiology and even lead to death.

Liver in the control fish had uniformly small sized cytoplasmic vacuoles in the hepatocytes and no observed lesions. Changes occurred with increase in concentration showing moderate congestion in the fish exposed to 0.033mg/l of calcium hypochlorite.

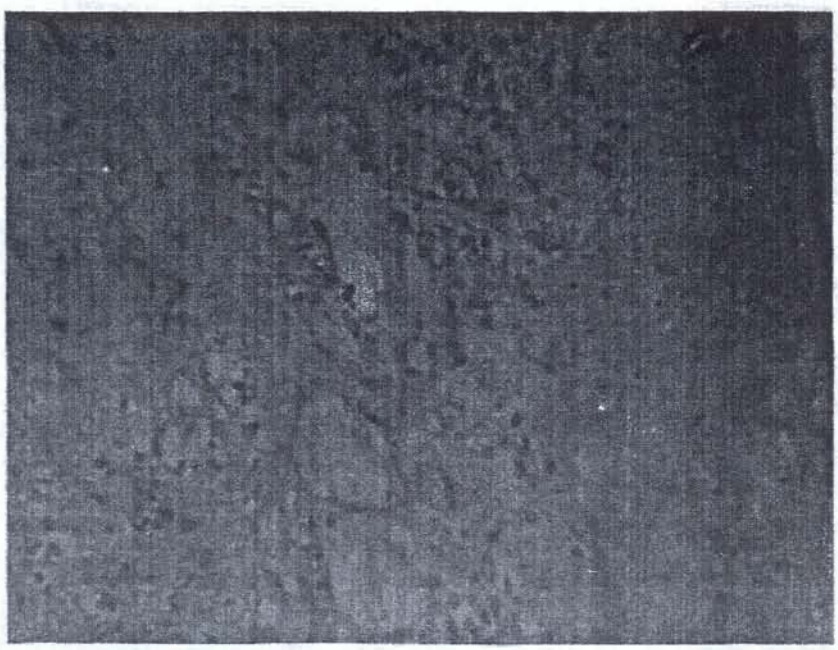

Plate 1.1: Photomicrograph of brain of *Clarias gariepinus* fingerlings in the control experiment (0.000) × 100, showing no visible lesion.

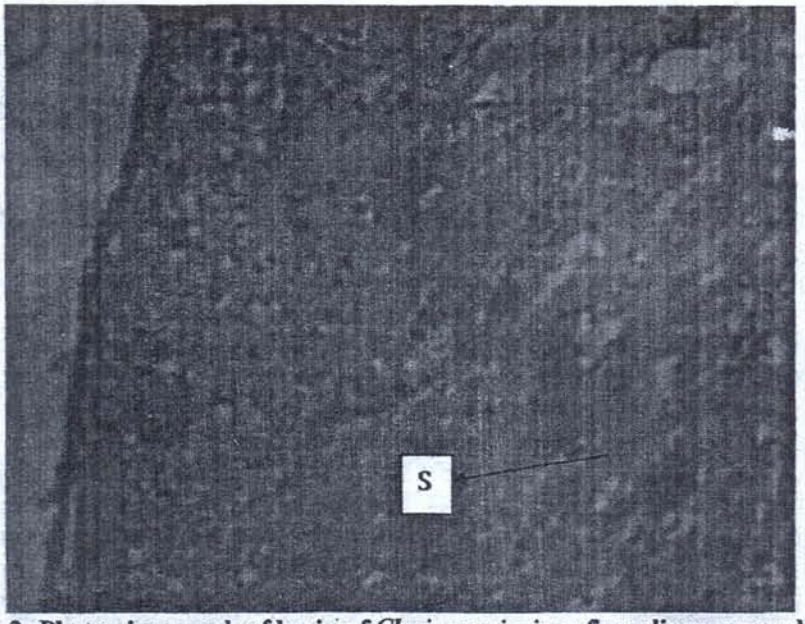

Plate 1.2: Photomicrograph of brain of *Clarias gariepinus* fingerlings exposed to 0.011mg/l of calcium hypochlorite × 100, showing spongiform vacoulations in the neurophil.

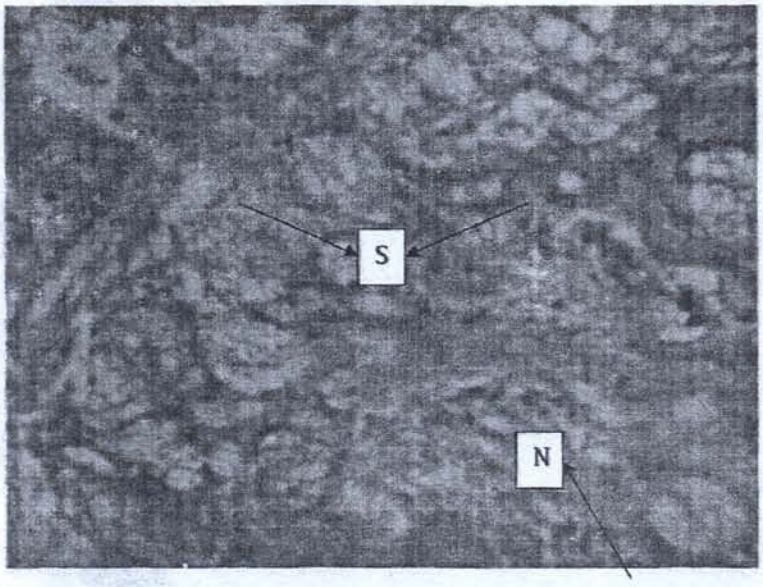

Plate 1.3: Photomicrograph of brain of *Clarias gariepinus* fingerlings exposed to 0.033mg/l of calcium hypochlorite × 100, showing numerous spongiform vacuolations of the neurons and neuropil. There is also moderate neuronal necrosis and degeneration.

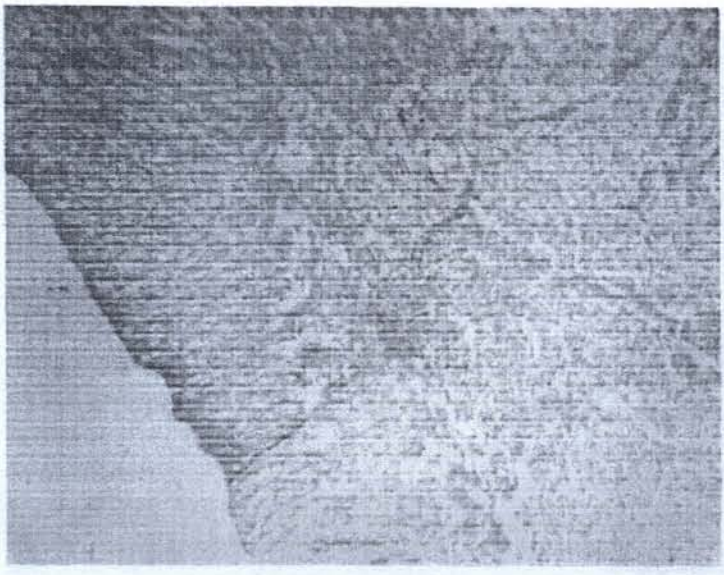

Plate 1.4: Photomicrograph of brain of *Clarias gariepinus* fingerlings exposed to 0.055mg/l of calcium hypochlorite × 100, showing widespread vacuolation of the neurophil.

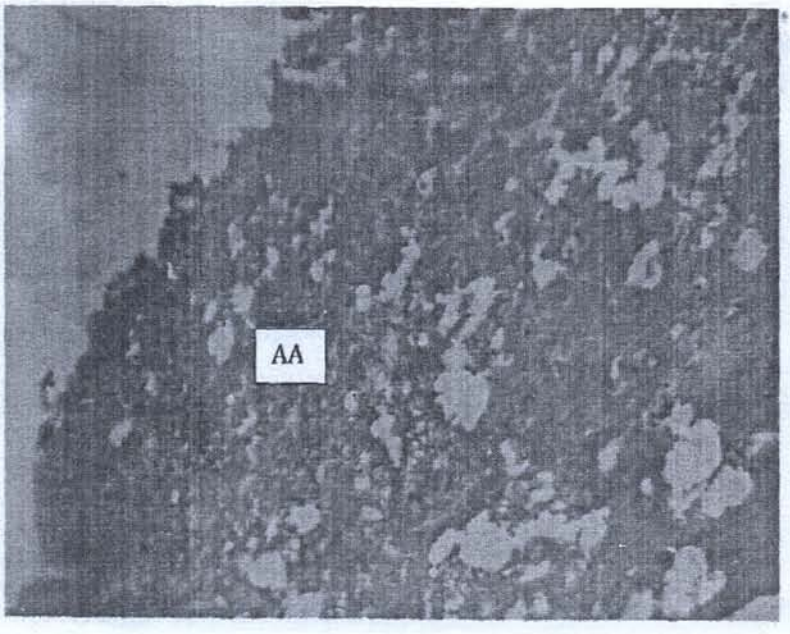

Plate 1.5: Photomicrograph of brain of *Clarias gariepinus* fingerlings exposed to 0.077mg/l of calcium hypochlorite × 100, showing autolysis (AA).

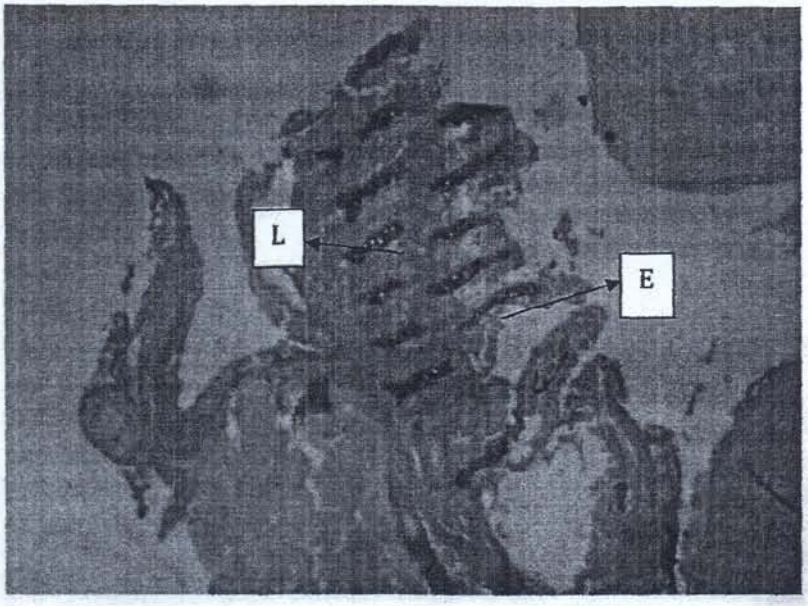

Plate 2.1: Photomicrograph of gill of *Clarias gariepinus* fingerlings in the control expeeriment × 100, showing well arranged lamella (L) and gill epithelium (E).

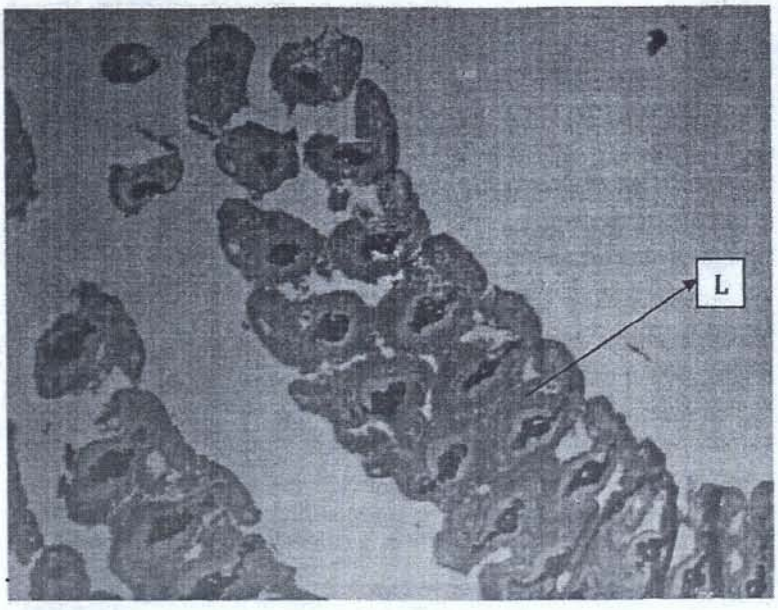

Plate 2.2: Photomicrograph of gill of *Clarias gariepinus* fingerlings exposed to 0.011mg/l of calcium hypochlorite × 100, showing slight thickening of gill lamella (L).

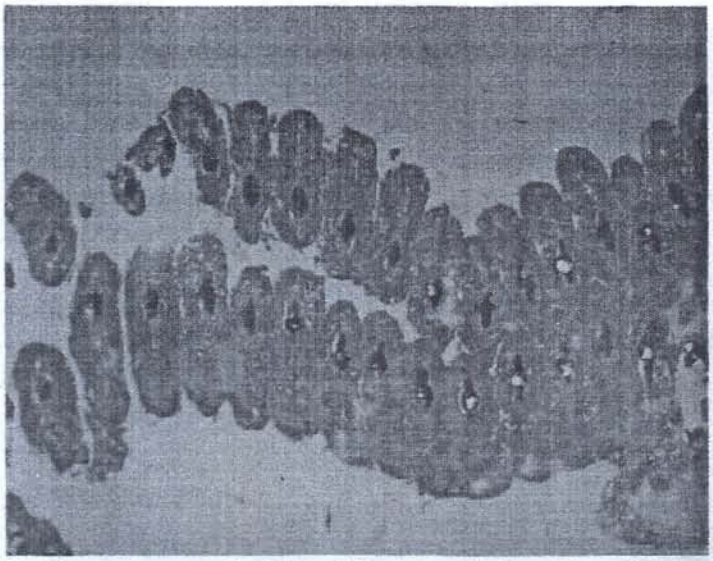

Plate 2.3: Photomicrograph of gill of *Clarias gariepinus* fingerlings exposed to 0.033mg/l of calcium hypochlorite × 100, showing moderate thickening of the primary and secondary gill lamellae. There is also moderate congestion of the gill capillaries.

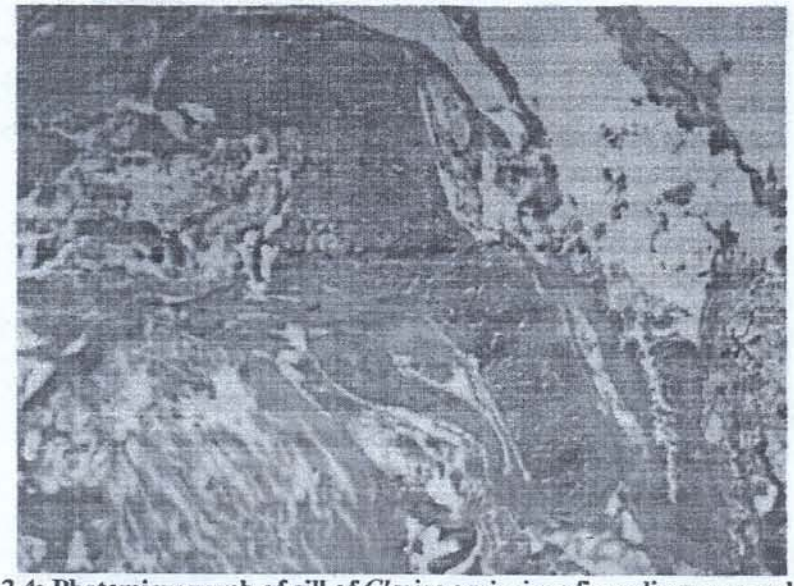

Plate 2.4: Photomicrograph of gill of *Clarias gariepinus* fingerlings exposed to 0.055mg/l of calcium hypochlorite × 100, showing moderate thickening of the gill lamellae and congestion of the gill capillaries.

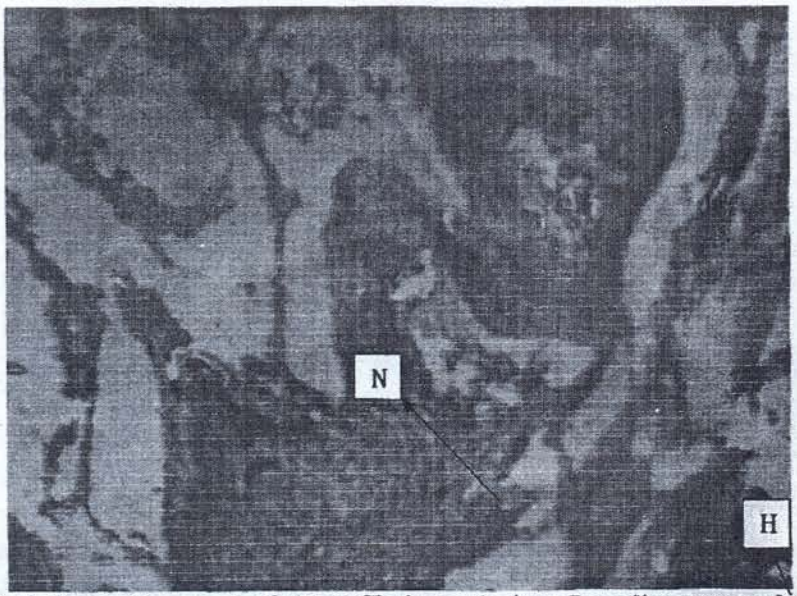

Plate 2.5: Photomicrograph of gill of *Clarias gariepinus* fingerlings exposed to 0.077mg/l of calcium hypochlorite × 100, showing necrosis (N) and degeneration of cells and Haemorrhage (H).

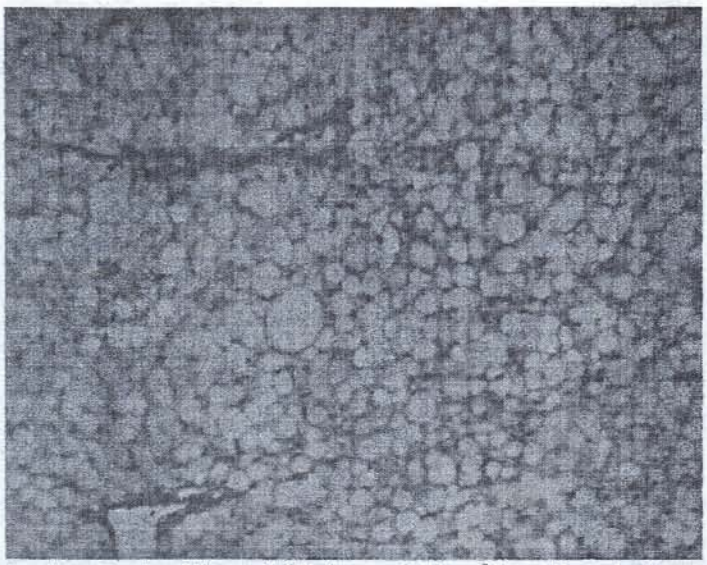

Plate 3.1: Photomicrograph of liver of *Clarias gariepinus* fingerlings in the control experiment × 100, showing uniformly small-sized cytoplasmic vacuoles in the hepatocytes.

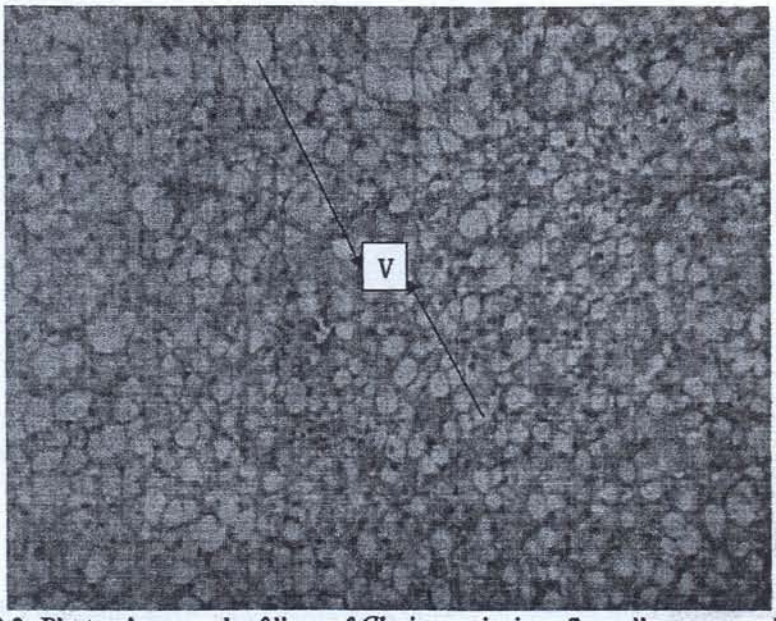

Plate 3.2: Photomicrograph of liver of *Clarias gariepinus* fingerlings exposed to 0.011mg/l of calcium hypochlorite × 100, showing variably-sized cytoplasmic vacuoles (ranging from very small to extremely large) in the hepatocytes

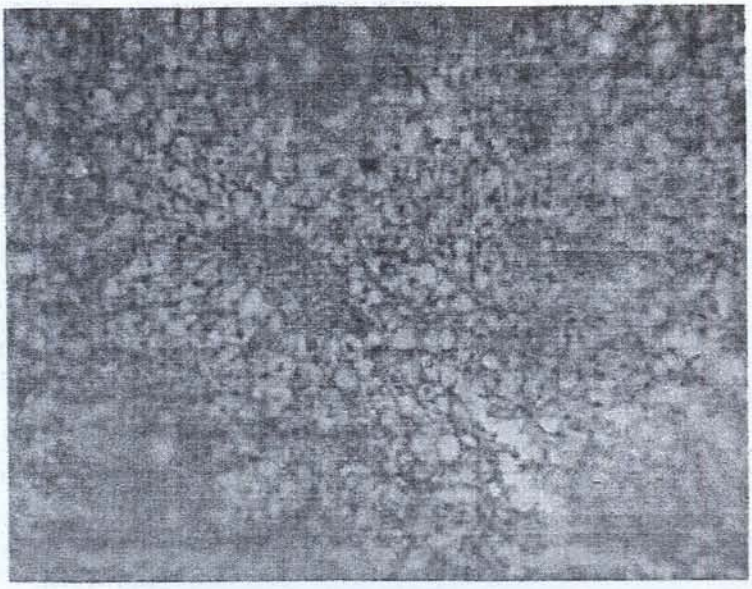

Plate 3.3: Photomicrograph of liver of *Clarias gariepinus* fingerlings exposed to 0.033mg/l of calcium hypochlorite × 100, showing variably-sized cytoplasmic vacuoles in the hepatocytes and moderate congestion in the central vein (C).

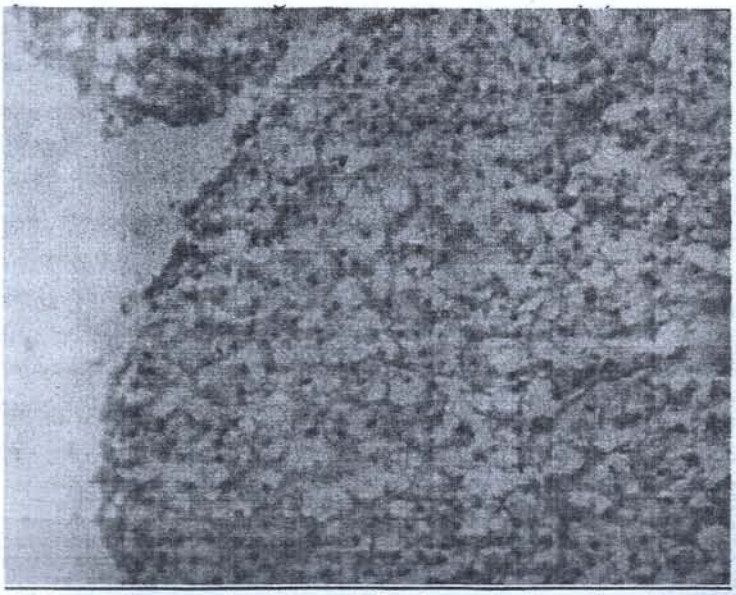

Plate 3.4: Photomicrograph of liver of *Clarias gariepinus* fingerlings exposed to 0.055 mg/l of calcium hypochlorite × 100, Hepatocytes contain moderately sized cytoplasmic vacuoles

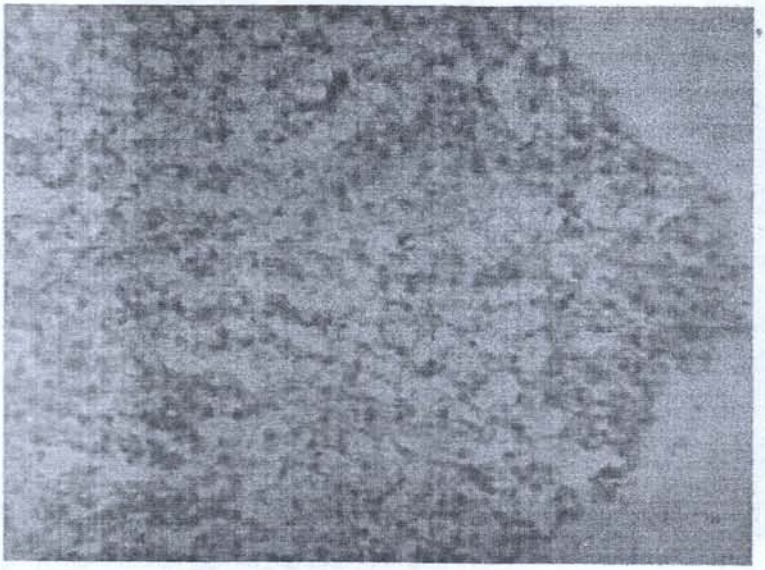

Plate 3.5: Photomicrograph of liver of *Clarias gariepinus* fingerlings exposed to 0.077 mg/l of calcium hypochlorite × 100, hepatocytes contain very large cytoplasmic vacuoles

Recommendations

A great variety of chemicals (disinfectants, therapeutants, pesticides and herbicides) affect majority of the water course which receive domestic, agricultural and industrial effluents. In this study, it was established that under short-term exposure of *C. gariepinus* fingerlings to calcium hypochlorite toxicity, negative potential alterations in the fish behavior, physical,-chemical, haematological and histological parameters resulted. See photo micrographs Therefore, in applying calcium hypochlorite as a disinfectant for water use in aquaculture, proper care must be taken to avoid excessive application.

Due to the knowledge of residual chlorine (0.2ppm) present in drinking water (tap water), it is therefore not suitable to culture fish with chlorinated water without proper treatment. There should be concentration standards that must be set for the level of residual chlorine that should be present in water meant for aquaculture. In accordance with this study, the residual chlorine level recommended for aquaculture should be less than 0.002ppm (Calcium hypochlorite of 0.011mg/l) as against the residual chlorine in municipal water supply (0.20 ppm). It is therefore recommended that the municipal water to be used if at all for aquaculture should be exposed for 24 to 48 hours to allow the evaporation of the residual chlorine present in it. However, Calcum hypochlorite should not be used indiscriminately in aqua cultural systems if it can be avoided.

Conclusions

It is evident from the results obtained in this study, that efforts should be made to reduce the chlorine content of municipal water to less than 0. 002ppm by / or in addition to exposing it to the atmosphere for 24 to 48 hours, whenever it is to be used for aquacultural fish production.

References

Adeogun, A.O. (2004). Effects of Methanolic Extract of *Raphia hookeri* (Mann and Wendl, 1804) on Life Stages of *Clarias gariepinus* (Burchell, 1862). *Ph.D Thesis,* University of Ibadan. 337pp.

Adesina, B.T. (2008). Toxicity of *Moringa oleifera* Extract to *Oreochromis niloticus* Fingerlings and Juveniles. Ph.D thesis. Department of Wildlife and Fisheries Management, University of Ibadan 272pp.

Adhikari, S. and Sarkar B. (2004). Effects of Cypermehrin and Carbofuran on Certain Haematological Parameters and Prediction of Their Recovery in Fresh Water Teleost, *Labeorohita*(Ham). Ecotoxicol. Environ. Safety 58: 220-226.

Ajani, F. (2006). Hormonal and Haematological Responses of Adult and Broodstock *Clarias gariepinus* (Burchell, 1822) to Ammonia and Nitrite Toxicity under Different Culture Environments. *African Journal of Biotechnology* 7, 19: 3466 – 3471.

Akpokodje, J.O., Mpama and Ibekwe, C.A. (2005).Basic Protocol for Histological Slide Preparation. Department of Veterinary Pathology, University of Ibadan.

Anderson, D. and Klontz, G.W. (1965). Basic Haematology for The Fish Culturist *Ann. Northwest Fish Culture Conf.* 16: 38–41.

Ayoola, S.O. (2008). Impact of Agrochemical Residue from Wetland Farming on Fish Resources in Oyo State, Nigeria. Ph.D Thesis Department of Wildlife and Fisheries Management, University of Ibadan.

Boyd, C.E. and Tucker, C.S. (1998). Pond Aquaculture Water Quality Management Kluwer Norwell, MA.

Ezeri, G.N.O. (2001). Haematological Response of *Clarias gariepinus* to Bacterial Infection and Prophylactic Treatment with Antibiotics. *J. Aquat. Sci.* 16: 22–24.

Fafioye, O.O. (2002). Lethal and Sub Lethal Effect of *Parkia biglobosa and Raphia vinifera*on some Freshwater Fauna. PhD Thesis, University of Ibadan 216pp.

Fagbenro, O.A. (2002). Tilapia: Fish for Thought. *Inaugural Lecture Series 32* delivered at the Federal University of Technology, Akure 23/07/07, 09–29.

Fryer J.D. (1977). Weed Control Handbook Vol. Make Peace Ed 384–389.

Gabriel, U.U., Alagoa, J.K. and Allison, M.E. (2001). Effects Of Dispersed Crude Oil Water Dispersion On The Haemoglobin and Haematocrit Of *Clarias gariepinus.J. Appl. Sci. Environ. Manage.* 5(2): 9–11.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A. and Okihiro, M.S. (1992). Histopathological Biomarkers. In: Huggett, R.J., Kimerle, R.A., Mehle, J.R., Bergman, H.L., Editors, Biomarkers. Biochemical, Physiological and Histological Markers of Anthropogenic Stress, Lewis Publishers, Boca Raton (1992), pp. 155–209.

Hunter, J.B. (1978). The Role Of Toxicity Testing on Water Pollution in Discharge Of Sewage From Sea Outfalls. A.I.A. Gameson Ed. Oxford Pergamon Press. 130pp.

Kock, G., Triendi, M. and Hofer, R. (1996). Seasonal Pattern of Metal Accumulation In Arctic Char (*Salvelinus alpines*) from an oligitriphicalopine lake Related To Temperature. *Can. J. Fish. Aquat. Sci.* 53: 780–786.

Maheswaran R., Devapaul A., Velmurugan B. and Ignacimuthu S. (2008). Haematological Studies of Freshwater Fish. *Clarias batrachus*(L.) Exposed to Mercuric Chloride. *Inter. J. Integr. Boil.* 2(1): 49–54.

Musa, S. and Omoregie E. (1999). Haematololgical Changes in Mudfish, *Clarias gariepinus* (Burchell, 1822) Exposed to Malachite Green *J. Aquat. Sci.* 14: 32–37.

Myers, M.S., Johnson, L.L., Olson, O.P., Sther, C.M., Homess, B.H., Collier, T.K. and McCain, B. (1998). Toxicopathic Hepatic Lesions as Biomarker of Chemical Contaminants Exposure and Effects in Marine Bottom Fish Species from The North Eastern and Pacific Coast. U.S.A. *Mar. Pollut. Bull.* 37, 92–113.

Odiete, W.O. (1999). Environmental Physiology of Animals and Pollution. Department of Zoology, Marine Biology and fisheries, University of Lagos. Diversified Resources Limited, 225–227.

Ogundiran, M.A., Fawole, O.O., Adewoye, S.O. and Ayandiran, T.A. (2007). Toxicological impact of detergent effluent on juveniles of African Catfish (*Clarias gariepinus*) (Burchell 1822). *Agric. and boil. Journal of North America* 1(3): 330.

Omitoyin, B.O., Ajani, E.K. and Fajinmi, A. (2006). Toxicity of gramoxone (paraquat) to juveniles of African Catfish, *Clarias gariepinus* (Burchell, 1822). *American J. Agric. and Environ. Sci.* 1.1: 26–30.

Omoregie, E., Eseyin, T.G., Ofojekwu, P.C. (1994). Chronic Effects of Formalin on Erythrocyte Counts and Plasma Glucose of the Nile tilapia *Oreochromis niloticus. Asian Fisheries Science* 7: 1–6.

Omoregie, E. and Wade, J.W. (2002). Toxicity of Cassava Effluent on the Nile Tilapia, *Oreochromis niloticus* under laboratory conditions. *Journal of Aquatic Sciences* 17, 2: 89–94.

Onuoha, S.O. (2010). Acute Toxicity Effects of Endosulfan (Organochlorine pesticide) on Fingerlings of *Clarias gariepinus* (Burchell, 1822). M.Sc., University of Ibadan, 98pp.

Rahmann, M.Z.Z., Hossain, N.F.R., Mellah and ahmed, G.U. (2002). Effect of Dizinon 60EC on *Anabustestudinensm, Channa punctatus* and *Barbades. Comp. Biochem. Physiol.* 134C: 109 – 121.

Sampath, K., Velamnial, S., Kennedy, I.J. and James, R. (1993). Haematological Changes and their Recovery in *Oreochromis mossambicus*as a Function of Exposure Period and Sublethal levels of Ekalus. *ActaHydrobiol.* 35: 73–83.

Wade, J.W., Omoregie, E. and Ezenwaka, F. (2002). Toxicology of Cassava (*Manihot esculanta*) Effluent on the Nile Tilapia, *Oreochromis niloticus* under Laboratory Conditions. *Journal of Aquatic Sciences* 17, 2: 89–94.

Warren, C.E. (1977). Biology and Water Pollution. Philadelphia: W.B. Sanders and Company 434pp.