Journal of Research in Forestry, Wildlife & Environment Vol. 11(4) December, 2019
E-mail: jrfwe2019@gmail.com; jfewr@yahoo.com
http://www.ajol.info/index.php/jrfwe
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ISBN: 2141 – 1778
Cheikyula et al., 2019

HISTOLOGICAL CHANGES IN JUVENILES OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO AMINO FORCE® (2, 4 D-DIMETHYLAMMONIUM SALT)

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ABSTRACT

The histological changes of exposed juveniles of African Catfish C. gariepinus to a commonly used herbicide, Amino force® was studied for 28 days. The toxicity was carried out using A hundred and twelve (112) healthy and active fish which were divided into four treatments with each treatment having ten fish and the setup was in triplicate. Some water quality parameters where evaluated during the period of the experiment. Chronic concentrations of Amino force® (0.0mg/L, 6.2mg/L, 18.5mg/L and 30.8mg/L) determined from the LC_{50} value of 360mg/L in an earlier study were used. Data for water quality parameters obtained were subjected to analysis of variance (ANOVA) at P< 0.05. Water quality results showed increase in TDS and EC, with decrease in DO as the concentrations increased while PH and temperature did not differ significantly (P>0.05). Histopathological changes in the liver were Necrosis, Vacuolation, Dilatation of the Hepatic cells and Blood congestion. In the gills, there were Distortion of the gill filaments, Blood congestion, epithelial cells Erosion, Destruction and Shortening of the lamellae. These histopathological changes observed showed increasing degrees of damage with increase in concentration of amino force®.

Keywords: Histology, African catfish, and Amino force®.

INTRODUCTION

Environmental contamination of air, water, soil and food threatens the continuous existence of many plants and animal communities and may ultimately hinder the survival of humans. The most important "in view" of the environmental pollution is the pollution of water with many contaminants including heavy metals (Abbas, 1998).

Herbicides are actively used in terrestrial and aquatic ecosystems to control unwanted weeds, and their use has generated serious concerns about the potential adverse effects of these chemicals on the environment (Oleh *et. al.*, 2009). Water pollution due to herbicides is a serious problem; due to their toxicity and persistence in the environment. More and more chemical formulations are widely used to control weeds of agricultural crops due to lack of suitable substitutes (Westernhagen *et. al.*, 1987). As a result of their usage, they find their way into the freshwater resources with the run-off water from agricultural land, or by direct application, spray drift, aerial spraying and by discharge of effluents from factories and sewage (Daabees, 1992).

Amino force belongs to the family of phenoxy carboxylic and is a selective hormone-type post emergence herbicide. It is selective for rice, maize, wheat, oil palm etc. The active ingredient contained in amino force is 720g/l 2, 4 D-Dimethylammonium salt is effective for controlling submerged aquatic plants. These compounds rapidly and completely decompose in about 3 weeks (Helfrich *et al.*, 2009). Amino force® is known to degrade rapidly in soil and natural water with DT 50 values ranging from 3-14 days in field and water. It inhibits plant growth through interference with the production of essential aromatic amino acids by inhibiting the enzyme, Enolpyruvyl Shikimate Phosphate Synthase (ESPS). This enzyme is responsible for the biosynthesis of

chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis (Pedron *et al.*, 2006).

The toxicity of Amino force® is considered to be low, according to data from (Oleh *et al.*, 2009). The toxicity of herbicides to fish depends on size and species (Noga, 2012). The African catfish, *Clarias gariepinus*, will be selected as the test organism in this study for its great aquaculture and commercial value in Nigeria and elsewhere in the developing world. *C. gariepinus* is a benthopelagic (bottom feeder), omnivorous feeder that occasionally feeds at the surface. Their diets include insects, crabs, plankton, snails and fish but also have been seen to consume young birds, rotten flesh, plants and fruits (Teugels, 1986). *C. gariepinus* also referred to as mudfish, is very hardy and tasty. They are able to tolerate adverse aquatic conditions where other cultivable fish species cannot survive (Olatunde, 1983). It is widely cultivated and used as an experimental fish (Musa and Omoregie, 1999).

The objectives of this study were to determine histological changes in the gills and liver of *Clarias gariepinus* and effects on the water quality parameters in exposures to different concentrations of Amino force®.

MATERIALS AND METHODS

Study Area

The experiment was carried out at the General Purpose Laboratory, Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria.

Collection of *Clarias gariepinus* juveniles.

A hundred and twelve (112) juveniles of *C. gariepinus* were obtained from Nigeria Army School of Military Engineering (NASME) fish farm North Bank, Makurdi. They were acclimatized for fourteen days in tanks of 60L capacity. During the period of acclimatization, the fish were fed at 5% of their body weight and water changed at intervals of two days.

Experimental Procedure

At the end of the acclimatization period, the tanks were filled with water to the 20 litre mark. The fish were randomly selected, weighed and stocked ten (10) per tank. The concentrations used were: 0.0mg/L ((control), 6.2 mg/L, 18.5mg/L, and 30.8 mg/L.

The various concentrations of herbicide used were measured using a micro-pipette and introduced into tanks containing 20 litres of water. The water quality parameters were checked before and after the introduction of the herbicide.

The fish were fed with Coppens twice daily at 5% of their body weight during the experiment. At the end of every week, weights of the fish were measured using an electronic weighing balance. The water was also changed with the renewal of the herbicide. The exposure lasted for a period of 28 days.

Determination of water quality parameters

Water quality parameters were checked before the introduction of the herbicide and at four (4) days intervals during the exposure period. The parameters determined were; Temperature, Hydrogen ion concentration(pH) , Total dissolved solids (TDS), Electrical conductivity (EC), all determined using Hanna multi parameter water tester Model HI 98129; this was done by inserting the probe into the water sample and setting the mode to read in °C ,PPM and µS/cm respectively by use of the MODE keypad. Dissolved oxygen (DO) was determined using Hanna dissolved oxygen meter Model HI 9418 by inserting the probe into the sample and recording the displayed value on the LCD after one minute.

Collection and assessment of histological samples

At the end of the exposure, the fish were randomly selected from each concentration and placed on a disserting board on which they were dissected using a dissecting scissors to remove the gills and liver.

The gills and liver extracted from the fish were placed in a 30ml Bijou bottles and 10% formaldehyde was added. The samples were taken to the laboratory for histological observations.

The histological assessments/observations of the samples include the following stages.

Dehydration: this was done by putting the liver and gills in various grades of alcohol over different periods ranging from 70% alcohol for 3-8 hours;

90% alcohol for 16 hours; absolute alcohol 1 for 2-3 hours; absolute alcohol 11 for 3 hours; to absolute alcohol 111 for 3 hours and finally into xylene for 17 hours. This process ensures hardening of the tissue and impregnation with wax 1 and wax 11.

Embedding: samples were embedded in wax. After solidifying, the samples were trimmed and mounted on wooden block to fit in microtome.

Sectioning: The microtome was used to section samples into 4-5µm for all histological samples.

Floatation: the samples were floated with warm water in a floating out bath to unfold the tissue. The tissue were picked, put on a slide and dried on a hot plate.

Staining: the samples were stained with hematoxylin and eosin and were cover-slipped by a thin glass plate. This process facilitates microscopic examination and photomicrography.

Microscopy: the samples were examined and read under a light microscope at different magnifications.

Data Analysis

Data obtained from the water quality parameters were subjected to a one-way analysis of variance (ANOVA), to determine if significant differences existed.

RESULTS

The effect of Amino Force® (2, 4 D-dimethylammonium salt) on the liver of *Clarias gariepinus* juveniles at 0.0, 6.2mg/L, 18.5mg/L, and 30.8mg/L. concentrations, resulted in the following changes; Dilation of hepatic cells(DHC), Necrosis, Hepatic cell Damage (HCD), Cytoplasmic Degenerations (CD), disarrangement of hepatic cells, and Vacoulation (V). These changes are shown in plates 1-4. Amino Force® (2, 4 D-dimethylammonium salt) on the gills of *Clarias gariepinus* juveniles at 0.0, 6.2mg/L, 18.5mg/L, and 30.8mg/L concentrations, had the following effects; Distortion of gill filaments (D), Epithelial cells Erosion and Destruction (EED), Blood congestion in the primary filament and Shortening of secondary lamellae. These changes are shown in plates 5-8.

Physico-chemical parameters of the water.

The physico-chemical parameters obtained during the experiment were significantly different ($P<0.05$) in Total dissolved solid (TDS) and Electrical Conductivity (EC) (Table 1). They increased with increase in concentrations while Dissolved oxygen decreased with increase in concentrations. Temperature and pH showed no significant difference ($P<0.05$).

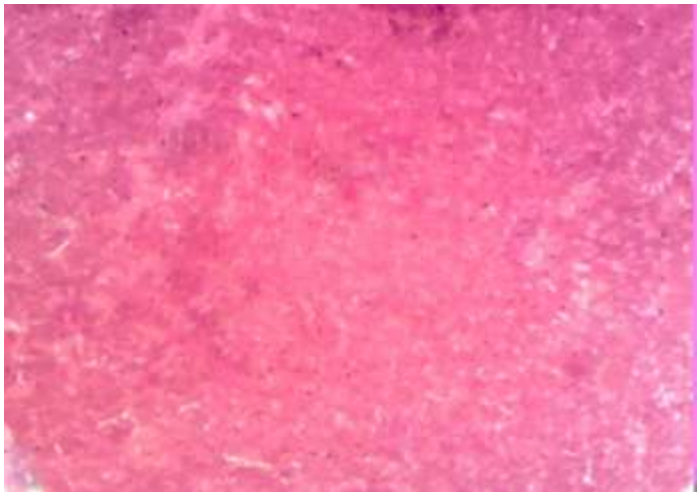

Plate 1: Photomicrograph of the Liver cells of *Clarias gariepinus* at control (0.0mg/L) showing hepatocytes with granular cytoplasm and nucleus. MagX400

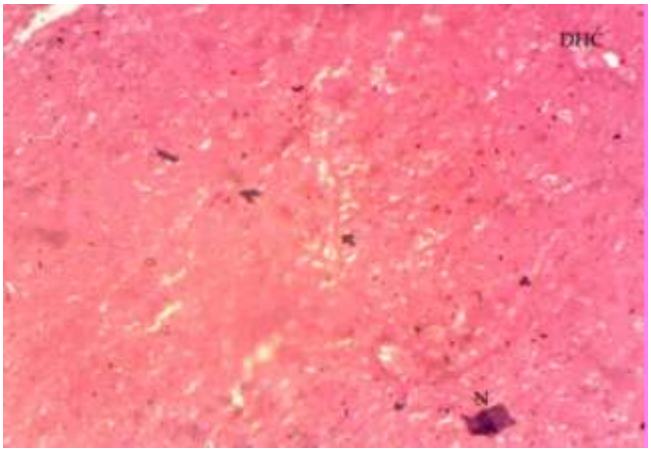

Plate 2: Photomicrograph of Liver cells of *Clarias gariepinus* juvenile at (6.2mg/L) with Dilation in hepatic cells (DHC) and Necrosis (N). MagX400.

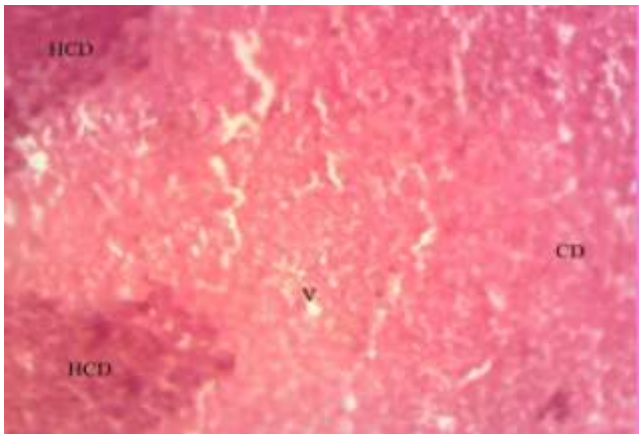

Plate 3: photomicrograph of Liver cells of *Clarias gariepinus* at (18.5mg/L) with Vacuolation (V), Cytoplasmic degenerations (CD) and Hepatic Cells Damage (HCD). MagX400.

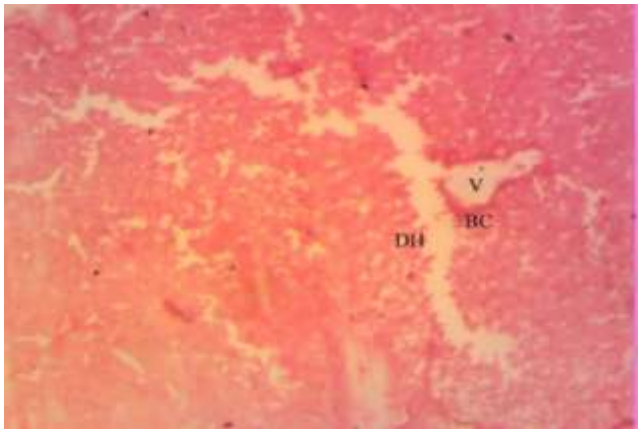

Plate 4: Photomicrograph of Liver cells of *Clarias gariepinus* at (30.8mg/L) showing Vacoulation (V), Disarrangement of hepatic cells (DH), and Blood congestion (BC). MagX400.

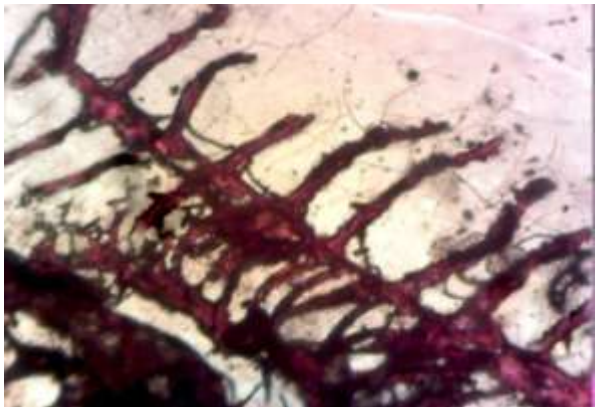

Plate 5: Photomicrograph of Gill cells of *Clarias gariepinus* juvenile at (0.0mg/L) with structure of the gill cell, primary and secondary lamellae. Mag. X400.

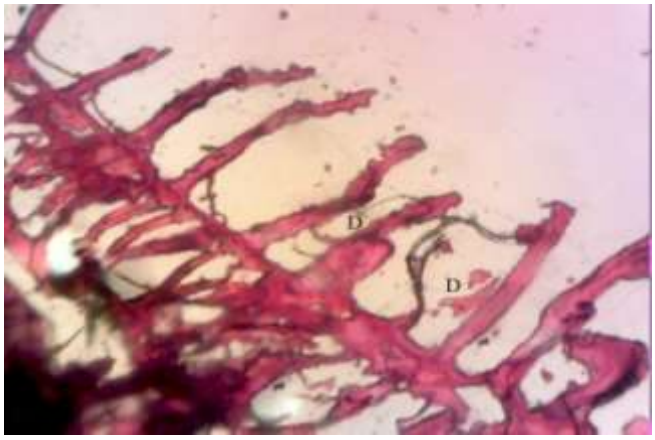

Plate 6 Photomicrograph of Gill cell of *Clarias gariepinus* juvenile at (6.2mg/L) with Distortion (D) of gill filaments. MagX400.

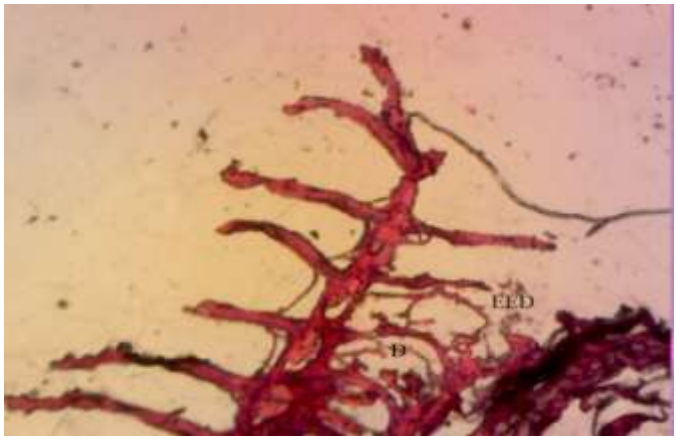

Plate 7: Photomicrograph of Gill cell of *Clarias gariepinus* juvenile (18.5mg/L) with Distortion of the gill filaments (D) and Epithelial cells Erosion and Destruction (EED). MagX400.

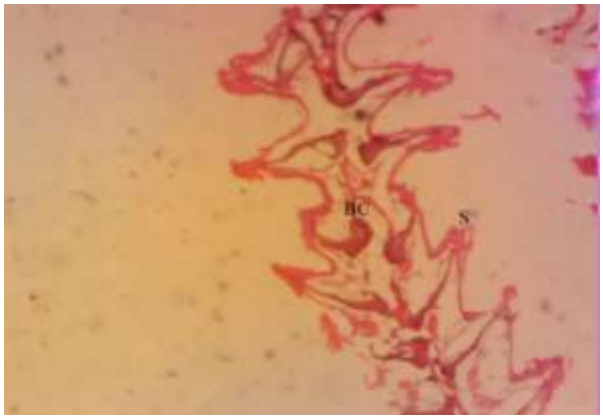

Plate 8: Photomicrograph of Gill cell of *Clarias gariepinus* juvenile at (30.8mg/L) with shortening of the secondary lamellae (S) and Blood Congestion (BC) in the filament. Mag X400.

Table 1. Mean physico-chemical parameters of the water during exposure of *Clarias gariepinus* juveniles to concentrations of AMINO FORCE® (2, 4 D-Dimethylammonium Salt)

Means in the same column with different superscript vary significantly (p<0.05); ns: not significant

Treatment (mg/L)	Temperature	Dissolved Oxygen	Total Dissolved Solid	Electrical Conductivity	pH
0.00	25.98 ± 1.00	6.04 ± 2.52^{a}	416.00 ± 19.05^{c}	827.00 ± 37.98^{d}	8.19 ± 0.23
6.20	26.24 ± 0.93	5.64 ± 2.63^{b}	437.00 ± 22.05^{ab}	844.00 ± 41.97^{c}	8.07 ± 0.19
18.50	25.92 ± 1.02	5.16 ± 2.75^{b}	450.80 ± 26.03^{a}	865.80 ± 46.98^{b}	7.96 ± 0.18
30.80	27.94 ± 1.84	3.86 ± 2.82^{c}	426.40 ± 40.49^{b}	915.20 ± 56.76^{a}	7.82 ± 0.21
p-value	0.635^{ns}	0.001	0.002	0.001	0.629^{ns}

DISCUSION

The histopathological examination of the gills and liver of *C. gariepinus* exposed to Amino force® shows that the organs were affected. In fish, gills are the critical organ for respiration, osmoregulation and excretory functions. Gills are generally considered as good indicators of water quality (Rankin *e.t al.*, 1982). They are the primary route for the entry of the herbicide and a major respiratory organ, all metabolic pathways depend upon the efficiency of the gills and damages to this vital organ cause a chain of destructive events which ultimately leads to respiratory distress (Magare, and Patil, 2000). Some damages of the gills exposed to Amino force® include; Distortion of gill filaments (D), Epithelial cells Erosion and Destruction (EED), Blood congestion in the primary filament and Shortening of secondary lamellae. Damages of the gills indicated that the chronic concentrations of the herbicide caused impairment in gaseous exchange efficiency of the gills and this is similar to the observation of Rahman et al., (2002), Omitoyin *et al*., (2006).

The liver is the main organ for detoxification (Dutta *et. al*., 1993) that suffers serious morphological alterations in fish exposed to toxicant. The liver exposed to Amino Force had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue were also observed which 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 cells might also have led to necrosis. Other damages observed are; Dilation of hepatic cells, Hepatic cell Damage, Cytoplasmic Degenerations, and Disarrangement of Hepatic cells. The water quality parameters of the test herbicide had little variations during the experiment and were within optimum range for fish culture as reported by Omoniyi *et. al*. (2006).

CONCLUSION

From this study, it can be concluded that Amino Force® (2, 4 D-Dimethylammonium salt) can induce several histopathological alterations in the gills and liver of *Clarias gariepinus.* Awareness should be made on the toxicity and use of herbicides in appropriate concentrations: dumping of herbicide containers in water bodies should be avoided as well as direct spraying of herbicides in aquatic environment.

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