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Full-Length Research Paper

Effect of Paraquat on the Growth of Catfish (*Clarias gariepinus*)

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ABSTRACT: Fish is sensitive to devastating consequences of oil contamination or deliberate discharge of chemicals to waterways. Histological changes in *Clarias gariepinus* fingerlings before death under exposure to different concentrations of toxicants was carried out. The study examined the effect of paraquat on the phsico-chemical parameters in water, determined the effect of paraquat on histopathology of catfish (Clarias *gariepinus*) and evaluation of the behavioral responses of fish at different concentration of paraquat. Three hundred and sixty *Clarias garinepinus* fingerlings of average weight 3.50± 0.01g and length 12 ± 0.000m was exposed to different concentrations of (0.01, 0.03 and 0.05 ml/L) of paraquat with fifteen (15) fishes per tank in two replicates. The treatment was subjected to 96 Hour bioassay test to determine its acute toxicity in a completely randomized design experiment. Changes in water quality and fish mortality in paraquat treatments for a period of six months were monitored daily in addition, behavioural and histological responses were also investigated. There was a significant difference ($p<0.05$) in values of temperature, electrical conductivity, salinity, pH and Dissolved Oxygen in the treatments with paraquat before and after the experiment. Mortality rate was highest (90%) in fish exposed to 0.05ml/L detergent water at 96hrs exposure. As concentration and time of exposure increased, the fish showed restlessness, rapid opercula movement, erratic swimming, discoloration and loss of reflex before death. Histological effects were hypertrophy, necrosis of hepatocyte, maliganancy, eroded gills, and degeneration of cells, hemorrhage, interstitial cell and secondary lamella of organs (kidney liver and gills).

Keywords: *Clarias gariepinus,* paraquat, mortality

INTRODUCTION

Paraquat, or N, N′-dimethyl-4,4′-bipyridinium dichloride, also known as methyl viologen, is an organic compound with the chemical formula $[(C_6H_7N)_2]Cl_2$. It is classified as a viologen, a family of redox-active heterocycles of similar structure. This salt is one of the most widely used herbicides (Wikipedia). Marine organisms, particularly fish, have been exposed to a variety of pollutants in aquatic systems such as rivers and ponds, including oil spillage, pesticides and fungicides, industrial discharge, and so on; many of these sources contain metals as part of their constituents, which has a direct impact on biomagnification (Storelli et al., 2015; Agatha, 2010; Oronsaye et al., 2010; Javed and Usman, 2011; Muiruri *et al*., 2012). Many academics, including Ozmen et al. (2004), Alaa and Werner (2010), Kumar et al. (2012), and Olowoyo et al. (2012), have expressed concerns about the risks of consuming marine species contaminated with hazardous metals such as cadmium, lead, chromium, manganese, nickel, and zinc. These metals are not only poisonous to fish and other marine organisms, but they are also indestructible and tend to bio collect in living tissues, where they can be transferred up the food chain and into the human body.

Catfish (*Clarias gariepinus*)

Catfish *Clarias gariepinus* of the family *claridae*is the most common Nigerian fresh water fish species and is prominent in aquaculture practice. They are easily cultured with large economic gains because of their air-breathing and hardy nature, suitable reproductive strategy, nutritional efficiency and attainment of large size in a short time (Fagbenro, 1993). Furthermore, the sharp tooth catfish (*Clarias gariepinus)* is one of the most important individual's species in traditional freshwater fisheries in Africa. It is widely attributed in Africa, where it occurs in almost any freshwater habitat but floodplains, large sluggish rivers, lakes and dams. The fish is omnivorous, feeding on fishes, birds, frogs, small mammal's reptiles, snails, crabs and other invertebrates. It is also capable of feeding on seeds and fruits. Catfish (*Clarias gariepinus) is* affected by heavy metals pollution in aquatic environment this has become a worldwide problem during past few decades. This fact is mainly attributed to their persistent stability (MacFarlane, 2000). Fish muscle is commonly analyzed to determine contaminant concentrations and to assess the health risks because it is the main part consumed by humans. Fish such as catfish (*Clarias gariepinus)* can be considered as one of the most significant indicators in freshwater systems for the impact of metal pollution (Begum, 2000).

Aim of the study

The aim of this study is to investigate the effect of paraquat toxicity on the growth of catfish (*Clarias gariepinus*).

MATERIAL AND METHODS

Study area

Study area is Keffi Local Government in Nasarawa state. It has an area of 138km and a population of 92,665 at 2006 census; resources used for the study were obtained from Nasarawa State University. The toxicological analysis was carried out in the Faculty of Agricultural Sciences, Nasarawa State University Lafia Campus. This study was carried out with the use of 12 plastics tanks in Keffi, Nasarawa state. Resources used for the study were obtained from Nasarawa State University located in Keffi.

Experimental fishes

Apparently five hundred and forty fishes of *Clarias gariepinus* fingerlings of mean weight 3.50g were obtained from Nasarawa State University Entrepreneur center Keffi and transported in an aerated container to the place where the experiment is going to take place at Dadin Kowa, Keffi, the fishes were acclimatize for at least 48h. During the period, the water was aerated with air pump (aerator). Fish were fed twice daily with aqual feeds (Ingredient composition - fish meal, wheat flour, soybean meal, corn meal, yeast, vitamins and mineral salt; Proximate analysis - crude protein mineral; 40%, crude fat mineral; 4%, crude fibers, maximum; 5% and moisture, maximum; 10%). Unconsumed feed and fecal wastes were removed and water replenished three times weekly as recommended by Oyelese and Faturoti (1995). Water quality of the test and reference solutions fish were monitored throughout the duration of the experiment.

Range finding test

The range finding test was conducted for accurate LC_{50} (Median lethal concentration that causes 90% mortality of exposed fish). Three lethal concentrations of 0.01ml/L, 0.03ml/L and 0.05ml/L were prepared by adding determined amount of toxicant to the tanks, Fifteen fishes were randomly placed in each tank and exposure lasted for 96 hours.

Experimental set up

Fish were stocked in twelve rectangular tanks of size 45.5 x 28 x 26cm, each had 15 fishes replicated twice in three concentrations (0.01ml/L, 0.03ml/L and 0.05ml/L) including the control. After determining the LC_{50}, for the Concentrations of 0.01ml/L, 0.03ml/L and 0.05ml/L toxicant and the control 0ml/L were used for the experiment. Water quality variables such as Temperature, pH, Electrical conductivity, Alkalinity, Hardness, Atmospheric temperature and Dissolved Oxygen (DO) were monitored for each concentration. Behavioral responses and mortality rate after 96 hours of exposure were determined.

Preparation of test medium

Twelve plastic tanks were used as test containers. The water soluble fraction of oil spillage was prepared by mixing 1L of oil obtained from a mechanic work shop and dilute with 4L of water in accordance to Esenowo and Ugwumba (2010). The mixture was stirred with a stick for 48h. It was made to stand for 12h before it was poured into a separating funnel and allowed to stand for 6h. The lower layer of the water was decanted into the plastic tanks (Afolabi *et al.,* 1985). It was repeated until sufficient quantity of water soluble fraction was obtained. 67g/L of paraquat was diluted in 4L of water to get stock solution needed for bioassay.

Acclimatization of fish

The fish were held in 42.5cm by 28cm by 26cm, plastic tank containing non chlorinated water. The fish were allowed to acclimatize for more than one week under laboratory conditions to allow fishes adapt to experimental conditions (27 ± 2°C). The period of acclimatization was extended beyond one week to ascertain the condition of the fish. Fish were inspected for disease conditions and general fitness. Fish were fed during the period of acclimatization and the water was changed every three days in order to remove faecal and unconsumed feeds.

Exposure of test organism

The water soluble fraction was made into three concentrations (0.01, 0.03 and 0.05mL, with two replicates each) for paraquat. Dilution was made with aerated water. Fifteen fingerlings were exposed each to three concentrations paraquat in 25L capacity plastic containers. Feeding was discontinued for 24hrs before the start of the experiment (Reish and Oshida, 1987). The volume of water to the weight of fishes was calculated in accordance with Reish and Oshida (1987) standard of 12g of fish to 5L. Catfish fingerlings were observed for 96hrs and any behavioral changes and mortality rate was recorded.

Long-term renewal toxicity test

Renewal toxicity test was conducted for a period of four days to study the effect of sub- lethal concentration of toxicant (paraquat) on the growth and survival. Fish were removed and place in clean water (containing non-toxicant) for the rest of the experiment period. Fish were well fed and weights and length were taken every week till the exposure period. The growth parameter which was obtained to determine the significant of toxicant (paraquat) on the growth of test organism was calculated using the formula:

$$\text{Relative Growth Rate (RGR) \%} = \frac{W_f - W_i \times 100}{\text{Time}}$$

$$K = \frac{100 \times W}{L^3}$$

Where;
RGR= Relative Growth Rate
K= Constant
W= Total Body Weight of the fish in g
L = standard length of the fish in centimeters
W_f = Final weight
W_i = Initial weight
T= Time

Physico-chemical parameters

The records of Temperature, pH, Dissolved oxygen, alkalinity, Hardness and Electrical conductivity were taken before and during the exposure period. Temperature, pH and electrical conductivity were measured using a T^o/pH/ Electrical Conductivity (EC) meter model HI 98129. The Dissolved oxygen was measured using a Dissolved oxygen meter Model 9150.

Histopathology

Gill, liver and kidney samples were collected from fish from Paraquat treatment and preserved in 10% formalin and processed for histological examinations using standard histological techniques. Sections of organs were cut at 5µm and stained with heamatoxylin and eosin. Permanent sections were read under light microscope.

Gill**:** Sections through the gill showed normal cellular pattern, ranging from gill arch, gill rakers, filament, Venus, sinus, cartilaginous support, pseudo-brachial lamella, ceratobrachial bone of the arch, mucous epithelium lining on the membrane and branches of the afferent and efferent arterioles, and nucleolus number of lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen. Moderate and severe areas of lesion, necrosis, malignancy, pigment and inclusion bodies were observed in fish exposed to Paraquat.

Liver**:** Transverse section through the liver showed normal cellular pattern, normal central vein, and space of dissect billary epithelium, hepatic plate and hepatocytes. No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen in the control. There were areas of slight lesion and necrosis.

Kidney**:** Histopathological section through the Control kidney transverse section through the kidney shows normal tissues where the blood cells are created, normal tissues and cells.

The organs (liver, gills and kidney) were removed and prepared for histhopathological examination. They were fixed in bouin's fluid for 24 hours, washed with 70% ethanol and dehydrated through a graded series of ethanol (Kelly, 1979). They were embedded in paraffin, sectioned at 4-5um thickness stained with haematoxylin and eosin and examined using light microscope and photomicrography.

Statistical analysis

The statistical analysis used in this research are; method of least significant difference (LSD) using the SPSS statistical package to analyzed the two variables of the physico-chemical parameters. Duncan Multiple Range Test was used to assess the toxicity effect at three levels of concentrations (0.01, 0.03 and 0.05ml/L) of the toxicant (paraquat) on weight and length of experimental fish. T-test was used to determine if a water toxicity level has significant difference on the weight and length of the fish (*C.gariepinus*). Scientific package for social sciences (SPSS) Inc. Chicago, USA, was employed to calculate the significance of the differences between control and experimental means and within various treatments P values of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

The control treatment shows low mortality rate,

Table 1: Effect of paraquat on physico-chemical parameters

Physico-chemical	Nov	Dec	Jan.	Feb.	Mar.	Apr.
	Mean/SD	Mean/SD	Mean/SD	Mean/SD	Mean/SD	Mean/SD
TEMP.(oc)	23.67±0.12	25.94±0.13	24.28±0.04	25.87±0.06	30.20±0.08	30.10±0.03
P^H	6.01±0.01	6.45±0.7	6.44±0.09	6.50±0.11	6.71±0.02	6.82±0.10
DO(mg/l)	4.98±0.09	5.12±0.17	4.20±0.18	3.73±0.15	4.05±0.24	4.32±0.14
EC(µs/cm)	320.02±0.00	419.38±0.22	419.69±0.12	430.06±0.02	430.13±0.24	440.03±0.08
ALK(PPM)	22.33±0.11	23.33±0.16	19.67±0.24	22.33±0.16	23.67±0.14	24.00±0.18
HD(ppm)	42.00±0.07	48.00±0.06	45.67±0.06	43.67±0.03	47.00±0.06	52.33±0.05
AT(0c)	28.33±0.11	25.00±0.11	24.67±0.01	27.67±0.08	24.33±0.09	29.00±0.09

Table 2: Effect of paraquat on growth of catfish (C. *gariepinus*).

Months	CONC.(ML)WI	CONTROL		PARAQUAT	
		W(g)	L(cm)	W(g)	L(cm)
	0.01	3.5	12	3.5	12
NOV	0.03	3.5	12	3.45	12
	0.05	3.5	12	3.49	12
	0.01	10.5	25	8.02	19.92
DEC.	0.03	12.03	25.05	7.99	19.56
	0.05	10.33	25.05	7.62	19.45
	0.01	14.55	29	9.04	20.01
JAN.	0.03	16.55	27	9.06	20.56
	0.05	16.55	25.05	8.56	19.89
	0.01	19.66	28.56	11	20
FEB.	0.03	20	29.05	10.73	20.01
	0.05	20	29	10.06	20.01
	0.01	23	30	12.08	21.04
MAR	0.03	21	34.5	10.98	21.01
	0.05	22	34.56	10.78	20.79
	0.01	25.55	33.98	12.55	22.05
APR	0.03	25.77	34.98	11.01	20.55
	0.05	26.71	35.45	11	20.52

observation shows that mortality was directly proportional to concentrations of toxicant (parquat) during exposure period. The mortality rate for control was minimal (6%) which shows the lowest mortality rate. Paraquat shows 20% mortality rate (Table 1).

Effect of toxicant (paraquat) on growth of *C. gariepinus*

At the beginning of the treatment, the average biomass of growth of fish in the container was almost similar, however, growth variations were observed weekly until the end of the investigation. These variations resulted in the daily growth or specific growth differences. This condition was probably because of different levels of concentrations. Fish which had high concentration level had ability to utilize their metabolic energy for growth. Oppositely, fishes which had a low concentration level would only utilize their metabolic energy for live not for their growth. As mentioned by Warren (1997) that aquatic organism had their own adaptation or homeostasis ability to with stand their internal conditions for their live and growth.

Mean values of physico-chemical parameter

Temperature of the control tank ranged between 26.03 ± 0.02 to 32.6 ± 0.5$(^{0C)}$, Temperature was high in March and April ranging from 31 ± 0.04to 32 ± 0.0$(^{0C})$, p^H of 7.5± 0.00 and 7.6± 0.01, while Dissolved oxygen was 4.50 ± 0.04 to 5.00 ± 0.09 which led to high mortality of the fishes (Table 1). Table 2 shows effect of toxicity on the growth of catfish from the month of November to April, were the weight and length of fish is taken at different concentration level. Paraquat exerted toxic effect on the fishes and toxicity increased as increased concentration. Abnormal behaviours such as incessant jumping and gasping of air, restlessness, loss of equilibrium, increase opercula activities, surface to bottom movement, sudden quick movement and resting at the bottom observed in this study. Fishes were stressed progressively with time before eventually dying, the stressful ailment of respiratory impairment due to toxic effect of Paraquat. The observed increasing state of inactivity with both increasing concentrations and exposure period agree with the report of Ayoola (2008).Water quality parameters had little variation, physico-chemical parameter

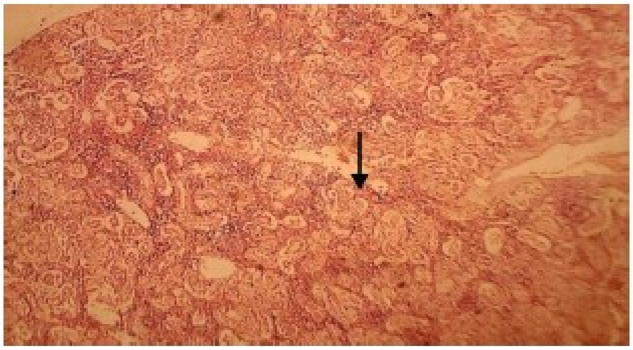


Plate 1: The photomicrograph of *clarias gariepinus* fingerlings, kidney in control tank (0ml/L) showing no pathological lesion (x4x6.7).

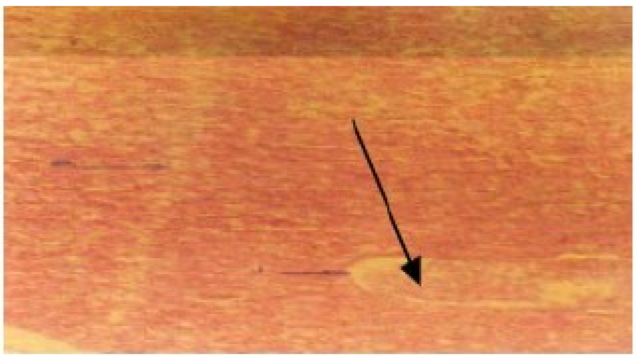


Plate 2: The photomicrograph of *Clarias gariepinus* fingerlings kidney exposed to 0.01ml/L of paraquat, showing mild pyknotic nuclei.

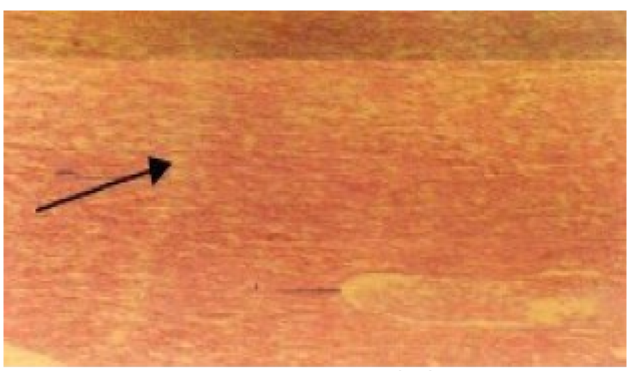


Plate 3: The photomicrograph of *Clarias gariepinus* fingerling, kidney exposed to 0.03ml/L of paraquat, showing inflammation.

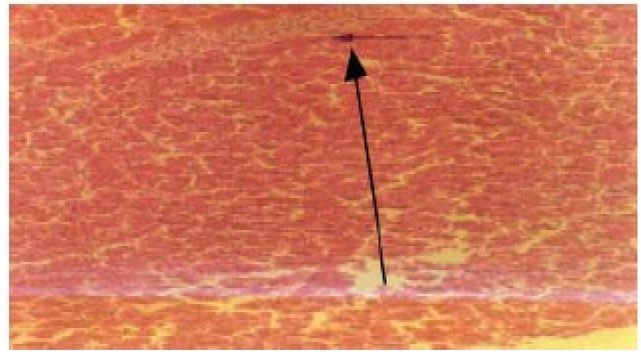


Plate 4: The photomicrograph of *Clarias gariepinus* fingerling, kidney exposed to 0.05ml/L of paraquat, showing homosiderosis and pyknosis.

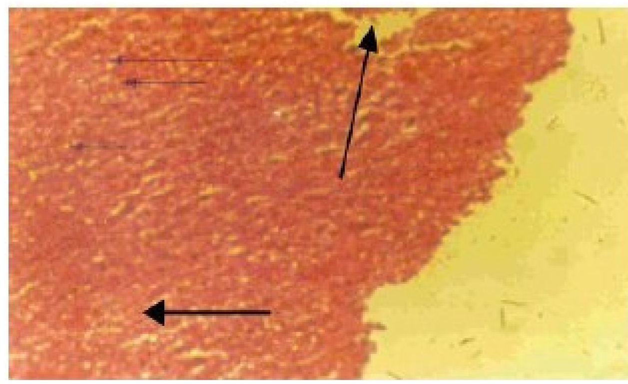


Plate 5: The photomicrograph of *Clarias gariepinus* liver exposed to 0.01ml/L of paraquat, showing glycogen vacuolation

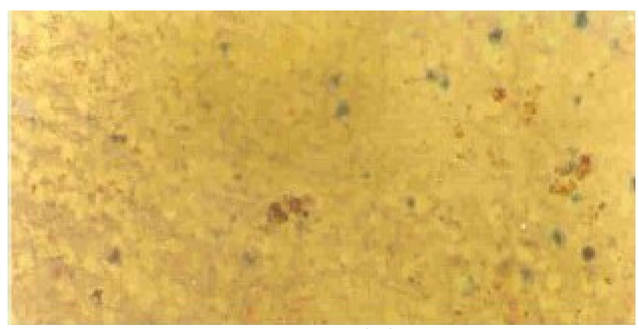


Plate 6: The photomicrograph of *Clarias gariepinus* liver exposed to 0.03ml/L of paraquat, showing fatty infiltration and hemosiderosis.

measured seemed to be within optimum range for fish culture as reported.

Histological examinations

Results of histological examinations are shown in Plates 1 to 12. Kidney histology of *Clarias gariepinus* exposed to different concentrations of paraquat are in (Plates 1-10). Control treatment (0ml/L) has no pathological lesion (Plate 1).Kidney of fish in (0.01ml/L) also showed no visible changes (Plate 2). There was degeneration in renal cells of fish in (0.03ml/L), while hydropic degeneration in renal cell was observed in fish treated with 0.05ml/L of water containing oil spillage.

Histological examination of *Clarias gariepinus* organ exposed to paraquat

Results of histological examination of *C. gariepinus* organs (kidney, liver and gills) are shown in plate 13-21,

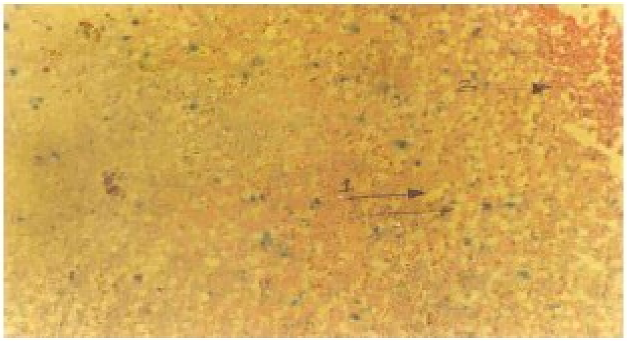


Plate 7: The photomicrograph of *Clarias gariepinus* liver exposed to 0.05ml/L of paraquat, showing darkly stained specks of necrotic, glycogen vacuolation and congested central vein.

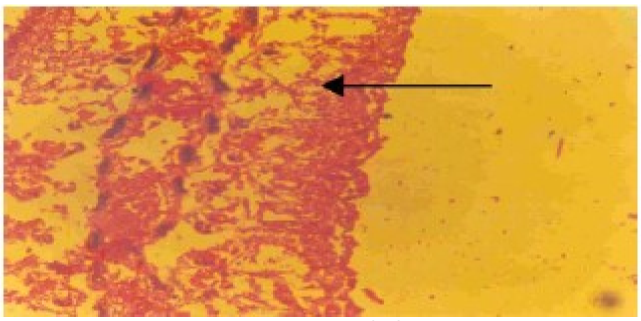


Plate 8: The photomicrograph of *Clarias gariepinus* gill exposed to 0.01ml/L of paraquat, showing eroding gill rakes and there is alteration in the gill rake.

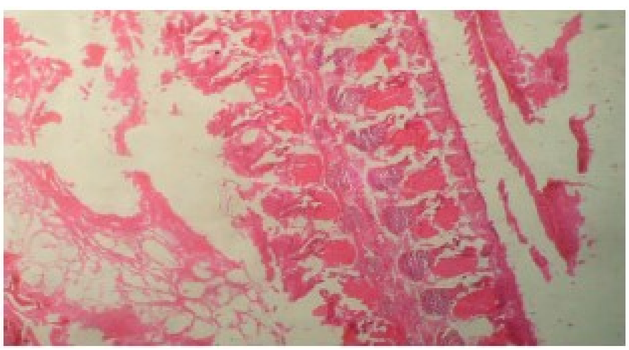
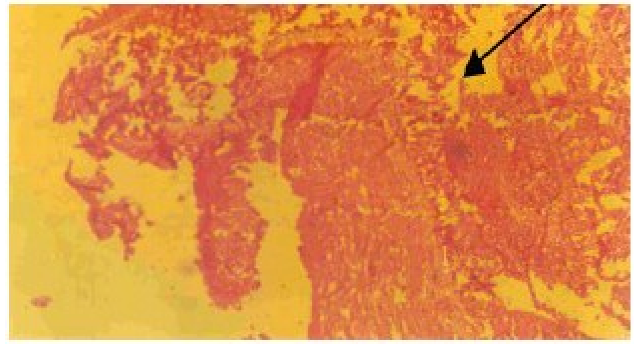


Plate 9: The photomicrograph of *Clarias gariepinus* gill exposed to 0.03ml/L of paraquat, showing swollen tip filament and maliganancy of gill cell.

showing different reaction of the organs exposed to different concentrations of paraquat. Control treatment has no significant or pathological lesion (plate 1.Kidney of fish in 0.01ml/L shows mild pyknotic nuclei (plate 13), 0.03ml/L shows mild pyknosis while it was observed that fish in 0.05ml/L shows homosiderosis and pyknosis. Liver of *C. gariepinus* subjected to different concentration of paraquat is shown in (Plates 2-10).

Conclusion

Conclusively, this study has revealed that, exposure of *C. gariepinus* fingerlings to 0.01 ml/L, 0.03ml/L and 0.05ml/L



concentrations of paraquat effluents is enough to induce various toxicological effects in terms of histological degradations in gill, liver, kidney and other organ structures. The damage induced on the test fish's gills, liver, and kidney was found to be concentration and exposure period dependent, with the pathologic lesion recorded in the fish's gill structures being most pronounced at higher concentrations (0.05ml/L) of the effluents in question. Toxic environmental conditions have been found to cause two types of structural changes in organism tissues. The first are the results of the pollutant's direct toxic effect, which causes degeneration and necrosis of vital organs at the cellular level. The second is the result of compensatory mechanisms that deal with environmental stressors (such as handling, poisoning), as seen in cellular hyperplasia.

Recommendations

The Government and Industries should adopt the proper effluent treatment technology which would ensure proper treatment of industrial effluent prior to their discharge into the environment. Environmental protection laws should be forced on the users of the environment so as to prevent water bodies from been polluted. Adequate water supply should be made available in all areas of the federation most especially in rural areas of the country.
Public Health Education Department of Local, State and Federal ministry of health education should organize a lecture to the general public on the effect of water pollution on aquatic organism especially fishes.

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