AJFARM
www.theajfarm.com

African Journal of Fisheries and Aquatic Resources Management
Volume 2, 2017
ISSN: 2672-4197
Pp 41-48

Histopathological Effects of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) on *Coptodon Zillii* (GERVAIS, 1848) Juveniles

[1]Ipinmoroti, M.O., [1]Iyiola, A.O. and [2]Fayiga, A.O.
[1]Department of Fisheries and Wildwife Management, College of Agriculture, Osun State University, Nigeria
[2]Department of Environmental Science and Geology, Stockton University, Galloway, USA
*Corresponding Author
E-mail: wumsco@yahoo.com

Abstract

Concerns have often been raised on the application of pesticides on croplands and the threats posed on aquatic organisms. These pesticides get to the aquatic system from runoff on croplands where there was excessive application This study was designed to investigate the lethal concentration of an agricultural pesticide; 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) on the physical and chemical properties of water as well as the liver, kidney and gills of *Coptodon zillii* juveniles. A total of 150 experimental fish (average length 6.2±0.32cm and weight 0.9±0.11g) were stocked in culture tanks (*43cm x 30cm) of 10litres water capacity at the rate of 1fish/L*. The bioassay media concentrations were 0mg/l (A), 0.33mg/l (B), 0.67mg/l (C), 1.0mg/l (D) and 1.3mg/litre (E) and representing 0mg/l, 3.3 x 10-^{6}mg/l, 6.7 x 10-^{6}mg/l, 1.0 x 10-^{5}mg/l and 1.3 x 10-^{5}mg/l of active ingredient Dichlorvos DDVP (1000g/L) EC respectively in three replicates of a Completely Randomized Design (CRD) experimental setup. Temperature, pH, Dissolved Oxygen (DO) and Chemical Oxygen Demand (COD) were measured before and after inclusion of the pesticide. Significant differences ($P<0.05$) were observed in mean concentration of DO and COD. The experimental fish exhibited restlessness and uncoordinated movement. These behavioural characteristics increased with increased concentration of DDVP and time of exposure. The LC_{50} for *C. zilli* juveniles after 96hours exposure was 0.39mg/l. Histological photomicrograph showed areas of lesions, necrosis, inflammatory bodies and malignancy in the gills, liver, kidney and muscle of the exposed experimental fish while the control was normal. The result indicated that DDVP is highly toxic to *C. zilli* and must be applied at recommended dosages for sustainability of the living communities and the general ecosystem.

Keywords: Agro-chemical, *C. zilli,* Histopathology, Water Quality, Sustainability.

Introduction

The use of pesticides in agricultural farmlands has been on the increase as a result of rapid urbanization and industrialization. It is intended for prevention or control of pests, vectors of human or animal diseases and unwanted plants or animals which can interfere with the production, processing, storage, transport or marketing of food (Omoniyi *et al.,* 2013; Umar *et al.,* 2010). Hazards arising during the application of pesticides are mainly due to lack of information, knowledge

and awareness, poor legislation or enforcement of legislation and sales of high pesticides in the open market (WHO 1991). The severity of any effect from exposure to pesticide depend on the dose, the route of exposure, the ease of absorption of the, the type of effect of the pesticides and its metabolites, the accumulation and persistence in the body and lastly, the health status of the individual (WHO 1991). Dichlorvos (DDVP) is a poisonous organophosphate pesticide commonly used on crops and stored products in developing countries, as an agent that kills larvae of flies, as an anti-worming agent for dogs and horses (USEPA 2000) and eradication of crustacean ectoparasites (Das, 2013). It is specifically used in the treatment of sea lice (*Lepophtheirus salmonis* and *Caliguse logatus*) on commercial salmon farms (Gupta *et al*. 2008). Despite these advantages, concerns have been raised on its application dosages because it has an adverse long term effect on the environment (Annue *et al.,* 1994; Fafioye *et al.,* 2011). It impedes the survival of aquatic life through impairment of metabolism and sometimes leading to death (Ogueji and Auta, 2007). Contamination from these toxicants is of great concern and their prolonged occurrence in aquatic environments can be dangerous for fish. The effects of these contaminants on fish are constantly assessed to determine the lethal and sub-lethal concentration and application of these pesticides in the environment.

Bioassay is widely accepted for toxicity testing (APHA *et al.,* 1985). Histological studies on liver, kidney, tissues, gills and reproductive organs can also exhibit the effects of these toxicants on the organs (Das and Gupta, 2012). Histopathological changes of gills and internal organs after the exposure of *Clarias gariepinus* to pesticides which was most evident on the liver of the fish because it is an organ that breakdown chemicals have been reported by Nowak (1992); Ladipo *et al.,* (2011) and Omoniyi *et al.,* (2013). Ayoola (2008) reported that glyphosphate pesticides resulted in various alterations like lesions, malignancy or inflammation on the cells and the gills of *O. niloticus.* This study was designed to investigate the effects of DDVP on the histopathology of *C. zillii* juveniles as well as its effects on the physical and chemical quality of the culture media.

Materials and Methods

Experimental Sample

A 96–hour short-term static bioassay was conducted to investigate the toxicity of DDVP on *C. zilli* juveniles and determine allowable concentrations for very short exposures. The choice of *C. zilli* was apt because it is ubiquitous to almost all aquatic systems and has high commercial value. A total of 150 *C. zilli* juveniles with average length 6.2±0.32cm and weight of 0.9±0.11g were collected from the Departmental fish pond for the experiment.

Source of Agrochemical

The pesticide was manufactured by Jubaili Agrotec, Ibadan and purchased from a reputable agro-chemical outlet in Ibadan. The active ingredient contained in 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) is Dichlorvos DDVP (1000g/L) EC.

Experimental Design

Experimental fish were randomly stocked in triplicates in fifteen culture tanks of size 43cm by 30cm each in 10 litres of non-chlorinated ground water. Experimental design was completely randomized and experimental fish were acclimatized for a period of two weeks during which they were monitored for signs of diseases, general fitness and survival rate was 95% (Subburaj *et al.,* 2018).

Experimental Feed and Feeding

Feed with crude protein level of 40% containing all essential nutrients in the required amount was formulated for the experimental animals using Pearson's method (Ayotunde *et al*., 2016). Diets were fed to fish at 5% body weight twice daily. Feeding was terminated 24 hours before the commencement of bioassay (Odiete, 2009).

Determination of Physical and Chemical Parameters of Water

Static renewal method was employed by changing water at 24 hours interval to prevent accumulation of toxic metabolites from faecal and unconsumed feeds (APHA, 1995). Physical and chemical parameters were measured before and after the inclusion of chemicals. Temperature was measured

insitu using a mercury-in-glass thermometer calibrated in degrees celcius and readings were taken when it was dipped into water to a depth of 10mm depth for about two minutes (Iyiola, 2015). Dissolved oxygen (DO) and pH were measured using appropriate kits manufactured by fresh innovative MULTITEC, NIFFR, Niger State following the manufacturer's protocol. Chemical Oxygen Demand (COD) was determined titrimetrically (APHA, AWWA, WEF, 2012).

Bioassay Techniques

The static bioassay procedure was done according to APHA (1995) and conducted as a short time toxicity test for 96 hours time frame. A range finding test was done prior to the experiment to determine the range of the toxicant that can be used for the experiment. During the range finding procedure, various concentrations between 0mg/L and 1.25mg/L of toxicant were prepared and standard methods as described by APHA (2005) were adopted to determine the toxicity of the chemicals. After the range test, the bioassay media test concentrations for the experiment were 0mg/L, 0.33mg/L, 0.67mg/L, 1.0 mg/L and 1.3mg/L and tanks were labelled accordingly (A- 0 mg/l; B – 0.3mg/l; C – 0.67mg/l; D – 1mg/l and E – 1.33mg/l). The amount of active ingredient contained in each milligram (mg) of test concentration was determined from the 1000g/L of dichlorvos formulation according to Ladipo *et al.,* (2011) (Equation 1; Table 1).

$$Y = \frac{x}{1000\ g/L(100000\ mg/L)} \quad \text{..... Equation 1}$$

Where: Y= amount of active ingredient in test concentration
X = test concentration

The behavioural pattern of the fish and other external changes in the body of fish were observed with mortality recorded. Dead fish were identified by an absolute lack of movement and were removed so as not to pollute the water (Nwani *et al.,* 2015).

Histopathological Studies

The organs (kidney, liver, gills and tissues) removed for histopathological analysis were washed in 70% ethanol and dehydrated through series of ethanol, embedded in paraffin and then stained with haematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989).

Statistical Analysis

The means of the physical and chemical parameters were compared using one way Analysis of Variance (ANOVA) at 5% probability. The dose response of mortality was analysed by Probit analysis (Finney 1971).

Results

Physical and Chemical Parameters of the Water

The mean physical and chemical results measured were almost even for temperature across the test concentrations, DO and pH reduced with increase in concentration of DDVP while COD increased with increased concentration of DDVP (Table 2).

Table 1: Concentration of active ingredient in each test concentration (mg/L)

Test concentration (mg/L)	Concentration of active ingredient
0	0
0.33	0.0000033 (3.3 x 10^{-6})
0.67	0.0000067 (6.7 x 10^{-6})
1.0	0.00001(1 x 10^{-5})
1 3	0.000013 (1.3 x 10^{-5})

Table 2: Mean physical and chemical parameters measured during the experiment

Parameters	A	B	C	D	E
Temperature (° C)	25±0.15^{a}	26±0.11^{a}	25.5±0.13^{a}	26±0.19^{a}	25.3±0.11^{a}
DO (mg/l)	6.5±0.12^{a}	6.0±0.14^{a}	5.4±0.11^{b}	3.9±0.24^{c}	3.2±0.21^{d}
pH	7.0±0.12^{a}	6.5±0.14^{b}	6.1± 0.15^{c}	6.0±0.13^{d}	5.9±0.11^{e}
COD (mg/l)	21.7± 0.11^{a}	21.5± 0.13^{a}	23.1±0.19^{b}	24.8±0.11^{c}	26.0±0.13^{d}

**Superscripts with different letters are significantly different (P<0.05)*

Acute Toxicity

Based on Probit analysis, the calculated LC_{50} for juvenile *C. zilli* after 96 hours experimental period was 0.39mg/L (Figure 1). Tables 3, 4 and 5 showed the rate of mortality of *C. zilli* juveniles for the 96 hours exposure time. Within few hours of test chemical inclusion, the experimental fish in facility E exhibited extremely weak syndrome and settled at the bottom (Table 3). Various abnormal behaviour, such as erratic swimming, sudden quick movements and restlessness were exhibited by experimental fish in B, C and D culture media. Mortality was found to increase with increase in test concentration (Table 4).

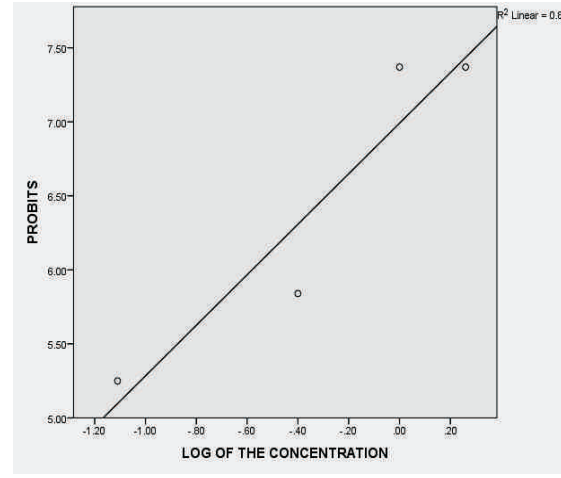

Figure 1: Linear relationship between Probit response and log concentration (mg/*L*) of Dichlorvos on *C. zilli* juveniles

Table 3: Rate of mortality of the exposed experimental fish to DDVP at the first two hours

Treatment	0 - 30 minutes	30 minutes to 1hour	1hour to 1:30 hours	1:30 hours to 2 hours
A	0	0	0	0
B	0	4	0	0
C	5	0	0	1
D	6	1	0	0
E	8	0	0	0

Table 4: Rate of mortality of exposed experimental fish to DDVP at 96 hours

Treatment	24 hours	48 hours	72 hours	96 hours	Mortality %
A	0	0	0	0	0
B	4	2	0	0	60
C	6	2	0	0	80
D	7	1	2	0	100
E	8	2	0	0	100

Table 5: Calculated log dose and probit values of DDVP

Treatment	Log dose	Mortality	Percentage	Probit
A	-	0	0	-
B	-1.11	1	60	5.25
C	-0.40	6	80	5.84
D	0.00	10	100	7.37
E	0.26	10	100	7.37

Histopathological Effects

The sections through the gill, tissues, liver and kidney of the control *C. zilli* juveniles showed normal cellular patterns (Plates 1A, 2A, 3A and 4A respectively) while the exposed gill (Plate 1B) showed severe areas of necrosis, lesions, malignancy, abnormal areas of inflammation inclusion bodies and blood congestion in secondary lamellae; exposed tissues showed moderate myofibre necrosis of muscle (Plate 2B), exposed liver showed moderate areas of necrosis, inflammation, inclusion bodies and severe periportal hepatic individualization (Plate 3B); in exposed kidney, tubular epithelia appeared ballooned, severe necrosis, inclusion bodies and an extensive fatty infiltration of the kidney parenchyma (plate 4B).

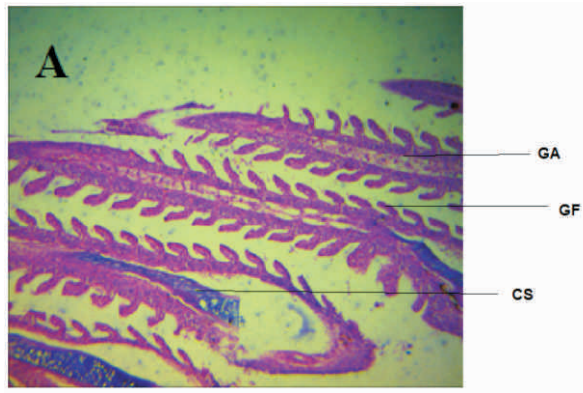

Plate 1A: Photomicrograph of Control gills showed normal Gill Arch (GA), Gill Filament (GF) and Cartilagenous Support (CS). No lesions, inclusion bodies or necrosis was observed (5µm thick; H&E staining; 200X)

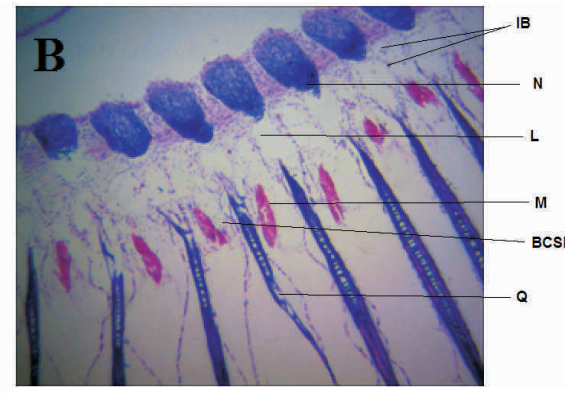

Plate 1B: Photomicrograph of exposed gills showed severe areas of Necrosis (N) and Lesions(L), Malignancy (M), abnormal areas of Inflammation (Q), Inclusion Bodies (IB) and Blood Congestion in Secondary Lamellae (BCSL) (5µm thick; H&E staining; 200X)

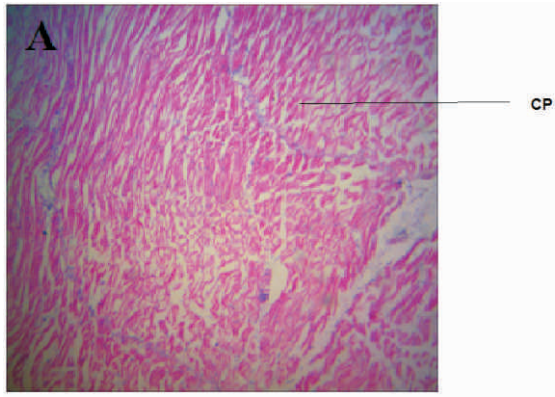

Plate 2A: Photomicrograph of control tissues showed normal Cell Pattern (CP), no lesions, necrosis or signs of inflammation (5µm thick; H&E staining; 200X)

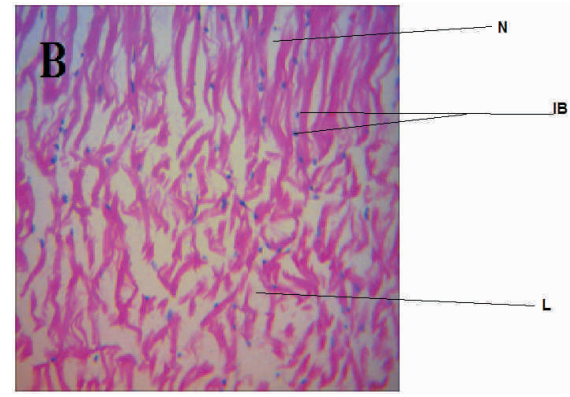

Plate 2B: Photomicrograph of exposed tissues showed areas of Lesions (L), Necrosis (N) and Inflammatory Bodies (IB) (5µm thick; H&E staining; 200X)

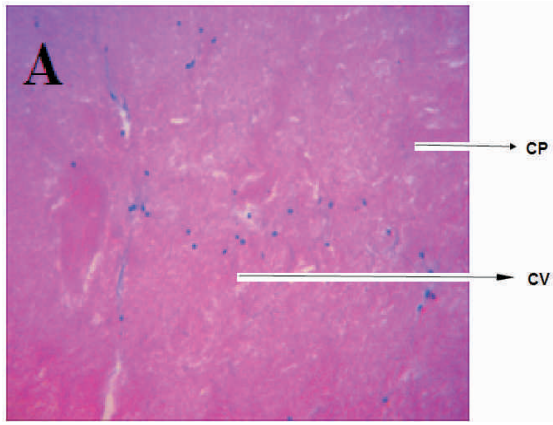

Plate 3A: Photomicrograph of Control liver; showed normal Cellular Pattern (CP), normal Central Vein (CV) with no lesions, necrosis, pigmentation and inclusion bodies (5µm thick; H&E staining; 200X)

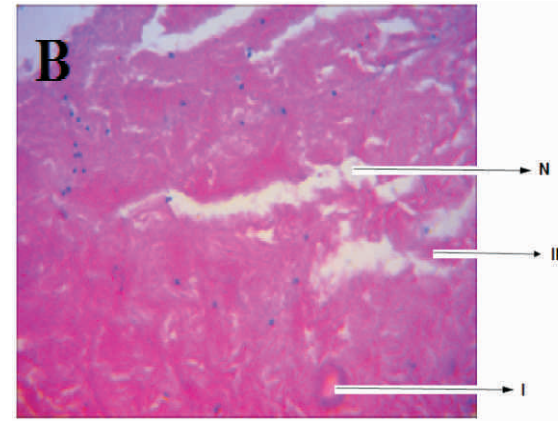

Plate 3B: Photomicrograph of exposed liver showed moderate areas of necrosis (N), Inflammation (IN), Inclusion bodies (I) and severe periportal hepatic individualization (5µm thick; H&E staining; 200X)

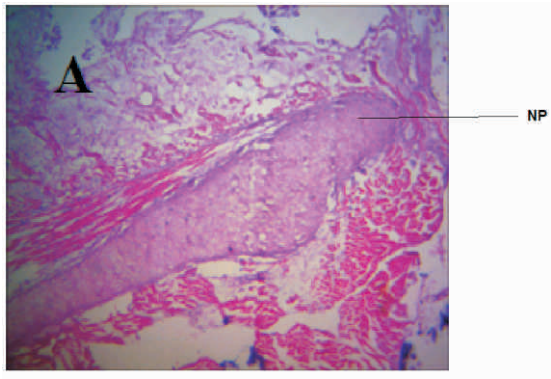

Plate 4A: Photomicrograph of control kidney showed Normal cellular Pattern (NP), no lesions, necrosis and fatty infiltration (5µm thick; H&E staining; 200X).

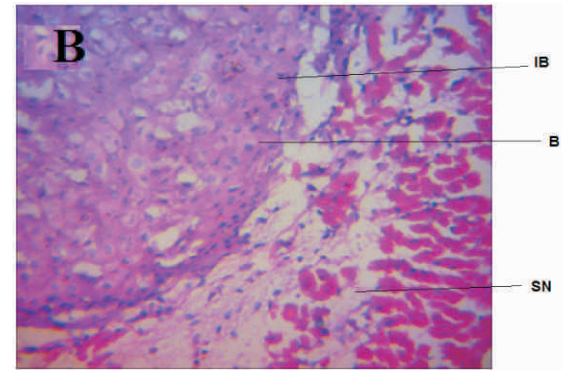

Plate 4B: Photomicrograph of exposed kidney showed tubular epithelia appeared Ballooned (B), Severe Necrosis (SN), Inclusion Bodies (IB) and an extensive fatty infiltration of the kidney parenchyma (5µm thick; H&E staining; 200X)

Discussion

The physical and chemical parameters measured before the inclusion of test concentration showed slight variations and were within the range for culture of fresh water fish as reported by Obot *et al.,* (2016), Omoregie *et al.,* (2009), Omitoyin *et al.,* (2006), and FAO (2000). The differences observed in DO, COD and pH among the test concentrations may be due to the fact that effects of the test chemical inhibited oxygen in culture media (Ladipo *et al.,* 2011). The extent of oxygen inhibition in culture medium translated to the degree of abnormality in behaviour such as erratic swimming, sudden quick movements and restlessness exhibited by experimental fish in the culture facility to different concentrations of DDVP. Nwani *et al.,* (2015) reported such restlessness and abnormal behaviour when *C.gariepinus* juveniles were exposed to DDVP and Ladipo *et al.,* (2011) reported such in *T. zilli* and *C. gariepinus* juveniles. Mortality then increased with increase in test concentration which occurred as a result of the mucus observed on the gills of the dead fish; the mucus inhibits gaseous exchange (Omoniyi *et al.,* 2011). Other organs such as the liver, kidney and the tissues of the experimental fish were also altered. The liver showed moderate areas of necrosis, inflammation, inclusion bodies and severe periportial hepatic

individualization similar to results by Ladipo *et al.,* (2011) in *T. zilli and* Ayoola (2008) in *Oreochromis niloticus*; the alteration in gills was similar to those reported in *Oreochromis mossambicus (*Subburaj *et al*., ,2018); *C. gariepinus* juveniles (Omoniyi *et al.,* 2013 & Nowak ,1992). Rahman *et al.,* (2002) also observed similar reports on the tissue of *C. punctatus* and *B. gonionotus.*

Conclusion

It was observed that the pesticide DDVP exhibited a direct effect on the vital organs of the fish and these effects were found to be directly related to the concentration of DDVP. As the use of the chemical is gaining more awareness in crop and animal husbandry, care must be taken on application and indiscriminate application should be avoided. This is especially true in integrated farms where DDVP is used against ants and termites. Public enlightenment on the effects of this pesticide on aquatic biota should be emphasized, the populace should be properly tutored and guided on the toxicity of the chemical to other useful organisms especially the aquatic environment.

Conflict of Interest: The authors declare that we have no conflict of interest.

References

Annune, P.A., Hbele, S.O and Oladimeji, A.A. (1994). Acute toxicity of cadmium to juvenile of *Clarias gariepinus* (Tuegls) and *Oreochromis niloticus* (Trewavas). *Journal of Environmental Science and Health*. 29:1357-1365.

American Public Health Association (APHA). (1995). Standard methods for the Examination for Water and Wastewater (19th edition). Byrd Prepress Springfield, Washington.

APHA, AWWA, WEF (2012). *Standard methods for examination of water and wastewater.* 22nd ed. Washington: American Public Health Association; 2012, 1360 pp. http://www.standardmethods.org

Ayoola, S.O. (2008). Toxicity of glyphosate herbicide on Nile tilapia (*O. niloticus*) juvenile. *African Journal of Agricultural Research.* 3:825-834.

Ayotunde, E.O., Ada, F.B and Udeh, G.N (2016). Effect of partial replacement of fishmeal with *Moringa oleifera* Leaf meal on the Haematology, carcass composition and growth perfomance of *Clarias gariepinus* (Burchell 1822) Fingerlings. *International Journal of Fisheries and Aquatic Studies.* 4(4):307-311.

Das, S. (2013): A review of Dichlorvos toxicity in Fish. *Current World Environment,* 8(1), 143-149.

Das, S. and Gupta, A., (2012). Effect of Malathion (EC50) on Gill Morphology of Indian Flying Barb, Esomus danricus (HamiltonBuchanan). *World Journal of Fish and Marine Sciences* 4: 626-628.

Fafioye, O.O., Adeogun, O.A., Olayinka, E.A and Ayoade A.A. (2001). Effect of sub-lethal concentrations of lead on growth of *C. gariepinus. Nigerian Experimental Biology.* 5(1):61-68.

Food and Agriculture Organization (FAO)(2000). Manuals of methods in aquatic environment Research. Part 9, Analysis of metals and organochlorides in fish. Fish Technical Paper 212pp.

Finney, D.J. (1971). *Probit Analysis,* 3rd edition. Cambridge University Press, Cambridge, 20.

Gupta, A.K., Verma, G.P and Jain, K.L. (2008). Acute toxicity of organophosphate insecticide, Dichlorvos in relation to selected water hardness for the freshwater zooplankters. *Journal of Environmental Biology.*_29:837-839.

Iyiola, A.O. (2015). Human impact on the water quality and benthic macro-invertebrate compositions in Ogunpa River, Nigeria. *Journal of Agriculture and Ecology Research International.* 2(2):120-128.

Keneko, J.J. (1989). *Clinical biochemistry of domestic animals.* 4th ed. Diego, Academic Press Inc. California, 132 pp.

Ladipo, M.K., Doherty, V.F and Oyebadejo, S.A. (2011). Acute toxicity, behavioural changes and histopathological effect of paraquat dichloride on tissues of catfish (*C. gariepinus*). *International Journal of Biology* 3(2):67-74.

Nowak, B. (1992). Histological changes in gills induced by residues of Endosulfan. *Journal of Aquatic toxicology.* 23:63-84.

Nwani, C.D., Ekwueme, H.I., Ejere, V.C., Onyeke, C.C., Chukwuka, C.O., Peace, O.S and Nwadinigwe, A.O. (2015). Physiological effects of paraquat in juvenile African catfish

C. gariepinus (Burchell 1822). *Journal of Coastal Life Medicine* 3(1):35-43.

Obot, O.I., Etim, L. and David, G.S. (2016). Review of some physical and chemical properties of some Nigerian creeks. *Nigerian Journal of Agriculture, Food and Environment* 14(4):82 – 90.

Odiete, W.O. (2009). *Environmental physiology of animals and pollution*. Diversified Resources, Lagos, Nigeria. 220-246pp.

Omitoyin, B.O., Ajani, E.K., Adeshina, B.T and Okuagu, C.N.F. (2006). Toxicity of Lindane (GammaHexachloro – CycloHexane) to *C. gariepinus* (Burchell 1822). *World Journal of Zoology* 1:57-63.

Omoniyi, I.T., Adeogun, K.L. and Obasa, S.O. (2013). Lethal effects of 2,2 Dichlorovinyl Dimethyl Phosphate (DDVP) on fingerling and Juvenile *C. gariepinus* (Burchell, 1822). *Croatian Journal of Fisheries* 71:19-24.

Omoniyi, I.T., Agbon, A.O. and Sodunke, S.A. (2011). Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobacum*) leaf dust extract on weight and hematological changes in *C. gariepinus* (Bruchell). *Journal of Applied Science Environmental Management* 6(2):37-41.

Omoregie, E., Malachy, N.O., Ajino, I., Romanus, I. and Kazimierz, W. (2009). Effect of single super-phosphate fertilizer on the survival and respiratory dynamics of *O. niloticus. Acuta Ichthyological et Piscatoria* 39(2): 103-110.

Ogueji, E.O. and Auta, J. (2007). Investigation of biochemical effects of acute concentrations of Lambda-cyhalothrin on African catfish *C. gariepinus*-Teugels. *Journal of Fish International* 2:86-90.

Rahman, M.Z., Houssain, Z., Mollaqh, M.F.A and Ahmed, G.U. (2002). Effect of Diazinon 60EC on Testudineus (*C. punctatus* and *B. gonionotus*). *The ICLARM Quarterly* 25(2):8-12.

Umar, M., Stephen, S.H., Abdullahi, M. and Garba, M. (2010). Dichlorvos concentrations in locally formulated pesticide (ota-piapia) utilized in North eastern Nigeria. *Scientific Research and Essay* 5(1):049-054.

Subburaj A, Jawahar P, Jayakumar N, Srinivasan A and Ahilan, B (2017). Acute toxicity bioassay of Malathion (EC 50%) on the fish, Oreochromis mossambicus (Tilapia) and associated histological alterations in gills. *Journal of Entomology and Zoology studies* 6(1): 103-107.

United States Environmental Protection Agency (USEPA) (2000). *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates.* 2th ed. Washington, D.C.: United State Environmental Protection Agency; 2000.

World Health Organization (WHO) (1991). Paraquat Health and Safety Guide. *Health and Safety Guide Publication No. 51*. Geneva Switzerland.