iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** III **Month of publication:** March 2019

DOI: http://doi.org/10.22214/ijraset.2019.3430

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue III, Mar 2019- Available at www.ijraset.com

Gill Toxicity and Biphasic Interaction of Antioxidant Enzymes in *Clarias Gariepinus* Exposed to Dichlovos

Ogunwole G.A.[1], Abiya S.E[2]
[1,2]*Department of Biology, School of Science, Federal University of Technology, Akure. P.M.B 704, Ondo, Nigeria*.

Abstract: ***The organophosphate, Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) is among the most utilized pesticide globally with a potential to cause harm to non-target organisms. This study assessed the sublethal effect of Dichlorvos concentrations on gills histology and biochemical parameters in Clarias gariepinus. In the acute studies, fish were distributed into five groups in triplicates and exposed to 0.40, 0.45, 0.50, 0.55 and 0.60 mg/L of Dichlorvos for 96 hours. Acute toxicity data of Dichlorvos to fish was observed to be 0.49mg/L. In chronic exposure, fishes were exposed to sub-lethal concentrations of 0.049 mg/L and 0.0049 mg/L of Dichlorvos and a control group devoid of the test chemical for 30 days. Liver and gills were harvested and evaluated for antioxidant activities and histopathology respectively. In the exposed animals, the activities of the enzyme GSH, SOD, CAT and GST were biphasic throughout the duration of study. There was a significant induction (P > 0.05) in the activity of GSH, SOD, CAT and GST on day 14 in fish when compared to day 0. While on day 30, there was a significant inhibition (P > 0.05) in the activity of GSH, SOD, CAT and GST respectively. The level of lipid peroxidation in exposed fish was significant induced (P > 0.05) throughout the study period. Histological sections of the gills revealed a decreasing incidence of necrosis induced lesion, disorganization of the whole length of the cartilaginous support of the primary lamellae, hypotrophy of the secondary lamellae, lamellae fusion and blood clot with increasing duration of the study. The reduced incidence of histopathological defects with increasing exposure suggest that the gills of C. gariepinus may be equipped with adaptive to counter prolonged exposure to environmental contaminants. The biphasic activity of antioxidant defense enzymes such as GSH, SOD, CAT and GST in fish suggest that short duration chronic studies may not be sufficient to give an overview of the overall health of an aquatic system.***

Keywords: Biphasic, anti-oxidant, histology, Dichlorvos, Clarias, gill.

I. INTRODUCTION

Extensive pollution of the aquatic ecosystems by indiscriminate and broad application of various pesticides by runoff, drift, leaching and drainage has globally become one of the most significant problems. A vast count of pesticides is frequently used to control numerous agricultural pests, amongst others, organophosphorus pesticides (OPs) pesticides have emerged as the most utilized class of insecticides due to their swift degradation ability (USEPA 2006), their low- persistent attributes in the environment (Oruc *et al.* 2006) and rapid biodegradability (Ye *et al.* 2010).

Dichlorvos is used as an anthelminthic and a diverse pest control of farm animals, man and plants. On plants it is deployed as a pre-harvest therapy for vegetable, fruits, rice and field crops while on livestock, it is usually deployed as aerosols, sprays and as anthelminthic pellets for oral administration of pigs, poultry, and horses (Deka and Mahanta, 2015). However, OPs are known to wreak havoc towards non-target organisms in the terrestrial and aquatic ecosystem with attendant severe, long term population effects, especially for the fishes (Mukhopadhyay and Dehadrai 1980a). The primary mechanism of action of Dichlorvos is inhibition of acetylcholinesterase (AChE), resulting to an upsurge in the level of acetylcholine in the synaptic cleft and hence, inducing anomalies like alteration in the swimming behavior, spasms, shaking palsy, impaired feeding, impaired reproductive behavior, low avoidance and escaping from predators, distorted spatial orientation of the species, and other unpleasant effects (Bretaud *et al.* 2000; Howard, 1991).

A study by Sisman (2010), showed that developmental abnormalities such cardiac edema, delayed hatching, no blood flow and vertebra malformations were observed in embryos and larvae of the zebra fish (Danio *rerio*) when exposed to Dichlorvos. Patar *et al.* (2015), Zhang *et al.* (2010) and Omoniyi *et al.* (2013) observed Dichlorvos induced discoloration in *Anabas testudineus* , *D. rerio* and *Clarias gariepinus* respectively.

Ecotoxicological risk assessment is an important tool in estimating the potential hazard pesticide and it associated metabolites poses to non-target organisms such as fish. It is also crucial in aquatic pollution management to access the relationship between the toxicant concentration and its effect on aquatic life (Olaifa *et al*. 2003). Histopathological defect is often deployed as biomarkers of the effects of pollutants on organisms and are suited as index of the overall ecosystem health (Velkova-Jordanoska and Kostoski, 2005). Biochemical parameters such as glutathione S-transferases, glutathione, alanine aminotransferase (ALT), superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (MDA), and aspartate aminotransferase (AST) have also been deployed to assess environmental stress in relation to existent pollutants (Suvetha *et al*. 2010).

However, studies on the chronic effects of Dichlovos on tropical fish focusing on durational responses have been insufficient. The African catfish (*Clarias gariepinus*) is an important source of animal protein in Nigeria due to its abundance in natural fresh water bodies, relative inexpensive cost, and ease of rearing them in local ponds. This species can be readily acclimatized to laboratory condition, thus, provides a suitable model for ecotoxicological research. The aim of the present study is to evaluate the sublethal effects of Dichlorvos on the histology and biochemical parameters *in C. gariepinus* over 30 days.

II. METHODOLOGY

A. Experimental Fish and Chemical

Juvenile African catfish (*C. gariepinus*) of average total length and weight of 16.5cm and 51g respectively was purchased from a commercial fish farm located at Agege local government area, Lagos state. The test animals were transferred to the Laboratory where they were stabilized and acclimatized for 7 days in a plastic tank ((36 x 30 x 48.5cm). The water utilized for stocking the organisms in the laboratory was sufficiently dechlorinated by aerating tap water in a plastic container with the aid of an aerator (Cosmo Aquarium, air pump 11,000) for 28 hours (Hr). This was done to accelerate vaporization of chlorine gas in the water. Throughout their acclimatization, the juveniles were fed with Coppens fish feed. They were fed twice daily at 12-hour intervals (morning and evening) and the holding water was changed once every two days to avoid accumulation of food residue and waste metabolite.

Feeding was terminated 24 hours before the commencement of the experiment as recommended by Ward and Parrish (1982), Reish and Oshida (1987). The test compound was bought from a local chemical store located at Alagomeji, Mainland local government, Lagos.

B. Measurement of Physico-Chemical Characteristics of Test Media

Physico-chemical parameters were measured at the inception of the experiment and at the end (that is, before change of test media). The parameters recorded are dissolved oxygen, pH, total dissolved solids, conductivity and salinity using appropriate digital instruments (Jenway).

C. Acute Toxicity Testing

Acute bioassay (96 Hr LC50) were conducted in 5 L glass tanks (36.5 x 25 x 28 cm) in a static system with the test medium kept constant throughout the study duration. A set of 10 fish per three replicates were randomly exposed to the test chemicals at 0.35, 0.40, 0.45, 0.50, 0.60mg/l respectively and an untreated control.

The quantal response (mortality) was evaluated every 24 hours over a period of 96 hours. Mortalities were recorded when they showed no response to mechanical stimulation when prodded with a glass rod. Dead specimens were removed to avoid pollution of the water during the experiment.

1) *Sublethal Effects of Dichorvos on C. gariepinus:* In the course of the experimental procedure, test organisms were exposed to sublethal concentrations (1/10th and 1/100th of 96 Hr LC50) of the test compound extrapolated from the acute toxicity bioassay. A semi static bioassay test protocol was adopted in which the test media was refreshed once every 24 hours with the same concentration and untreated control. At the end of the experiment- period on day 14 and 28, test organisms were retrieved, Fish specimens were anesthetized with tricaine methane sulfonate (MS-222) to enable dissection to obtain gill and liver organ required for histological studies and biochemical assays respectively.

D. Preparation of Tissue Homogenates

At a pre-determined day, gills of test samples were extracted from sacrificed organisms. They were washed free of all blood residues in ice cold isolation medium (0.25M sucrose, 5mM tris HCL), lightly blotted and weighed. The gills were sliced into fragments and homogenized (9% W/V) in 100% methanol and centrifuged at 10,000xg for 15min at 40C after method described by Hermes-Lima *et al*. (1995). The supernatant was collected for assays.

E. Antioxidant Assays

The Reduced Glutathione (GSH): The concentration of reduced glutathione (GSH) was determined according to the method of Beutler et al. (1963), by the reaction of glutathione with the color reagent 5,5-dithiobis- 2-nitrobenzoic acid (DTNB), forming a thiolate anion (TNB), which was measured at 412 nm. The GSH concentration was expressed in µg GSH.mg protein-1.

Superoxide Dismutase (SOD): SOD enzyme activity was analyzed using the method adopted by Sun and Zigman (1978). The SOD enzyme assay measured the difference between superoxide anion disintegration and synthesis i.e, its ability to repress the autoxidation of epinephrine. Enzyme action was observed at absorbance level of 450nm. Concentrations are presented as U/mg or SOD-Unit/mg protein, where one unit is expressed as the level of enzyme required to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25^0c and pH 7.8.

1) *Catalase (CAT):* Catalase activity was analyzed following the protocol adopted by Cohen et al. (1970). The protocol focuses on measuring the rate of H2O2 disintegration at absorbance levels of 240nm. The results were presented as U/mg or CAT-units/mg protein, where one unit is the level of enzyme that hydrolyzes 1 µmol of H_2O_2 per minute and per milligram of protein at 30^oC and pH 8.0.
2) *Glutathione-S-Transferase (GST):* The level of GST activity was analyzed according to the protocol adopted by Habig and Jakoby (1981). The measurement of GST activity was carried out by monitoring at absorbance level of 340nm, the induction of a conjugate between 1mM GSH and 1mM 1-chloro-2, 4-dinitrobenzene (CDBN). The results were presented as U/mg or GST unit/mg protein, where one unit is expressed as the amount of enzyme that conjugates 1 µmol of CDBN per minute and per milligram of proteins at 25^0C and pH 7.4.
3) *Lipid Peroxidation (LPO) Assay:* The levels of homogenized tissue malondialdehyde (MDA), as an index of lipid peroxidation were analyzed by thiobarbituric acid reaction (TBARS Assay). Using the protocol adopted by Yagi (1998). In this method, malondialdehyde is evaluated spectrophotometrically at absorbance levels of 535nm to assay for the amount of lipid peroxidation in a sample.

F. Statistical Analysis

Acute toxicity data involving quantal response (mortality) were analyzed using the probit analysis after Finney (1971.The anti-oxidant activity and lipid peroxidation assessment data were subjected to a one-way analysis of variance (ANOVA) using graphpad prism 7 statistical package to compare means and to determine the significance differences at a 5% probability level.

III. RESULTS

A. Acute Toxicity

Computed 96 hours LC_{50} for fish exposed to Dichlorvos is presented in table 1. The data revealed LC_{50} of Diclorvos to fish to be 0.5mg/L

Table 1: Computed 96 hours LC_{50} for fish exposed to Dichlorvos

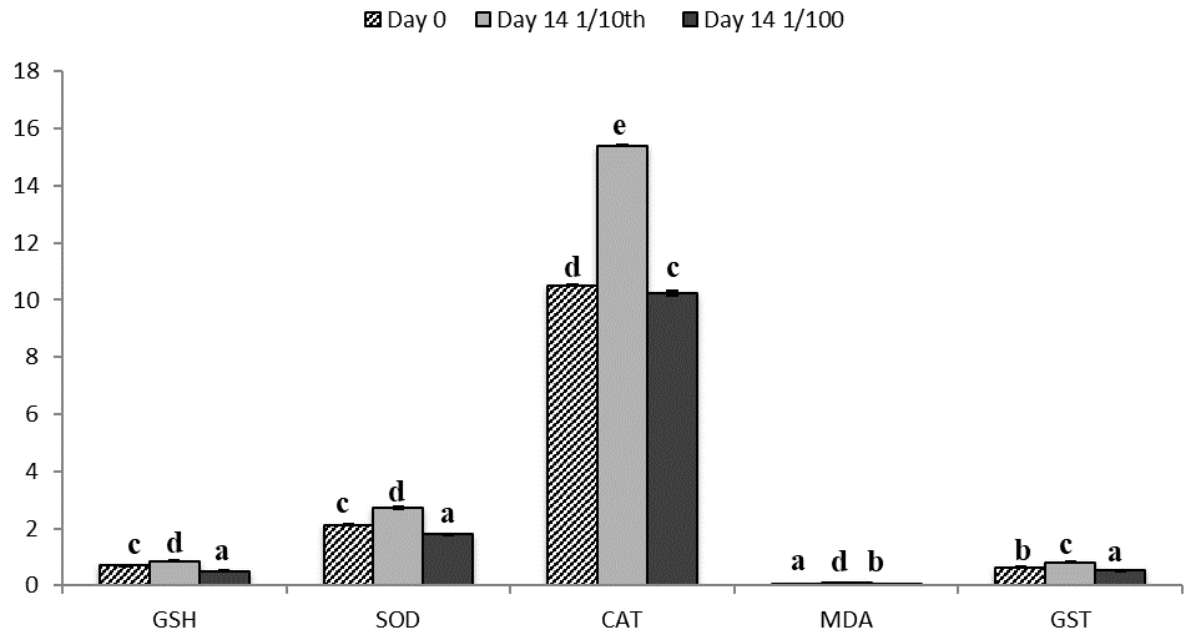
	LC_{05}(95% CL) (mg/L)	**LC_{50} (95% CL)** (mg/L)	**LC_{95} (95% CL)** % (mg/L)	**SLOPE + S.E**	**d.f**	**PROBIT EQUATION**
Dichlorvos	0.329 (0.380–0.107)	0.49 (0.476–0.381)	0.585(9.692 - 3.066)	0.267±5.729	2	y=12.5 * x + 4

KEY: cl = Confidence limit
df = Degree of freedom

B. Biochemical Results

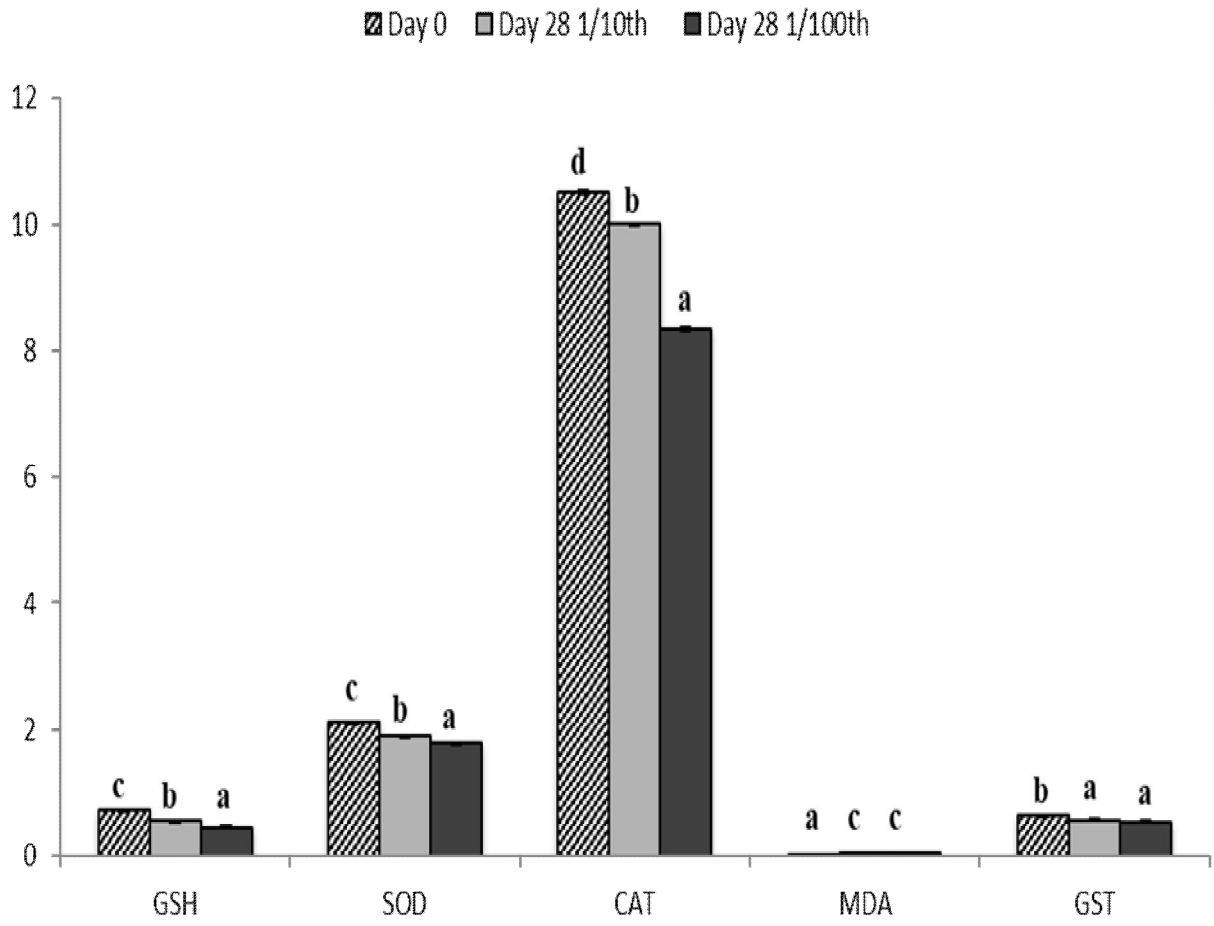
All antioxidants investigated in this study exhibited a biphasic behavior with respect to day 14 and day 28 respectively when compared to day 0.

1) *Reduced Glutathione transferase (GSH):* The enzyme activity of GSH on Day 14 was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (0.84µ/mg) and 1/100 (0.46µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.72µ/mg) (Figure 1). On day 28, the level of activity of GSH enzyme was significantly inhibited ($P < 0.05$) in fish respectively exposed to 1/10 (0.55µ/mg) and 1/100 (0.45µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.72µ/mg) (Figure 2).
2) *Superoxide dismutase (SOD):* The activity of the enzyme SOD on Day 14 was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (2.78µ/mg) and 1/100 (1.83µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (2.13µ/mg) (Figure 1). While on day 28, the level of activity of SOD enzyme was significantly inhibited ($P < 0.05$) in fish respectively exposed to 1/10 (1.90µ/mg) and 1/100 (1.78µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (2.13µ/mg) (Figure 2).
3) *Catalase (CAT):* The enzyme activity of CAT on Day 14 was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (15.37µ/mg) and 1/100 (10.31µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (10.50µ/mg) (Figure 1). While, on day 28, the level of activity of CAT enzyme was significantly inhibited ($P < 0.05$) in fish respectively exposed to 1/10 (10.03µ/mg) and 1/100 (8.35µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (10.50µ/mg) (Figure 2).
4) *Lipid Peroxidation (LPO) Assay:* The enzyme activity of MDA on Day 14 was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (0.081µ/mg) and 1/100 (0.055µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.033µ/mg) (Figure 1). On day 28 as well, the level of activity of MDA enzyme was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (0.063µ/mg) and 1/100 (0.062µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.033µ/mg) (Figure 2).
5) *Reduced Glutathione Transferase (GST):* The activity of the enzyme GST on Day 14 was significantly induced ($P < 0.05$) in fish respectively exposed to 1/10 (0.84µ/mg) and 1/100 (0.53µ/mg) 96Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.64µ/mg) (Figure 1).While on day 28, the level of activity of GST enzyme was significantly inhibited ($P < 0.05$) in fish respectively exposed to 1/10 (0.55µ/mg) and 1/100 (0.53µ/mg) 96 Hr LC_{50} sublethal concentration of the test chemical when compared to Day 0 (0.64µ/mg) (Figure 2).



Charts followed by the same alphabets are not significantly different ($P < 0.05$)

Figure 1: Biochemical parameters of *C. gariepinus* exposed to sublethal concentration of Dichlorvos on Day 14.



Charts followed by the same alphabets are not significantly different (P< 0.05)
Figure 2: Biochemical parameters of *C. gariepinus* exposed to sublethal concentration of Dichlorvos on Day 28

C. Histology

Sectioned gill at day 0 revealed a flawless state of the gill tissue showing the chondrocyte cells(A), entire length of the extracellular cartilaginous support of the primary lamellae (B), long primary lamellae (C), epithelium lining on the membrane (D) and long comb-like secondary lamellae (E) (Fig. 3). There were no observable gill defects such as blood congestion, lesion, epithelial lifting of lamellae, hyperplasia of the gill epithelium, lamellar fusion, lamellar disorganization and hypertrophy of the gill epithelium (Fig. 3). On day 14 at $1/10^{th}$ 96 Hr $LC_{50,}$ the histologic section of the gill filament revealed a severe degree of disorganization of the whole length of the cartilaginous support of the primary lamellae (A) , hypotrophy of the secondary lamellae (B), and incidence of epithelial lifting (D)(Figure 4) while on day 14 at $1/100^{th}$ 96 Hr LC_{50}, the histologic section of the gill filament revealed a mild degree of lesion (A), Blood clot (B) (Figure 5). On day 28, at 1/10th 96 Hr LC_{50}, the histologic section of the gill filament revealed gills with mild degree of lesion (A), incidence of lamellae fusion (B), and Hyperplasia of the secondary lamellae (C). (Figure 6), while on day 28 at $1/100^{th}$ 96 Hr LC_{50}, the histologic section of the gill filament revealed a pristine state of the entire length of the extracellular cartilaginous support of the primary lamellae (A) , long primary lamellae (B) very similar to what was obtained in the control group. (Figure 7). Interestingly, with increasing duration of exposure on day 28, gill tissues began to recover to pre exposure status.

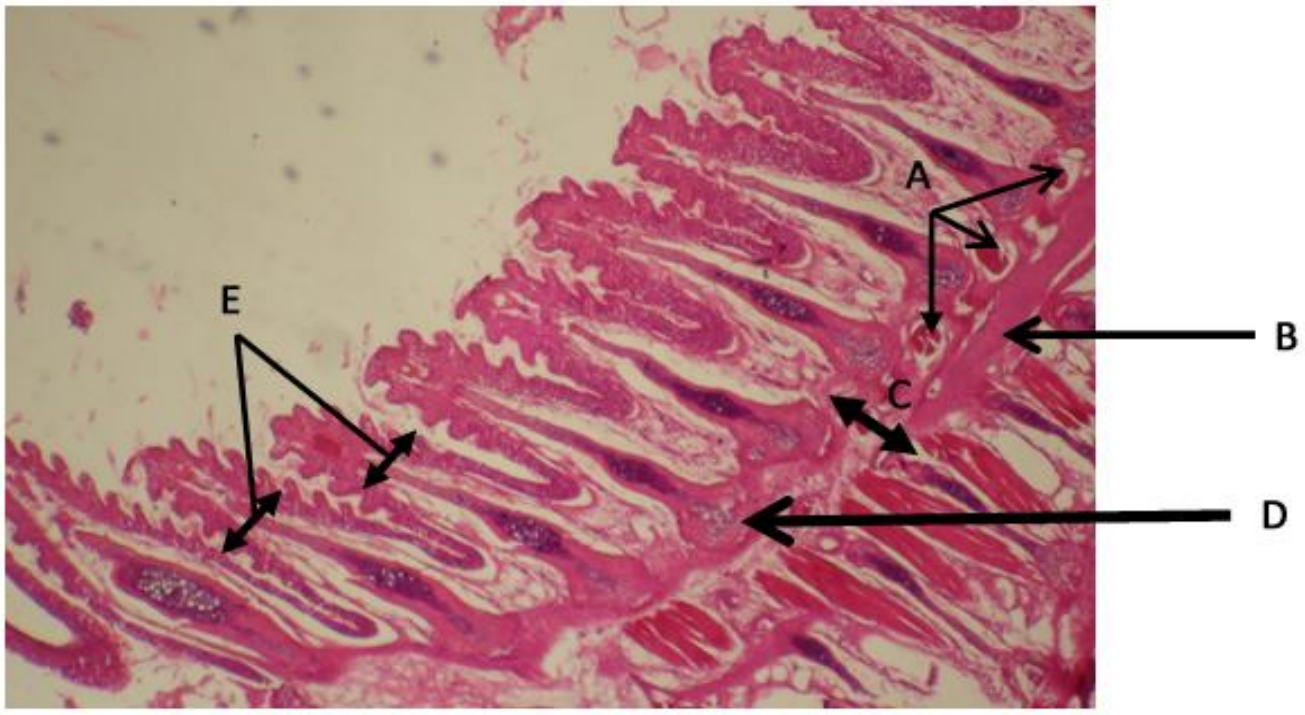


Figure 3 at Mg X100: A photomicrograph showing a pristine state of the gill showing the chondrocyte cells (A), entire length of the extracellular cartilaginous support of the primary lamellae (B), long primary lamellae (C) and long comb-like secondary lamellae (E) projecting from both sides of each primary lamella, epithelium lining on the membrane (D) .

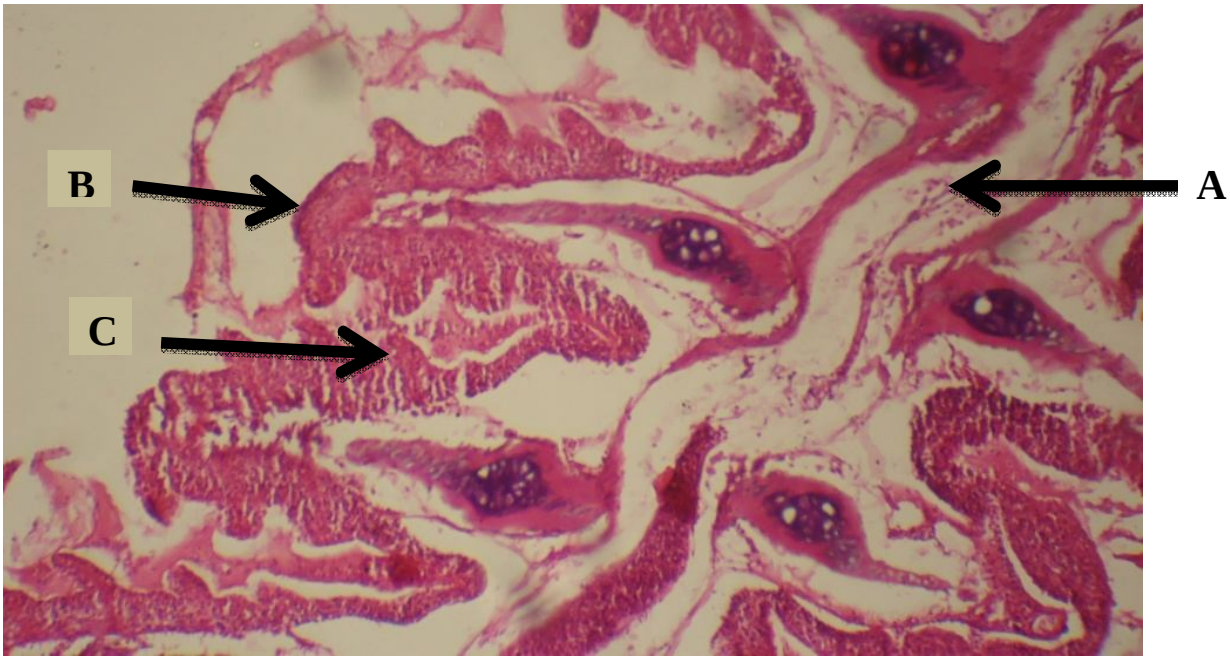


Figure 4 at Mg X100: A photomicrograph of 1/10th 96 Hr LC_{50} Day 14 gills revealing severe disorganization of the whole length of the cartilaginous support of the primary lamellae (A) , hypotrophy of the secondary lamellae (B), and incidence of epithelial lifting (C).

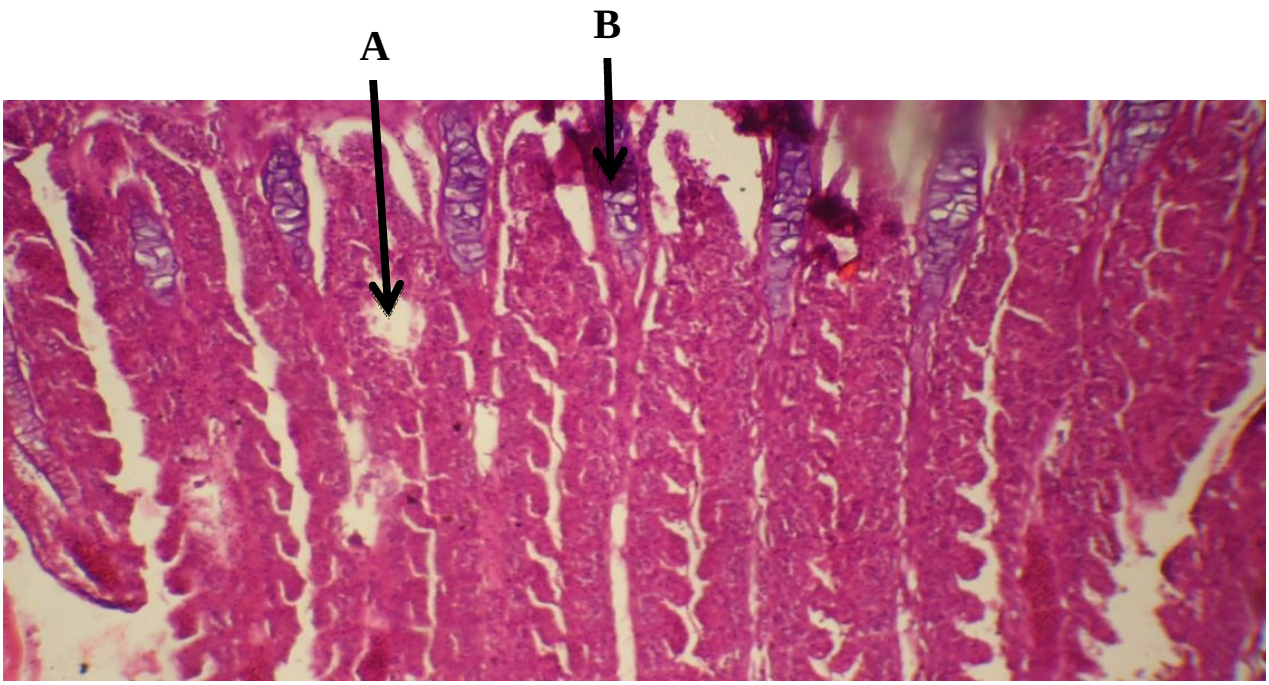


Figure 5 at Mg X100: A photomicrograph of Day 14 1/100th 96 Hr LC_{50} gills revealing mild degree of lesion (A), Blood clot (B)

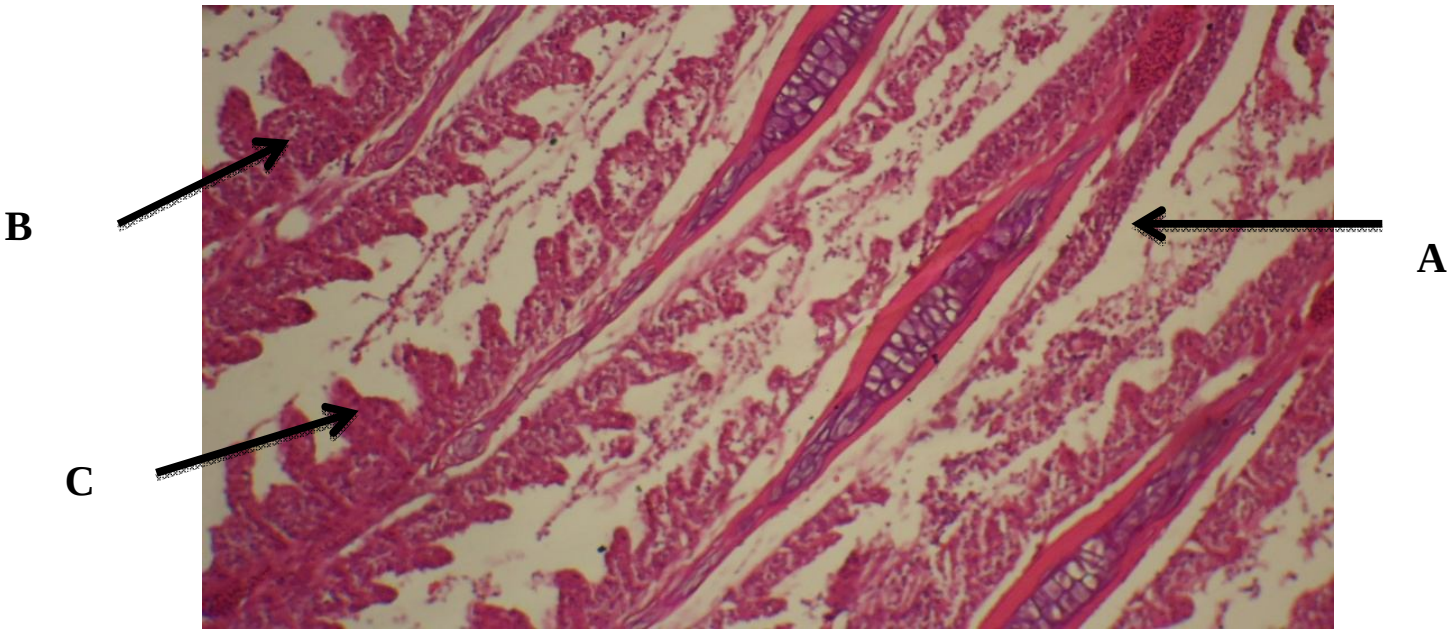


Figure 6 at Mg X100: Day 28 1/10th 96 Hr LC_{50} gills revealing mild degree of lesion (A), incidence of lamellae fusion (B), and Hyperplasia of the secondary lamellae (C).

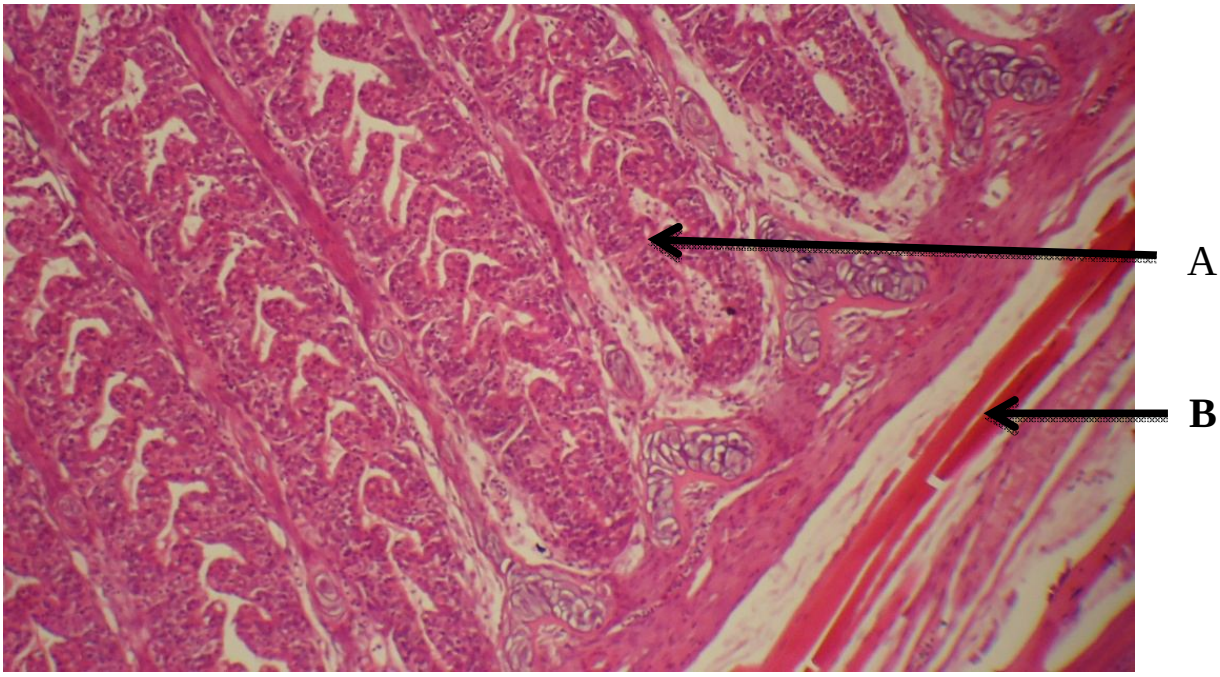


Figure 7 at Mg X100: Day 28 1/100th 96 Hr LC_{50} gills showing a pristine state of the entire length of the extracellular cartilaginous support of the primary lamellae (A) , long primary lamellae (B) very similar to what was obtained in the control group.

IV. DISCUSSION

Acute toxicity derived 96 Hr LC_{50} is one of the most important aspects for evaluating toxicity of pollutants in ecotoxicological studies. In this study, the 96 h LC_{50} value (i.e. 0.490 mg/L) obtained from *C. gariepinus* exposed to Dichorvos, suggests that this pesticide is highly toxic to fish. This conforms to the World Health Organization (WHO) report of 1989 stating that dichlorvos is highly toxic (LC_{50} in the range 0.1-1mg/L) to moderately toxic (LC_{50} in the range 1 to 10mg/L) to fish. This finding also agrees with the study of Omoniyi *et al.* (2013) who reported a 96 Hr LC_{50} value of 0.492mg/L of *C. gariepinus* exposed to Dichlovos. Toxic aspects of pesticides on organism is influenced by age, sex, size, species strain, water quality, formulation of test chemical and temperature (Nwani *et al.* 2013).

Enzymes engaged in detoxifying xenobiotics and their metabolites, such as biotransformation and antioxidant defense enzymes are amongst the most intensely studied biochemical biomarkers in fish. Antioxidant enzymes activity such as SOD, CAT, GSH, MDA and GST could be induced or inhibited by exposure to environmental contaminants depending on the duration and intensity of the stimulant. The susceptibility of the exposed organisms/species is also an important factor. Ferbal *et al.* (2012)

Glutathione (GSH), a tripeptide is the first line of cellular defense against damage elicited by oxidants. It plays an important role in the detoxification of many toxic agents, reactions of oxidation/reduction and amino acid transport (Van der Oost *et al.* 2003). The significant increase in GSH activity in the liver observed on day 14 suggest an increase in the production of reactive oxygen species (ROS), since GSH is primary protective response of cells against ROS (Parvez and Raisuddin, 2006). This observation agrees with a study by Pandey *et al.* (2006) who reported an increase in GSH activity under short-term exposure of *Corydoras punctatus* to chlorinated insecticide. The significant inhibition of GSH activity in the liver on day 28, maybe due to the utilization of GSH to counter the propagation of oxidative stress induced by ROS due to exposure to Dichlorvos. Similar observation of decreased GSH levels were observed by Jin *et al.* (2011) and Achudume *et al.* (2010). The decreased GSH in the exposed fish indicates that cellular mechanisms against ROS damage is required (Shabnam and badre, 2013).

SOD accelerates the dismutation of the superoxide anion radical to H_2O_2 and H_2O respectively, which is further detoxified by CAT and GSH-Px activities. Due to the repressive outcome on ROS formation, the SOD–CAT system proffers the first shield against oxygen toxicity and usually deployed as an indirect biomarker of ROS production (Pandey *et al.* 2003; Van der Oost *et al.* 2003). In this study, the initial increase of SOD activity in the liver of fish on day 14 indicates Dichlorvos induced adaptive response of fish to counter the O2- production. This reaction is linked to the synthesis of excess ROS which activated the biosynthesis of SOD, better

suited to shield the cells against oxidant damage (Zhang *et al.* 2004a, Zhang *et al.* 2013). This aligns with previous studies on induction of SOD in aquatic organisms. For example, Ogunwole *et al.* (2018) reported an increase in SOD level in liver of *C. gariepinus* exposed to paraquat and Oruc & Usta (2007) who described an increase of SOD content, in the gill and muscle of *Cyprinus carpio* after diazinon exposure respectively. The significant inhibition of SOD with continuous exposure on day 28 could indicate a high level of ROS production by the test compound in the exposed organism, ultimately leading to a decrease in the activities of SOD. Gisela *et al.* (2008). In addition, excess synthesis of superoxide radicals or aftermath transformation to H_2O_2 causes an oxidation of the cysteine in the enzyme which deactivates SOD (Dimitrova *et al.* 1994).

CAT is the basic enzyme responsible for eradicating the ROS formed during bioactivation of toxicants in hepatic tissues (Sk and Bhattacharya, 2006). It functions by catalyzing the degradation of H O to molecular O and H_2O (Shabnam and badre, 2014). The significant increase in CAT activity with initial exposure of the organisms at day 14 may be due to an increase in antioxidant cellular shield to eradicate ROS majorly formed during the metabolism and biotransformation of chemical compounds. Similar significant increase in CAT activity was noticed in liver of *Brycon cephalus* (Monteiro *et al.* 2006); liver and kidney of *Carassius auratus* exposed to Glyphosate (Lushchak *et al.* 2009, Zhang *et al.* 2004). The significant decrease in CAT activity with increasing exposure of the test compound on day 28 could also be due to decrease in reaction rates emanating from the surplus production of H_2O_2 (Shabnam and badre 2014). This could have been because of the flux of superoxide radicals, which has been shown to inhibit CAT activity (Ahmad *et al.* 2000). Other reasons could be that harsh oxidative stress could hinder antioxidant shield enzymes from functioning optimally (Zhang *et al.* 2003), binding of Dichlorvos to CAT or by inhibiting CAT synthesis as reported by Tripathi and Verma (2004) who suggested the binding affinity of alphamethrin to CAT, thus inhibiting it.

MDA is one of the major processes enhanced by oxidative stress and the first step of cellular damage caused by OP insecticides (Kavitha and Rao, 2008). The significant induction of MDA throughout the duration of the study could be attributed to either excessive oxidative stress (Buyukokuroglu *et al.*, 2002) or metabolism of the Dichlorvos pesticide resulting to the peroxidation of membrane lipids in the liver renowned as a site of multiple oxidative reactions and maximum free radical generation (Atli *et al.* 2006). The significant elevation of MDA in this study conforms with the findings of Vadhba and Hasan, (1986), who reported a significant elevation in MDA content in *Heteropneustes fossilis* exposed to Dichlorvos; Hai *et al.*, (1997) reported a significant elevation of MDA in *Cyprinus carpio* and *Ictalurus nebulosus* exposed to Dichlorvo. Oruc (2011) also reported similar findings in Diazinon exposure in the liver of *C. carpio.*

Glutathione-S-transferase (GST) is crucial in the guiding against damage from potentially reactive compounds, coupling them with endogenous molecules such as reduced glutathione (GSH), to be later excreted by the body. (Van der Oost *et al.* 2003). The significant elevation of GST activity on day 14 can be associated with the presence of of xenobiotics that are metabolized by conjugation with GSH, to be excreted from the body. The significant decline of GST with increasing exposure to the Dichlorvos on day 28 agrees with the finding of Otitoloju and Olagoke 2009 who reported that some conjugation products catalyzed by GST may impede this process, indicating that oxidative products can infact hamper the conjugation capabilities.

The gills of fish are essential organs for gaseous exchange, acid-base balance, osmoregulation and nitrogenous waste excretion (Heath 1987). This places the organ as an entry point for diverse environmental variables (Baskar 2014, Haaparanta *et al.* 1997). By virtue of their external location and close contact with the aquatic media, the gills are among the most vulnerable structures of teleosts making them liable to damage by any toxic agent in the environment often leading to cell pathology (Mallatt 1985, Roberts 1978).

In this study, the most common histopathology observed was lesion which can be attributed to either fish defense system or direct damage by irritants. These lesions increase the distance between the blood and the environment thus extending the barrier for environmental interactions (Baskar 2014, Poleksic and Mitrovic-Tutundzic, 1994). Futhermore, lesions are capable of impeding the primary physiological functions of the gills such as osmoregulatory mechanisms and antioxidant defense. Hyperplasia of the gills is a function of high proliferation of the epithelia filament which increase the thickness. Continuous cell proliferation with thickening of the gill filament may result into lamellar fusion evident in this study (Kantham and Richards 1995). These impairments can be attributed to either a defense mechanism trigger or the effect of the toxic agent which modifies glycoprotein in the mucus covering of the cells, ultimately affecting the negative charge of the epithelium and favoring adhesion to adjacent lamellae (Ferguson 1989). The benefit of these histological alterations is an increase in the gap between blood and the external medium. Ultimately, serving as a barrier to xenobiotics (Fernandes & Mazon 2003). However, the impact of the increased distance between water and blood is oxygen uptake impairment. Fishes counteract this limitation by elevating their ventilation rate to compensate for low oxygen uptake

(Fernandes & Mazon 2003). The gills pathology are nonspecific and can be activated by diverse group of toxic agents (Mallatt, 1985). Further studies is required to explain the gill tissue recovery of *C. gariepinus* with increasing exposure to DDVP.

V. CONCLUSION

The findings of this study reinforced the fact that Dichlovors exposure disrupts normal cellular function leading to alterations in the fundamental histology and biochemical mechanisms in fish. The reduced incidence of histopathological defects with increasing exposure suggests that the gills of *C. gariepinus* are equipped with adaptive mechanisms to counter prolonged exposure to environmental contaminants.

The biphasic activity of antioxidant defense enzymes such as GSH, SOD,CAT and GST in fish suggest that short duration chronic studies may not be sufficient to give an overview of the overall health of an aquatic system.

REFERENCES

[1] Achudume, A.C., Nwoha P.U. and Ibe, J.N. (2010). Effects of dietary exposure to insecticide “Raid” on the survival, growth and inhibition of metabolic processes in Wistar rats – J. Environ. Chem. Ecotoxicol. 2: 120-125.
[2] Adebayo, O. and Oluwatoyin, O. (2011). Lipid peroxidation and antioxidant defense enzymes in Clarias gariepinus as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. Environmental Monitoring and Assessment 182(1-4):205-13
[3] Atli, G., Alptekin, O., Tukel, S.and Canli, M. (2006) Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of fresh water fish Oreochromis niloticus. CompBiochemPhysiol 143:218–224
[4] Baskar, T. (2014).Impact of nitrite toxicity on histopathological profile to freshwater fish, Cirrhinus mrigala.The International Journal of Engineering and Science. 3(4), 42–47.
[5] Bretaud, S. and Toutant, J.P. (2000). Saglio P. Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (Carassius auratus). Ecotoxicol Environ Saf. 47: 117-124.
[6] Buyukokuroglu, M.E., Taysi, S., Polat, F. and Gocer, F. (2002) Mechanism of the beneficial effects of dantrolene sodium on ethanol-induced acute gastric mucosal injury in rats. Pharmacol Res. ;45:421–425.
[7] Cohen, D., Dembiec, D. and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. Annals Biochemistry 34, 30–38.
[8] Deka, S and Mahanta, R. (2015). Dichlorvos toxicity on fish - a review. European Journal. 5 (3): 78-85.
[9] Dimitrova, M.S.T., Tsinova, V. and Velcheva, V. (1994) Combined effect of zinc and lead on the hepatic superoxide dismutase– catalase system in carp (Cyprinus carpio). Comp Biochem Phys. 108:43–46
[10] EPA. Registration eligibility decision for dichlorvos (DDVP). United States Environmental Protection Agency, Washington D.C., 20460. 2006. www.epa.gov/pesticides/reregistration/REDs/ddvp_i red.pdf
[11] Ferbal, O., Suna, G.G., Mehmet, B., Arzu, H. and Serap, Y. (2012). The protective role of ascorbic acid (vitamin C) against chlorpyrifos-induced oxidative stress in Oreochromis niloticus. Fish Physiol Biochem. 38:635–643.
[12] Ferguson, H.W. (1989). Gills and pseudobranchs. In: Ferguson, H.W., (ed.), Text book of systemic pathology of fish, 1st ed., Lowa state University press, pp. 18-20. Amer, Lowa 500/0. Canada.
[13] Fernandes, M. N. and Mazon, A. F. (2003). Environmental pollution and fish gill morphology. In: Val, A. L. & B. G. Kapoor (Eds.), pp. 203-231. Fish adaptations. Enfield, Science Publishers
[14] Finney, D. J. (1971). Probit Analysis, 3rd edn. Cambridge University Press, Cambridge, 20.
[15] Gisela, K., Noemí, R., Verrengia, G. and Adriana, C. C. (2008). Effects of azinphos-methyl exposure on enzymatic and non-enzymatic antioxidant defenses in Biomphalaria glabrata and Lumbriculus variegatus. Chemosphere 72 (2008) 1333–1339.
[16] Haaparanta, A., Valtonen, E. T., and Hofmann, R. W. (1997). Gill anomalies of perch and roach from four lakes differing in water quality. Journal of Fish Biology, 50(3), 575–591.
[17] Habig, W.H. and Jakoby, W.B. (1981) Assays for di¡erentiation of glutathione S-transferases. Methods Med. Res. 77, 398^405.
[18] Hai, D.Q., Varga, S.I. and Matkovics, B. (1997).Organophosphate effects of antioxidant system of carp (Cyprinus carpio) and catfish (Ictalurus nebulosus).CompBiochem Physiol. 117: 83–88.
[19] Heath, A.G. (1987). Water pollution and fish physiology. CRC Press, Florida.
[20] Hermes-Lima, M., Castilho, R.F., Meinicke, A.R And Vercesi, A.E. (1995). Characteristics of Fe(II)- ATP complex-induced damage to the rat liver mitochondria. Mol Cell Biochem. 145: 53–60.
[21] http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation93/dichlorv.pdf
[22] Jin, Y., Zheng S., Pu, Y., Shu, L., Sun, L., Liu, W. and Fu Z. (2011). Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (Danio rerio) – Chemosphere 82: 398-404.
[23] Kantham, K.P. and Richards, R.H. (1995). Effect of buffers on the gill structure of common carp, Cyprinus carpio and rainbow trout, Oncorhynchus mykiss. Journal of fish diseases, 18, 411-423.
[24] Kavitha P, Rao V (2008) Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, Gambusia affinis. Environ Toxicol Phar 26:192–198
[25] Kelly, W.R. (1979).Veterinary clinical diagnosis 2nd ed. Balliere Tindall, London. pp. 266-279.
[26] Keneko, J.J. (1989). Clinical Biochemistry of domestic animals. 4th ed. Diego, Academic press Inc. California,132 pp.
[27] Kono, Y. and Fridovich I (1982). Superoxide radical inhibits catalase. J Biol Chem. 257: 5751– 5754.

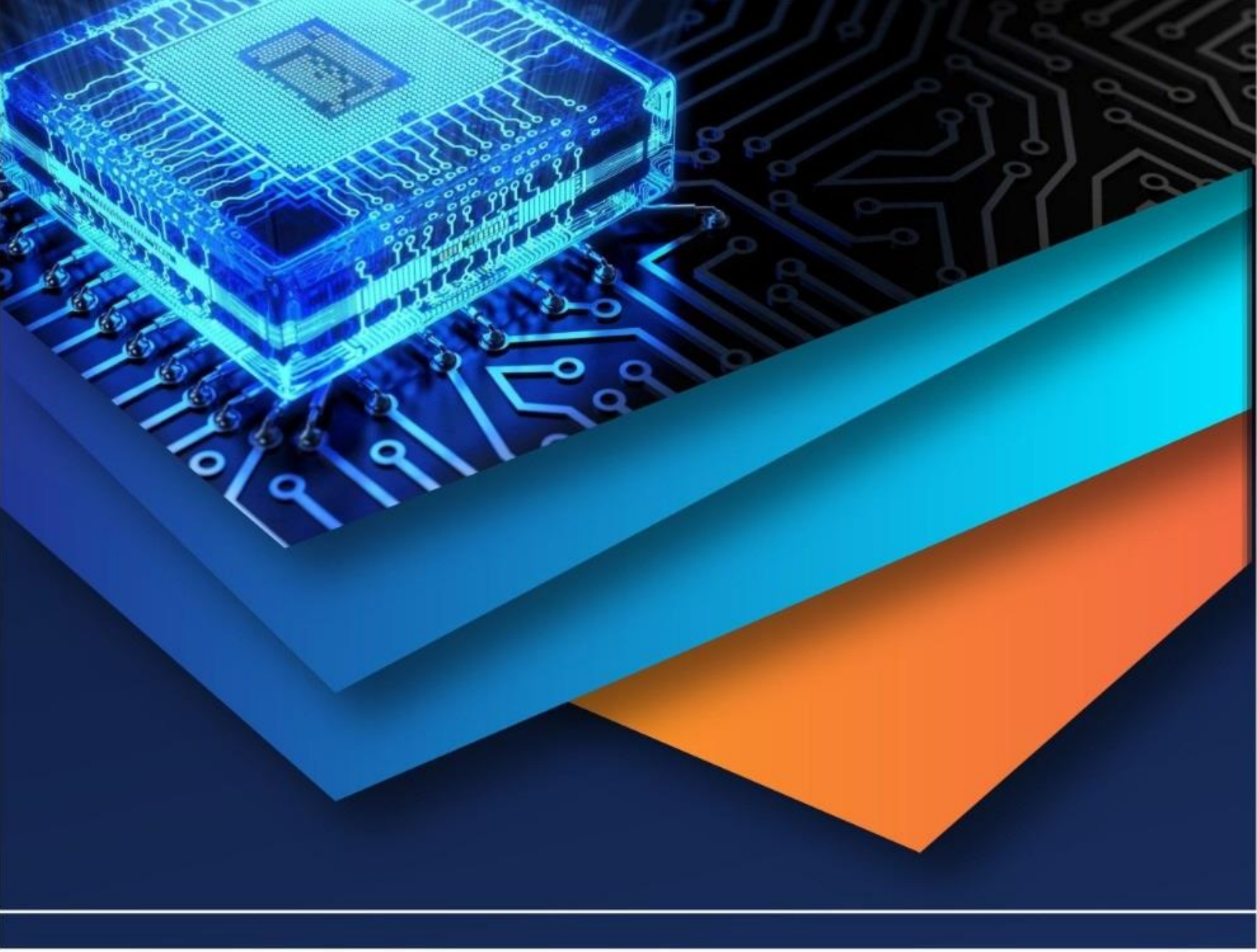
[28] Lindalva, P. and Claudia, B.R. M (2014). Integrated biomarker response index using a Neotropical fish to assess the water quality in agricultural areas. Neotropical Ichthyology, 12(1): 153-164/
[29] Lushchak, O.V., Olha, I., Kubrak, O.I., Storey, J.M., Storey, K.B. and Lushchak, V.I (2009) Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76:932–937
[30] Mallatt J. (1985). Fish gill structural changes induced by toxicants and other irritants; a statistical review. Canadian Journal of Fisheries and Aquatic Sciences, 42, 630-648
[31] Monteiro, D.A., Almeida, J.A., Rantin, F.T. and Kalinin, A.L (2006). Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Com Biochem Phys C 143:141–149
[32] Mukhopadhyay, P.K. and Dehadrai P.V. (1980). Studies on air-breathing catfish Clarias batrachus (Linn.) under sublethal malathion exposure. Indian. J. Exp.Biol.,18: 400-404 (
[33] Nwani, C.D., Ama, U.I, Okoh, F., Oji, U.O., Ogbonyealu, R.C. and Agha, A. (2013).Acute toxicity of the chloroacetanilide herbicide butachlor and its effectson the behavior of the freshwater fish Tilapia zillii.Afr J Biotechnol. 12: 499-503.
[34] Ogunwole, G.A ,Uju, S. and Saliu, J.K (2018). Paraquat Toxicity on Selected Biomarkers in Clarias gariepinus. IOSR Journal of Environmental Science, Toxicology and Food Technology . Volume 12, Issue 5 Ver. I (May. 2018), PP 66-75
[35] Olaifa, F.E., Olaifa, A.K. and Lewis, O.O. (2003). Toxic Stress of Lead on Clarias gariepinus (African catfish) Fingerlings. African Journal of Biomedical Research. 6: 101 –104.
[36] Omoniyi, I.T., Adeogun, K.L and Obasa, S.O. (2013). Lethal effects of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) on fingerling and juvenile Clarias gariepinus (Burchell, 1822). Croat. J. Fish., 71: 19-24.
[37] Oruc, E.Ö., Üner, N., Sevgiler, Y., Usta, D. and Drumaz, H. (2006). Sublethal effects of organophosphate diazinon on the brain of Cyprinus carpio. Drug Chem. Toxicol., 29: 57-67.
[38] Oruc, E. (2011). Effects of diazinon on antioxidant defense system and lipid peroxidation in the liver of Cyprinus carpio (L.) Environ Toxicol.;26:571–8.
[39] Oruc, E.O. and Usta, D. (2007). Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of Cyprinus carpio.Environ ToxicolPharmacol. 23: 48–55.
[40] Pandey, S., Parvez, S., Sayeed, I., Haquer, R., Bin-Hafeez, B. and Raisuddin, S. (2003) Biomarkers of oxidative stress: a comparative study of river Yamunafish Wallagoattu (Bl. Schn). Sci Total Environ 309:105–115
[41] Pandey, S., N. S. Nagpure, R. Kumar, S. Sharma, S. K. Srivastava & M. S. Verma. 2006. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost Channa punctatus (Bloch) by alkaline single-cell gel electrophoresis. Ecotoxicology and Environmental Safety, 65: 56-61.
[42] Parvez, S. and Raisuddin, S. (2006). Effects of paraquat on the freshwater fish Channa punctata (Bloch): non-enzymatic antioxidants as biomarkers of exposure. Arch Environ Contam Toxicol. 2006 .50(3):392-7
[43] Patar, A.A., Hassan, W.R.M., Hashim, N. and Yusof, F.Z.M. (2015). Toxicity Effects of Malathion, Dichlorvos and Temephos on acetylcholinesterase in climbing perch (Anabas testudineus). Adv. Env. Biol., 9(21): 81-86.
[44] Poleksic, V. and Mitrovic-Tutundzic, V. (1994). Fish gills as a monitor. Science of the Total Environment of sublethal and chronic effects of pollution.In R. Muller & R. Lloyd (Eds.), Sublethal and chronic effects of pollution on freshwater fish (pp. 339–352). Oxford: Fishing News Books Ltd.
[45] Reish, D.L. and Oshida, P.S (1987). Manual of methods in aquatic environment research, Part 10: short-term static bioassays. Rome: Food and Agriculture Organization of the United Nations: p. 62.
[46] Roberts, J.R. (1978). The pathophysiology and systematic pathology of teleosts. In fish pathology. 1st ed. pp. 67-70. Bailliere Tindall, London
[47] Schalm, O. W., Jain, N.C and Carrol, E.J. (1975). Veterinary Haematology, 3rd Edn., Lea and Febiger, Philadelphia, pp. 197-199.
[48] Shabnam, A. and Badre, A. A. (2013). Toxic Effect of Alphamethrin on Catalase, Reduced Glutathione and Lipid Peroxidation in the Gill and Liver of Zebrafish, Danio rerio. World Journal of Zoology 9 (3): 155-161, 2014.
[49] Shabnam Ansari, Badre Alam Ansari. (2014). Temporal variations of CAT, GSH, and LPO in gills and livers of zebrafish, Danio rerio, exposed to dimethoate. Arch. Pol. Fish. (2014) 22: 101-109
[50] Sisman, T. (2010). Dichlorvos-induced developmental toxicity in Zebrafish. Toxicol Industr Health. 2010; 26, 567.
[51] Sk, H.U. and Bhattacharya, S. (2006). Prevention of cadmium induced lipid peroxidation, depletion of some antioxidantive enzymes and glutathione by series of organoselenocyanates – Environ. Toxicol. Pharmacol. 22: 298-308.
[52] Sun, M. and Zigman, S. (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Analytical Biochemistry. 1;90(1):81-9.
[53] Suvetha, L., Ramesh, M. and Saravanan, M. (2010). Influence of cypermethrin toxicity on ionic regulation and gill Na+/K+-ATPase activity of a freshwater teleost fish Cyprinus carpio. Environ Toxicol Pharmacol. 29: 44-49.
[54] Tripathi, G. and Verma, P. (2004). Fenvalerate-induced changes in catfish, Clarias batrachus: metabolic enzymes, RNA and protein. Comparative Biochemistry and Physiology Part C, 138: 75-79.
[55] Vadhva, P. and Hasan, M. (1986). Organophosphate dichlorvos induced dose-related differential alterations in lipid levels and lipid peroxidation in various regions of the fish brain and spinal cord. J Environ Sci Health B. 21:413–24
[56] Van der Oost, R., Beyer, J. and Vermeulen, N.P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Phar 13:57–149
[57] Velkova-Jordanoska, L. and Kostoski, G. (2005). Histopathological analysis of liver in fresh(Barbus meridionalis petenyi Hekel) in reservoir Trebenista. Natura Croatica. 14: 147-153.
[58] Ward, G.S. and Parrish, P.R. (1982) Manual of methods in aquatic environment research. Part 6: toxicity tests (FAO fisheries technical paper). Rome: Food and Agriculture Organization of the United Nations. No 185 FIRI/T185.
[59] WHO. (1989). Dichlorvos. Environmental Health Criteria 79. World Health Organisation, Geneva, Switzerland..
[60] Yagi, K. (1998). Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. Methods in Molecular Biology, 108, 107–110.
[61] Ye, J., Zhao, M., Liu, J. and Liu W, 2010. Enantio selectivity in environmental risk assessment of modern chiral pesticides. Env. Poll., 158(7): 2371-2383.

[62] Zhang, Z.Y., Yu, X.Y., Wang, D.L., Yan, H.J and Liu, X.J. (2010). Acute toxicity to zebrafish of two organophosphates and four pyrethroids and their binary mixtures. Pest Manage. Sci., 66: 84-89.

[63] Zhang, J., H. Shen, X. Wang, J. Wu and Y. Xue, 2003. Effect of choric exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish, Carassius auratus. Chemosphere, 55: 167-174.

[64] Zhang, J.F, Wang, X.R., Guo, H.Y., Wu, J.C. and Xue, Y.O (2004a). Effects of water- soluble fractions of diesel oil on the antioxidant defences of the goldfish (Carassius auratus).Ecotox Environ Saf. 58: 110–116.

[65] Zhang, Q., Lusheng, Z., Jun, W., Hui, X., Jinhua , W., Yingnan, H. and Jinhui, Y. (2013). Oxidative stress and lipid peroxidation in the earthworm Eiseniafetida induced by low doses of fomesafen.Environ SciPollutRes . 20:201–208.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY