

## Paraquat Toxicity on Selected Biomarkers in *Clarias gariepinus*

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**Abstract:** Pesticides are major sources of environmental pollutants utilized to control or eradicate pests negatively impacting on man agricultural resources. Amid the varied classes of pesticides, Paraquat dichloride, an organochloride is widely deployed against varieties of agricultural and domestic pest with potential consequences. This study examined the impact of Paraquat (1,1-dimethyl 4, 4-bipyridiniumdichloride) on the gills and liver of *Clarias gariepinus*. In the acute studies, fish were allocated into five groups and exposed to 50, 100, 150, 250 and 250 mg L<sup>-1</sup> of Paraquat for 96 h. In chronic exposure, fish were distributed into 2 groups and exposed to 1/10<sup>th</sup> and 1/100<sup>th</sup> 96 Hr LC50 value of (128.03 mg L<sup>-1</sup>) of Paraquat for 30 days. Liver and gills were harvested and evaluated for antioxidant parameters and histopathology respectively. There was no significant induction ( $P > 0.05$ ) in the activity of SOD activity in exposed organisms while CAT enzyme activity was significantly ( $P < 0.05$ ) induced in exposed organisms when compared to the control group respectively. GSH and GST activity was significantly ( $P < 0.05$ ) inhibited, while MDA was significantly ( $P < 0.05$ ) induced in the exposed organisms throughout the duration of the study. Histology of the gills revealed increasing incidence of 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 histological alterations in the gill tissue suggest they were induced by increased concentrations of the test chemical. Therefore, combined antioxidant parameters and histopathological studies are imperative for the assessment and evaluation of potential pollutants in the aquatic environment).

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### I. Introduction

Pesticides comprise of heterogeneous groups of chemical products basically used to stave off, control or eradicate pests negatively impacting on man agricultural resources. Ultimately, man has derived great benefits by the increased yield from agricultural products pesticide usage offers (Cavalcante, Martinez & Sofia 2008; Atamanalp, *et al.*, 2001). Despite its role in crop yield boost, pesticides have been reported to evoke negative consequences on the ecosystem. Pesticides find their way into the environment, either through water runoff and or as aerosols carried by winds from the indiscriminate application of pesticides in the field or from reckless storage and disposal of pesticide containers. The regular incursion of farmland effluents into fresh water sources often leads to diverse contamination incidences which become significant when considering pollution (Mason, 1991). The presence of pesticides in aquatic bodies often lead to a modification of the physico-chemical properties of water (Richardson, 1988). High levels of pesticide contamination in aquatic ecosystems results into poisoning, oxygen depletion and resultant mass mortality of aqua-fauna (Atamanalp, *et al.*, 2001; Seiyaboh *et al.*, 2013).

Among all classes of pesticides, Herbicides account for the gross use, symbolizing over 80 percent of pesticides utilized in farmlands globally (Gerald *et al.*, 1995). Herbicides have the potential to accumulate in water bodies to levels detrimental to the survival of zooplankton which are main source of food for small and young fishes (Doherty *et al.*, 2011).

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is one of the most broadly used herbicidal product globally and is currently accessible in more than 130 countries (Kimbrough 1974; Calderbank 1975; Dasta 1978; Haley 1979; Hughes 1988; Suntutres, 2002; Wang *et al.*, 2012). It is a principal element of the bipyridylum family of non-selective herbicide developed first commercialized for agricultural use in the United Kingdom in 1962 (Chia *et al.*, 1982). Paraquat readily disintegrates and can persist in the environment absorbed to soil particles (Seiyaboh *et al.*, 2013). Even at minor levels of concentrations, it still could elicit deleterious effects, such as cytogenetic damage, physiological effects, and even death to exposed non-target species (Dinis-oliveira *et al.*, 2008). Studies have shown that Paraquat has the potential to impede the growth and weight of *Oreochromis niloticus* (Babatunde and Olajimeji, 2014), negatively impact on the blood plasma activities of *Clarias gariepinus* (Seiyaboh *et al.*, 2013), induce Respiratory stress, erratic swimming and instant death of fish

(Doherty *et al.*, 2011), impair the physiological processes in *Clarias gariepinus* by significantly increasing the level of white blood cells, glucose, aspartate aminotransferase, and alanine aminotransferase (Nwani *et al.*, 2015), impacts on the immune and growth of the rainbow trout, *Oncorhynchus mykiss* (Amir *et al.*, 2014). At high levels, Paraquat inhibits the photosynthetic ability of some algae in stream water thus disrupting the food web necessary for ecological balance (Kenneth, 1990).

The use of bioassay techniques to evaluate the toxicity of chemicals on animals has been used as suitable models to predict hazards posed by chemicals to man. Aquatic bioassay most especially is very crucial in water pollution management to verify if a potential toxicant is perilous to aquatic fauna and if so, to establish the relationship between the toxicant concentration and its effect on aquatic life (Olaifa *et al.*, 2003).

Histopathological deformities can be used as biomarkers of the effects of anthropogenic pollutants on organisms and are suitable as indicators of the overall ecosystem health (Velkova-Jordanoska and Kostoski, 2005). These histological distortions are intimately linked to diverse biomarkers of stress because pollutants usually undergo metabolic activation in order to induce cellular modifications in the affected organism. Biochemical parameters have also been deployed to assess environmental burdens in response to prevailing pollutants, these parameters include the levels of plasma proteins, glucose, and other enzymes, like alanine aminotransferase (ALT), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases, glutathione, lipid peroxidation (MDA), and aspartate aminotransferase (AST) (El Sayed *et al.*, 2007 ; Suvetha *et al.*, 2010).

However, studies on the chronic impacts of Paraquat on tropical fish have been largely insufficient. The African catfish (*Clarias gariepinus*) is a major source of animal protein in Nigeria due to its relative abundance in natural fresh water bodies, inexpensive cost, and ease of rearing them in local ponds. This fish species can easily be acclimatized to laboratory condition, thus, provides an excellent model for ecotoxicological studies. The aim of the present study is to evaluate the sublethal effects of Paraquat on the histology and biochemical parameters in *C. gariepinus*.

## **II. Materials And Methods**

### **2.1. Experimental fish and chemical**

Juvenile African catfish (*C. gariepinus*) of average weight and total length of 52g and 17 cm, respectively was collected from a fish farm located in Agege local government area, Lagos state and transported to the Laboratory where they were acclimatized for 7 days in a plastic tank ((36 x 30 x 48.5cm). The water used for stocking the test organisms in the laboratory was adequately dechlorinated by aerating tap water in a plastic container with the aid of an aerator (Cosmo Aquarium, air pump 11,000) for 27 hours. This was done to enhance swift vaporization of chlorine gas in the water. The fingerlings were fed with Coppens fish feed throughout their acclimatization. The organism were fed twice daily at 12 hour intervals (morning and evening) and the holding water was changed once every two days to avoid the 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 trademark name of the Paraquat used in this study was Paraeorce manufactured by Red Sun Group Corporation, China.

### **2.2. Measurement of Physico-Chemical Characteristics of Test Media**

Measurement of the Physico-chemical characteristics measurements were taken 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).

### **2.3. Acute toxicity testing**

96 h LC<sub>50</sub> value acute bioassay were conducted in 25 L glass tanks (36.5 x 25 x 28 cm) in a static laboratory system with the test solution kept constant throughout the duration of the study. A set of 10 fish per three replicates were randomly exposed to the test chemicals at 50, 100, 150, 200, 250mg/l respectively and an untreated control.

The quantal response (mortality) was assessed 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, fish behavior was observed for opercula count.

### **2.4 Sublethal effects of paraquat on *Clarias gariepinus***

In the course of the experiment test organisms were exposed to sublethal concentrations (1/10th and 1/100th of 96h LC<sub>50</sub>) of the test compound extrapolated from the acute toxicity bioassay. A semi static bioassay test protocol was utilized in which the test media were changed once every 24 hours to fresh media of the same concentration and untreated control. At the end of the experimental procedures on day 28, test

organisms were retrieved, sacrificed and dissected to obtain gill tissues required for histological studies and biochemical assays and.

### 2.5 Preparation of Tissue Homogenates

At a pre-determined day, gills of test samples were harvested 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 cut 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.

### 2.6 Enzymes Activity Assays

**Superoxide Dismutase (SOD):** SOD enzyme activity was analyzed using the method as described 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 °c and pH 7.8.

**Catalase (CAT):** Catalase activity was analyzed following the protocol adopted by Cohen *et al.*, (1970). The protocol focuses on measuring the rate of H<sub>2</sub>O<sub>2</sub> disintegration at absorbance levels of 240nm. The results were presented asU/mg or CAT-units/mg protein, where one unit is the level of enzyme that hydrolyzes 1 μmol of H<sub>2</sub>O<sub>2</sub> per minute and per milligram of protein at 30°C and pH 8.0.

**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 250C and pH 7.4.

### 2.7 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 byYagi (1998). In this method, malondialdehyde is evaluated spectrophotometrically at absorbance levels of 535nm to assay for the amount of lipid peroxidation in a sample.

### 2.8 Histopathology studies

Test specimen organs were extracted and processed for histopathological review. They were fixed in bouin’s fluid for 24 hours, rinsed with 70 percent ethanol and dehydrated through a graded series of ethanol (Schalm *et al*, 1975, Kelly, 1979). They were embedded in paraffin, sectioned at 4-5 um thickness stained with haematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989).

### 2.9 Statistical Analysis

Acute toxicity data involving quantal response (mortality) were analyzed using the probit analysis after Finney (1971).The enzyme 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

### 3.1 Acute Toxicity

Computed 96hours LC<sub>50</sub> for Paraquat exposed to fish was 128mg/L(Table 1).

	LC <sub>50</sub> (95% CL) % (mg/L)	SLOPE + S.E	d.f	PROBIT EQUATION
Paraquat	128.124 (172.827– 89.506)	4.940 ± 1.575	2	y = 3.75 * x + -7.5

**KEY:**            *cl* = Confidence limit  
                      *df* = Degree of freedom

### 3.2 Biochemical Analysis

#### 3.2.1 Superoxide dismutase (SOD)

The activity of the enzyme SOD in all exposed groups was inhibited ( $P > 0.05$ ) on day 14 (1.91 – 2.02  $\mu\text{mg}$ ) and induced ( $P > 0.05$ ) on day 28 (1.79 - 2.35  $\mu\text{mg}$ ) when compared to day 0 (2.13  $\mu\text{mg}$ ). There was no significant difference ( $P > 0.05$ ) in the activity of SOD on day 14 and day 28 when compared with each other (Figure 1).

#### 3.2.2 Catalase (CAT):

The activity of the enzyme CAT in exposed fish was induced ( $P > 0.05$ ) on day 14 (9.25-10.1  $\mu\text{mg}$ ) and day 28 (7.73-11.11  $\mu\text{mg}$ ) when compared to day 0 (2.4  $\mu\text{mg}$ ). The induction of the enzyme of CAT on day 28 was significant ( $P < 0.05$ ) when compared to day 0 and day 14 respectively (Figure 1).

#### 3.2.3 Reduced Glutathione transferase (GSH)

The enzyme activity of GSH was significantly inhibited ( $P < 0.05$ ) in exposed groups on day 14 (0.47- 0.53  $\mu\text{mg}$ ) and day 28 (0.34-0.58  $\mu\text{mg}$ ) when compared to day 0 (0.72 $\mu\text{mg}$ ). Furthermore, there was a significant difference ( $P < 0.05$ ) in the inhibition of GSH activity between sublethal concentrations on day 14 and 28 when compared to each other (Figure 1).

#### 3.2.4 Lipid Peroxidation (LPO) Assay

The activity of the enzyme MDA was significantly induced ( $P < 0.05$ ) in exposed fish on day 14 (0.083- 0.093 $\mu\text{mg}$ ) and day 28 (0.082-0.093 $\mu\text{mg}$ ) when compared to day 0 (0.034) (Figure 1).

#### 3.2.5 Glutathione-S-Transferase (GST):

The activity of the enzyme GST was inhibited in exposed fish on day 14 (0.57-0.61 $\mu\text{mg}$ ) and day 28 (0.54- 0.61 $\mu\text{mg}$ ) when compared to day 0 (0.64). GST activity on day 14 and day 28 in all exposed groups was not significant ( $P > 0.05$ ) when compared with each other and with day 0 (Figure 1).

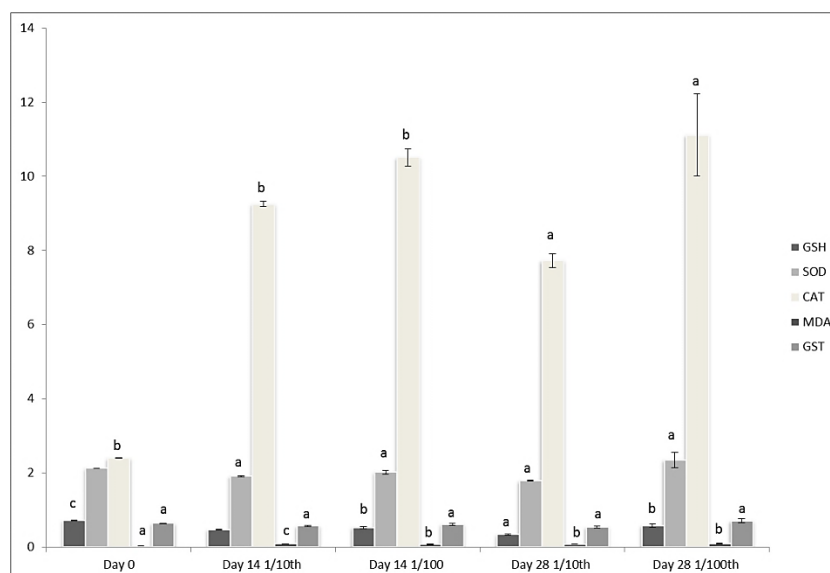


Figure 1 Biochemical parameters of *C. gariepinus* exposed to sublethal concentration of Paraquat.

### 3.3 Histology

Sections through the gill at day 0 revealed a pristine state of the 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) and chondrocyte cells (A) (Fig. 2). There was no observable gill defects such as blood congestion, hyperplasia of the gill epithelium, lesion, epithelial lifting of lamellae, lamellar fusion, lamellar disorganization and hypertrophy of the gill epithelium (Fig. 2). At day 14, the histologic section of the gill filament revealed a mild degree of lesion (A), disorganization of the whole length of the cartilaginous support of the primary lamellae (B), hypotrophy of the secondary lamellae (C), and incidence of lamellae fusion (D) (Figure 3). At day 28, exposed gills of fish to the test chemical showed a severe degree of lesion (E), lamellae disorganisation and blood congestion (F) (Figure 4).

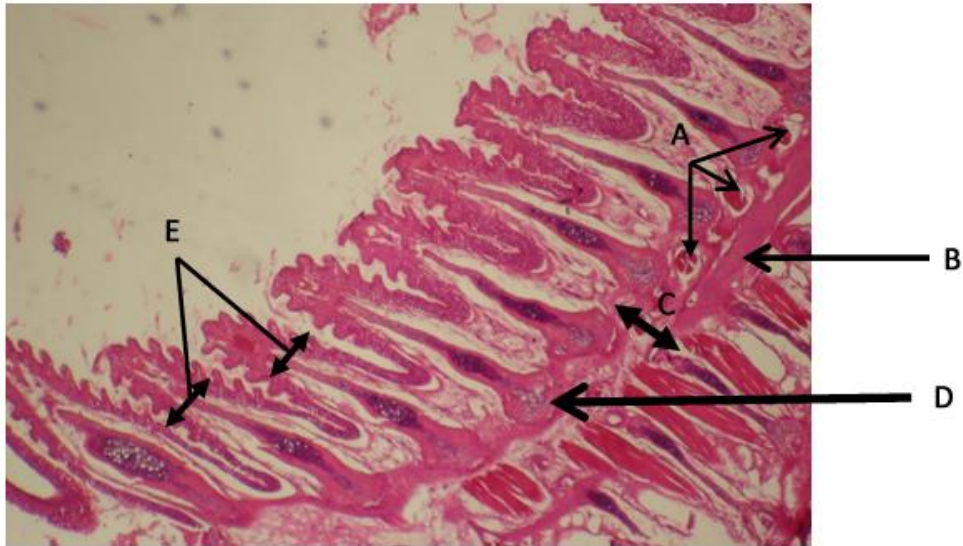


Figure 2: Showing a pristine state of the entire length of the extracellular cartilaginous support of the primary lamellae (B) , long primary lamellae (C) and long comb-like secondary lamellae (E) projecting from both sides of each primary lamella, epithelium lining on the membrane (D) and chondrocyte cells ( A)

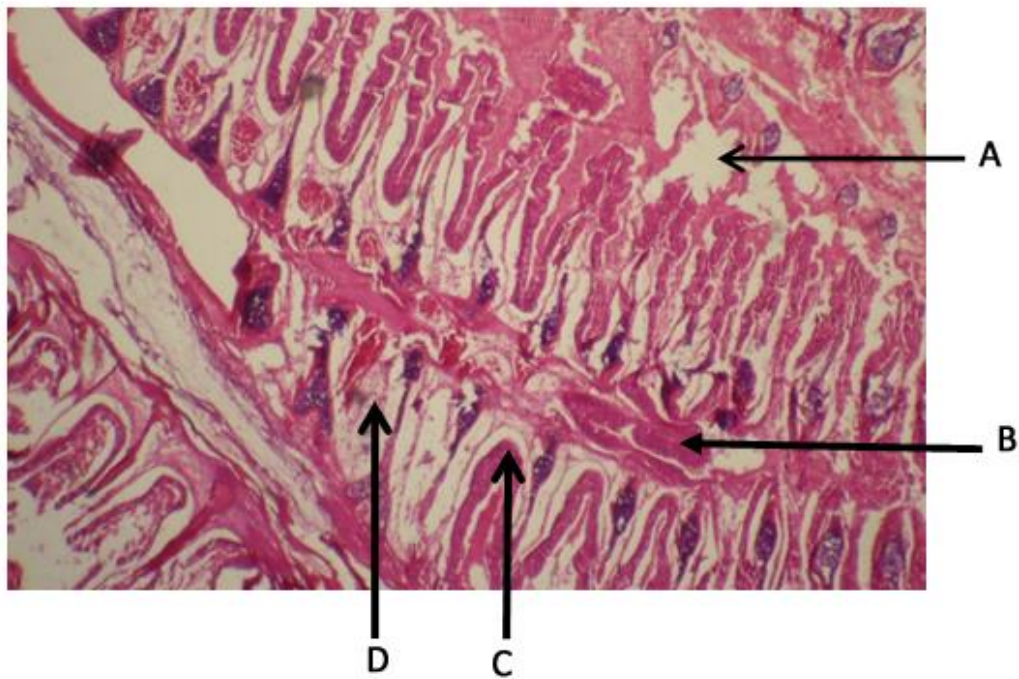


Figure 3: Day 14 exposed gills to toxicants revealing mild degree of lesion (A), disorganization of the whole length of the cartilaginous support of the primary lamellae (B) , hypotrophy of the secondary lamellae (C), and incidence of lamellae fusion (D).



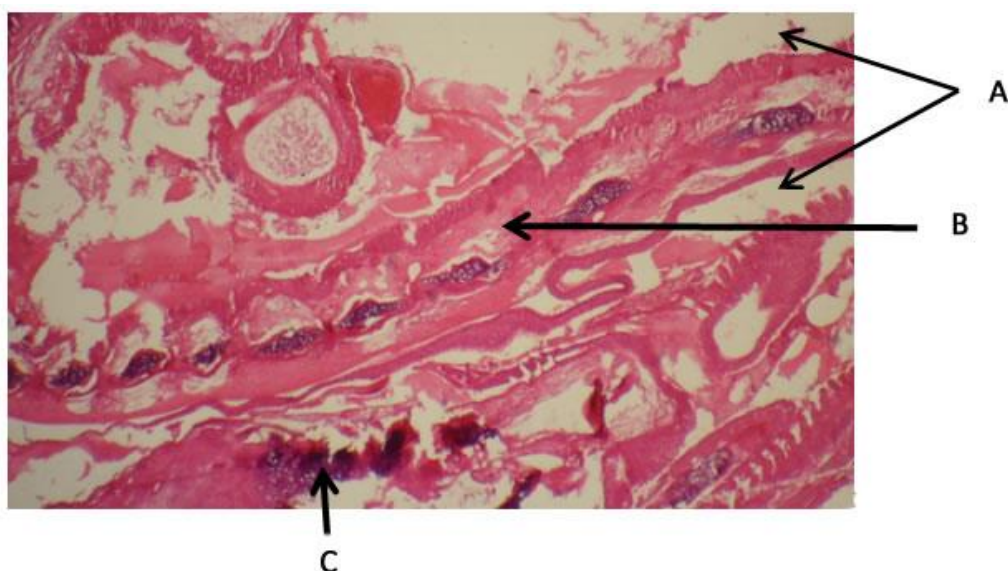


Figure 4. Histologic section of gill filament at day 28 with severe degree of lesion (E), lamellae disorganisation and blood congestion (F).

#### IV. Discussion

In ecotoxicological studies, the 96 hr LC<sub>50</sub> is one of the most essential parameters for evaluating the toxicity of pollutants. Herein, the 96 h LC<sub>50</sub> value (i.e. 128 mg/L) obtained from *C. gariepinus* exposed to Paraquat, suggests that this herbicide is moderately toxic to fish. This is in contrast to previous studies of Paraquat exposed to *C. gariepinus* (27.46mg/L) (Nwani *et al.*, 2015); *Mesopotamichthys sharpeyi* (1.48mg/L) (Safahieh *et al.*, 2012); *Trichogaster trichopterus* (1.41mg/L) (Banaee *et al.*, 2012); and *Oreochromis niloticus* 7.00mg/L) (Ada *et al.*, 2012). Pesticide toxicity on an organism is affected by age, sex, size, species strain, water quality, species strain, formulation of test chemical and temperature (Pandey *et al.*, 2005; Nwani *et al.*, 2013).

Antioxidant enzymes such as SOD, CAT, GSH, MDA and GST perform a crucial role in the adaption of organisms to environmental stress through the riddance of Reactive oxygen species (ROS) (Barata *et al.*, 2005; Zelikoff *et al.*, 1996). The first line of defense commences with the activity of the enzyme SOD which converts hydroxyl ion(O<sub>2</sub><sup>-</sup>) into oxygen(O<sub>2</sub>) and hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>) (Singh *et al.*, 2006. H<sub>2</sub>O<sub>2</sub> is further detoxified by other enzymes such as CAT. CAT is located in the peroxisomes, cytosol and mitochondria and is capable of scavenging H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> respectively (Zhang *et al.*, 2007a).

In the present study, the activity of SOD in fish was triggered with increasing exposure to Paraquat. This reaction is linked to the generation 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 Oruc & Usta (2007) who described an increase of SOD content, in the gill and muscle of *Cyprinus carpio* after diazinon exposure. This increase in the SOD activity indicates the increase in .O<sub>2</sub>- production (Zhang *et al.*, 2004a).

CAT is responsible for the breakdown of H<sub>2</sub>O<sub>2</sub> to water and oxygen, protecting the cell from further damaging action of H<sub>2</sub>O<sub>2</sub> and the hydroxyl radical. The significant induction (P < 0.05) in the activity of CAT after 28 days mirrors the high H<sub>2</sub>O<sub>2</sub> production from the activity of SOD and the increased oxygen consumption (Ritola *et al.*, 2002a). Similar findings have been reported by Atif *et al.*, (2005); Bouraoui *et al.*, (2008); Dabas *et al.*, (2012).

GSH is an endogenous antioxidant, which counters cellular components impairment byperoxides and ROS (Pompella ,2003).Despite it activity as an express free-radical hunter, GSH also acts as a substrate for GST. The significant decline in GSH level may be attributed to its deployment to arrest the invasive ROS generated from Paraquat oxidative trigger. Decline in the GSH level has already been observed in fresh water fish exposed to other organophosphate methyl parathion (Monteiro , 2006).

GST is a microsomal enzyme that neutralizes a vast range of endogenous metabolic by-products and xenobiotics via enzymatic glutathione conjugation, glutathione-dependent peroxidase activity or isomerisation reactions (Hayes *et al.*, 2005).Therefore, it also performs a valuable role in safeguarding tissues from oxidative stress (Fournier *et al.*, 1992; Jifa *et al.*, 2006). The enzyme GST has been reported to exhibit varying response to different compounds, for example, inhibition of the enzyme was reported in animals exposed to lead and zinc (Awoyemi *et al.*, 2014), dimethoate (Hamed *et al.*, 1999), Trichlorfon (Thomaz *et al.*, 2009). In contrast, statistically significant induction in GST was reported in organisms exposed to oxidative stress of 2,4-

dichlorophenol (Zhang *et al.*, 2004), Methylmercury (Neto *et al.*, 2008), Endosulfan (Kono & Fridovich, 1982) and Carbaryl (Ferrari *et al.* 2007). In this study, the activity of GST was reduced in the examined liver of fish tissues exposed to Paraquat when compared to control. This result aligns to the finding by Saliu and Bawa-Allah (2012), who reported a decrease in the level of GST-GSH activity in the liver of post-juvenile *C. gariepinus* exposed to Pb(NO<sub>3</sub>)<sub>2</sub> after 30 days. The decrease in GSH level and GST activity was caused by the ROS overproduction which depleted GSH and inactivated GST (Farombi *et al.*, 2008)

The significant increase in MDA is a pointer of intense oxidative stress (Buyukokuroglu *et al.*, 2002). This observation is in agreement with a study by Vadhbha and Hasan, (1986), who reported a significant increase in MDA content in *Heteropneustes fossilis* exposed to Dichlorvos. Similar elevation of MDA level was reported in *Cyprinus carpio* and *Ictalurus nebulosus* exposed to Dichlorvos (Hai *et al.*, 1997). Oruc, (2011) also reported similar findings in Diazinon exposure in the liver of *C. carpio*.

The gills of fish have a thin, expansive surface directly in contact with the aquatic medium, thus making the organ an entry point for numerous environmental stimulants (Haaparanta *et al.* 1997; Baskar 2014; Poleksic and Mitrovic - Tutundzic, 1994). Furthermore, the gills is the principal site for responding to unfavourable environmental conditions (Benli and Ozkul 2008). These peculiarities imply that the gill epithelium is highly exposed to environmental xenobiotics. In this study, the severity of lesions with increasing duration suggests a reflection of the direct reaction of the toxic compound on the tissue (Temmink *et al.*, 1983; Marina and Claudia, 2007). Lesions impacts on the gill morphology resulting in the disruption of basic physiological processes, such as antioxidant defence or osmoregulatory mechanisms (Yancheva *et al.*, 2015). Deformities such as hypertrophy of the epithelial cells, fusion of some secondary lamellae may indicate trigger of defense mechanisms, since, in general, these culminates into an increase of the space between the external medium and the blood. Thus, serving as a barrier to contaminant interactions (Mallatt, 1985; Hinton & Laurén, 1990; Poleksic & Mitrovic-Tutundzic, 1994; Fernandes & Mazon, 2003). The aftermath of the increased distance between water and blood due to the deformities is the impairment of oxygen uptake. Fishes are capable of countering this effect by increasing their ventilation rate, to compensate low oxygen uptake (Fernandes & Mazon, 2003). The gills alterations are nonspecific and can be triggered by different groups of toxicants (Mallatt, 1985). Similar gills deformities have also been reported in the fishes exposed to organic contaminants (Rosety-Rodríguez *et al.*, 2002; Fanta *et al.*, 2003) and metals (Oliveira Ribeiro *et al.*, 2000; Cerqueira & Fernandes, 2002; Martinez *et al.*, 2004).

These gills distortions occur very early in fish than physical and behavioral changes (Yancheva *et al.*, 2015). Thus, they are useful as effective biomarker for estimating the impact of extensive herbicide application on *Clarias gariepinus*.

## References

- [1]. Ada, F.B., Ekpenyong, E. and Ayotunde, E.O (2012). Haematological, biological and behavioural changes in *Oreochromis niloticus* (Linne 1757) juveniles exposed to Paraquat herbicide. *J Environ Chem Ecotoxicol*. 4: 64-74.
- [2]. Amir, T., Jafar, R., Vahid, N. and Najmeh, S. (2014). Effect of acute and chronic toxicity of paraquat on immune system and growth performance in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research*. 45: 1737-1743.
- [3]. Atamanalp, M., Keles, M.S. and Aras, H.I. (2001). The Effects of Cypermethrin (a Synthetic Pyrethroid) on some Biochemical exposed to organophosphorus insecticide Folisuper 600 (methylparathion). *Comp Biochem Physiol C Toxicol Pharmacol*. 143: 141-149.
- [4]. Atif, F., Parvez, S., Pandey S, Ali, M., Kaur, M. and Rehman, H. (2005). Modulatory effect of cadmium exposure on deltamethrin-induced oxidative stress in *Channapunctata Bloch*. *Arch Environ Contam Toxicol*. 49:371-7.
- [5]. Atli, G., Alptekin, O., Tukul, S. and Canli, M. (2006) Response of catalase activity to Ag<sup>+</sup>, Cd<sup>2+</sup>, Cr<sup>6+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> in five tissues of fresh water fish *Oreochromis niloticus*. *Comp Biochem Physiol* 143:218-224
- [6]. Babatunde, M.M. and Oladimeji, A., A. (2014). Effect of paraquat on weight and behavior of *Oreochromis niloticus*. *Environmental Research Journal*. 8(2):44-47.
- [7]. Banaee, M., Davoodi, M.H. and Zoheiri, F. (2013). Histopathological changes induced by Paraquat on some tissues of gourami fish (*Trichogaster trichopterus*). *Open Vet J*. 3: 36-42.
- [8]. Barata, C., Calbet, A., Saiz, E., Ortiz, L., Bayona, J.M., 2005. Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. *Environ. Toxicol. Chem*. 24, 2992-2999.
- [9]. Basha, P.M. and Sujitha, N.S (2012). Combined influence of intermittent exercise and temperature stress on the modulation of fluoride toxicity. *Biol Trace Elem Res*. 148:69-75.
- [10]. 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.
- [11]. Benli, A. C. K., & Ozkul, G. (2008). Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): effect on gill, liver and kidney histology. *Chemosphere*, 72(9), 1355-1358.
- [12]. Bouraoui, Z., Banni, M., Ghedira, J., Clerandau, H., Guerbej, H., Narbonne, J.F. and Bousseta, H. (2008). Acute effects of cadmium on liver phase I and II enzymes and metallothionein accumulation on *abream Sparus aurata*. *Fish Physiol. Biochem*. 34 (3) pp. 201-207.
- [13]. 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.
- [14]. Calderbank, A. (1975). Environmental effects of the herbicide, paraquat. Pages 136-139 in F. Coulston and F. Korte, eds. *Environmental quality and safety*, Vol. 4. Academic Press, New York.
- [15]. Cavalcante D.G.S.M., Martinez C.B.R. and Sofia S.H. (2008) Genotoxic effects of Roundup® on the fish (*Prochilodus lineatus*). *Mutation Research*. 655: 41-46.

- [16]. Cerqueira, C. C. C. & M. N. Fernandes. 2002. Gill tissue recovery after cooper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicology and Environmental Safety*.52: 83-91.
- [17]. Chia, L.S., McRae, D.G. and Thompson, J.E. (1982). Light-dependence of paraquat- initiated membrane deterioration in bean plants. Evidence for the involvement of superoxide. *Plant Physiology*. 56: 492-499.
- [18]. Christopher, D. N., Henry, I. E., Vincent, C. E., Christopher, C. O., Christian, O. C., Onas, S. P. and Alfreda, O. N.(2015). Physiological effects of paraquat in juvenile African catfish *Clarias gariepinus* (Burchell 1822). *Journal of Coastal Life Medicine*.doi: 10.12980/JCLM.3.2015JCLM-2014-0113
- [19]. Cohen, D., Dembiec, D. and Marcus, J.(1970). Measurement of catalase activity in tissue extracts. *Annals Biochemistry* 34, 30–38.
- [20]. Dabas, A., Nagpure, N., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W. (2012) Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, *Channapunctatus*. *Fish PhysiolBiochem*. 38:469–482.
- [21]. Dasta, J. F. (1978). Paraquat poisoning: a review. *Am. J. Hosp. Phar.* 35: 1368 - 1372.
- [22]. Dinis-Oliveira, R.J., Duarte, J.A., Sánchez-Navarro, A., Remião, F., Bastos, M.L. and Carvalho, F. (2008). Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Crit Rev Toxicol*.38: 13-71.
- [23]. Doherty V. F., Ladipo M. K., and Oyebadajo S. A. (2011). Acute Toxicity, Behavioural Changes and Histopathological Effect of Paraquat Dichloride on Tissues of Catfish (*Clarias Gariepinus*). *International Journal of Biology*. Vol. 3, No. 2.
- [24]. El-Sayed, Y.S., Saad, T.T. and El-Bahr, S.M. (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. *Environ ToxicolPharmacol*. 24: 212-217.
- [25]. Ensibi, C., Pérez-López, M., Soler, R. F., Míguez-Santiyán, M.P., Yahya, M.N., Hernández-Moreno, D. (2013). Effects of deltamethrin on biometric parameters and liver biomarkers in common carp (*Cyprinus carpio* L.). *Environ ToxicolPharmacol*.36: 384-391.
- [26]. Fang, J.K., Au, D.W., Wu, R.S., Chan, A.K., Mok, H.O., Shin, P.K. (2009). The use of physiological indices in rabbitfish *Siganus oramin* for monitoring of coastal pollution. *Mar Pollut Bull* 58: 1229-1235.
- [27]. Fanta, E., Rios, F. S., Romão, S., Vianna, A. C. C. and Freiberger, S. (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety*.54: 119-130.
- [28]. Faramobi, E. O., Adewole, O. A., and Ajimoko, Y. R. (2007). Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, 4(2): 158-165.
- [29]. Fernandes, M. N. & Mazon, A. F. (2003). Environmental pollution and fish gill morphology. In: Val, A. L. & B. G. Kapoor (Eds.). *Fish adaptations*. Enfield, Science Publishers, 203-231.
- [30]. Ferrari A, Venturino A, Pechén de D'Angelo A M (2007). Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. *Pest Biochem Physiol*. 88: 134–142.
- [31]. Filipak Neto F, Zanata SM, Silva de Assis HC, Nakao LS, Randi MAF, Oliveira Ribeiro CA (2008). Toxic effects of DDT and methylmercury on the hepatocytes from *Hoplias malabaricus* F. *Toxicol in Vitro*. 22: 1705–1713.
- [32]. Finney, D. J. (1971). *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge, 20.
- [33]. Fournier, D., Bride, J.M., Poirie, M., Berge, J.B. and Plapp, F.W. 1992. Insect glutathione S-transferases. Biochemical characteristics of the major forms of houseflies susceptible and resistant to insecticides. *J. Biol. Chem.*, 267, 1840–1845.
- [34]. Fryer, J.D. (1977). *Weed control Handbook* edited by Make Peace GESAMP, 1, 384–389.
- [35]. Gerald, W., Biing-Hwan, L. and Utpal, V. (1995). Restricting Pesticide Use: The Impact on Profitability by Farm Size. *J Agr. and Applied Econ*. 27 (2):352-362
- [36]. Gwathway, C.O. and Craig, Jr. C. (2007). Defoliants for cotton. In: Pimentel D, editor. *Encyclopedia of pest management. Boca Raton: CRC Press*.p. 135-137.
- [37]. 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.
- [38]. Habig, W.H. and Jakoby, W.B. (1981) Assays for differentiation of glutathione S-transferases. *Methods Med. Res.* 77, 398–405.
- [39]. Hai, D.Q., Varga, S.I. and Matkovic, B. (1997). Organophosphate effects of antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). *Comp Biochem Physiol*. 117: 83–88.
- [40]. Haley, T J. (1979). Review of the toxicology of paraquat (1, 1'-dimethyl-4, 4'-bipyridinium chloride). *Clin. Toxicol*. 14:1-46.
- [41]. Hamed, R.R., Elawa, S.E., Farid, N.M. and Ataya, F.S. (1999). Evaluation of detoxification enzyme levels in Egyptian catfish, *Clarias lazera*, exposed to dimethoate. *Bull Environ Contam Toxicol*. 63:789–796.
- [42]. Hayes, J.D., Flanagan, J.U. and Jowsey, I.R (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol*. 45:51–88.
- [43]. He, X., Wang, L., Szklarz, G., Bi, Y. and Ma, Q (2012). Resveratrol inhibits paraquat induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J Pharmacol Exp Ther*.342: 81-90.
- [44]. 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.
- [45]. Hinton, D. E. and Laurén, D. J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. Pp. 51-65. In: McCarthy, J.F. & L.R. Shugart (Eds.). *Biomarkers of Environmental Contamination. Boca Raton, Lewis Publishers*.
- [46]. Hughes, J. T (1988). Brain damage due to paraquat poisoning: a fatal case with neuropathological examination of the brain. *Neurotoxicology*.9:243 - 248.
- [47]. Jifa, W., Zhiming, Y., Xiuxian, S. and You, W. (2006). Response of integrated biomarkers of fish (*Lateolabrax japonicus*) exposed to benzo [a] pyrene and sodium dodecylbenzenesulfonate. *Ecotoxicol. Environ. Saf*. 65: 230-236.
- [48]. Kelly, W.R. (1979). *Veterinary clinical diagnosis* 2nd ed. Balliere Tindall, London. pp. 266-279.
- [49]. Keneko, J.J. (1989). *Clinical Biochemistry of domestic animals*. 4th ed. Diego, Academic press Inc. California, 132 pp.
- [50]. Kenneth, A.H. (1990). *The Biochemistry and Uses of Pesticides*, 2nd Edition, Wiley-Blackwell publishings, U.S.A, pp. 10-11.
- [51]. Kono Y, Fridovich I (1982). Superoxide radical inhibits catalase. *J Biol Chem*. 257: 5751– 5754.
- [52]. Li, Z.H., Zlabek, V., Velisek, J., Grabic, R., Machova, J. and Kolarova, J. (2011). Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): effects on antioxidant responses, hematological parameters and hepatic EROD. *Ecotoxicol Environ Saf*.74: 319-327.
- [53]. Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fish and Aquatic Sciences*. 42: 630-648.



- [54]. Marina M. P. C. and Cláudia B. R. M. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*, 5(3):327-336.
- [55]. Marin-Morales, M.A., Ventura-Camargo, B.C. and Hoshina, M.M. (2013) Toxicity of herbicides: impact on aquatic and soil biota and human health. InTech. doi: 10.5772/55851.
- [56]. Martinez, C. B. R., M. Y. Nagae, C. T. B. V. Zaia & D. A. M. Zaia. 2004. Morphological and physiological acute effects of lead in the neotropical fish *Prochilodus lineatus*. *Brazilian Journal of Biology*, 64 (4): 797-807.
- [57]. Mason, C.F. (1991). *Biology of Freshwater Pollution*. 2nd Edition, Longman Scientific and Technical U.K. 351pp.
- [58]. Monteiro, D.A., Almeida, J.A., Rantin, F.T., Kalinin, A.L. (2006). Oxidative stress biomarkers in the freshwater characid fish, *Bryconcephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). *Comp Biochem Physiol C Toxicol Pharmacol*. 143:141-9.
- [59]. Nwani, C.D., Ama, U.I, Okoh, F., Oji, U.O., Ogbonyeau, R.C. and Agha, A. (2013). Acute toxicity of the chloroacetanilide herbicide butachlor and its effect on the behavior of the freshwater fish *Tilapia zillii*. *Afr J Biotechnol*. 12: 499-503.
- [60]. 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.
- [61]. Oliveira Ribeiro, C. A., Pelletier, E., Pfeiffer, W. C. and Rouleau, W. C. (2000). Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. *Environmental Research*. 83: 286-292.
- [62]. Olushola, M. A., Kafilat, A. B. and Adebayo, A. O. (2014). Accumulation and Anti-oxidant Enzymes as Biomarkers of Heavy Metal Exposure in *Clarias gariepinus* and *Oreochromis niloticus*. *Applied Ecology and Environmental Sciences*. Vol. 2, No. 5, 114-122
- [63]. 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.
- [64]. Oruc, E.O. and Usta, D. (2007). Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ Toxicol Pharmacol*. 23: 48-55.
- [65]. Pandey, S, Kumar, R., Sharma, S., Nagpure, N.S., Srivastava, S.K. and Verma, M.S (2005). Acute toxicity bioassays of mercuric chloride and malathion on airbreathing fish *Channa punctatus* (Bloch). *Ecotoxicol Environ Saf*. 61: 114-120.
- [66]. Poleksic, V., & 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.
- [67]. Pompella, A., Visvikis, A., Paolicchi, A., De, T.V. and Casini, A.F (2003). The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol*. 66:1499-1503. 10.1016/S0006-2952(03)00504-5.
- [68]. 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.
- [69]. Richardson, M. L. (1988). Risk assessment of chemicals in Environment UK, RSC publication.
- [70]. Ritola, O., Livingstone, D.R., Peters, L.D. and Lindstrom, S.P (2002a). Antioxidant processes are affected in juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to ozone and oxygen-supersaturated water. *Aquaculture*. 210: 1-19.
- [71]. Rosety-Rodríguez, M., Ordoñez, F. J., Rosety, M., Rosety, J. M., Ribelles, A. and Carrasco, C. (2002). Morpho-histochemical changes in the gills of turbot, *Scophthalmus maximus* L., induced by sodium dodecyl sulfate. *Ecotoxicology and Environmental Safety*, 51: 223-228.
- [72]. Safahieh, A., Jaddi, Y., Yavari, V. and Zadeh, R.S. (2012) Sub-lethal effects of herbicide paraquat on hematological parameters of benny fish *Mesopotamichthys sharpeyi*. In: 2nd International Conference on Biotechnology and Environment Management; 2012; Singapore: IACSIT Press; p.141-145.
- [73]. Saliu, J. K. and Bawa-Allah K. A. (2012). Toxicological Effects of Lead and Zinc on the Antioxidant Enzyme Activities of Post Juvenile *Clarias gariepinus*. *Resources and Environment*, 2(1): 21-26.
- [74]. Schalm, O. W., Jain, N.C and Carrol, E.J. (1975). *Veterinary Haematology*, 3rd Edn., Lea and Febiger, Philadelphia, pp. 197-199.
- [75]. Seiyaboh, E.I., Inyang, I.R., Gijo, A.H. and Adobeni, G.D. (2013). Acute Toxicity of Paraquat Dichloride on Blood Plasma Indices of *Clarias gariepinus*. *Journal of Environmental Science, Toxicology And Food Technology*. Vol: 7 (6): PP 15-17.
- [76]. Singh, S., Eapen, S. and D'Souza, S.F. (2006). Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopamonnieri* L. *Chemosphere* 62:233-246.
- [77]. Summers, L.A. (1980). *The bipyridinium herbicides*. London: Academic Press.
- [78]. Suntres, Z.E. (2002). Role of antioxidants in paraquat toxicity. *Toxicology*. 180: 65-77.
- [79]. Suvetha, L., Ramesh, M. and Saravanan, M. (2010). Influence of cypermethrin toxicity on ionic regulation and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity of a freshwater teleost fish *Cyprinus carpio*. *Environ Toxicol Pharmacol*. 29: 44-49.
- [80]. Temmink, J., Bowmeister, P., Jong, P. and Van der Berg, J. (1983). An ultrastructural study of chromate-induced hyperplasia in the gill of rainbow trout, *Salmo gairdneri*. *Aquatic Toxicology*. 4: 165-179.
- [81]. Thomaz, J.M., Martins, N.D., Monteiro, D.A., Rantin, F.T. and Kalinin, A.L (2009). Cardio-respiratory function and oxidative stress biomarkers in Nile tilapia exposed to the organophosphate insecticide trichlorfon (NEGUVON®). *Ecotox Environ Saf*. 72: 1413-1424
- [82]. United State Environmental Protection Agency (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.
- [83]. 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.
- [84]. Velkova-Jordanoska, L. and Kostoski, G. (2005). Histopathological analysis of liver in fresh (*Barbus meridionalis petenyi* Heckel) in reservoir *Trebenista*. *Natura Croatica*. 14: 147-153.
- [85]. Wang, Y., Zhu, N., Fu, J., Gao, Y., Ssebugere, P. and Jiang, G. (2013). Hexabromocyclododecane in alpine fish from the Tibetan Plateau, China. *Environmental Pollution*. 181: 7-13
- [86]. 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.
- [87]. Yagi, K. (1998). Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. *Methods in Molecular Biology*, 108, 107-110.
- [88]. Yancheva, V., Velcheva, I., Stoyanova, S. and Georgieva, E. (2015). Fish in ecotoxicological studies. *Ecologica Balkanica*. 7(1), 149-169.
- [89]. Zelikoff, J.T., Wang, W., Islam, N., Twerdok, L.E., Curry, M., Beaman, J. and Flescher, E. (1996) Assays of reactive oxygen intermediates and antioxidant enzymes: potential biomarkers for predicting effects of environmental pollution. In: Ostrand GK (ed) *Techniques in aquatic toxicology*. Lewis Publishers, Boca Raton, pp 287-306.

- [90]. Zhang, F.Q., Wang, Y.S., Lou, Z.P. and Dong, J.D (2007a) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandeliacandel* and *Bruguieragymnorrhiza*). *Chemosphere*. 67:44–50.
- [91]. 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.
- [92]. Zhang, Q., Lusheng, Z., Jun, W., Hui, X., Jinhua , W., Yingnan, H. and Jinhui, Y. (2013). Oxidative stress and lipid peroxidation in the earthworm *Eisenia fetida* induced by low doses of fomesafen. *Environ Sci Pollut Res* . 20:201–208.

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