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In conclusion, this current study results revealed that glyphosate is very toxic leading not only to abnormal behavioral responses and tissue alterations, but might cause mass extinction of fish species. Therefore, glyphosate should be used carefully in/or near aquatic systems to avoid extinctions of life forms, particularly *Aphanius dispar*. Thence, protecting species diversity, which is a key issue for stability and resiliency of aquatic ecosystems.

Keywords: glyphosate, fish toxicity, aphanius dispar, behavioral changes, histology, acute toxicity, environmental health.

I. Introduction

Glyphosate herbicide (Tiller 480 SL), which is a broad spectrum non-selective herbicide is used excessively to control and inhibit a great variety of annual, biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs in agricultural, industrial, urban, forestry, aquatic ecosystems, fish ponds, lakes, and canals (Cavas and Konen, 2007; Langiano and Martinez, 2008; Sani and Idris, 2016; Tsui and Chu, 2008). Glyphosate has been reported to be the most important herbicide ever developed (WHO, 1994) to particularly be applied in plant varieties that are genetically modified to be better able to tolerate glyphosate treatment during weed control (Langiano and Martinez, 2008), without affecting crops (Sani and Idris, 2016). Glyphosate is not only used directly to control noxious weeds in aquatic systems, but also reported to reach aquatic systems after application in agricultural fields, thus affect non-targeted organisms indirectly like invertebrate, fish and other life forms from the first level up higher the food chains (Jofre et al., 2013), thence reduce species diversity, community structure affecting the stability and resilience of aquatic ecosystems (Perez et al., 2011).

Glyphosate mode of action is through competitive inhibition of phosphoenolpyruvate (PEP) on the active site of 5-enolpyruvylshikimate-3-phosphatethensate, an enzyme involved in the biosynthesis of aromatic amino acids (phenylalanine, Tyrosine, and tryptophan), which are essential for protein synthesis (Mallory-Smith, 2013, Tu et al., 2001). Glyphosate and its formulations, especially those containing surfactants are considered hazardous to the aquatic environment, which showed higher toxicity to most aquatic organisms than the active ingredient itself, which has been classified into very slight to high toxicants to aquatic organisms including fish species (WHO, 1994; Perez et al., 2011) due to its higher solubility varying from 10,000 to 15,000 mg/l at 25oC (Nwani et al., 2013). Glyphosate and its formulations acute and chronic effects on aquatic organisms including fish species have been reported to involve behavioral, histopathological, biochemical, and physiological changes (Langiano and Martinez, 2016; Jiraungkoorskul et al., 2002; Thanomsit et al., 2016), reflecting slight to severe concentration-related alterations over a short period of time (Perez et al., 2011). Several studies on fish species have reported variations for the acute toxicity of glyphosate concentrations of which: 10 mg/l for Asian sea bass, *Lates Calcarifer*, 13.69 mg/l for the Neotropical fish, *Prochilodus lineatus* (Langiano and Martinez, 2008), and 8.3 mg/l for *Oncorhynchus mykiss*, (Waynon, 1980), 16.8 mg/l for nile tilapia, *Oreochromis niloticus* (Jiraungkoorskul et al., 2002), 97.47 mg/l for catchama blanca, *Piaractus brachypomus* (Ramirez-Duarte et al.,

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2008), and 211.80 mg/L for *Tilapia zilli* (Nwani et al., 2013). *Aphanius dispar* juveniles has been reported to respond differently to different pesticides upon 24h acute exposure to different concentrations of synthetic pyrethroid pesticides, fenpropathrin and fenvalerate (Shoaib et al., 2013) expressing differently low LC50 values, indicating high sensitivity of fish juveniles to different pesticides as many environmental factors influence the bioassay results (Shoaib et al., 2012).

Fish behavior has been indicated to be the most sensitive indicators upon exposure to environmental stressors particularly in fish species (Banace et al., 2011; Zarei et al., 2013). Fish were observed in previous studies to express various abnormal behavioral changes such as hyperactivity, loss of schooling, overcrowding, hypoactivity, breathing difficulties, jumping out of water, surfacing, jerky swimming, rapid opercula movements, loss of righting response convulsion, loss of balance, after exposure to a number of environmental toxicants, thus their living standards is affected overwhelmingly before extinctions occur (Banace et al., 2011; Ba-Omar and Al-Jardani, 2011; Kumar et al., 2015; Zarei et al., 2013; Sani and Idris, 2016; Nwani et al., 2013). *Aphanius dispar* exposed to temphos, which is an organophosaphate pesticide expressed common behavioral changes such as restlessness, erratic swimming, convulsion, and loss of balance (Ba-Omar and Al-Jardani, 2011). Furthermore, the reported effects of glyphosate exposure in inducing histopathological changes of gills and liver tissues of fish included epithelial uplifting, interlamellar hyperplasia, hypertrophy of epithelial cells, shortening and folding of lamellae, necrosis of lamellar epithelium, lamellar fusion, aneurism, hyperplasia of chloride cells and mucus cells in the interlamellar spaces, clubbing, edema, and degeneration of filaments of the gills (Akinsorotan and Olele, 2013; ayoola, 2008; Deivasigamani, 2015; Jiraungkoorskul et al., 2002). while, they observed histopathological changes of the liver to include cytoplasmic and nuclear degeneration, hyperplasia, vacuolization of the cytoplasm, mild to severe infilteration of leukocytes, pyknotic nuclei, hypertrophy of hepatocytes, necrosis and bile stagnation. Ba-Omar and Al-Jardani, (2011) found lamellar damages including degradation of chloride cells, desquamation, epithelial uplifting, sloughing of epithelial cells, hypertrophy of lamellae, fusion of secondary lamellae, curling, tearing and collapsing of the lamellae after exposure of *Aphanius dispar* to the organophate temphos.

Aphanius dispar (Ruppell 1829), which also is known by the common name Arabian killifish has a wide distribution throughout Africa, Asia, and coast line of the red sea including Saudi Arabia. However, Arabian killifish population represents a single species, but with many color variations and patterns depending on locality. In Saudi Arabia, *Aphanius dispar* can breed all year round with vivid coloration of the males that attract females and can tolerate in their habitats a wide range of temperature, salinity, and other factors making *Aphanius dispar* able to tolerate stressors, which might jeopardize their existence due to reduced food availability, habitat degradation, exotic species, chemical contamination, and exploitation.

Since *Aphanius dispar* existence is threatened not only by their habitat degradation and food availability, but also by various environmental stressors (Saeed et al., 2015), the focus of this current study was to determine the acute toxicity of the commercially formulated glyphosate and its effects on behavior and histology of the gills and liver tissues in light of the excessive use of glyphosate in agriculture.

II. Materials and Methods

a) Chemicals

A commercial formulation of glyphosate (480 g/l glyphoste-isopropylamine salt) with trade name (Tiller 480 SL) manufactured by Astra Chem., Tabuk, KSA, was used in this current study.

b) Experimental Fish

Male and female juvenile Arabian killifish, *Aphanius dispar* were collected from southwestern Saudi Arabia, specifically from sadder Weila valley, through netting using a hand net. The juveniles were transported in a clean-aerated freshwater to the laboratory with care to lessen stress. *Aphanius dispar* juveniles mean weight was 1.5 ± 0.3 g and 4.5 ± 0.5 cm of length were allocated to aquaria randomly and left to be acclimatized under laboratory conditions for two weeks before running the static bioassay. Fish were fed commercial diet (flaked- food) once daily. The average values of water quality were (temperature 22 ± 1.0˚C, pH 7.2 ± 0.1, dissolved oxygen 7.03± .02 mg/L, and total hardness 220 ± 2 mg/l). The light and dark cycle of 12 h: 12 h was maintained throughout the whole study duration.

c) Acute Toxicity Test

The bioassay test was conducted according to the US EPA guidelines 712-C-6-118 (1996) to determine the 96-h LC50 values of commercial formulation of glyphosate (Tiller 480 SL). Fish were starved for 24 hours prior to and during the bioassay. The test was conducted in plastic aquaria (30 + 30 + 15 cm) containing 8 L of static water (10 fish per aquarium). Seven different concentrations of glyphosate (60, 90, 120, 150, 180, 210 and 240 mg/L) with two replicates plus the control were used for running the acute toxicity test. Fish mortality in each aquarium was recorded and dead fish were removed immediately throughout the bioassay duration. The LC50 value of the fish was determined using the Probit analysis method (Finney,1971), and their behavior was monitored daily

for any abnormal behavioral changes throughout the bioassay.

d) Sub-Acute Toxicity Test

In order to investigate the histopathological effect of glyphosate on the gill and liver tissues, fish were exposed to 1/4 th of the 96-h LC50 glyphosate dose for 14 days. At the end of the exposure, fish were sacrificed and the gill and liver tissues of the control and treated fish were immediately excised and fixed in Bouin's solution for 48 h at room temperature. After fixation, the tissues were washed with tap water, dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin wax. Sections of 6 µm were cut using a microtome (American Optical Co., USA), and stained with hematoxylin and eosin (Bancroft J and Steven A 1996). The stained sections were then examined for histopathological changes and photographed using Olympus light microscope (Olympus, Tokyo, Japan) equipped with a digital camera.

e) Statistical Analysis

The 96-h LC50 value of the Aphanius dispar was calculated using the Probit analysis method (Finney, 1971). One way ANOVA was performed using SPSS software to detect significant differences among groups. *P* value < 0.05 was considered statistically significant.

III. Results

a) Acute Toxicity Test

The 96-h LC50 value (Fig.1) of glyphosate upon exposure of *Aphanius dispar* to different glyphosate concentrations (60, 120, 150, 200, 180, 210 and 240 mg/L, was determined to be 115.25 mg/L using the probit analysis method.

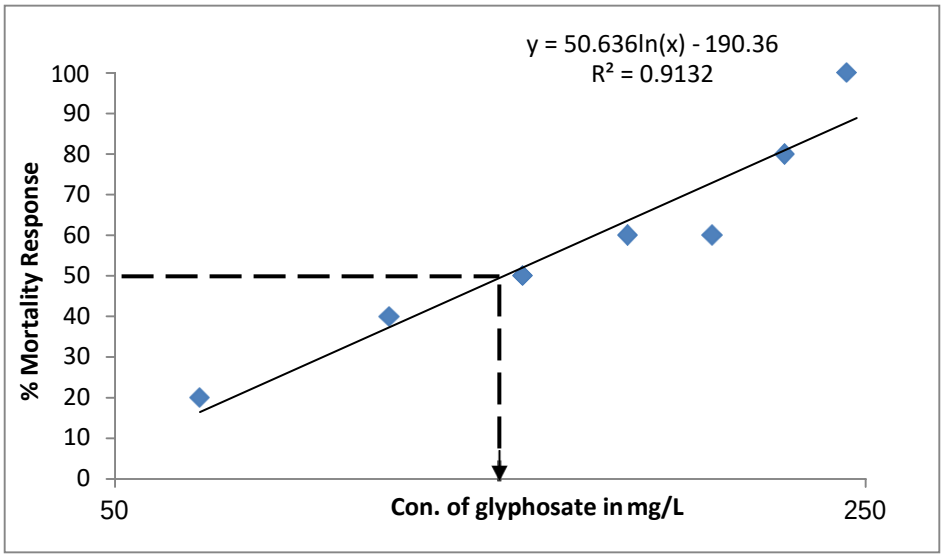

Fig. 1: The relationship between glyphosate concentrations and mortality percentages

b) Fish behavior

Unexposed fish group to glyphosate did not exhibit any adverse abnormal behavioral responses or any mortality throughout the duration of the bioassay as compared to the treated fish. However, exposed fish to glyphosate exhibited various abnormal behavioral responses, which were concentration-related as shown in Table1.

Table 1: Behavioral responses of the Arabian killifish, Aphanius dispar after acute exposure to different concentrations (mg/L) of glyphosate.

Concentration mg/L	Behavioral responses of Aphanius dispar
0	Normal activity to mild hyperactivity on the first day, Normal feeding behavior
60-89	Mild hyperactivity associated with loss of schooling into schools Hyperactive
90-119	surfacing to the tank top as an avoidance response associated with rapid opercula movements
120-149	Erratic movements associated with opercula movements and rapid mouth movement rate gulping for air
150-179	Schooling on and off, frequent surfacing and jumping outside the aquaria, cannibalism, loss of balance associated with hanging vertically in the water column head-up tail-down, swirling with rapid speed

180-209	Exhaustion associated with hypoactivity, settling on the bottom of the tanks with less opercula and mouth movements
210-240	Exhaustion associated with hypoactivity and mortality

c) Histopathological Study

Mild focal changes of the Arabian killifish, *Aphanius dispar* gill lamellae were observed in the control group (Fig. 2-a), while there were no observed histopathological changes in the liver tissues. However, exposed fish to the herbicide glyphosate (1/4 96 hLC50) concentration exhibited a wide range of mild to excessive histopathological alterations of the gills (Fig. 2, b-e) and Liver (Fig. 3, b-d) (Fig. 3-a).).

The gills of exposed fish exhibited histopathological changes of which epithelial uplifting, edema, epithelial hyperplasia, fusion of lamellae, clubbing of the tips of secondary lamellae, and thickening of lamellar epithelium (Figure 2, b-e). While, the liver organ exhibited histopathological changes of significance including deterioration and necrosis of the liver hepatocytes, hypertrophy of hepatocytes, eccentric nuclei and pyknosis, as well as mild to extensive vacuolization of hepatocytes with a foamy appearance (Figure 3, b-d).

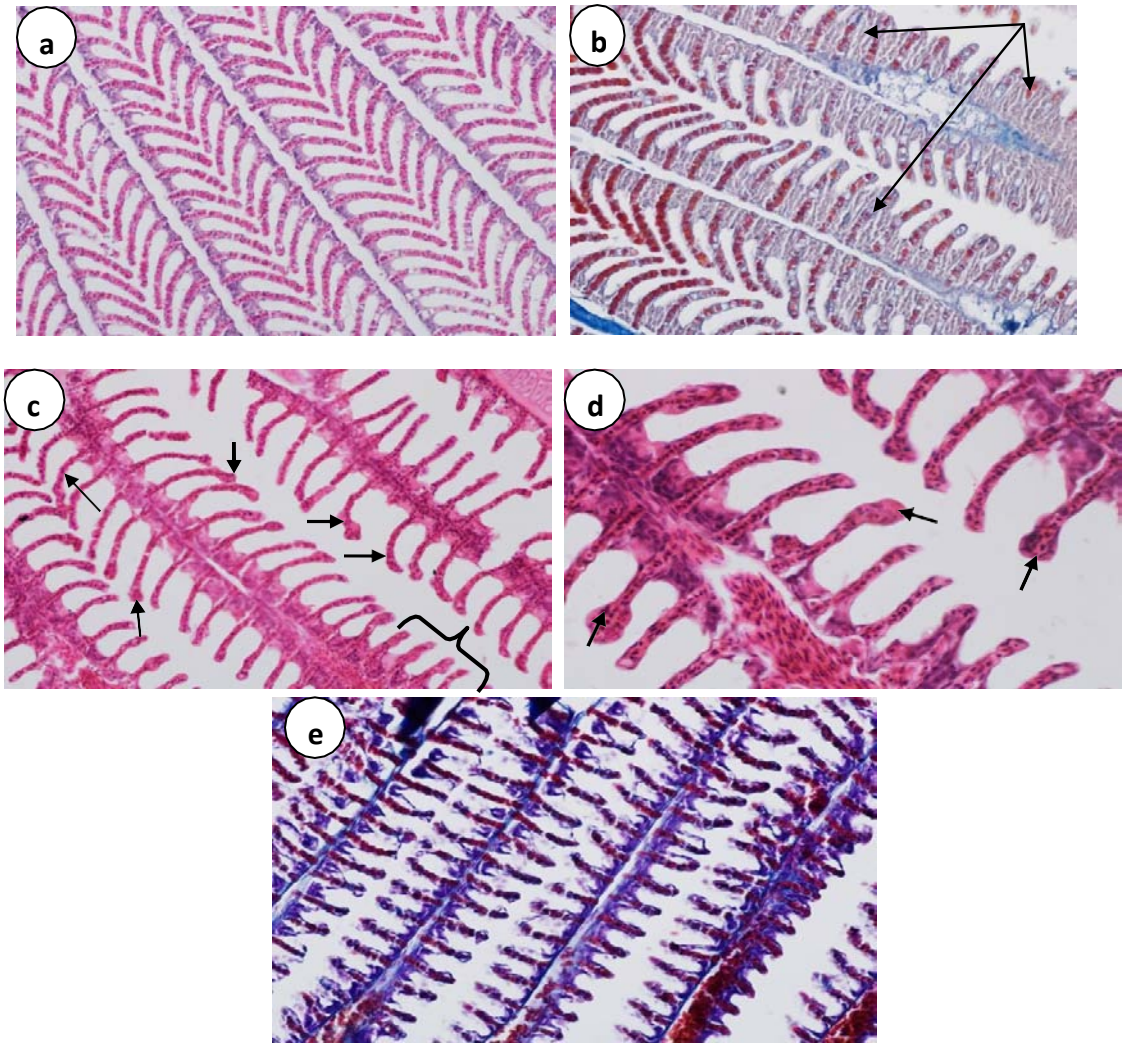

Fig. 2 (a-e): Histopathological changes of *Aphanius dispar* gills: Control group (a) and glyphosate treated (b-e). a: normal gill filaments and secondary lamellae. b: interlamellar hyperplasia of the filaments leading to fusion of secondary lamellae (arrows), c: shortening of secondary lamellae (bracket), elongation associated with curling, clubbing and elongation of secondary lamellae ((small arrows), d: clubbing of the secondary lamellae (arrows). e: gills showing generalized excessive epithelial uplifting from the basement membrane. (H & E) X 20.

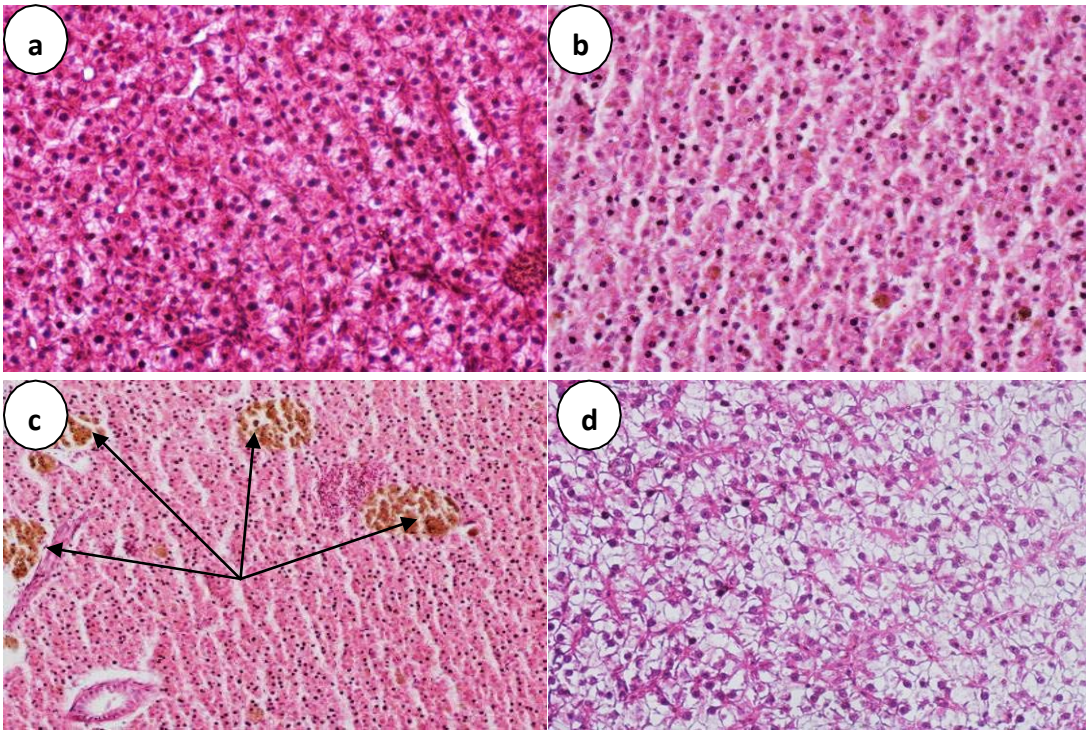

Fig. 3: Histopathological changes of Aphanius dispar liver: Control group (a) and glyphosate treated (b-d). a: normal liver of the control group with normal polygonal hepatocytes associated with very mild deterioration. b: deterioration of the hepatocytes and pyknosis of nucleolus. c: extensive deterioration and excessive bile stagnation (arrows). d: excessive cytoplasmic vacuolization with a foamy appearance . (H & E) X 20.

IV. Discussion

a) Acute Toxicity

Mortality and extinction of many life forms is imposed by myriads of chemical pollutants including herbicides. The impacts of chemical pollutants reflect their concentrations, duration of exposure, environmental factors, and sensitivity of life forms. Thus determination of the lethal dose (LC50), the dose that might kill half of any population within a short period of time, is considered the first step prior to any physiological studies as with regard to chemical pollutants. In this current study, glyphosate formulation (Tiller 480 SL) 96 h LC50 of the Arabian killifish, *Aphanius dispar* juveniles valued at 115.25 mg/l (Figure. 1). The 96h LC50 values of glyphosate have been investigated on different fish species at different environmental conditions, indicating variation in concentrations (Neskovic et al., 1996; Jiraungkoorskul et al., 2002). Previously very limited studies have stated that *Aphanius dispar* juveniles responded differently to different pesticides (Shoaib et al., 2013), indicating variability in sensitivity to different pesticides being influenced by various environmental factors (Shoaib et al., 2012; Shoaib et al., 2013). Futhermore, Nwani et al. (2013) reported the 96 h LC50 for *Tilapia Zilli* at 211.80 mg/l upon exposure to glyphosate formulation (Forceup). The reported LC 50 values which are close to the results of this current study upon exposure to glyphosate for 96 h were found to be 97.47 mg/L for cachama blanca, *Piaractus brachypomus* (Ramirez-Duarte et al., 2008) and 86 mgl for the common carp, *Cyprinus carpio* (Deivasigamani, 2015). Additionally, lower 96 h LC50 values were recorded: 43.65 mg/L for the African catfish, *Clarias gariepinus* (Akinsorotan, 2013),13.69 mg/l for Neotropis fish, *Pochilodn lineatus* (Landgiano and Martinez, 2008), 10.0 mg/l for the Asian Bass, *Lates calcarifer* (Thunomsit et al. 2016), 1.05 mg/l for nile tilapia, *Oreochromis niloticus* (Ayoola 2008) and 0.05 mg/l for the African catfish, Clarias gariepinus (Ayanda et al., 2015).

Therefore, from the determined 96 h LC50 values (115.25 mg/l), we do believe that *Aphanius dispar* juveniles are very sensitive to glyphosate and its toxicity might be exacerbated further upon exposure under harsher environmental factors. Thus, as we compare this current study 96 h LC50 value to the previously reported studies, 96 h LC50 higher and lower values on different fish species and different environmental conditions, we believe that the determined 96 h LC50 value in our study on might be influenced by the ambient environmental conditions for this species.

b) Fish behavior

Behavioral responses have been indicated to be the most sensitive indicators upon exposure to potential toxic effects in fish species (Banace et al., 2011; Ba-Omar and Al-Jardani 2011). Our results

showed that the unexposed fish group did not reveal any adverse abnormal behavioral responses or mortality throughout the duration of the bioassay as compared to the treated fish groups other than mild hyperactivity at the onset of the experimental execution, which might be attributed to fish handling during allocation. On the contrary, exposed fish to glyphosate exhibited various abnormal behavioral responses (Table1) and were concentration-related similar to the previously reported observations after exposure of different fish species to the herbicide glyphosate (Akinsorotan et al., 2013; Ayoola 2008; Okayi et al., 2010). Abnormal behavioral changes such as mild to moderate erratic swimming, rapid rate of opercular and mouth movements, infrequent surfacing were observed at low to moderate concentrations, while at higher concentrations Aphanius dispar exhibited rapid swimming associated with frequent surfacing and jumping outside of the aquaria, hanging head-up tail-down position, and hypoactivity before the fish became weak, hypoactive, and settled at the bottom followed by exhaustion and death. These observations were consistent with the previously reported abnormal behavioral changes after exposure of various fish species to the herbicide glyphosate: Clarias gariepinus adult (Akinsorotan et al., 2013), Clarias gariepinus fingerlings (Okayi et al., 2010), juvenile African catfish, Clarias gariepinsus (Ayoola, 2008), the common carp, Cyprinus carpio (Deivasigamani, 2015), and Asian sea bass, Lates calcarifer (Thanomsit et al., 2016). Similarly, Apanius dispar juveniles exposed to the organophosphate temphos expressed abnormal behavioral changes (Ba-Omar and Al-Jardani 2011). These previously mentioned authors reported such abnormal behavioral changes and mortality to occur after acute and chronic toxicity indicating respiratory failure inflected by the effects of the glyphosate on the gills. Thus, fish mortalities observed in this study could be due to the destruction of gill tissues and impairment of gas-exchange capacity after fish became very lethargic and exhausted. Furthermore, fish respiratory failure might be an indication of physiological distress on juveniles resulting from potential progressive energy expenditure with time preceding mortality of fish.

Furthermore, according to Kumar et al. (2015), Zarei et al. (2013), Nwani et al., (2013), and Okayi et al., (2010) mucus secretion observed in this current study at the water surface at higher concentrations might suggest excessive impacts of glyphosate on fish gills forming a mucus film on the gills interrupting gaseous exchange and causing death of fish following exhaustion and lethargic responses. While, Pandey et al., (1990) attributed the secretion of mucus to dysfunction of the endocrine gland under toxic stress thus changes in the number and area of mucus glands and chromatophores. On the other hand, Sani and Idris, (2016) the previously reported behavioral changes have been indicated to occur as a result of not only metabolic dysfunction but also due to nervous disorder upon exposure to toxic glyphosate. Thus we do believe that fish exhibiting such abnormal behavioral changes and mucus secretion in Aphanius dispar in this current study might have been due to the toxic effects of glyphosate on gill tissues and respiration impairment.

c) Histopathology

Aphanius dispar upon exposure to glyphosate revealed gill and liver tissue alterations which were concentration-and-time related. Literatures on the impacts of noxious chemicals on fish histopathology of Aphanius dispar gills, liver, kidney and all other levels of biological organizations are scarce and very current. For example, the acute and chronic impacts of 3,4-dichloroaniline (DCA), sodium dodecyl sulfate, and zinc sulfate and chlorine on Aphanius dispar embryos development were studied (Saeed et al., 2015). While, the effects of the organophosphate temphos on the gills of Aphanius dispar revealed various concentration-related gill damages including hemorrhage of lamellae, epithelial uplifting, epithelial hypertrophay, swelling at the base and tips of lamellae, fusion of lamellae, tears in the filaments, sloughing of epithelial cells from the filaments and lamellae (Ba-Omar and Al-Jardani, 2011).

The gill organs of fish, which are very complex structure essential for gaseous exchange, acid-base balance, excretion, and osmoregulatory function are in contact with the outside environment. There was no recognizable alterations in the gills of the control fish. However fish exposed to 1/4th 96 h LC50 glyphosate exhibited various tissue alterations including fusion of secondary lamellae, epithelial uplifting, minor clubbing of the secondary lamella tips, hyperplasia of the primary filaments and secondary lamellae, and curling of the secondary lamellae. Similarly, previous studies have reported wide spectrum of gill histopathological changes after exposure of fish species to variety of noxious agents including glyphosate. Fish exposure to glyphosate for 96 h of Clarius gariepinus (Akinsorotan et al., 2013), Juveniles African catfish, Clarias gariepinus (Ayoola, 2008), common carp, Cyprinus carpio (Deivasigaman, 2015), Cyprinus carpio (Neskovic et al., 1996), Nile tilapia, Oreochromis niloticus (Jiraungkoorskul et al., 2002) and Aphanius dispar to temphos (Ba-Omar and Al-Jardani (2011) caused wide spectrum of gill histopathological changes of which epithelial hyperplasia, edema, lifting of epithelium, epithelial hyperplasia thickening of primary lamellar epithelium, clubbing, fusion of lamellae and secretion of mucus etc. The pronounced variety of insults of the gill organs which have been observed in this current study followed by exhaustion, lethargy and death of fish are clear indication of the impairment of gaseous exchange and reduced functional efficiency of the gills before suffocation of fish occurred. Therefore, the histopathological changes of fish gills can impair the

respiratory function by reducing the total surface area available for oxygen uptake and increase the diffusion distance between the external environment and the blood inside the lamellae preventing gaseous exchanges, which cause suffocation of fish and death (Ayoola 2008; Ba-Omar and Al-Jardani, 2011; Jiraungkoorskul et al., 2002;).

On the other hand, liver, which is an organ performing various functions associated with the metabolism of xenobiotics (Langiano et al., 2008) exhibited various histopathological alterations after exposure to glyphosate used in this current study including vacuolization of the cells cytoplasm, hypertrophy of hepatocytes, degeneration of hepatocytes, and bile stagnation. Similar findings on the impacts of glyphosate on fish species at varios concentrations were reported (Langiano et la., 2008; Ayola 2008; Akinsorotan et al., 2013; Jiraungkoorskul et al., 2002; Deivasigamani 2015; Neskovic et al., 1996). It is believed that the vacuolization of liver cells might indicate evidence of fatty degeneration (Deivasigamani 2015; Jiraungkoorskul et al., 2002; Ayoola 2008). The localized necrosis of hepatocytes suggest excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification as well as failure of the liver cells to regenerate due to continuous exposure to noxious agents (Deivasigamani 2015; Ayoola 2008). Bile stagnation was observed in the control and treated Neotropical fish Prochilodus lineatus as manifestation of a physiopatholoical conditions caused by a lack of bile metabolism and secretion (Langiano et la., 2008; Ayoola 2008; Deivasigamani 2015), whereas we found that bile secretion by hepatocytes observed within the cells as yellowish droplets in the exposed fish, however, bile droplets were very intense and the degree of intensity was observed in the treated fish with higher glyphosate concentrations, which might indicate glyphosate effects as opposed to suggested nutritional deficiency by Langiano et la., (2008) since we were prompt on feeding the fish once daily and on time.

In conclusion, the results of this current study asserts the toxic impacts of glyphosate on fish behavior and histopathology of the gill and liver tissues. Thus, impairment of gills and liver functional efficiencies before exhaustion, suffocation, and death occurred. Therefore, we recommend regulating glyphosate usage in/or near aquatic environment and the importance of establishing environmental monitoring commission guidelines to regulate or discourage the use of glyphosate and other harmful chemicals. Thence, protecting not only life forms like fish from extinction, but also ensures biological diversity and stability for healthier ecosystems.

References Références Referencias

1. Al-Akel AS , Elamin MS. 2011. Biological Control agent for mosquito larvae: Review on the killifish, Aphanius dispar (Ruppel, 1829). Afr J Biotech 10(44): 8683-8688.
2. Akinsorotan AM, S. Zelibe AA, Olele NF. 2013. Histopathological effects of acutely toxic levels of dizensate (glyphosate herbicide) on gill and liver of Clarias gariepinus adult. Inter J Engi Res 4(3): 2229-5518.
3. Ayanda OI, Oniye SJ, Auta J, Ajibola, VO. 2015. Acute toxicity of glyphosate and paraquat to the African catfish (Clarias gariepinus, Teugels 1986) using some biochemical indicators. Tropical Zool 28 (4): 152-162.
4. Ayoola SO. 2008. Histopathological effects of Glyphosate on juveniles African catfish (Clarias gariepinus). Amer-Euras. J Agri Environ Sci 4(3): 362-367.
5. Ayoola SO. 2008. Toxicity of glyphosate herbicide on Nile tilapia (Oreochromisniloticus) juvenile. African J Artic Res. 3: 825-834.
6. Banace M, Sureda A, Mirvaghefi AR, Ahmadi K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). Pest Biochem Physiol 99: 1-6
7. Bancroft J, Stevens A. 1996: Theory and practice of histological techniques. 4th Edition. Churchill Livingstone, Edinburgh.
8. Ba-Omar TA, Al-Jardani RV. 2011. Effects of pesticide temphos on the gills of Aphanius dispar (Pisces : Cyprnodontidae). Tissue and Cell. 43: 29-38.
9. Cavas T, Konen S. 2007. Detection of cytogenic and DNA damage in the peripheral goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and comet assay. Mutagenesis 22: 263-268.
10. Deivasigamani S. 2015. Effects of herbicides on fish and histological evaluation of common carp (Cyprinus carpio). Intern J Appli Res 1(7): 437-440. Gonzalo LP, Vera MS, Leandro AM. 2011. Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems. Herbicdes and environment, Dr Andreas Kortekamp (Ed). ISBN: 978-953-307-476-4, InTech, Available from: http://www.intechopen.com/book/herbicide-and-environment/effects-of-herbicde-glyphosate-and-glyphosate-based-formulations-on-aquatic-ecosystems.
11. Jofre MJ, Garcia MJG, Salcedo R, Morales M, Alfarez M, Enriz D, Gianni F. 2013. Fish Toxicity of commercial herbiceds formulated with glyphsate. J Environ Anal Toxicol 4:199. Doi: 10,2172/2161-0525, 1000199.
12. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus). Environ Toxicol 18, 260–267.

13. Finney DJ. 1971. Probit analysis. Cambridge: the University press.
14. Kumar M, Kumar P, Devi S. 2015. Toxicity of copper sulphate on behavioural parameter and respiratory surveillance in freshwater catfish, Clarias batrachus (Linn.). Intern J Fauana and Biologic Stud. 2(4): 80-85.
15. Langiano VC, Martinez CBR. 2008.Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish Prochiloduslineatus. Comp Biochem Physiol C 147:222–231.
16. Neskovic NK, Poleksic V, Elezovic I, Karan V, Budimir M. 1996. Biochemical and histopathological effects of Glyphosate on Carp, Cyprinus carpio L. Bull Environ Contam Toxicol 56: 295-302.
17. Nwani GD, Nagpure NS, Kumar R, Kushwaha B, Kumar B, Lakra WS. 2010. Lethal concentration and toxicity stress of Garbosulfan, Glysophate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch). Int Aquatic Res 2:105-111.
18. Mallory-Smith C. 2013. Multiple-resistant Italian ryegrass (Lolium Perenne Spp. Multiflorum) population in Oregon. Oregan State University. Master thesis, 65pp.
19. Okayi RG, Annune PA,Tachia MU, Oshoke OJ. 2010. Acute toxicity of glyphosate on Clarias gariepinus fingerlings. J Res Fores Wildlife Environ 2 (2): 150-155.
20. Pandey A, Kunwar GK, Munshi JSD. 1990. Integumentary chromatophores and mucus glands of fish as indicator of heavy metal pollution. J Fresh Biol 2: 117-121.
21. Ramirez-Duarte W. F, Rondon-Barragan LS, Eslava-Mocha PR. 2008. Acute toxicity and Histopathological alterations of roundup herbicide on "cachama blanca" (Piaractus brachypomus)1. Pesq Vet Bras 28 (11): 547-554.
22. Saeed S, Al-Naema, N, Buttler JD, Febbo EJ. 2015. Arabian Killifish (Aphanius dispar) embryo: A model organism for the risk assessment of the Arabian Gulf coastal waters. Environ Toxicol Chem 34(12): 2898-905.
23. Sani A, Idris MK. 2016. Acute toxicity of herbicide (glyphosate) in Clarias gariepinus juveniles. Toxicol Rep 3: 513- 515.
24. Servizi JA, Gordon RW, Martens DW. 1987. Acute toxicity of Garlon 4 and Roundup herbicides to salmon, daphnia, and trout. Bull Environ Contam Toxicol 39:15-22.
25. Shoaib N, Pirzada JA, Amjad A. 2012. Acute toxic effects of organophosphate pesticdes on killifish (Aphanius dispar) juveniles. Pakistan J Zool 44(2): 569-572.
26. Shoaib N, Pirzada JAS, Khalid H. 2013. Toxicity of synthetic pyrethroid pesticides, Fenpropathrin Fenv- alerate, on killifish Aphanius dispar juveniles. Pakistan J Zool 45(4): 1160-1164.
27. Thanomsit C, Wattanakorsiri A, Nanthanawat P. 2016. Efects of glyphosate on fish behavior and histologic-
al alteration og gills in Asian Sea Bass (Lates calcarifer).
28. Tsui MT, Chu LM. 2008. Environmental fate and non-target impact of glyphosate-based herbicides (roundup) in a subtropical wetland. Chemosph 71:439-446.
29. Tu M, Hurd, C, Randall JM. 2001. Weed control method handbook: tools and techniques for use in natural areas. California: the nature Conservatory. US Environmental protection Agency (USEPA). 1993. Registration eligibility decision (RED): Glyphosate. US Environmental Protection Agency. Office of prevention, pesticides and toxic substances, Washington, DC.
30. Waynon J, Finley MT. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. US Fish and Wildlife Service Publication, No. 137, Washington, DC.
31. World Health Organization (WHO). 1994. Glyphosate. Environmental Health Criteria, Publication NO 159, Geneva, Switzerland.
32. Zarei I, Pourkhabbaz A, Alipour H, Khazaei SH. 2013. Acute toxicity and the effects of copper sulphate (CuSO4.5H2O) on the behavior of the black fish (Capoeta fusca). Iran J Toxicol 6(19): 771-778.