International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

ACUTE TOXICITY OF GLYPHOSATE AMMONIUM SALT AND PARAQUAT COMMONLY USED IN NGAOUNDERE (CAMEROON) ON FRY OF *Oreochromis niloticus* (CICHLIDAE) LINNAEUS, 1758

Toua Vroumsia*, Ressong-Vivien Mbilba

Department of Biological Sciences, Faculty of Science,
University of Ngaoundere, PO Box 454, Ngaoundere, Cameroon

*Corresponding author

ABSTRACT

Herbicides, when not applied following standards of use, contribute to environmental degradation, mainly water quality, and can disrupt the ecological balance of non-target species. The aim of this study is to evaluate the acute toxicity of two herbicides (ammonium salt of glyphosate and paraquat) frequently used by the farmers of Ngaoundere, on the fry of *Oreochromis niloticus*. Surveys were undertaken to estimate the frequency of sold herbicides and their use in the fields, respectively, with the sellers of plant protection products and farmers in Ngaoundere. A static acute toxicity test was carried out, to determine the LC50 of commercial formulations of glyphosate (Super Machette 888 WSG) and paraquat (Supraxone Royal) on the freshwater *Oreochromis niloticus* and its behaviours, during the exposition. The fishes were exposed to increasing concentrations of the two herbicides, in a non-renewed oxygenated microcosm medium. The numbers of dead fishes were recorded, after 24, 48, 72 and 96 h and the behaviours observed, during the exposition. Surveys results indicate that glyphosate (64.99%) and paraquat (15.77%) herbicides are the most commonly used herbicides in Ngaoundere and that 56 % of the respondent farmers do not use the herbicides according to the recommended standards. The 96h-LC50 values, estimated by probit analysis, were of 8.78 and 14.87 mgL^{-1}, for glyphosate ammonium salt and paraquat, respectively. Lowest Observed Effect Concentration (LOEC) means were of 6 $mg.L^{-1}$ for ammonium salt of glyphosate and 10 $mg.L^{-1}$ for paraquat. The cocktail glyphosate ammonium salt + paraquat showed no mortality at the concentration of 3 + 5 $mg.L^{-1}$, while the mortality rate was of 100% at the concentration of 5.25 + 9.5 $mg.L^{-1}$. The fry showed unbalanced behaviours as concentration increased: erratic swimming, increased surfacing activities and opercular movements, loss of balance, immobility and finally death. The ammonium salt of glyphosate was found to be moderately toxic, while paraquat is slightly toxic.

It is therefore necessary to establish quality standards on the use of glyphosate and paraquat in the ecosystem, and to put in place a phytopharmaco-vigilance and public awareness mechanism.

Keywords: herbicides, glyphosate ammonium salt, paraquat, *Oreochromis niloticus*, acute toxicity.

1. INTRODUCTION

The need to optimize agricultural yields has always pushed humans to use chemicals. The use of plant protection products was very quickly established in agricultural practices. These have been used routinely and in increasing numbers around the world for years [1]. These products are well recognized for their effectiveness in controlling and stemming unwanted organisms considered harmful [2, 3]. They are mainly used in agriculture to eliminate weeds (herbicides) and to control pests (insecticides) or the development of pathogenic fungi (fungicides) [4]. To preserve yields, farmers tend to increase herbicide rates. This weed control introduces chemical species that can be harmful for both the environment and humans [5].

Pesticides often end up in the aquatic environment, which acts as the final receptacle for a number of anthropogenic pollutants. They may then cause a point or diffuse contamination. This diffuse pollution appears to be the dominant source of pesticide input to surface and groundwater [6]. This can be associated with several transport mechanisms that allow pesticides to leave the agricultural parcel and end up in the marine environment. The two main mechanisms for transporting pesticides to surface and groundwater are runoff (pesticides dissolved or bound to soil particles) and leaching [6]. The supply of fertilizers, pesticides and organic matter to watercourses is an environmental issue related to agricultural practices [7]. The consequences of this diffuse pollution are numerous, both environmental and economic. These include water eutrophication, increased filtration costs for drinking water, loss of organic soil, loss of water-related uses, and adverse effects of these pollutants on ecosystem and human health. In addition, of the top 10 global sources of pollution, pollution from pesticides used in agriculture, and pollution from manufacturing and processing storage of these pesticides are ranked, respectively, third and tenth by the Blacksmith Institute [8].

In a natural ecosystem, the various species present, are in relation to each other (intra and interspecific relations such as competition, commensalism, symbiosis, predation, etc.) and the disappearance or decrease of the density of a given species, can lead to significant upheavals throughout the biocenosis [1]. Thus, misuse of these pesticides can lead to toxicity problems at the level of non-target organisms [9, 10].

Glyphosate is a broad-spectrum herbicide widely used to kill unwanted plants both in agriculture

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

and in non-agricultural landscapes. It has been called “extremely persistent” by the USEPA, and half- lives of over 100 days have been measured in field tests in Iowa and New York [11]. Glyphosate has been found in streams following agricultural, urban, and forestry applications [12]. Glyphosate- containing products are acutely toxic to animals, including humans [13, 14].

Paraquat is one of the most widely used herbicides in the world. It is described and referred to by the USEPA as extremely active and toxic to plants and animal and by the New Zealand Environmental Risk Management Authority as very ecotoxic for the environment [15].

Fish is considered as a model organism in the experimentation of toxicological and pharmacological studies [16]. Potential application of research findings on human and other environmental health issues has made fish a more attractive model organism in toxicology research [17]. Tilapia (*Oreochromis niloticus*) is a useful model for ecotoxicological studies [18]. This fish can spawn in all types of water, withstand extreme water temperatures and low dissolved oxygen amount [19].

The relief of Ngaoundere presents flat-bottomed valleys with sloping parts and mountains. Rainfall is greater than or equal to 1400 mm with a rainy season of 7 to 8 months depending on the year [20]. The relief and climate are factors that favour the transfer of agricultural products to the bottom, thus feeding the river systems, which are often full of fish and other aquatic organisms. There are several fish farms in Ngaoundere, which benefit from the hydrographic network as a source of water.

Thus it becomes necessary to ensure that herbicides, when used for the intended purpose, have no deleterious effect on fish and aquatic ecosystems, particularly, when it is known that agricultural pollution caused by plant protection products is often implicated in the pollution of aquatic environments [21]. To date, there are no studies dealing with the effect of herbicides on the aquatic ecosystems in Ngaoundere region. Moreover, most of the existing studies evolving glyphosate and paraquat, [22-25], focused on the active ingredients separately, giving lethal concentrations for fishes species, yet measurements carried out in aquatic environments highlight situations of mixtures of two or more pesticides that can exert an increased toxic activity [26,27]. Studies assessing, separately or in mixture, the effects of multiple stresses on fish in their natural environment are then needed. It is with this in mind that this work, which objective is to evaluate the acute toxicity of two herbicides (ammonium salt of glyphosate and paraquat), regularly used at Ngaoundere, on fry of *Oreochromis niloticus*, was undertaken.

2. MATERIAL AND METHODS

2.1 Survey

Surveys studies were undertaken to estimate the frequency of sold herbicides and their use in the fields, respectively, with the sellers of plant protection products and farmers in Ngaoundere. To gather the views of people, we prepared a set of questions. Globally, the sellers (40 respondents) set were questioned about their identification and the nouns and number of herbicides units sold, while farmers (300 persons) were interrogated on their identification, their usage of herbicides and their knowledge of the impacts of this use on the environment.

2.2 Fish acclimatization

The study was conducted in laboratory microcosms in the Aquaculture Station of Ngaoundere (Adamawa Region, Cameroon). The freshwater *Oreochromis niloticus* fingerlings, with a mean length of 6.10 ± 0.80 cm and a mean weight of 3.80 ± 1.41 g, were obtained from the Aquaculture Station of Ngaoundere (Adamawa Region, Cameroon). Fingerlings were transferred to cylindrical plastic aquariums, with a capacity of 21 liters filled with local drilling water and acclimated to laboratory conditions for 4 days, prior to experimentation. A group of 10 fry was introduced into each aquarium. They were fed, once a day, with small granules of a size starting at 3 mm, supplied by the Aquaculture Station and the medium was change daily. The fry feeding was stopped 24 hours before the test [28]. The daily ration was of 8% of the biomass present in each aquarium. The aquariums were ventilated by an electromagnetic air pump (RESUN ACO 004 ISO 9001: 2008).

2.3 Acute toxicity tests

Toxicity tests were conducted in accordance with the static test model of [29] and [30] protocols. Preliminary tests were conducted, to find out the range of concentrations to be used in the bioassays [28]. Stock solutions of glyphosate ammonium salt and paraquat were prepared by diluting their respective commercial formulations (obtained from Ngaoundere market) Super Machette 888 WSG (888 g/kg of glyphosate ammonium salt, Kesai Eagrow, Cameroon) and Supraxone Royal (200 g/l of paraquat, Louis Dreyfus Commodities, Cameroon) with boring water. The nominal test concentrations were of 0, 6, 7.5, 9, 10.5 and 12 $mg.L^{-1}$, for glyphosate ammonium salt and of 0, 10, 13, 16, 19 and 22 $mg.L^{-1}$, for paraquat. In both cases, each concentration was tested in triplicate and 10 specimens of fish were placed in a 21-litres transparent plastic aquarium, filled with 12 litres of test solution. Small mesh net was used to cover the aquariums, to prevent the specimens jumping out of the test solutions. The behaviour of the fry was observed every 3 hours and death was recorded after 24, 48, 72 and 96 hours. The fish are considered dead, if no movement is observed (for example, the movement of the ears) and if the touching of the caudal peduncle produces no reaction. The dead fishes were promptly removed and preserved in a healthy medium for observations. An essay with the binary mixture glyphosate ammonium salt-paraquat was also, run, to evaluate the potential effects of the mixture on fish.

Some physicochemical parameters of the experimental drilling water were measured. Temperature was taken with an Exteck EC 400 model digital apparatus. Levels of TDS (Total Dissolved Solids) and electrical conductivity were measured, using a Wagtech model, 3937-40 series digital conductivimeter. The pH was measured, using a Wagtech model, 3916-55 series digital pH-meter.

Free and total Chlorine levels were measured by calorimetry with a Wagtech model, series 1024-C3 el photometer, and total hardness was measured, according to [29].

2.4 Data Analysis

The LC_{50} was calculated according to the method of [31]. Percent mortality was calculated and the values obtained were transferred into probit scale. Regression lines of probit against logarithmic transformation of concentrations were made, using the Microsoft Office Excel 2007 software. 95%- Confidential limits (upper and lower) of the CL_{50}, with chi square test were calculated, according to the method of [31] also.

3. RESULTS AND DISCUSSION

3.1 Survey

The results of this part of the study show that glyphosate (64.99%) and paraquat (15.77%) are the most sold herbicides in Ngaoundere and, consequently, the most commonly used (Figure 1). It can be noticed that selective herbicides like atrazine, nicosulfuron and diuron are not extensively utilized as total herbicides. This could be explained by the efficacy of the latters and their total mode of action, and so their non-selective broad-spectrum.

It was also found that most of the respondent farmers (56%) do not use the herbicides according to the recommended standards. Normally, a more than 50 m safety area must be established between the treated fields and surface water, in order to reduce in-flight drift and water carrying along of the herbicides [15]. The survey revealed that about 44% of the investigated farmers do not follow this recommendation (Figure 2A), 94 % know nothing about this safety zone (Figure 2B), and 30% gargle their sprayer apparatus, outright, in surface water close by their fields

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

(Figure 2C).

3.2 Experimental water quality

The physico-chemical parameters of the drilling water are shown in Table 1. These parameters show that the water is of an enough good quality, as the [32] recommends the use of a water with a total hardness between 10 and 250 mgL^{-1} of CaCO3, a pH between 6.0 and 8.5 and a residual chlorine less than 10 μgL^{-1}. We can say that the used waters have physico-chemical characteristics tolerable by *O. niloticus* and. Moreover, the absence of deaths in the control aquaria, during testing, strengthen this fact and shows that the experimental water supplies the demands of the fingerlings for their survival and that our experimental conditions can be validated.

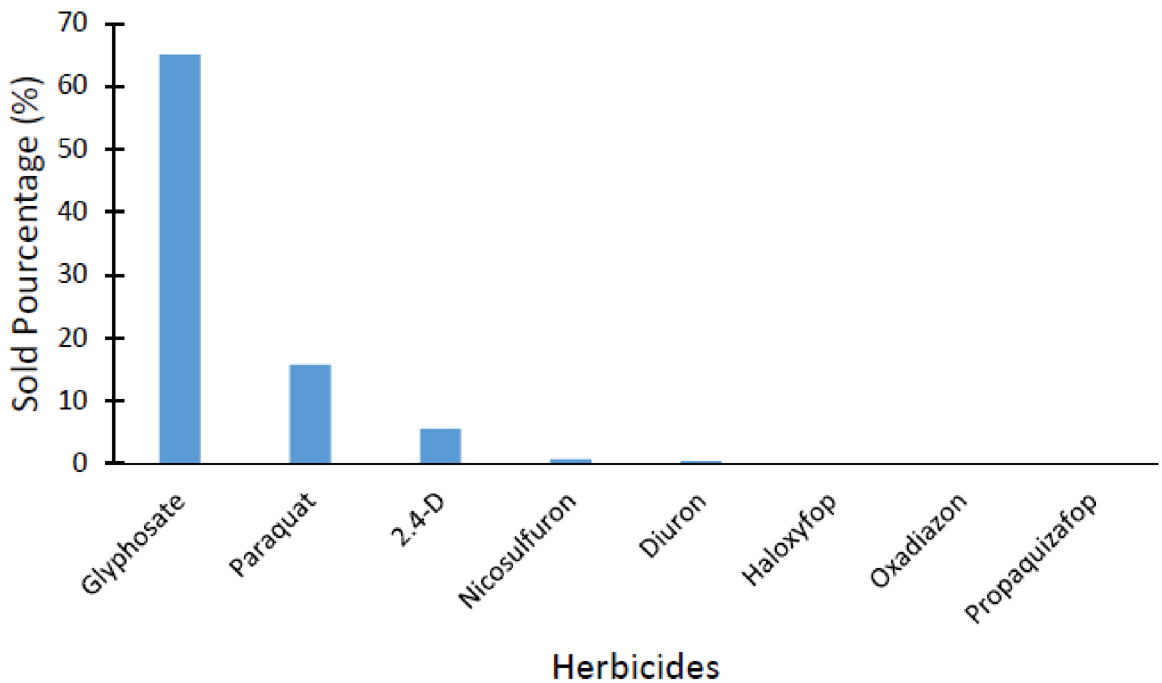

Figure 1: Frequency of sold herbicides in Ngaoundere (Cameroon)

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

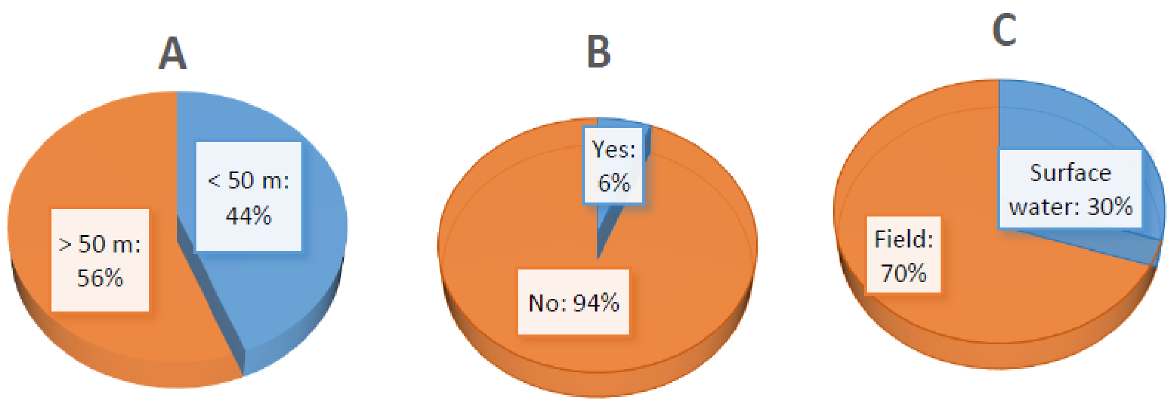

Figure 2: Frequency of the investigated farmers according to their respect of the safety area (A), their knowledge of this zone (B) and the rising site of their sprayers (C)

Table 1: Physico-chemical parameters of the drilling experimental water

Physico-chemical parameters	Values
Temperature (°C)	22.52 ± 0,1
pH	8.1 ± 0,24
Electrical conductivity (µS/cm)	358.55 ± 24,25
TDS (ppm)	179.55 ± 10,1
Free chlorine ($mg.L^{-1}$)	0.065 ± 0.02
Total hardness ($mg.L^{-1}$ of $CaCO_3$)	195.43 ± 1.7
Total chlorine ($mg.L^{-1}$)	0.18 ± 0.05

3.3 Observation of fish behaviours

Behavioural responses of fry exposure to glyphosate ammonium salt, paraquat and ammonium glyphosate-paraquat mixture were observed and compared to those of controls. Reactions such as air absorption, gill-cover movements, cascade swimming and immobility have been observed. Lastly, dead fry with open mouth and erected fins were observed. The intensity of these symptoms were positively related to the xenobiotics concentration and varied with herbicides nature (Tables 2-4). Respiratory stress, erratic swimming and instant death of fish were also observed by [22], while studying toxicity of glyphosate herbicide on Nile tilapia.

[33] identified four main phases of fish reaction to toxic substances: the contact phase (short period of excitability), visible efforts (fast swimming, jumping, and attempting to jump out of the toxic zone), the phase of loss of balance and mortality, when the opercular movement and the

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

response to tactile stimuli cease completely. Responses such as hyperactivity, increased ventilation of the operculum, erected fins and cascade swimming, lead to fatigue (immobility) and stress [33]. The increased movement of the operculum and the absorption of air at the surface show a lack of dissolved oxygen [25]. The fry use enough energy to adapt to their environment [34]. To compensate for this lack of oxygen, surface air absorption and hyperventilation of the operculum were observed [35, 36]. Oxygen exchange through the gills may have been altered as evidenced by the disturbance of the gill structure [33]. The cascade swimming of the fry is in order to change the environment [34]. [37] observed that the behavioural changes such as swimming patterns shows sign of neurotoxicity. The death of exposed test fish can be attributed to the destruction of organs such as the gills, liver, kidneys, brain, blood system and pancreas [38].

Table 2: Fry behaviours in the presence of glyphosate ammonium salt

Symptoms	Concentrations ($mg.L^{-1}$)					
	0	6	7.5	9	10.5	12
Air absorption at surface	-	-	+	+	+	++
Gill-cover movements	-	-	+	++	++	++
Cascade swimming	-	-	-	-	-	+
Immobility	-	-	+	+	+	++
Dead fry with open mouth	-	-	-	+	++	+++
Dead fry with erected fins	-	-	-	-	+	+++

Key: None (-), Weak (+), Moderate (++), Strong (+++)

Table 3: Fry behaviours in the presence of paraquat

Symptoms	Concentrations ($mg.L^{-1}$)					
	0	10	13	16	19	22
Air absorption at surface	-	-	-	+	++	+++
Gill-cover movements	-	-	+	+	++	+++
Cascade swimming	-	-	-	-	+	+
Immobility	-	-	+	+	++	++
Dead fry with open mouth	-	-	+	+	++	+++
Dead fry with erected fins	-	-	+	+	+	++

Key: None (-), Weak (+), Moderate (++), Strong (+++)

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

Table 4: Fry behaviours in the presence of glyphosate ammonium salt-paraquat mixture

Symptoms	Concentrations ($mg.L^{-1}$)					
	0	3 + 5	3.75 + 6.5	4.5 + 8	5.25 + 9.5	6 + 11
Air absorption at surface	-	-	-	+	+	+
Gill-cover movements	-	-	-	-	+	+
Cascade swimming	-	-	-	-	-	-
Immobility	-	-	-	+	+	++
Dead fry with open mouth	-	-	-	-	+++	+++
Dead fry with erected fins	-	-	-	-	+++	+++

Key: None (-), Weak (+), Moderate (++), Strong (+++)

3.4 Assessment of acute toxicity

According to the dose-response curves, after 96 h of exposition of *Oreochromis niloticus* to glyphosate ammonium salt (Figure 3) and to paraquat (Figure 4), it appears that the LOEC is of 6 $mg.L^{-1}$, for the first pesticide and 10 $mg.L^{-1}$, for the second. The results obtained, do not give the exact values of the NOEC, however, indicate that it is in the [0- 6]$mg.L^{-1}$ concentrations range, for glyphosate ammonium salt and in the [0-10]$mg.L^{-1}$ concentrations range, for paraquat. The curves have the appearance of a sigmoid and indicate that mortality rate of exposed fishes increased, as the concentration of herbicides increased. Based on the log concentration-probit regression lines (Figures 5 and 6), 96h-LC50 were found to be 8.78 $mg.L^{-1}$, with 95%-confidential limits of [7.69-10.02], for glyphosate ammonium salt and 14.87 $mg.L^{-1}$, with 95%-confidential limits of [12.77-17.30], for paraquat (Table 5).

Depending on the [39] table of pesticides toxicity on fish and aquatic invertebrates classification, the ammonium salt of glyphosate is moderately toxic to fry of *Oreochromis niloticus*. Studying toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile [22] found a 96h-LC50 of 1.05 $mg.L^{-1}$. This important difference can be explained by the physiologic parameters of the used fishes, the test conditions and the formulation of the herbicides [40-42]. In other fish species, glyphosate 96h-LC50 of 13.6 $mg.L^{-1}$ [43], 9.76 $mg.L^{-1}$ [44], 32.54 $mg.L^{-1}$ [45], 18.6 $mg.L^{-1}$ [46] and 3.74 $mg.L^{-1}$ [23] were found, respectively, on *Prochilodus leneatus*, guppies fry (*Poecilia reticulata*) for the second and third values, rainbow trout (*Salmo gairdneri*), and bluegill harlequin (*Piaractus mesopotamicus*. This discrepancy in the results may be due to the fact that the toxicity of pollutants in aquatic organisms has been shown to be influenced by age, species type, water quality, herbicide concentration and formulation [35, 47, 48].

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

With a 96h-LC50 of 14.87 mg.L^{-1}, paraquat is slightly toxic [39]. Previous studies dealing with acute paraquat toxicity studies on *Oreochromis niloticus* fry revealed 96h-LC50 values of 12.25 mg.L^{-1} [24] and 11.84 mg.L^{-1}, with dichloride as a surfactant [49].

In other fish species, 96h-LC50 values of 13 and 26.07 mg.L^{-1}, for *Crapet arlequin*, were found respectively by [50] and [25]. For *Trichogaster trichopterus*, the 96h-LC50 amont was of 1.41 ± 0.17 mg.L^{-1} [51]. According to [52], the 96h-LC50 values of paraquat for game fish (*Fundulus similis*), Gambusia affinis, zebrafish (*Brachydanio rerio*), molly (*Poecilia mexicana*), medaka (*Oryzias latipes*), *Lepomis macrochirus*, guppy (*Poecilia reticulata*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), catfish (*Ictalurus punctatus*) were found to be of 1, 3, 60, 7, 5, 12, 13, 15, 25 and more than 100 mg.L^{-1}, respectively. As can be seen, the sensitivity of the species varies.

Generally, the effects of chemical compounds on ecosystems are studied individually. However, the need to understand the toxicity of mixed pesticides is currently well established [53]. Several herbicides can be found in a marine environment and, in order to study their toxicity to fish, the fry were exposed to a mixture of glyphosate and paraquat at different concentrations. The results of this test are shown in Table 6.

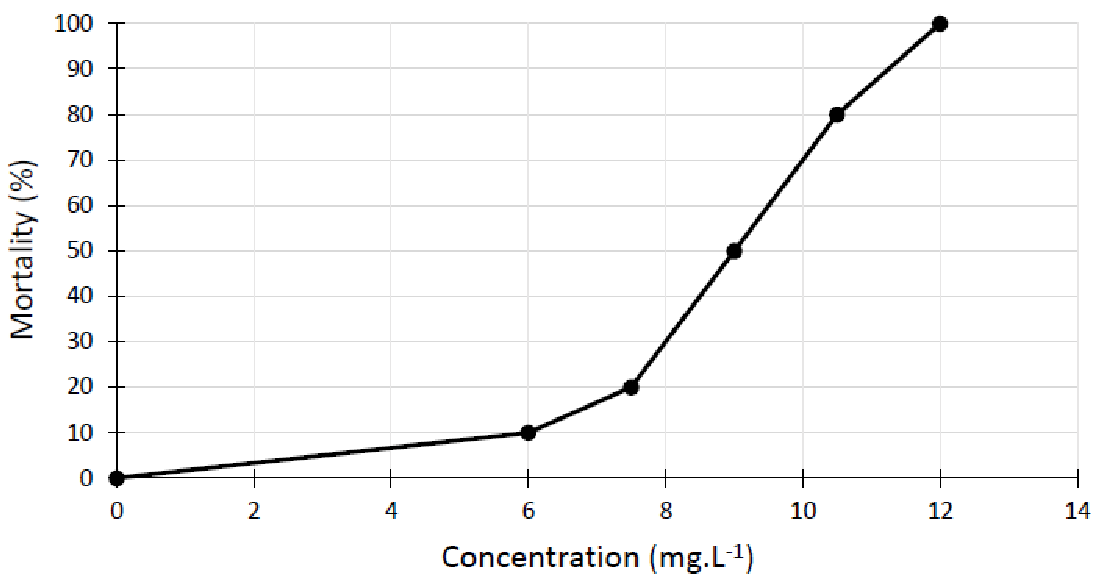

Figure 3: Dose-response curve, after 96 h of exposition of *Oreochromis niloticus* to glyphosate ammonium salt

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

The mixture of ammonium salt of glyphosate and paraquat showed no mortality with concentrations of 3 + 5, 3.75 + 6.5 and 4.5 + 8 $mg.L^{-1}$, while the mortality rate was of 100%, since the concentration of 5.25 + 9.5 $mg.L^{-1}$. It seems that before these values of the concentration, the two xenobiotics had an antagonism effect, as there were no mortality, even at the mixture concentration of 4.5 + 8 $mg.L^{-1}$ close to the values of the half of the respective 96h-LC_{50} of each pesticide (4.39 and 7.44 $mg.L^{-1}$).

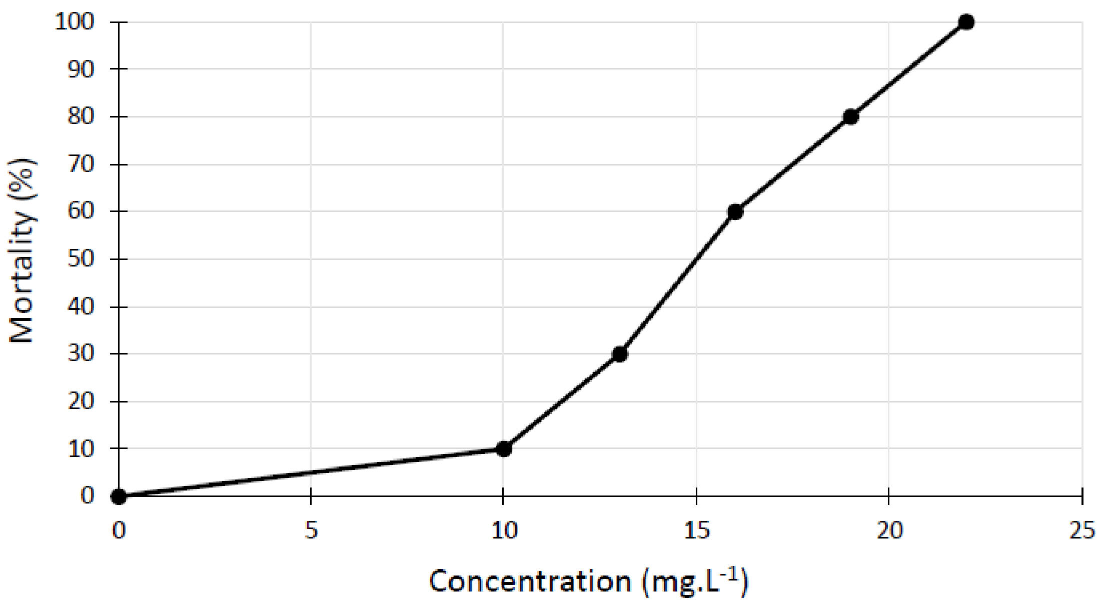

Figure 4: Dose-response curve, after 96 h of exposition of *Oreochromis niloticus* to paraquat

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

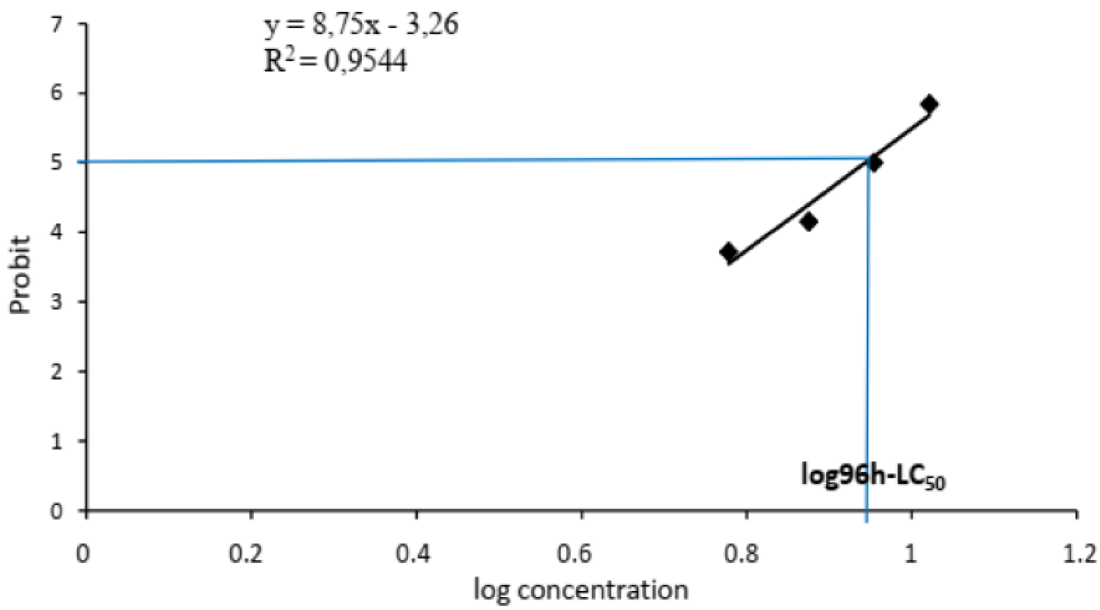

Figure 5: Regression line log concentration-Probit, after 96 h of exposition of *Oreochromis niloticus* to glyphosate ammonium salt

This tendency may be related to the fact that toxicities additivity may be reduced for a binary mixture of products in which each is at the half of its LC50 amount [54]. These preliminary results lead us to think that the cocktail of glyphosate ammonium salt-paraquat, at 5.25 (almost the LOEC of 6 mg.L^{-1}) + 9.5 mg.L^{-1} (almost the LOEC of 10 mg.L^{-1}) give rise to an additional effect on the fry, with however, positive interactions, maybe synergetic effects. The toxicity of the mixture is the result of the combined effects of its components. The toxicity of the compounds alone is not sufficient to predict that of the mixture [55]. It was proven that mixtures of plant protection substances showed combined synergy effects, antagonism or no interaction [56, 57].

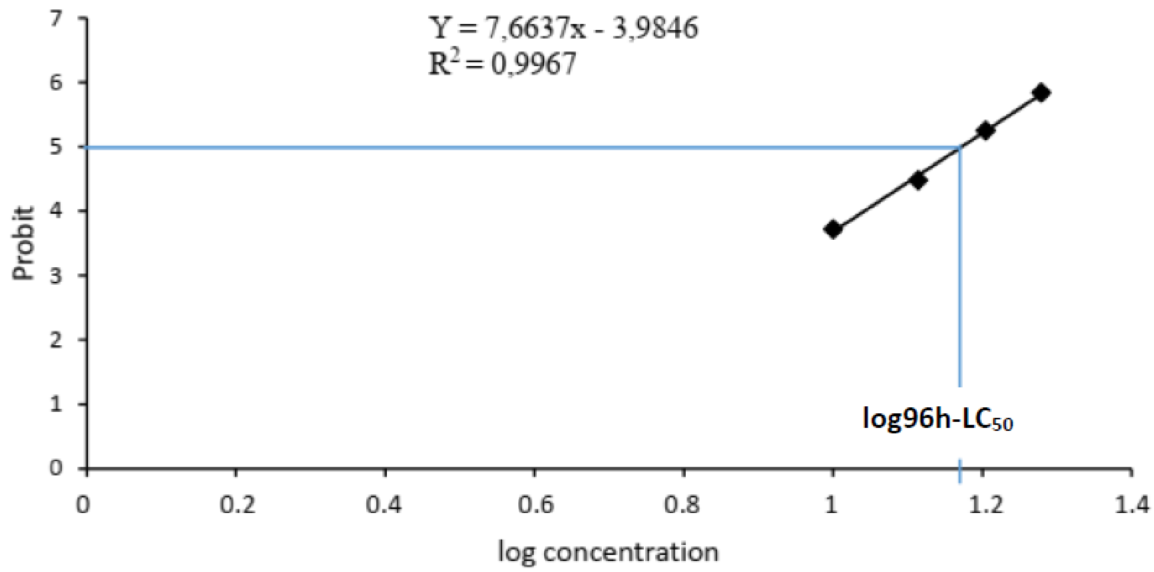

Figure 6: Regression line log concentration-Probit, after 96 h of exposition of *Oreochromis niloticus* to paraquat

Table 5: 96h-LC50 values of glyphosate ammonium salt and paraquat to *Oreochromis niloticus*

Herbicide	96h-LC_{50} (mgL-1)	95%-Confidential limits		Calculated χ2	Table χ2 value
		Lower	Upper		
Glyphosate ammonium salt	8.78	7.69	10.02	1.817	7.815
Paraquat	14.87	12.77	17.30	0.120	7.815

Table 6: Mortality of *Oreochromis niloticus* fry exposed to glyphosate ammonium salt-paraquat mixture at different concentrations

Aquarium	Exposed Fry number	Concentration		Dead fry number after			
		Glyphosate ($mg.L^{-1}$)	Paraquat ($mg.L^{-1}$)	24 h	48 h	72 h	96 h
Control	10	0	0	0	0	0	0
1	10	3	5	0	0	0	0
2	10	3.75	6,50	0	0	0	0
3	10	4.5	8	0	0	0	0
4	10	5.25	9.50	9±0.57	10±0	10±0	10±0
5	10	6	11	10±0	10±0	10±0	10±0

4. CONCLUSION

This study enable us to gather information about the use of herbicides in Ngaoundere and the toxicity of glyphosate ammonium salt and that of paraquat on *Oreochromis niloticus* fry. It appeared that glyphosate (64.99%) and paraquat (15.77%) are the most sold and consequently, the most widely used herbicides in Ngaoundere and that some farmers in the locality of Ngaoundere do not respect the safe use patterns of pesticides: they spray herbicides near surface water (44%) and rinse spray equipment in these same waters (30%). Ammonium salt of glyphosate with a 96h-LC50 mean value of 8.78 $mg.L^{-1}$ and paraquat with a 96h-LC50 mean amount of 14.87 $mg.L^{-1}$, were found, respectively, moderately and slightly toxic for fish and aquatic invertebrates. The effect of the glyphosate ammonium salt-paraquat mix showed a mortality rate of 100% at 5.25 + 9.5 $mg.L^{-1}$ concentration, suggesting synergetic effects of the mixture, at this concentration. Fry abnormal behaviours such as jerky swimming, frequently air absorption at surface, increased operculum movements, immobility and finally death have been observed, during the exposition to the herbicides.

Given these results, the use of glyphosate and paraquat must be done following the recommended standards and farmer must be aware of the environmental impacts of their misuse.

ACKNOWLEDGEMENTS

The authors, greatly, thank the Director of the Aquaculture Station of Ngaoundere (Cameroon) who opened his doors for the realization of the tests within his station and for the materials

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

disposal, as well as his collaborators and the neighbouring fishermen, for the fry supply.

REFERENCES

1. Amara A., 2012. Evaluation de la toxicité de pesticides sur quatre niveaux trophiques marins: microalgues, échinodermes, bivalves et poissons. Thèse de Doctorat, Université de Bretagne occidentale-Brest, France, 133 p.

2. Liu Y., Xu Z., Wu X., Gui W. and Zhu G., 2010. Adsorption and desorption behaviour of herbicide diuron on various Chinese cultivated soils. *J. Hazard. Mater.* 178: 462-468.

3. Narita K., Matsui Y., Iwao K., Kamata M., Matsushita T. and Shirasaki N., 2014. Selecting pesticides for inclusion in drinking water quality guidelines on the basis of detection probability and ranking. *Environ. Int.* 63: 114-120.

4. Giroux I., 2003. Contamination de l'eau souterraine par les pesticides et les nitrates dans les régions en culture de pommes de terre. Québec, Ministère de l'Environnement, Direction du suivi de l'état de l'environnement, Envirodoq: ENV/2003/0233, 34 p. http://www.mddep.gouv.qc.ca/pesticides/pomme_terre/Pesticides_pomme_terre.pdf.

5. Boschetto G., 2013. Evaluation de la pertinence de l'utilisation des herbicides en lien avec le développement durable. Centre universitaire de formation en environnement, 86 p. https://www.usherbrooke.ca/environnement/fileadmin/sites/environnement/documents/Essais_2013/Boschetto_G_2013-03-15_.pdf.

6. Gilliom R., Barbash J., Crawford C., Hamilton P., Martin D., Nakagaki N., Nowell L., Scott l., Stackelberg P., Thelin G. and Wolock D., 2006. Pesticides in the nation's streams and ground water, 1992–2001. National Water-Quality Assessment Program, Circular 1291, U.S. Department of the Interior, U.S. Geological Survey, 184 p. https://pubs.usgs.gov/circ/2005/1291/pdf/circ1291.pdf.

7. OCDE (Organisation de Coopération et de Développement Economiques), 2008. La performance environnementale de l'agriculture dans les pays de l'OCDE depuis 1990, Paris, France, www.oecd.org/tad/env/indicateurs.

8. Harris, J. and McCartor A., 2011. The world's worst toxic pollution problems report 2011: The top ten of the Toxic Twenty. Blacksmith Institute, New-York, 76 p. http://worstpolluted.org/docs/TopTen2011.pdf

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

9. Ramade F., 1992. Précis d'Ecotoxicologie. Ed. Masson, 1992, Paris, France, 300 p.

10. Toe A. M., Kinane M. L., Kone S. et Sanfo-Boyarm E., 2004. Le non-respect des bonnes pratiques agricoles dans l'utilisation de l'endosulfan comme insecticide en culture cotonnière au Burkina Faso: quelques conséquences pour la santé humaine et l'environnement. *Rev. Afric. San. Prod. Anim.* 2: 275-280.

11. USEPA (United Stated Environmental Protection Agency), 1993. Pesticide environmental fate one line summary: Glyphosate. Environmental Fate and Effects Division, Washington, D.C.

12. Vereecken H., 2005. Mobility and leaching of the glyphosate: a review. *Pest. Manag. Sci.* 61: 1139-1151.

13. Cox C, 1998, Herbicide factsheet: Glyphosate. *J. Pestic. Reform* 18: 3-17.

14. Portier C. J. *et al.*, 2016. Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA). *J. Epidemiol. Community Hea*0: 1-5.

15. Meriel W., 2011. Paraquat. Pesticide Action Network Asia and the Pacific. http:// www.stop- paraquat.

16. Boudou A. D. and Ribeyre F. D., 1989. Fish as "biological model" for experimental studies in Ecotoxicology. *In:* Aquatic Ecotoxicology fundamental concepts and methodologies (Vol. II). Boudou A. D. and Ribeyre F. D. (Eds.), CRC press inc., Boca Raton, Florida, pp 127-157.

17. Govind P., 2011. Un examen sur le modèle de poisson en pharmacologie expérimentale, *Int. Res. J. Pharm.* 2: 33-36.

18. Jos A., Pichardo S., Prieto A. I., Repetto G., Vazquez C. M., Moreno I. and Camean A. M., 2005. oxic cyanobacterial cells containing microcystins induce oxidative stress in exposed tilapia fish (*Oreochromis* sp.) under laboratory conditions. *Aquat. Toxicol.* 72: 261-271.

19. Assiah V. F., 1996. La pisciculture en eau douce à petite échelle. AGRODOK/AGROMISA 6 700 AA, Centre technique de coopération agricole et rurale ACP/CEE/CTA, Wageningen, Pays-Bas, 81 p.

20. Tchotsoua M., Boutrais J. et Bonvallot J., 2002. Dynamique des usages des vallées péri-urbaines de Ngaoundéré. Cas de la plaine inondable de Marza. *In* : Gestion intégrée des ressources naturelles en zones inondables tropicales, Orange D, Arfi R., Kuper M., Morand P., Poncet Y. (Dir.) ; IRD Eds., pp 587-601.

21. Le Mer C., 2009. Effets des expositions aux herbicides atrazine et glyphosate sur des larves d'épinoches à trois épines, *Gasterosteus aculeatus*. Mémoire de Maîtrise, Université du Québec, Rimouski, Canada, 121 p.

22. Ayoola S. O., 2008. Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. *Afr. J. Agr. Res*. 3: 825-834.

23. Shiogiri, N. S., Paulino S. P., Carraschi F. G., Baraldi C., Cruz and Fernandes M. N., 2012. Acute exposure to glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus*. *Environ. Toxicol. Pharmacol*. 34: 388-396.

24. Babatunde M. M. and Oladimeji A. A., 2014. Effect of paraquat on the growth and behaviour of *Oreochromis niloticus* (Trewavas) in Nigeria. *Environ. Res. J.* 8: 44-47.

25. Hassan M., Shah Norhan N. A., Shah Norhan H., and Karim N. U. 2015. Behavioral and histopathological changes of Common Carp (*Cyprinus carpio)* exposed to Paraquat. *J. Fisheries. Livest. Prod.* 3: 131. doi: 10.4172/2332-2608.1000131.

26. Boobis A., Budinsky R., Collie S., Crofton K., Embry M., Felter S., Hertzberg R., Kopp D., Mihlan G., Mumtaz M., Price P., Solomon K., Teuschler L., Yang R. and Zaleski R., 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. *Crit. Rev. Toxicol.*, 41: 369-383.

27. European Food Safety Authority, 2013. International framework dealing with human Risk assessment of combined exposure to multiple chemicals. *EFSA J.* 11: 3313. [69 pp.] doi:10.2903/j.efsa.2013.3313.

28. OCDE, 1992. Fish, Acute toxicity test, Report of test Guideline N° 203, OCDE Guidelines for Testing of Chemicals, Paris, France, 10 p.

29. APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. APHA, AWWA, WPCF (Eds.), 16th Edition, Washington DC, USA, 1268 p.

30. USEPA, 1996. Ecological effects test guidelines OPPTS 850.1075 Fish acute toxicity

Test, freshwater and marine, EPA 712–C–96–118, EPA Public Access Gopher. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100G6QF.PDF?Dockey=P100G6QF.PDF.

31. Litchfield J. T. and Wilcoxon F., 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96: 99-113.

32. OCDE, 2007. Report of the OCDE guidelines for testing of chemicals, Full List of Test Guidelines, Paris, France, 11 pp.

33. Ujagwung G. U. et Erondu, E. D., 2010. Toxicité du roundup (un produit glyphosate) sur les alevins *Clarias gariepinus. Anim. Res. Int.* 7: 1184-1193.

34. Benli A. C. K. and Özkul A., 2010, Acute toxicity and histopathological effects of sublethal fenitrothion on Nile tilapia, *Oreochromis niloticus*. *Pestic. Biochem. Physiol.* 97: 32-35.

35. Chumlong A. and Hiroshi K., 1992. Acute toxicity of paraquat to various developmental stages of common carp *Cyprinus carpio* Linnaeus. *Gyobyo Kenkyu* 27: 63-68.

36. Patil V. K. and David M., 2008. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). *Turk. J. Fish. Aquat. Sci.* 8: 233-237.

37. Osti S. C., Varoli F. M. F., Matushima E. R. and Bernardi M. M., 2007. Comparative studies of delthametrin acute toxicity in exotic and Brazilian fish. *J. Braz. Soc. Ecotoxicol.* 2: 101-106.

38. Okayi R. G., Annune P., Tachia A. M. and Oshoke O. J., 2010. Acute toxicity of glyphosate on *Clarias gariepinus* fingerlings. *J. Res. For., Wildlife. Environ.* 2: 150-155.

39. RECORD, 2007. Etat de connaissance sur le devenir des polluants organiques dans le sol lors de la biodégradation naturelle après un biotraitement: identification des composés « métabolites » et des cinétiques, Rapport final N° 05-0513/1A, 148 p.

40. Beggel S., Werner I., Connon R. E. and Geist J. P., 2010. Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (*Pimephales promelas*). *Sci. Total. Environ.* 408: 3169-3175.

41. Bhattacharjee D. and Das S., 2013. Toxicity of organochlorine pesticide, Lindane to fish: A review. *J. Chem. Pharm. Res.* 5: 90-96.

42. Das S, 2013. A review of Dichlorvos toxicity in fish. *Curr. World Environ.* 8: 143-149.

43. Langiano, V. C. and Martinez C. B. R., 2008. Toxicity and effects of glyphosate-based herbicide n the Neotropical fish *Prochilodus lineatus*. *Comp. Biochem. Physiol.* Part C 147: 222-231.

44. Chandrasekera W. U. and Weeratunga N. P., 2011. The Lethal impacts of Roundup® (glyphosate) on the fingerlings of Guppy, *Poecilia reticulata* Peters, 1859. *Asian Fish. Sci.* 24: 367-378.

45. Nwani C. D., Naresh S. N., Ravindra K., Basdeo K., Pavan K. et Wazir S. L., 2010. Concentration létale et stress toxique du carbosulfan, glyphosate et atrazine pour les poissons respirant l'air frais *Channa punctatus* (Bloch). *Int. Aquat. Res.* 2: 105-111.

46. Monsanto, 2003. Backgrounder Glyphosate and water quality. http://www.monsanto.com/products/Documents/glyphosate-background-materials/gly_water_bkg.

47. Gupta P. K., Khangarot B. S. and Durve V. S., 1981. The temperature dependence of the acute toxicity of copper to a freshwater pond snail, *Viviparus bengalensis* L. *Hydrobiologia* 83: 461-464.

48. Kumar R., Nagpure N. S., Kushwaha B., Srivastava S. K. and Lakra W. S., 2009. Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. *Arch. Environ. Contam. Toxicol.* 58: 123-130.

49. Babatunde M. M., Oladimeji A. A. and Balogun J. K., 2001. Acute toxicity of gramoxone to *Oreochromis niloticus* (Trewavas). *Water Air Soil Poll*. 131: 1-10.

50. Syngenta, 2009. Fiche technique santé-sécurité Syngenta Canada inc. 140 Research Lane, Research Park Guelph (Ontario) N1G 4Z3, 7 p.

51. 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.

52. Eisler R., 1990. Paraquat hazards to Fish, wildlife, and invertebrates. A Synoptic Review. Biological Report 85(1.22), August 1990. U.S. Patuxent Wildlife Research Center, Laurel, Maryland 20708, Fish and Wildlife Service, pp 1-38.

ISSN: 2455-6939

Volume:04, Issue:03 "May-June 2018"

53. Babut M. P., Delmas H., Bray M., Durrieu C., Perrodin Y. and Garric J. 2006. Characterizing the risks to aquatic ecosystems: a tentative approach in the context of freshwater dredged material disposal. *Integr. Environ. Assess. Manag.* 2: 330-343.

54. Warne M. St J., and Hawker D. W., 1995. The number of components in a mixture determines whether synergistic and antagonistic or additive toxicity predominate: The funnel hypothesis. *Ecotoxicol. Environ. Safe.* 31: 23-28.

55. Legrand E., 2016. Couplage d'approches écotoxicogénomiques chez le copépode estuarien *Eurytemora affinis* et d'outils bio-analytiques pour l'évaluation du caractère perturbateur endocrinien des contaminants aquatiques: exemple de deux pesticides modèles: le pyriproxyfène et la chlordécone, seuls et en mélange. Thèse de Doctorat, Université du Havre, France, 282 p.

56. Bianchi L., Zannoli A., Pizzala R., Stivala L. A. and Chiesara E., 1994. Genotoxicity assay of five pesticides and their mixtures in *Saccharomyces cerevisiae* D7. *Mutat. Res.* 321: 203-211.

57. Bianchi-Santamaria A., Gobbi M., Cembran M., and Arnaboldi A., 1997. Human lymphocyte micronucleus genotoxicity test with mixtures of phytochemicals in environmental concentrations. *Mutat. Res.-Gen. Tox. Env.* 338: 27-32.

