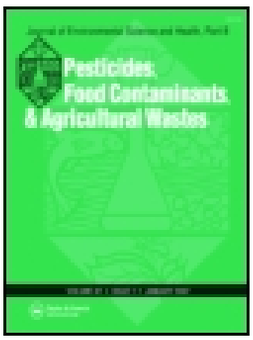

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Evaluation of acute toxicity and histology effect on liver of glyphosate and atrazine in the African catfish *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

Aquatic organisms are exposed to chemical pesticides including glyphosate (Sharp 480 SL) and atrazine (Atraforce), two phytocidal molecules used for agriculture purposes in Benin. In this study, we assessed the acute toxicity of these two herbicides with emphasis on their histopathological effects on the liver of catfish *Clarias gariepinus*. One hundred and eighty juveniles of *C. gariepinus* (mean length 7.26 ± 0.59 cm and mean weight 5.21 ± 3.22 g) were exposed over 96 h to increasing concentrations of each phytocide. The values of 96 h-LC_{50} were 6.175×10^3 and 3.165 ppm, respectively for Sharp 480 SL and Atraforce. This indicates that Sharp 480 SL was nontoxic, while Atraforce displayed a moderate toxicity to *C. gariepinus* juveniles. During the tests, the behavioral responses (hyperexcitation, lethargy, loss of balance, discoloration of skin, etc.) that usually precede death were observed in exposed fishes, confirming the neurotoxicity of these phytocides. Histological alterations observed in liver of contaminated fishes were regressive changes, such as necrosis, hepatocyte vacuolation, nuclear degeneration, hepatocytes degeneration, sinusoids dilatation, etc. These results indicate that exposure to these herbicides had destructive effects on the liver of *C. gariepinus*.

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Toxicity; herbicides; bioassay; *Clarias gariepinus*; behavioral responses; liver histological changes; mortality

Introduction

Pesticides and related chemicals used during human activities, such as agriculture are poured directly or indirectly into watercourses.[1] Indeed, pesticides are frequently used to effectively control crop pests and improve yields.[2–6] These products include herbicides sometimes called weedkillers.[7] These are active ingredients or formulated products having the property of killing plants.[2,7] The two molecules with phytocidal properties that are commonly used for farming in Africa, particularly in the western part are glyphosate and atrazine. In Benin, these two herbicides are widely used in cotton cultivation, especially in the north of the country.[8]

Derived from glycine, glyphosate is a systemic, total foliar herbicide, penetrating through the leaves and then transported to the roots.[9] Glyphosate, or *N*-phosphonomethyl glycine, is a broad spectrum herbicide, meaning a nonselective one that belongs to the Aminophosphonate family.[10,11] It is an analogue of glycine, a natural amino acid on which a hydrogen atom has been substituted by a phosphonomethyl group at the primary amine function level (R-NH2). Glyphosate is moderately soluble in water (10–12 g L^{-1} at 20 °C at pH = 2) and insoluble in most organic solvents.[12,13] Its Henry constant (2.1×10^{-7} Pa·m^{-3} mol^{-1}) indicates its low volatility in aqueous solution.[11,14] Glyphosate dissipates quickly in water where its half-life varies from a few days to several weeks.[15] Glyphosate has a very high organic carbon-water partition coefficient (28,000 mL g^{-1}), which explains its strong reversible tendency to preferentially switch from water to sediments.[12,13] According to the very low octanol/water partition coefficient (log kow = −4.59 to −1.7) and its low fat solubility, glyphosate is not expected to bioaccumulate in aquatic organisms.[15] The 96 h-LC_{50} reported were 30.015 mg L^{-1} for Channel catfish (*Ictalurus punctatusis*),[12] 0.05–43.65 mg L^{-1} for African catfish (*Clarias gariepinus*),[16] 975 mg L^{-1} for Dusky millions fish (*Phalloceros caudimaculatus*),[17] and 211.80 mg L^{-1} for Redbelly tilapia (*Tilapia zillii*).[18] In Benin where this weedkiller is widely used more than 1,241,644 T/L of total herbicides and 1,359,000 T/L of selective herbicides, all based on glyphosate, were ordered in 2013–2014 for the cotton cultivation only.[19] Previous studies performed in Benin, have reported the presence of aminophosphates residues in the water and sediments of the Agbado river in Savalou, as well as glyphosate with quantities ranging from 0.10 to 1.316 ppb and up to 8.62 ppb, respectively.[20] In Djidja, the levels of glyphosate residues in the water varied from 0.105 to 0.193 g L^{-1}.[21]

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-1-3,5-triazine) is a synthetic herbicide belonging to the class of Triazines. It is a selective systemic herbicide, absorbed mainly by the roots but also by the leaves, with translocation toward the apex via the xylem and an accumulation in the apical meristem and the leaves. Atrazine inhibits

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photosynthesis and interacts with other enzymatic processes.[22] Atrazine is sparingly soluble in water (30 mg L^{-1} at 20 °C), its octanol/water partition coefficient (log Kow = 2.75) close to 3 also indicates its low liposolubility.[23] Its vapor pressure is 2.89×10^{-7} mm Hg at 25 °C and its Henry's constant is 2.36×10^{-9} atm-m^3 mol^{-1}. Atrazine is considered very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment.[23] The acute toxicity of atrazine generally varied between 3 and 45 mg L^{-1} for fish.[24] The species that turned out to be the most sensitive are Rainbow trout (*Oncorhynchus mykiss*) in freshwater with a 96 h-LC_{50} = 5.3 mg L^{-1} and Drum croca (*Leiostomus xanthurus*) in estuarine/marine environments with an 96 h-LC_{50} = 8.5 mg L^{-1}.[25] Gbaguidi et al.[20] reported levels of atrazine of up to 5 ppb in the water of the Agbado river in Benin.

As the two phytocides (glyphosate and atrazine) are increasingly used for crop cultivation in Benin, their concentration in aquatic ecosystems could also increase. The overall objective of this study is to determine, under local laboratory conditions, the effects of the acute concentrations of each herbicide in *C. gariepinus*, an important species of aquatic ecosystems receiving pollutants from the cotton basin of northern Benin. This involves determining the behavioral responses of fish to each herbicide, the 96 h-LC_{50} for each pesticide, and the histological responses of fish through the state of the liver, the primary organ for metabolism, detoxification of xenobiotics, and excretion of harmful substances.

Materials and methods

Animals and maintenance

One hundred and eighty healthy fresh water teleosts, *C. gariepinus* juveniles (mean length 7.26 ± 0.59 cm and mean weight 5.21 ± 3.22 g) were obtained from Benin Aquaculture Research and Incubation Center located in Abomey-Calavi. They were transported to the Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq) at the University of Parakou (Benin) in plastic bags which were sufficiently aired in the day (7.00–9.00 a.m.). Laboratory stock of fish was maintained in glass aquarium containing dechlorinated aerated tap water (capacity: 1000 L). The quality of water was assessed before exposure. Fishes were acclimatized for twelve days (after an initial 48 h stabilization period as required by OECD Test 203 Guidelines) under laboratory conditions (ambient temperature 28 ± 0.5 °C; relative humidity 70 ± 5%; Light: Darkness, 12:12 h) and fed with TOP FEEDS (3 mm and 45% protein) feed twice daily *ad libitum*. During this period, the stock water was changed once every other day to prevent the accumulation of decaying food particles and waste metabolites. The acclimatization tank was continuously aerated with 220 V air pumps and air stones throughout the period.

Chemicals

Two herbicides namely Sharp 480 SL (480 g L^{-1} of glyphosate) and Atraforce (80% atrazine) were used for acute toxicity studies. These chemicals used in the present study were procured from a major pesticide market at Banikoara (Benin). The test solutions for each herbicide were obtained by mixing the products directly with dechlorinated tap water, which is usually done in farming environment. All working stock solutions were made immediately before the tests.

Determination of 96 h-LC_{50}

Feeding was stopped 24 h before the start of the experiment. Each test was performed according to OECD guidelines 203 with some minor adaptations. Ten fishes were placed in each of the 18 glass aquaria containing 25 L of water. After that, Sharp 480 SL (glyphosate) was added as per the following nominal concentrations 0.0; 4.0; 6.0; 8.0; 10.0; and 15.0 mL L^{-1} in triplicate. The tests were performed in semi-static systems with daily renewal of 50% of the aquarium water. The stock solution of glyphosate used in this study was always freshly prepared when needed. After that, the same procedure was carried out for the exposure to Atraforce (atrazine) at different concentrations (0.0; 1.0; 3.0; 9.0; 15.0; and 21.0 mg L^{-1}).

Initial range-finding tests were performed for the two herbicides to ascertain the concentration range to be used in the definitive tests. The test was performed over a 96 h treatment period and dead fishes were removed as the test continued. The number of dead fishes per group was recorded against the time of their death in a tabular form as specified by Sprague.[26] The 96 h-LC_{50} value of glyphosate and atrazine was calculated using CETIS™ v1.9.2.7 (Mckinleyville, CA, USA). The behavioral pattern of fish was monitored regularly under suggested treatment conditions.[27] Before and during the exposure, water-quality parameters were measured daily in all aquaria using standard methods (temperature 26.74 ± 0.05 °C, pH 7.27 ± 0.03, dissolved oxygen 5.78 ± 0.08 mg L^{-1}).

Nominal concentrations were not confirmed by chemical analyses. Accurate measurement of the actual concentrations was considered to be of minor importance in these series of increasing concentrations. In addition, the half-lives of glyphosate (2–83.4 days),[12,13] and atrazine (84 days at 20 °C),[23] in neutral environments are greater than the water-renewal times in our experiments. Furthermore, the active components are not very volatile (vapor pressures: glyphosate $<10^{-5}$ Pa,[11,14] and atrazine 3×10^{-7} mm Hg (0.4×10^{-7} kPa) all at 20 °C.[23] We, therefore, did not expect a significant quantity of these compounds to be lost by volatilization during the study.

Liver histology

Fish livers were removed after dissection according to ethical standards during the test and kept in bottles containing

formalin (4%), are transported to the Research Unit in Environmental and Evolutionary Biology (University of Namur, Belgium) where the histological analyzes were performed. Indeed, a sample (mid-section) of each liver was dehydrated through a graded series of methanol, cleared with toluene, and embedded in paraffin. Each liver was cut at 5 µm. For histological analyses, sections were mounted on glass slides and stained with Hematoxylin, Eosin, and Safran (HES) and examined using light microscopy in a range of magnifications (10–40×) to identify lesions. To assess the effect of pollutants on the degree of utilization of glycogen (energy) in the liver in response to these chemical stress, slides were stained with Periodic Acid-Schiff (PAS) and examined using light microscopy in a range of magnifications (10–40×). A qualitative histopathological assessment was performed using a multi-headed Olympus light microscope to make the results more objective. The results were also semi--quantitatively assessed using a scoring system, modified from the protocol by Bernet et al.[28] The score value and the importance factor for each alteration were multiplied and these results for all the alterations identified were then summed to give a Liver index (I_L) per fish. According to Bernet et al.,[28] the formula used to calculate this indice is:

$$I_L = \sum_{\text{rp}} \sum_{\text{alt}} \left(\alpha_{\text{liv rp alt}} \times \omega_{\text{liv rp alt}}\right)$$

where Liv = liver (constant), rp = reaction pattern, alt = alteration, α = score value and ω = importance factor.

This index was used to compare the severity of the occurrence of liver histological alterations between fish from each treatment. To classify the I_L results according to the severity of the histological response, the results were evaluated according to a classification system,[29,30] based on the scoring scheme by Zimmerli et al.[31]

Class 1 (index <10): Normal tissue structure with slight histological alterations
Class 2 (index 10–25): Normal tissue structures with moderate histological alterations
Class 3 (index 26–35): Pronounced alterations of organ tissue
Class 4 (index >35): Severe alterations of organ tissue

Besides the indices calculated (score value) and pathological importance (importance factor) of lesions, a further point of interest is the prevalence of histopathological features. The prevalence of every alteration was calculated as the percentage of occurrence of an alteration within all fish of each treatment. The formula was:

$$\begin{aligned}&\text{Prevalenceofhistologicalalteration}\\&= (\text{Numberoffishwiththealteration})\\&\times 100/\text{Totalnumberoffish}\end{aligned}$$

Data analysis

Survival values were transformed into logits and normality was checked by the Shapiro-Wilk test, then 96 h-LC_{50} values and 95% confidence intervals of each herbicide were determined. No-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were determined using the Dunnett comparison test. All these values were determined using the computer program CETIS version 1.9.2.[32]

Results

Behavioral responses of exposed fish

Fish exposed to glyphosate and atrazine expressed behavioral abnormalities, such as hyperexcitation, loss of balance, lethargy, surfacing activity, low rate of swimming, vertical position, and color of skin (only in glyphosate treated group) (Table 1). At the beginning of exposure, the fishes were found to be healthy and very active. During the experiment, they tried to avoid the test water for some time by swimming fast, jumping, and other random movements in treated groups. At high concentrations of glyphosate and atrazine, fish expressed hyperexcitation with surfacing activity followed by depth activity. There was loss of balance, exhaustion, and lethargy owing to respiratory incumbency. At last, they took a vertical position with mouth near the water surface and tail downwards direction to gulp the air. Soon, they settled down passively at the bottom of the tank, and then they died. In glyphosate treated group fish became pale as intensity of body color decreased.

Acute toxicity

The times of appearance of the first mortalities during exposure to the various herbicides are shown in Table 2. For the two tested pesticides the first mortalities were observed with the highest concentrations. The first mortality was observed in the glyphosate treatments at 3.33 min and in the

Table 1. Behavioral responses of *Clarias gariepinus* juveniles exposed to Sharp 480 SL and Atraforce up to 96 h.

	Sharp 480 SL (1000 ppm)						Atraforce (ppm)					
Behavior	0.0	4.0	6.0	8.0	10.0	15.0	0.0	1.0	3.0	9.0	15.0	21.0
Hyperexcitation	−	−	+	++	++	++	−	+	++	++	+++	+++
Loss of balance	−	−	+	++	++	++	−	+	+	++	+++	+++
Lethargy	−	−	+	++	++	+++	−	+	+	++	+++	+++
Surfacing activity	−	−	+	++	++	+++	−	+	+	+++	+++	+++
Depth activity	−	−	+	++	+++	+++	−	+	+	++	+++	+++
Low rate of swimming	−	−	+	++	++	+++	−	+	++	+++	+++	+++
Vertical position	−	−	+	++	++	+++	−	+	+	++	+++	+++
Color of skin	−	+	++	+++	+++	+++	−	−	−	−	−	−

The sign (+) indicates that 1–30% of the fish are affected by the alteration, (++) = 40–60% of fish affected, (+++) = 70–100% of fish affected.

Table 2. Times (min) of the first mortality and cumulative 96 h mortality rate by concentration tested for each herbicide.

Sharp 480 SL			Atraforce		
Treatments (1000 ppm)	First mortality (min)	96h mortality rate (%)	Treatments (ppm)	First mortality (min)	96h mortality rate (%)
0.0	–	0	0.0	–	0
4.0	80.33	16.67	1.0	32.33	26.67
6.0	28	36.67	3.0	23.33	43.33
8.0	23.66	73.33	9.0	19.33	63.33
10.0	4.33	100	15.0	19.33	90
15.0	3.33	100	21.0	7.66	100
Probability		$P = 0.05$	Probability		$P = 0.1$

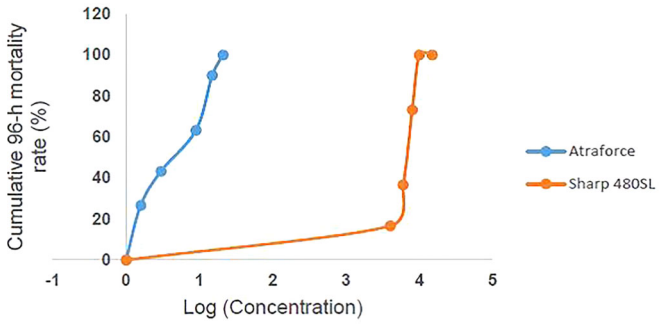

Figure 1. Cumulative 96 h mortalities of *Clarias gariepinus* juveniles *vs.* herbicide concentration.

atrazine treatments at 7.66 min. Cumulative 96 h mortalities of *C. gariepinus* juveniles versus herbicide concentration are presented in Figure 1. Lethal concentrations of Sharp 480 SL and Atraforce for *C. gariepinus* as well as NOEC and LOEC are indicated in Table 3. As the herbicide concentration increases, the mortality rate also increases. The 96 h-LC_{50} value of Sharp 480 SL was 6.175×10^3 ppm. That of Atraforce was 3.165 ppm. The NOEC and LOEC obtained for Sharp 480 SL were respectively 4×10^3 and 6×10^3 ppm and those of Atraforce, respectively <1 and 1 ppm.

Liver histology

Prevalence of liver alterations

Table 4 shows the prevalence of the various histological lesions observed in the liver of fish exposed to various herbicides. A total of seven alterations were observed in the exposed fish, all regressive lesions. The most prevalent lesions regardless of the herbicide were hepatocyte vacuolation and glycogenic depletion (up to 100% in the highest concentrations). Only 1–2 alterations were observed (Vacuolation and Melano-macrophagic Centers) at very low rates (5%) in unexposed fish. Some images of these alterations are presented in Figure 2.

Semi-quantitative analysis of liver alterations

The Liver index I_L of control fishes remained in Class 1 ($I_L < 10$). The I_L of all herbicides treated fish reached Class 1 or 2 ($10 < I_L \leq 35$). In general, I_L appeared higher in high concentrations of herbicides (Fig. 3). The most toxic herbicides to *C. gariepinus* juveniles created more liver damage and these changes are proportional to the toxicity level.

Table 3. Lethal concentrations (LC_{5-90}) of Sharp 480 SL and Atraforce for *Clarias gariepinus* as well as NOEC and LOEC.

Lethal concentrations	Sharp 480 SL (×1000)	Atraforce
LC5	3.512 (n/a to 4.072)	0.2778 (n/a to 0.5493)
LC10	4.053 (2.9 to 4.623)	0.515 (0.08279 to 0.9447)
LC15	4.428 (3.516 to 5.001)	0.7549 (0.2419 to 1.319)
LC20	4.734 (3.946 to 5.306)	1.007 (0.4248 to 1.692)
LC25	5.002 (4.295 to 5.572)	1.277 (0.6329 to 2.077)
LC40	5.713 (5.138 to 6.284)	2.264 (1.438 to 3.404)
LC50	6.175 (5.634 to 6.768)	3.165 (2.179 to 4.596)
LC90	9.407 (8.428 to 11.64)	19.45 (12.27 to 46.23)
Slope	6.95 (4.998 to 8.903)	1.691(1.189 to 2.194)
Intercept	−5.463 (−7.086 to −3.839)	−0.9413 (−1.276 to −0.4152)
P-value	<0.05	<0.05
NOEC	4	<1
LOEC	6	1

NOEC: no-observed effect concentration; LOEC: lowest observed effect concentration; n/a: <detection limit.

Discussion

Alterations of behavioral patterns

The behavioral study gives direct responses of the fish to the pesticides and related chemicals.[1] Radhaiah et al.[33] and Warner et al.[34] stated that the behavioral activities of an organism represents the final integrated results of a diversified biochemical and physiological processes. The fish from the control groups were free from such type of behavioral changes. However, the increase in these different reactions when the toxicant increases in the environment, confirms that these behavioral changes are induced by these herbicides. These neurobiological signs show the neurotoxicity of these two tested phytocides. These behavioral responses found in this study were also reported in *C. gariepinus* while exposed to acetamiprid, lambdacyhalothrin and Acer 35 EC.[35] These reactions were found in Spotted snake head (*Channa punctatus*) contamined to mercurichloride and Malathion,[36] hexavalent chromium,[37] and cypermethrin and λ-cyhalothrin.[38] Changes, such as hyperexcitation, loss of balance, lethargy, surfacing activity, depth activity, low rate of swimming and vertical position, were induced in *C. gariepinus* by the two herbicides tested in this study. Hyperexcitation in some fish is a reflex directly linked to the nervous system.[39] It has been reported that under stress condition, the fishes become hyperactive, perhaps to get out of the stressful environment and would require an increased amount of oxygen to meet their energy demand.[40] Surfacing activity and vertical position in fish can express hypoxic stress caused by toxicants,[41,42] or respiratory distress which is one of the first symptoms of pesticide poisoning.[43] Low rate of swimming is also associated with the

Table 4. Prevalence (%) of histological alterations identified in the liver of *Clarias gariepinus* exposed to increasing acute concentrations of Sharp 480 SL and Atraforce.

	Sharp 480 SL (1000 ppm)						Atraforce (ppm)					
Alterations	0.0	4.0	6.0	8.0	10.0	15.0	0.0	1.0	3.0	9.0	15.0	21.0
Necrosis	–	–	–	10.0	15.0	20.0	–	10.0	10.0	20.0	25.0	25.0
Hepatocyte degeneration	–	–	–	–	–	–	–	–	–	10.0	10.0	25.0
Vacuolation	5.0	20.0	50.0	100	100	100	5.0	100	100	100	100	100
Nuclear degeneration	–	–	–	–	–	–	–	–	–	–	5.0	15.0
MMC	5.0	10.0	10.0	30.0	20.0	30.0	–	30.0	30.0	30.0	90.0	95.0
Sinusoids dilatation	–	–	–	–	–	–	–	–	–	10.0	10.0	25.0
Glycogenic depletion	–	100	100	100	100	100	–	100	100	100	100	100

MMC: melano-macrophagic centers.

possible nervous disorder.[44] The loss of balance, depth activity and then lethargy are the last steps before the fish dies. Skin discoloration was observed in this study only in the glyphosate group. Similar changes in coloring were reported in guppy fish (*Poecilia reticulate*)[45] and *O. mykiss* juveniles[46] exposed to alpha-cypermethrin and deltamethrin, respectively. Skin discoloration is also observed in *C. gariepinus*[35] and in Nil tilapia (*Oreochromis niloticus*)[47] exposed to acetamiprid and Acer. The change in skin color has also been reported in Barramundi (*Lates calcarifer*) when treated with glyphosate.[48] Adewoye[49] explained this discoloration of the skin observed with the fingerlings and the adults of *C. gariepinus* exposed to soap and detergent effluents to the weakening or inhibition of melanin production, due to the extent of the depletion of the oxygen content in major constituents of melanin. However, Pandey et al.[50] explained that the loss of pigmentation was attributed to dysfunction of the endocrine/pituitary gland under toxic stress, causing changes in the number and area of mucus glands and chromatophores.

After these successive behavioral responses, most of the fish died, especially at the high level of pollutants concentrations. Indeed, chemical stress induced by toxicants and behavioral responses, such as hyperexcitation, rate of swimming, depth activity, surfacing activity, etc., generate metabolic costs that only fish in an adequate physiological state, therefore having a reserve of energy to be allocated to this activity can ensure.[51,52] However, during this time of exposure to acute concentrations, the fish are deprived of food, so the death of the fish could be assimilated to the depletion of their energy reserves.[53]

Mortalities of *C. gariepinus* juveniles exposed to acute concentrations of pesticides

The median lethal concentration (96 h-LC_{50}) is one of the most important parameters for evaluating the toxic effects of pollutants. The fish in the control aquarium were observed to be healthy and normal and no mortality was recorded in it. The mortality of fish increased in line with the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration applied. The LC_{50} for Sharp 480 SL, after 96 h treatment was found in a present study to be 6.175×10^3 ppm for *C. gariepinus* juveniles. Sharp 480 SL's active ingredient being glyphosate at a level of 480 g L^{-1}, an LC_{50} for glyphosate is calculated to be 2964 ppm. Sharp 480 SL and its active component can thus be considered as nontoxic to juveniles of *C. gariepinus* following the international classification of toxicity of substances based on their median lethal concentration.[54] Previously reported LC_{50} values of glyphosate in *C. gariepinus*, *T. zillii*, *O. niloticus*, *C. punctatus*, Arabian pupfish (*Aphanius dispar*), Sheepshead minnow (*Cyprinodon variegatus*), and *P. caudimaculatus* were 0.05–43.65,[16] 211.80,[18] 16,8,[55] 32.540,[56] 115.25,[57] 240,[58] and 975 ppm,[59] respectively. The present study also showed that the glyphosate is nontoxic to *C. gariepinus*, in comparison to other pesticides, such as LC_{50} of cypermethrin at 0.063 ppm,[60] diethyl phthalate at 3.931 ppm,[44] diazinon at 7.3 ppm,[61] fenthion at 39.97 ppm,[62] chlorpyrifos at 160 ppb,[63] acetamiprid at 265.7 ppm, lambdacyhalothrin at 0.00083 ppm and Acer at 0.210 ppm.[35]

The 96 h-LC_{50} of Atraforce was calculated and found to be 3.165 ppm. As Atraforce is a formulation that contains 80% atrazine, an $LC_{50} = 2.532$ ppm is calculated for atrazine. The toxicity of Atraforce or atrazine in the present study can be considered moderately to *C. gariepinus*, following the international classification of toxicity of substances based on their median lethal concentration.[54] Previously reported LC_{50} values of atrazine in *C. punctatus*, Caspian kutum (*Rutilis frisii kutum*), Blue gill (*Lepomis macrochirus*), Common carp (*Cyprinus carpio*), *O. niloticus*, Grass carp (*Ctenopharhyngodon idella*), Mangar (*Luciobarbus esocinus*), and *C. gariepinus* were 42.38,[56] 24.95,[64] 16.0,[65] 18.8,[66] 9.37,[67] 80.0,[68] 44.30,[69] and 183.0 ppm.[70] The present study showed that the atrazine is moderately toxic to *C. gariepinus*, in comparison to other pesticides, such as LC_{50} of cypermethrin at 0.063 ppm,[60] chlorpyrifos at 160 ppb,[63] lambdacyhalothrin at 0.00083 ppm and Acer at 0.210 ppm,[35] and Thionex 350 EC at 0.22 ppm.[53]

Based on LC_{50}, NOEC, and LOEC, the relative acute toxicity of these herbicides tested were: Atraforce > Sharp 480 SL. Indeed, the LC_{50} of Sharp 480 SL is 1951 times the one of Atraforce. Regarding the physico-chemical characteristics of each of the two phytocides, particularly their respective octanol/water partition coefficient, atrazine is potentially more toxic than glyphosate. In addition, the major metabolite of glyphosate in water under aerobic or anaerobic conditions, aminomethylphosphonic acid (AMPA), is also nontoxic with respect to these physico-chemical characteristics.[13] Glyphosate is only an analogue of glycine, a natural amino acid on which a hydrogen atom has been substituted by a phosphonomethyl group at the primary amine function R-NH2.[71]

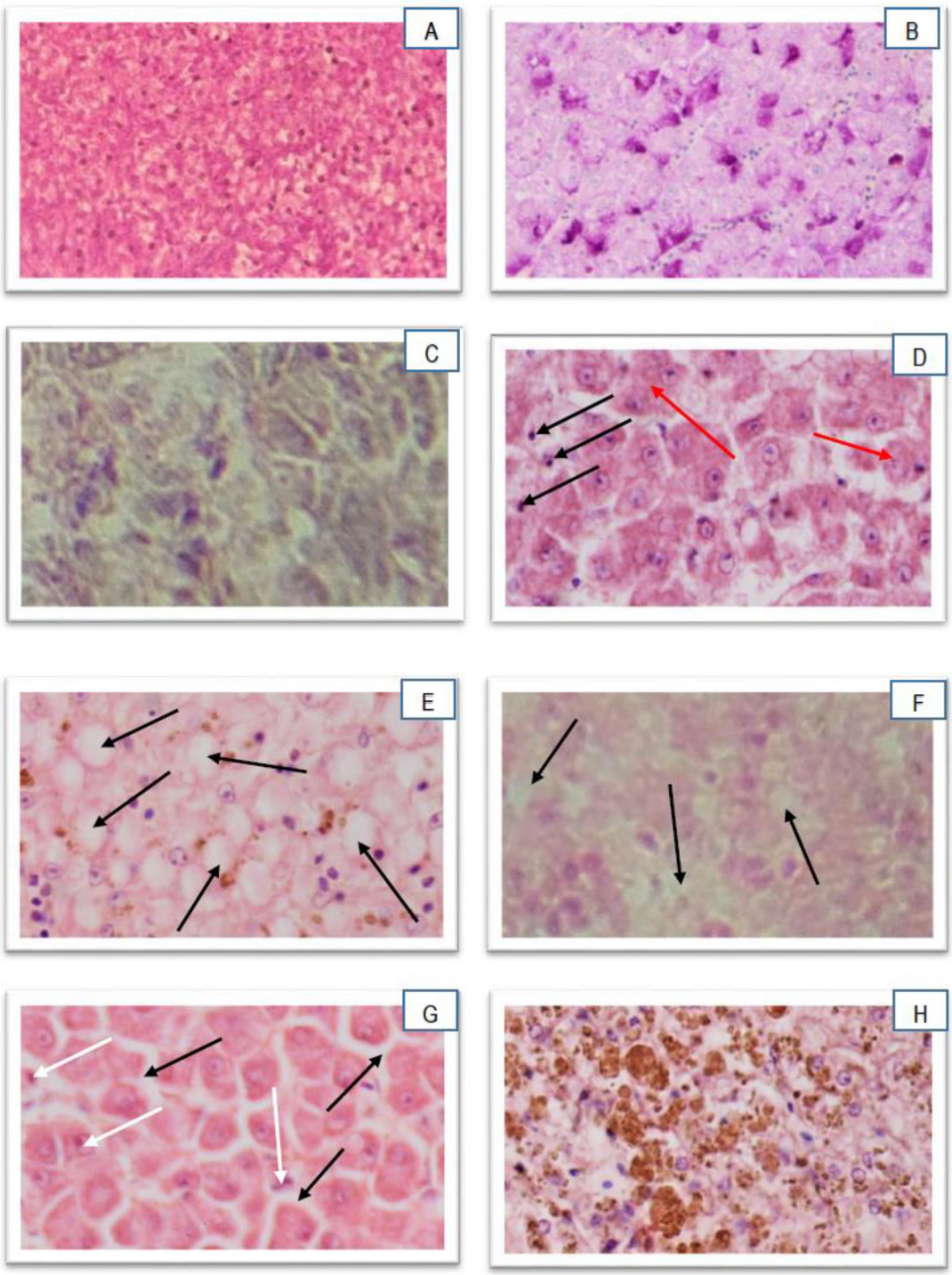

Figure 2. Light micrographs of liver sections in *Clarias gariepinus* exposed to different concentrations of Sharp 480 SL or Atraforce for 96 h (40× objective): (A) Control, exposed to 0.0 ppm of Sharp 480 SL, showing normal liver; (B) Control, exposed to 0.0 ppm of Sharp 480 SL, showing normal liver with PAS positive; (C) Fish exposed to 15,000 ppm of Sharp 480 SL showing liver section with PAS negative; (D) Fish exposed to 15.0 ppm of Atraforce showing liver section with nuclear degeneration (black arrows) and hepatocyte degeneration (red arrows); (E) Fish exposed to 9.0 ppm of Atraforce showing liver section with hepatocyte vacuolation (black arrows), (F) Fish exposed to 10,000 ppm of Sharp 480 SL showing liver section with foci of necrosis (black circle); (G) Fish exposed to 3.0 ppm of Atraforce showing liver section with hepatocyte degeneration (white arrows) and sinusoids dilatation (black arrows); (H) Fish exposed to 4000 ppm of Sharp 480 SL showing liver section with MMC. (A,D–H) Stained with HES and (B,C) stained with PAS.

Based on the order of the first mortalities found during exposure to different herbicides, the following classification of the pesticides studied can be made: Sharp 480 SL > Atraforce. The first mortalities appeared in the highest concentrations of glyphosate and atrazine in the first 10 min post-exposure. This time to the appearance of the first mortalities is very short and <2 h post-exposure of the first mortalities found by Guedegba et al.[47] in the exposure of juveniles of *O. niloticus* to Acer and its active ingredients lambdacyhalothrin and acetamiprid. The very short time-frame obtained in the present study is justified on one hand by the fact that *C. gariepinus* is a species without scales

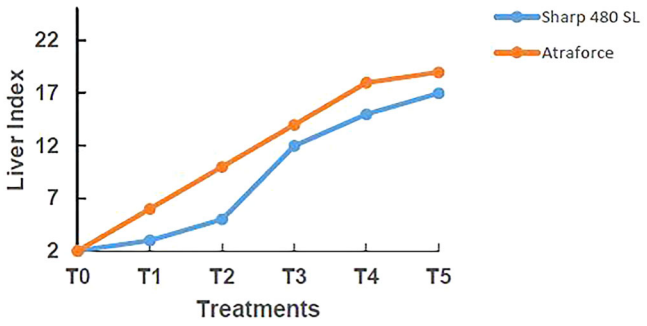

Figure 3. Liver index in *Clarias gariepinus* juveniles exposed to increasing acute concentrations of Sharp 480 SL and Atraforce.

which therefore offers more entry surface for pollutants and, on the other hand, by the fact that the toxicants are not the same and thus their modes of action are different.

Liver histology

The liver is the main organ for detoxification[72] that suffers serious morphological alterations in fish exposed to pesticides.[73] Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. In another study, necrosis, hepatocyte degeneration, vacuolation, nuclear degeneration, CMM (Melano-macrophagic center), sinusoids dilatation and glycogenic depletion have been reported in the *C. punctatus* exposed to chlorpyrifos,[74] in *C. gariepinus* exposed to diazinon,[61] *C. carpio* subjected to chlorpyrifos,[75] and in Liver catfish (*Heteropneuste fossilis*) subjected to pentachlorophenol.[76] Vacuolation, degeneration of hepatocytes and focal necrosis have been reported in *P. conchonius* exposed to carbaryl and dimethoate.[77] In the present study, glycogen depletion were the most prevalent alteration, observed in the fish livers exposed to each of these two herbicides. The glycogen content was significantly reduced in the liver of contaminated fish regardless of the pesticide, as found by Agbohessi et al.[78] in an exposure of *C. gariepinus* to endosulfan and by Pandey and Dubey[76] in an exposure of *H. fossilis* to pentachlorophenol. Similar observations were also reported in other fish species, such as *C. punctata*, *O. mykiss*, Zebra danio (*Danio rerio*), and Grey mullet (*Liza ramada*), following exposure to several toxicants.[79,80] This may be due to either increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity[81] or reduced intestinal absorption of carbohydrates.[82] The second prevalent lesion was vacuolation, observed in the livers of fish exposed to each of these two pesticides tested. This alteration is due to an accumulation of lipids within hepatocytes forming lipidic vacuoles. These vacuoles appear empty of foam as the lipids are removed during processing.[83] Fat infiltration also occurs within the liver as fat is deposited within the liver. This alteration has been identified in fish as a result of fat imbalances and nutrient deficiencies.[84] In our study, fish were not fed during the test making this hypothesis plausible. For Gingerich,[85] the vacuolation of hepatocytes might indicate an imbalance between the rate of synthesis of substances and the rate of their release into circulation. This enhanced number of lipid droplets indicated a decline of protein synthesis in the cytoplasm, which ultimately blocks the utilization of lipid–protein conjugation.[86] Another alteration was the presence of single necrotic cells and foci of necrosis. Necrosis of some portions of the liver tissue is characteristic of the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification and similar to the observation of Rahman et al.,[87] Ayoola and Ajani,[60] Agbohessi et al.[78,88] The inability of the fish to regenerate new liver cells may also have led to necrosis. Nuclear degeneration and hepatocytes degeneration, observed in our study, lead to necrotic liver cells. Another prevalent alteration in the present study was the presence of MMC. MMC have detoxification and recycling function, as they break down endogenous and exogenous materials, thus acting as metabolic dumps. Metabolic pigments normally make up the majority of a center and each pigment can be specially stained for. In this study, the presence of vacuolation and MMC in control groups when no pesticide was applied suggests that these liver alterations are occurring quite naturally within fish populations and are not necessarily induced by pollutants, but it's the increase in its size and frequency in fish exposed to agricultural pesticides which is pathologic.[89] When the pollutant content of the environment is high and the fish liver has to surpass itself to detoxify these toxicants, the vessels responsible for supplying the liver with blood are under great strain. To adapt to this intense strain on the liver, the hepatic sinusoids can also dilate for easier circulation of the blood, as found by Agbohessi et al.[88] in *C. gariepinus* exposed to endosulfan.

From T3 group for Sharp 480 SL and from T2 group to T5 for Atraforce the most toxic of the two herbicides, the mean liver index values calculated according to Bernet et al.[28] for each treatment fell within Class 2 of normal tissue structures with moderate histological alterations. Based on liver histology, the results showed that fish exposed to these herbicides were in bad shape, particularly those exposed to Atraforce. Similar liver indexes were also found by Agbohessi et al.[78] in *C. gariepinus* exposed to endosulfan and Tihan.

Conclusion

In conclusion, glyphosate is nontoxic while atrazine is moderately toxic to juvenile of *Clarias gariepinus*. They have the potential to damage the physiology of *C. gariepinus* leading to observed changes in behavioral pattern with resultant dose dependent mortality. Histopathological results clearly indicate that acute concentrations of these pesticides have destructive effects in the liver tissues of *C. gariepinus*. Exposures of *C. gariepinus* to sublethal concentrations of these herbicides must be carried out in order to know the effects on growth and reproduction, a guarantee of the renewal of fish stocks and the maintenance of aquatic biodiversity.

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Data availability statement

The data used to write this article are available and will be provided as far as is reasonable.

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