

Comparative Toxicological Effects of the Herbicide, Atrazine, on Fingerlings and Juveniles of African Catfish, *Clarias gariepinus* (Burchell, 1822)

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Abstract

This research was aimed at evaluating the toxic effects of atrazine, a commonly used herbicide on acetylcholinesterase (ACHE) activity, lipid peroxidation and testosterone levels in different growth stages of African catfish, *Clarias gariepinus* (Burchell, 1822). The acute and the chronic evaluations were conducted while activities of acetylcholinesterase, lipid peroxidation and testosterone levels were determined using predetermined sub-lethal concentrations of the herbicide. The mean 96-h lethal concentration (LC50) of atrazine exposed to catfish fingerlings and juveniles were 0.350 mg.L⁻¹ and 0.553 mg.L⁻¹, respectively. Acetylcholinesterase activity was higher in the brain of juveniles (25.0 μmol.mL⁻¹) exposed to the herbicide than that of the fingerlings (17.0 μmol.mL⁻¹), and a similar pattern was observed in the liver. Malondialdehyde was higher in the fingerlings (liver and brain) (2.7 and 2.0 μmol.mL⁻¹) exposed to the herbicide than the juveniles (1.8 μmol.mL⁻¹ and 1.6 μmol.mL⁻¹). Testosterone was not detected in the serum of fingerlings exposed to atrazine herbicide. The results of this study elucidated potential biomarkers for monitoring fish health in rivers receiving runoffs of the herbicide, atrazine, and the risk of loss of fisheries productivity attributable to the anti-androgenic properties of the herbicide.

Keywords: farm runoffs, agrochemicals, lipid peroxidation, acetylcholinesterase, endocrine disruption

Introduction

As developing countries strive for self-sufficiency in agriculture, it is expected that the use of agrochemicals such as herbicides will also increase as farmers try to optimise productivity per hectare of land. Agrochemicals pose a considerable threat to aquatic life due to runoffs which enter into nearby lakes, rivers and streams. Atrazine is commonly used as agrochemicals and it has the capacity to be transported through long distance, as a result their residues have been detected in food and households (Mahmood et al. 2016). The cause of toxicity they pose to aquatic and other non-target fauna cannot be excluded from over application and poor agro-extension services to educate farmers.

Atrazines are classified under the group of herbicides known as the S-triazine group, and they are important water contaminants which pollute various water

bodies, including marine and freshwater (Tasli et al. 2009). Its mechanism of activity in weeds and other plants has been reported to include disruption of biological structures and processes, or the promotion of uncontrolled growth and inhibition of photosynthesis (William et al. 2008; Dodge et al. 2010; Giddings et al. 2011). Atrazine can remain in water bodies over long periods, especially in bodies of water with high pH. Several reports have paid attention to the route of entry of atrazine in water (Wauchope 1978; Ren et al. 2002). Atrazine is known to be an endocrine disruptor which is potent at environmentally low levels, and it has been shown to affect the larval development process of amphibians in a study conducted by Hayes et al. (2010). Atrazine alters biochemical and physiological processes and enzymatic activities of fish in polluted waters containing different levels of the herbicide (dos Santos and Martinez 2014).

Agrochemical exposures have been associated with effects on non-target organisms, which include nervous system disruptions and other physiological effects. Inhibition of acetylcholinesterase (ACHE) activity in the synapse, as well as a few other enzymes, has become the gold standard in evaluating the effects of these chemicals on biota (Chambers and Carr 1993). Sex hormones are known to play very important roles in many physiological activities in vertebrates, especially in reproduction. Testosterone, which is a serum hormone, is abundantly produced in gonadal tissues under the control of pituitary gonadotropins (GTH) and is important for reproductive success and survival of aquatic species (Taghizadeh et al. 2013). Solomon et al. (2008) reported that several studies had evaluated atrazine effects on sex steroids in fish; however, most studies have been conducted on adult fish. But there is a dearth of information comparing the effects of this herbicide on both fingerlings and juveniles.

In this study, the effect of exposure to sub-lethal of atrazine on the ACHE enzymes, lipid peroxidation of cell membranes (using malondialdehyde) and changes in testosterone level were investigated in the African catfish fingerlings and juveniles as a way of determining locally relevant biomarkers to protect our valuable freshwater fishes. Investigating these toxic effects is important in determining the sensitivity of the aquatic species to atrazine, which is useful in determining biological indicators of this herbicide due to chronic exposures to very small concentrations alongside runoffs. The information from this study will help fill some knowledge gaps in understanding effects and streamlining useful biomarkers to help with environmental monitoring and risk assessment studies of herbicides around farming communities.

Materials and Methods

Experimental design

One hundred fingerlings and one hundred juveniles of *Clarias gariepinus* (Burchell, 1822) were obtained from Premier Fisheries Limited, Ikorodu, Lagos state, which is a major supplier of farm-bred fishes for households. Since the fishes are sensitive to changes in water quality, they were transported in aerated bags to minimise stress and avoid mortality. Ice was added to the bags to decrease the temperature of the water. The catfish were taken to the laboratory and transferred into an acclimatisation tank (60 × 30 × 30 cm) three-quarters filled with dechlorinated tap water. The water temperature change was minimised during the transport of fish and in the receiving tanks to reduce stress. The mean weight and length of the fingerlings and juveniles were 4.2 ± 1.23 g, 0.95 ± 0.11 cm and 9.8 ± 2.10 g and 1.35 ± 0.15 cm, respectively. The test organisms were acclimatised for 2 weeks during which the water was renewed daily to minimise the toxic effects of metabolic waste products. The

fishes were fed twice daily with fish pellet (Copens® Feed, Nigeria) during the period of acclimatisation. Feeding was discontinued during the 96-h test period. The toxicity test was then carried out in two phases, i.e. the acute and the chronic evaluations.

The herbicide, atrazine is a white, crystalline solid product of Ciba-Geigy Corp. Greensboro, NC. Commercial powdered formulation of atrazine was obtained and used for this study from the local pesticide market in Lagos, Nigeria.

Physico-chemical conditions in bioassay during toxicity testing

During the bioassay, the pH, temperature and dissolved oxygen (DO) values remained fairly constant at 7.4 ± 0.3 , 26 ± 3 °C and 5.7 ± 0.4 mg.L⁻¹, respectively, from the beginning to the end. These parameters were measured with the aid of digital instruments, Jenway products model 3000 series of pH and DO meter.

Acute toxicity evaluation

After acclimatisation, the toxicity range to establish the specific concentrations of atrazine that would be used for the acute experiment was determined following the procedures of ASTM (1997). Five concentrations of atrazine were established in replicates for exposure of fingerlings and juveniles; these were 0.25 mg.L⁻¹, 0.30 mg.L⁻¹, 0.35 mg.L⁻¹, 0.40 mg.L⁻¹, 0.50 mg.L⁻¹ and the control. The herbicide, atrazine, was dissolved in distilled water, and used in the bioassay, according to Pluta (1989). Eight fish were introduced into each concentration, including a control experiment to determine the toxicity on fingerlings and juveniles. Mortalities were recorded at the end of 96-h to determine the 96-h lethal concentration (96-h LC50). The fish were considered dead when they did not respond to prodding with a glass rod after being observed for almost 15 min.

Chronic toxicity evaluation

After determining the acute toxicity, the fish were then exposed to 1/100th of the 96-h LC50 values which was 0.035 and 0.055 for fingerlings and juvenile respectively. A control experiment was also set up. The herbicide mixture was changed every 48 h to prevent toxic effects due to waste accumulation for the 14 days. Glass aquaria measuring 60 × 30 × 30 cm with 40 L of dechlorinated tap water were used for the experiment (Nwani et al. 2010). Ten fishes of each age group were together introduced into separate glass containers and used for the different assays in replicates. The control set up was maintained with the fresh water with no atrazine concentration. Mortality was not recorded in the sub-lethal exposures of the herbicides.

Determination of acetylcholinesterase activity and lipid peroxidation level

Tissue collection and preparation

After the 14-days assay, the exposed and control fishes were removed from the experimental medium, handled carefully, and the desired organs and tissues (liver and brain) were collected to be analysed for the biochemical assays. Five fish were sacrificed by cervical decapitation per test concentration. The liver was dissected out carefully, washed in ice-cold 1.15 % potassium chloride (KCl) solution, blotted and weighed. They were then homogenised in homogenising buffer (50m M Tris-HCl mixed with 1.15 KCL and pH adjusted to 7.4), using a motor-driven Teflon Potter-Elvehjem homogeniser. The resulting homogenate was centrifuged at $10,000 \times g$ for 20 min in a refrigerated centrifuge (2900 Uniscope, United Kingdom) at 4 °C following the method of Hermes-Lima et al. (1995), Nwani et al. (2010) and Doherty et al. (2016). The supernatant was subsequently used for the enzyme assays.

Extraction of acetylcholinesterase

Tissue samples collected from the fish were homogenised (Model-RL 1036, India) using sodium phosphate buffer and phenylmethylsulfonyl fluoride in ice at 4 °C for an hour. The supernatant from the tissue preparation was added into a 96-well microplate containing 50 μ L of Cu^{2+} solution, and the samples were incubated at 25 °C for 30 min. The activity of ACHE in the exposed fish was investigated using the adapted procedure of Ellman et al. (1961). The absorbance was measured using a 96-well microplate at a wavelength of 405 nm. Next, 200 μ L of sodium phosphate buffer (0.1 M, pH 7.0), 20 μ L of 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB) (0.1M) and 10 μ L of ACHE were loaded into the well and incubated for 15 min. After 15 min of incubation, 20 μ L of substrates - 5.0 mM acetylthiocholine iodide (ATC), butyrylthiocholine iodide (BTC) and propionylthiocholine iodide (PTC) were added into the mixture and incubated for another 10 min to allow for the reaction to occur. The ACHE activity was expressed as the quantity of substrate (μ M) hydrolysed by ACHE per minute. A darkroom was used to carry out the experiment (Hayat et al. 2016).

Assay for lipid peroxidation

Lipid peroxidation analysis of liver and brain samples was done following the procedure of Ohkawa et al. (1979). Liver and brain were weighed and homogenised manually in a glass tissue grinder using 0.05M phosphate buffer solution as a homogenising medium to yield a concentration of 100 mg net tissue weight per mL of the homogenising medium. A 1.0 mL of homogenate prepared in KCl solution was incubated at 37 °C for 30 min. Proteins were precipitated by adding 1 mL of 10 % trichloroacetic acid (TCA) and

then centrifuged at $2,000 \times g$ for 15 min. The organic layer was obtained, and the absorbance measured spectrophotometrically at 535 nm.

Testosterone analysis procedure

Blood samples were collected from the caudal vein using a non-heparinized syringe. Serum was collected from blood samples and stored at -20 °C until the time of analysis. The levels of testosterone were determined using non-heparinized blood samples, which were centrifuged for 5 min at $3000 \times g$, according to Mohammad et al. (2012). The levels of the testosterone were determined with an automatic analyser (Persige 24 I, Anthos 2020, Stat fax).

Statistical analysis

The recorded mortality values after the acute toxicity experiment were subjected to probit analysis using SPSS software (SPSS 2004). The susceptibility of the life stages to the pesticides was determined as follows:

Susceptibility factor (S.F.) = (96-h LC50 of other test organisms)/(96-h LC50 of most sensitive organism)

Data obtained from the sublethal assay were subjected to one-way analysis of variance, and Duncan post hoc tests to compare means at $P < 0.05$ (SPSS 2004). Results were presented as mean \pm standard deviation. The P value of less than 0.05 was declared significant.

Quality assurance

To ensure the reliability of the results, quality assurance precautions and procedures were taken by ensuring that all experiments were controlled and organs for biochemical analysis were blind tested to remove biasness. Experiments were carried out in line with the principles guiding animal use in toxicology.

Results

Median lethal concentration (LC50)

Abnormal and uncoordinated swimming behaviour were observed once the experimental tanks were dosed with respective concentrations of atrazine. At the start of the experiment, the fish stop swimming and remained in a static position trying to acclimatise to the changing environment. In tanks with higher concentrations of atrazine (0.35 mg.L⁻¹, 0.40 mg.L⁻¹ and 0.50 mg.L⁻¹) the fish were seen gasping for air. Their erratic behaviours continued until mortality resulted for some within the 96 h. The LC50 value of atrazine was determined to be 0.350 mg.L⁻¹ for fingerlings, and 0.553 mg.L⁻¹ for juveniles (Table 1). The control experiment did not record any mortality.

Susceptibility factor (SF)

The susceptibility factor of the catfish to atrazine was determined to be 1.57 for juveniles and 1.0 for fingerlings. The fingerlings were, therefore, 1.57 times more susceptible than the juveniles (Table 1).

Table 1. Susceptibility factor (S.F.) of the test organisms exposed to atrazine.

Test organism	LC50(mg.L ⁻¹)	S.F.
Juvenile	0.55	1.57
Fingerlings	0.35	1.00

Activity of ACHE in the exposed and control fingerlings and juveniles (liver and brain)

Fingerlings of *C. gariepinus* exposed to atrazine herbicide recorded significantly lower ($P < 0.05$) activity of ACHE (20.6 $\mu\text{mol.mL}^{-1}$) in their liver than the control (23.5 $\mu\text{mol.mL}^{-1}$) (Fig. 1) whereas the activity of ACHE was significantly higher ($P < 0.05$) in the liver samples of exposed juveniles (21.1 $\mu\text{mol.mL}^{-1}$) compared to control (18.8 $\mu\text{mol.mL}^{-1}$).

The activity of ACHE was significantly higher ($P < 0.05$) in the brains of control fingerlings (18.7 $\mu\text{mol.mL}^{-1}$) than those exposed (17.0 $\mu\text{mol.mL}^{-1}$) to atrazine herbicide. In contrast, ACHE activity was significantly lower ($P < 0.05$) in the brain of control juveniles (11.7 $\mu\text{mol.mL}^{-1}$) than those exposed to 25.0 $\mu\text{mol.mL}^{-1}$. However, ACHE activity was significantly higher in the brains of juveniles than the fingerlings exposed to the herbicide (Fig. 1).

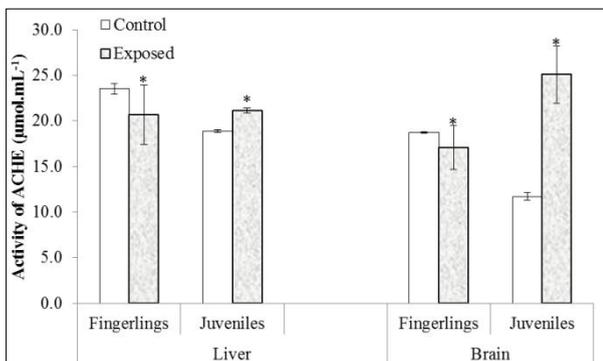


Fig. 1. Activity of acetylcholinesterase (ACHE) in the juveniles and fingerlings of *Clarias gariepinus* exposed to atrazine herbicide. * in figure indicates 'significantly different' values in the exposed and control *C. gariepinus*. Error bars in the figure represent the standard deviation.

Comparatively, in the liver and brain of exposed catfishes, the activity of acetylcholinesterase was

significantly higher in the juveniles when compared with the activity in the fingerlings.

Oxidative stress marker in exposed fingerlings and juveniles (liver and brain)

The levels of lipid peroxidation product (malondialdehyde - MDA) in the juveniles and fingerlings (liver and brain) exposed to sub-lethal concentrations of atrazine herbicide is presented in Figure 2. The mean MDA levels were significantly higher ($P < 0.05$) in the liver samples of fingerlings exposed to atrazine (2.7 $\mu\text{mol.mL}^{-1}$) than the control (1.7 $\mu\text{mol.mL}^{-1}$). Similarly, mean MDA levels were significantly higher ($P < 0.05$) in the liver of juveniles (1.8 $\mu\text{mol.mL}^{-1}$) than the control (0.8 $\mu\text{mol.mL}^{-1}$). MDA was also significantly ($P < 0.05$) higher in the liver of fingerlings than those of juveniles.

The levels of lipid peroxidation were observed to be significantly higher in the brains of exposed fingerlings (2.0 $\mu\text{mol.mL}^{-1}$) than the control (0.88 $\mu\text{mol.mL}^{-1}$). A similar result was also obtained in the exposed juveniles, which had MDA levels of 1.6 $\mu\text{mol.mL}^{-1}$ compared to 0.65 $\mu\text{mol.mL}^{-1}$ in the control (Fig. 2). Lipid peroxidation product (MDA) was, however higher in the brains of fingerlings exposed to the herbicide than the juveniles.

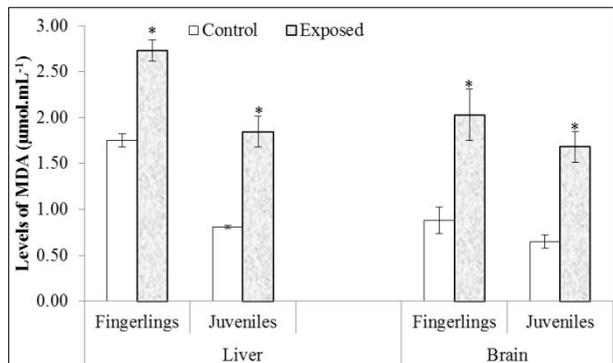


Fig. 2. Levels of lipid peroxidation (MDA) in juvenile and fingerlings of *Clarias gariepinus* exposed to atrazine herbicide. * in figure indicates 'significantly different' values in the exposed and control *C. gariepinus*. Error bars in the figure represent the standard deviation.

Level of testosterone in the serum

Testosterone was not detected in the serum of exposed fingerlings (Fig. 3). However, in the exposed juveniles, a very low concentration of 0.002 ng.mL^{-1} was recorded. Testosterone was however recorded in the control fingerlings (0.06 ng.mL^{-1}) and juveniles (0.14 ng.mL^{-1}).

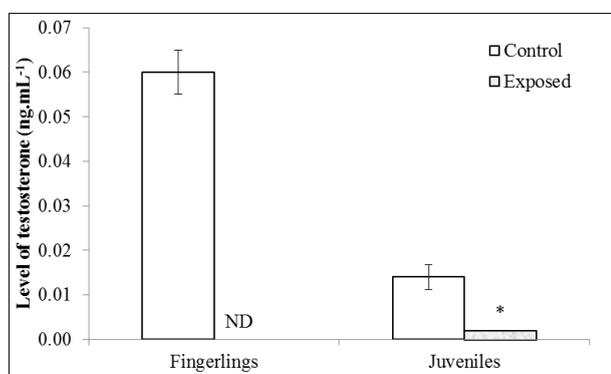


Fig. 3. Level of testosterone in the serum of juvenile and fingerlings of *Clarias gariepinus* exposed to atrazine. * in the figure indicates 'significantly different' between the exposed and control *C. gariepinus*. Error bars in the figure represent the standard deviation. ND means not detected.

Discussion

Fishes play important roles in the aquatic food web; they respond to low levels of toxicants and can bioaccumulate contaminants. These characteristics make them useful as sentinel organisms in toxicological studies (Pandey et al. 2018). The physicochemical parameters of the test water (containing atrazine) measured during the bioassay were within acceptable values for the growth of *C. gariepinus*. Ajiboye et al. (2015) stated that the optimum pH level for growth of fish should be within the range of 6.5 and 9, and the value recorded from this study was within that range. The temperature and dissolved oxygen values were within the normal temperature range in water bodies in the tropics (FEPA 1991). This implies that the changes in fish behaviour and mortality observed during bioassay were not due to poor water quality but due to the herbicide.

The findings from the toxicity evaluation indicated a dose-dependent relationship to mortality and sublethal effects associated with exposure to concentrations of atrazine. This is similar to the findings of Obiezue et al. (2014) in which a direct relationship between mortality in *C. gariepinus* and concentration of diethyl phthalate was recorded.

Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Santos and Martinez 2012). The present study shows that the LC50 for atrazine at 96 h was 0.350 mg.L⁻¹ and 0.553 mg.L⁻¹ for fingerlings and juvenile. This result is lower when compared to 24.35 mg.L⁻¹ obtained by Santos and Martinez (2012) for *Rutilus frisii kutum* (Kamensky, 1901) exposed to atrazine. Tomlin (2000) and Brodeur et al. (2009) recorded LC50 values of atrazine for bluegill sunfish *Lepomis macrochirus* Rafinesque, 1819 and toads *Rhinella arenarum* (Hensel, 1867) to be 16 mg.L⁻¹ and 14.41 mg.L⁻¹, respectively. Furthermore the results also were markedly different compared to

those reported by Hussein et al. (1996) and Kreutz et al. (2008) who reported that the LC50 96-h of atrazine were 9.37, 76, 4.3 and 10.2 mg.L⁻¹ *Oreochromis niloticus* (Linnaeus, 1758), *Cyprinus carpio* (Linnaeus, 1758), *Poecilia reticulata* (Peters, 1859) and *Rhamdia quelen* (Quoy & Gaimard, 1824), respectively. These previous investigations when compared with the results of the present studies, indicates that the fingerlings and juveniles of catfishes are very susceptible to the toxic effects of atrazine.

The findings from this study also showed that the fingerlings were more susceptible to atrazine. According to Abdul-Farah et al. (2004), the differences in results of the toxic effects may be attributed to age, size, health and fish species. Varying LC50 values of agrochemicals occur in different fish species, and this results in the difference in susceptible or tolerance to the toxicant, which is also associated with the process of accumulation, biological transformation and excretion of toxicants (Singh 2013). The concept of age-dependent response and susceptibility to toxicity as observed in this study agrees with the findings of Kousar et al. (2016) who noted based on determined 96-h LC50 values that 90-day old fishes showed the highest sensitivity followed by 120-day olds and 150-day olds to aluminium, revealing that the younger fishes were most sensitive. Results from this study also revealed that atrazine could elicit abnormal swimming behaviours in the exposed fishes. This agrees with the findings of Mohammad et al. (2012), who observed similar behavioural changes in fish exposed to diazinon. This could be attributed to an increase in oxygen demand and an attempt by the fish to offset the effect of atrazine in the water by gasping for air.

Malondialdehyde (MDA) is a clinical marker of oxidative stress, specifically lipid peroxidation (LPO) which occurs in organisms exposed to toxicants. The occurrence of lipid peroxidation is a result of the damaging effects of reactive oxygen species (ROS) on the cell membrane. ROS and oxidative stress have been demonstrated to be triggers of apoptosis (Nwani et al. 2010) and the resulting levels of the commonly measured aldehyde; malondialdehyde has become a notable measure of oxidative damage.

The levels of MDA in the liver samples analysed was observed to be significantly higher ($P < 0.05$) in the brains of *C. gariepinus* fingerlings and juveniles exposed to the atrazine herbicide than the control. The liver is noted as a site of multiple oxidative reactions and maximal free radical generation (Nwani et al., 2010) and thus has been a consistent focus for studies determining the effects of oxidative stress on biological systems. Malondialdehyde was, however, significantly different in the brains and livers of fingerlings exposed to the herbicide than the juveniles. This is attributable to the sensitivity of the fingerlings being younger and not fully developed

compared to the juveniles. These results are in tandem with the findings of Santos and Martinez (2012) for the liver, spleen and gills of *Plotosus lineatus* (Thunberg, 1787) exposed to atrazine. In contrast to this study, Lushchak et al. (2009) did not observe high levels of lipid peroxidation in the brain and liver of goldfish *Crassius auratus* (Linnaeus, 1758) exposed to chronic levels of Roundup®, an agrochemical. The different responses probably are functions of differences in species susceptibility to agrochemicals exposure, the time of exposure, type and concentration of stressors. MDA is considered a useful biomarker of exposure to toxicants because of their consistency in various studies involving exposure to a toxicant. They represent a dose-dependent parameter whose release is always associated with higher levels of oxidative stress, correlating consistently with inhibition or excessive release of antioxidative stress enzymes such as catalase and superoxide dismutase (Amaeze et al. 2015).

In this study, high activity of ACHE was observed in the exposed juvenile catfishes compared to control, while lower activity was observed in fingerlings indicating inhibition of synaptic activities in this case. The inhibition of ACHE in the fingerlings is consistent with the report of Doherty et al. (2016) who observed the lower activity of ACHE after catfish exposure to the pesticides, phostoxin and DD Force. Acetylcholinesterase is the classical indicator of effects of organophosphate pesticides. Therefore the observed inhibition observed in the fingerlings exposed to atrazine in this study may imply that herbicides also can influence processes within the synapse.

Also, with respect to testosterone, levels recorded in the control fingerlings were significantly lower in the catfishes to atrazine. This result is similar to the one reported by Mohammad et al. (2012) where *Rutilus frisii kutum*, was exposed to diazinon pesticides, which caused a decrease in testosterone. According to Hayes et al. (2010), salmon exposed to atrazine (≥ 6 ppb) showed a dose-dependent decrease in androgens and reduction in sperm production. The lower levels of testosterone detected in the exposed fish suggests the herbicide could be acting as an anti-testosterone endocrine disruptor. The non-detection of the hormone in the exposed fingerlings is a cause for concern, and this has serious implication for reproductive success. Hecker et al. (2005) also reported that atrazine reduced the level of testosterone in plasma of adult male *Xenopus laevis* (Daudin, 1802). However, these effects are not always consistent when compared with existing literature. For instance, Span'ó et al. (2004) reported that there was no effect on testosterone levels when adult goldfish *Carassius auratus* were exposed to atrazine at $100 \mu\text{g}\cdot\text{L}^{-1}$ for 21 days but a decrease was observed at a higher concentration of $1000 \mu\text{g}\cdot\text{L}^{-1}$. McDaniel et al. (2008) on the other hand also reported no

correlation between levels of testosterone and atrazine concentrations in the plasma of *Rana clamitans* (Latreille, 1801) and *Rana pipiens* (Schreber, 1782) from wetland areas in Ontario. The inhibition of testosterone observed for catfishes in this study suggest its use as a potential biomarker for the detection of adverse effects of exposure to atrazine on reproduction and sexual development. This should elicit concerns giving the importance of the role of reproduction in the sustainability of the aquatic population. The aquatic risk caused by endocrine disruptors can lead to changes in the community structure, reducing the most sensitive species and increasing the most resistant, with the consequent effect on biodiversity (da Silva et al. 2018).

Conclusion

The findings from this study show that atrazine has toxic effect on non-target organisms inhabiting freshwater habitats such as catfishes. Their ability to inhibit ACHE in catfishes though age-dependent is a cause of concern, especially given their ability to also result in damage to cell membranes. Most importantly, atrazine is an endocrine disruptor with potential effects on fisheries resources productivity in the long run. There is, therefore, need to complement the demands of increased agricultural productivity with the protection and availability of fisheries resources which are ecologically important and a key source of proteins in human diets. Farmers also need to be enlightened on the harmful effects of the indiscriminate discharge of these herbicides into nearby streams and ponds.

References

- Abdul-Farah, M., B. Ateeq, M.N. Ali and W. Ahmad. 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere* 55:257-265. <https://doi.org/10.1016/j.chemosphere.2003.10.063>
- Ajiboye, A. O., A.A. Awogbade and O.A. Babalola. 2015. Effects of water exchange on water quality parameters, nutrient utilization and growth of African catfish (*Clarias gariepinus*). *International Journal of Livestock Production* 6:57-60.
- Amaeze N.H, R.O. Adeyemi and A.O. Adebessin. 2015. Oxidative stress, heats shock protein and histopathological effects in the gills Induced by bridge runoffs in African catfish, *Clarias gariepinus*. *Environmental Monitoring and Assessment* 187:1-16.
- American Society for Testing and Materials (ASTM). 1997. Method D2887-93, 'Test method for boiling range distribution of petroleum fractions by gas chromatography'. In: Annual book of ASTM standards. ASTM, Philadelphia, PA. 27 pp.
- Brodeur, J.C., G. Svartz, C. S. Perez-coll, D.J. Marino and J. Herkovits. 2009. Comparative susceptibility to atrazine of three developmental stages of *Rhinella arenarum* and influence on metamorphosis: Non-monotonous acceleration of the time to climax and delayed tail resorption. *Aquatic Toxicology* 91:161-170.

- da Silva, A. P. A., C. D. L. de Oliveira, A.M.S. Quirino, F.D.M. da Silva, R. de Aquino Saraiva and J.S. Silva-Cavalcanti. 2018. Endocrine disruptors in aquatic environment: Effects and consequences on the biodiversity of fish and amphibian species. *Aquatic Science and Technology* 6:35-51. <https://doi.org/10.5296/ast.v6i1.12565>
- Dodge, J.A., A.L. Glasebrook, D.E. Magee, D.L. Phillips, M. Sato, L.L. Short and H.U. Bryant. 2010. Environmental estrogens: Effects on cholesterol lowering and bone in the ovariectomized rat. *Journal of Steroid Biochemistry and Molecular Biology* 59:155-161. [https://doi.org/10.1016/S0960-0760\(96\)00104-5](https://doi.org/10.1016/S0960-0760(96)00104-5)
- Doherty, V. F., M. K. Ladipo, I.A. Aneyo, A. Adeola and W.Y. Odulele. 2016. Histopathological alterations, biochemical responses and acetylcholinesterase levels in *Clarias gariepinus* as biomarkers of exposure to organophosphates pesticides. *Environmental Monitoring and Assessment* 188:312. <https://doi.org/10.1007/s10661-016-5299-y>
- dos Santos, K.C. and C.B. Martinez. 2014. Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicology and Environmental Safety* 100:7-14.
- Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7:88-95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- Federal Environmental Protection Agency (FEPA). 1991. Guidelines and standards for environmental pollution control in Nigeria. Federal Environmental Protection Agency, Lagos. 238 pp.
- Giddings, J.M., T.A. Anderson, L.W. Hall Jr, R.J. Kendall, R.P. Richards, K.R. Solomon and W.M. Williams. 2011. A probabilistic aquatic ecological risk assessment of atrazine in North American surface waters. SETAC Press, Pensacola, FL, USA. 516 pp.
- Hayat, N.M., N.A. Shamaan, M.K. Sabullah, M.Y. Shukor, M.A. Syed, A. Khalid and S.A. Ahmad. 2016. The use of *Lates calcarifer* as a biomarker for heavy metals detection. *Rendiconti Lincei* 27:463-472. <https://doi.org/10.1007/s12210-015-0501-7>
- Hayes, T.B., V. Khoury, A. Narayan, A. M. Nazir, A. Park, T. Brown, L. Adame, E. Chan, D. Buchholz, T. Stueve and S. Gallipeau. 2010. Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). *Proceedings of the National Academy of Sciences of the United States of America* 107:4612-4617. <https://doi.org/10.1073/pnas.0909519107>
- Hecker, M., J.W. Park, M.B. Murphy, P.D. Jones, K.R. Solomon, G.J. Van Der Kraak, J.A. Carr, E.E. Smith, L.H. Du Preez, R.J. Kendall and J.P. Giesy. 2005. Effects of atrazine on CYP19 gene expression and aromatase activity in testes and on sex steroid concentrations in plasma of male African clawed frogs (*Xenopus laevis*). *Toxicological Sciences* 86:273-280. <https://doi.org/10.1093/toxsci/kfi203>
- Hermes-Lima, M., W.G. Willmore and K.B. Storey. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. *Free Radical Biology and Medicine* 19:271-280. [https://doi.org/10.1016/0891-5849\(95\)00020-X](https://doi.org/10.1016/0891-5849(95)00020-X)
- Hussein, S. Y., M.A. El -Nasser and S.M. Ahmed. 1996. Comparative studies on the effect of herbicide atrazine on freshwater fish, *Oreochromis niloticus* and *Chrysiichthys auratus*. *Bulletin of Environmental Contamination and Toxicology* 57:503-510. <https://doi.org/10.1007/s001289900218>
- Kousar, S., M. Javed and R. Ilyas. 2016. Assessment of acute toxicity of aluminum to the four freshwater fish species (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*). *Pakistan Journal of Agricultural Science* 53:491-495. <https://doi.org/10.21162/PAKJAS/16.2369>
- Kreutz, L.C., L.G. Barcellos, T.O. Silva, D. Anziliero, D. Martins, M. Lorenson, A. Marteninghe and L.B. da Silva. 2008. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings. *Ciência Rural Santa Maria* 38:1050-1055. <https://doi.org/10.1590/S0103-84782008000400022>
- Lushchak, O.V., O.I. Kubrak, M.J. Storey, B.K. Storey and I.V. Lushchak. 2009. Low toxic herbicide roundup induces mild oxidative stress in gold fish. *Chemosphere* 76:932-937. <https://doi.org/10.1016/j.chemosphere.2009.04.045>
- Mahmood, I., R.I. Sameen, S. Kanwal, G. Alvina and R.H. Khalid. 2016. Effects of pesticides on environment. *Plants Soil and Microbes* 4:254-269. https://doi.org/10.1007/978-3-319-27455-3_13
- McDaniel, T., P.A. Martin, J. Struger, J. Sherry, C.H. Marvin, M.E. McMaster, S. Clarence and G. Tetreault. 2008. Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture. *Aquatic Toxicology* 88:230-242. <https://doi.org/10.1016/j.aquatox.2008.05.002>
- Mohammad, N.M., M. Soltani, A. Kamali, M.R. Imanpoor, I. Sharifpour and H. Khara. 2012. Effects of organophosphate, diazinon on some haematological and biochemical changes in *Rutilus frisii kutum* (Kamensky, 1901) male brood stocks. *Iranian Journal of Fisheries Sciences* 11:105-117.
- Nwani, C.D., W.S. Lakra, N.S. Nagpure, R. Kumar, B. Kushwaha and K.S. Srivastava. 2010. Toxicity of the herbicide atrazine: Effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa Punctatus* (Bloch). *International Journal of Environmental Research and Public Health* 7:3298-3312. <https://doi.org/10.3390/ijerph7083298>
- Obiezue, R.N., C.B. Ikele, B.O. Mgbenka, I.C. Okoye, G.N. Attamah, C. Uchendu, E. Ezeamachi, and C.Q. Onyia. 2014. Toxicity study of diethyl phthalate on *Clarias gariepinus* fingerlings. *African Journal of Biotechnology* 13:884-896. <https://doi.org/10.5897/ajb2013.13210>
- Ohkawa, H., N. Ohishi and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. *Analytical Biochemistry* 95:351-358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Pandey, A.K., N.S. Nagpure, and S.P. Trivedi. 2018. Genotoxicity assessment of pesticide profenofos in freshwater fish *Channa punctatus* (Bloch) using comet assay and random amplified polymorphic DNA (RAPD). *Chemosphere* 211:316-323. <https://doi.org/10.1016/j.chemosphere.2018.07.182>
- Pluta, H.J. 1989. Toxicity of several xenobiotics and waste water effluents measured with a new fish early life stage test. *Ger. Journal of Basic and Applied Zoology* 76:195-220.
- Ren, J., K. Jiang and H. Zhou. 2002. The concentration and source of atrazine residue in water of Guanting reservoir. *Huan Jing Ke Xue* 23:126-128 (in Mandarin).
- Santos, T.G and C.R. Martinez. 2012. Atrazine promotes biochemical changes and DNA damage in a Neotropical fish species. *Chemosphere* 89:1118-1125. <https://doi.org/10.1016/j.chemosphere.2012.05.096>
- Singh, R.N. 2013. Acute toxicity of an organophosphate, dimethoate to an air breathing fish, *Colisa fasciatus* (Bl. & Schn.). *Indian Journal of Scientific Research* 4:97-100.
- Solomon, K.R., J.A. Carr, H.L. Du Preez, J.P. Giesy, J.K. Ronald, E.E. Smith and G.J. Van Der Kraak. 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: A critical review. *Critical Reviews in Toxicology* 38:721-772. <https://doi.org/10.1080/10408440802116496>

- Span'o, L., C.R. Tyler, R. Van Aerle, P. Devos, S.N.M. Mandiki, J.P. Thom'e and P. Kestemont. 2004. Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carasius auratus*). *Aquatic Toxicology* 66:369-379. <https://doi.org/10.1016/j.aquatox.2003.10.009>
- Statistical Package for Social Sciences (SPSS). 2004. Windows release 14.0.0, 19. Standard version, SPSS Inc.
- Taghizadeh V., M.R. Imanpoor, N. Mehdinejad. 2013. Study the seasonal steroid hormones of common carp in Caspian Sea, Iran. *SpringerPlus* 2:193. <https://doi.org/10.1186/2193-1801-2-193>
- Tasli, S., L. Patty, H. Boetti, P. Ravanel, G. Vachaud and C. Scharif. 2009. Persistence and Leaching of atrazine in corn culture in the experimental site of Lacote Saint Andr'e (Is'ere, France). *Archives of Environmental Contamination and Toxicology* 30:203-212. <https://doi.org/10.1007/BF00215799>
- Tomlin, C. 2000. *The pesticide manual: A world compendium*. 12th ed. The British Crop Protection Council. Farnham, Surrey, UK. 1250 pp.
- Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields - A review. *Journal of Environmental Quality* 7:459-472. <https://doi.org/10.2134/jeq1978.00472425000700040001x>
- William, R.D., L.C. Burrill, D. Ball, T.L. Miller, R. Parker, K. Al-Khatib, R.H. Callihan, C. Eberlein and D.W. Morishita. 2008. *Pacific Northwest weed control handbook*. Oregon State University Extension Service, Corvallis, OR. 358 pp.