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Influence of Atrazine on some antioxidant enzymes activities in sub-adult *Clarias gariepinus*

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Abstract

Atrazine is a pre-emergence herbicide that is widely used in Nigeria to control weeds. Its influence on oxidative stress enzymes activities were investigated in some tissues (liver, kidney, gills and heart) of sub adult *Clarias gariepinus*. The LC50 24 hours, 48 hours, 72 hours and 96 hours of the fish was investigated after which the fish were sacrificed to remove the tissues. These were homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4), using a Teflon homogenizer. The homogenate was centrifuged at 10,000g for 20 minutes in a Beckman L5-50B centrifuge at 4 °C to obtain the post mitochondrial supernatant fraction for enzyme analysis. Enzymes concentrations were measured using colorimetric photometry. Analysis of variance showed that oxidative enzymes concentrations were higher in treated groups compared to control ($\alpha = 0.05$). These higher enzymes concentrations in the tissues were as a result of oxidative stress imposed on the tissues by Atrazine.

Keywords: Atrazine, Antioxidant enzymes, Sub-adults, *Clarias gariepinus*, Herbicide.

1. Introduction

Nigerian government imports average of 1.9 million tonnes of fish valued at \$500 million to augment the short fall of fish in the country per year. Nigeria is one of the highest importers of fish in Africa. Increase importation will likely lead to serious depletion of nation's financial resources if not properly controlled. Importation could be reduced by increase in food fish production, which involves taking care of environmental and other predisposing factors that will affect its production. The use of herbicides to control aquatic weeds has been applied in weed management and they are used in aquatic habitat especially rice fields and some fish farms (Wu *et al.*, 2010) [43]. Atrazine is considered the main aquatic herbicide used and is the most commonly detected in ground and surface water (U.S.A. EPA, 2002) [36].

African catfish is one of the most cultured fish in Nigeria due to its ability to withstand some adverse conditions in control environment. It is rated the third most cultured (Offem *et al.*, 2010) [28]. Due to high rate of herbicides use and the fact that the rate of use will continue to increase, it is believed that this will hamper Aquaculture industry in future. The modes of effects on several fishes have been reported (Weeks *et al.*, 1986; Vijayan *et al.* 2001; Visoottiviseth *et al.*, 1999; Wany *et al.*, 1992) [41, 37, 38, 39]. Lethality recorded often arise due to several reasons. Investigation of what occurs in essential organs needs to be carried out. Fish is affected mostly in the liver, kidney, spleen, etc. This makes it necessary to investigate the activities of antioxidants enzymes in these organs of African catfish when exposed to a commonly used herbicide, Atrazine.

2. Materials and methods

2.1. Specimen collection and acclimation

Clarias sub-adults were obtained from the Department of Fisheries and Aquatic Science fish farm located in Obubra campus of CRUTECH, Cross River State. These were transported in plastic Jeri cans in the early hours (between 7.00am and 9.00am of the day) to the laboratory of the same department for acclimation.

The fish were batch weighed using a spring balance (Arca) to the nearest mg (to reduce stress), while the standard length (SL) was measured using measuring board to the nearest mm (Thomas *et al.*, 2003) [33]. The fish were fed at 6 % of their

Body weight per day using pellet size 1.8 to 2mm obtained from COPPENS, www.coppens.eu.

The daily ration was divided into two (3 % body weight) and fed at 10 am and 4 pm (Gilbert, 1996; Ajani *et al.*, 2007; Mills, 1986) [17, 5, 26]. During the two weeks acclimation in the laboratory were fed with industrially made feed pellets containing zero percent concentration of pesticides. The fish were not fed for 48 hours prior to the commencement of the experiment and during the experiment (Omitoyin *et al.*, 2006 and Mills, 1986) [29, 26].

Plastic aquaria of 52 cm length, 38 cm width and 30 cm height were filled with 10 litre of rain water per tank. These were subjected to five different concentrations of Atrazine; and replicated three times. Ten fish with a parent population of weight 76.26 ± 0.92 and standard length (SL) of 22.50 ± 61 cm were selected randomly and stocked in each aquarium (APHA, 1981; Cengiz *et al.*, 2001; Adeyemo, 2005; Ayoola, 2008) [7, 12, 4, 8].

2.2. Biological observations of fish

From the start, the fish were observed every 30 minutes for abnormal behaviours. Interval of observation increased to every one hour after 12 hours for the remaining period of the experiment. Death fish were immediately removed and preserves in 10 % formaldehyde (Ayoola, 2008) [8].

2.3. Water quality parameters

Temperature was measured using mercury in glass thermometer and electronically by WTW OXI 196 to the nearest degree Celsius. Oxygen and pH values were measured electronically using meter model WTW PH 90.

2.4. Determination of antioxidant enzyme activities in fish tissues

The fish were sacrificed by decapitation and dissected to remove the liver, kidney, gills and heart. The tissues were washed in ice cold 1.15% KCl solution, blotted and weighed. These were homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4), using a Teflon homogenizer. The homogenate was centrifuged at 10,000g for 20 minutes in a Beckman L5-50B centrifuge at 4 °C to obtain the post mitochondrial supernatant fraction for enzyme analysis.

Alanine Transaminase (ALT) and Aspartate aminotransferase (AST) were determined by colorimetric methods and Alkaline Phosphatase (ALP) by methods of Bowers & McComb, (1966) [11] and Tietz *et al.* (1983) [34].

Activity of catalase (CAT) was determined as described by (Clairborne, 1995) [14] following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 25°C. Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation as described by (Gutteridge and Wilkins, 1982) [18]. Malondialdehyde is one of the most widely used tests for determining the extent of lipid oxidation in a sample. This measures the concentration of aldehydes. A pink colour is produced from a reaction between thiobarbituric acid and aldehyde when heated for 20 minute. The intensity of pink is directly related to TBA- aldehyde complex. Color intensity was determined by measuring its absorbance at 540 nm using a UV-visible spectrophotometer. The principle source of color is the formation of a complex between TBA and malondialdehyde (MDA). One gram tissue was weighed out, chopped into small pieces and homogenized in a pre-cooled pestle in a mortar placed in a bowl of ice chips. The homogenized tissue was further diluted to obtain a 1:5 dilution. This was stored in freezer at - 4 °C prior to analysis. The precipitate was removed by centrifuging at 2500 rpm for

10 minutes. Absorbance of the clear supernatant was read at 546 nm at 37 °C. The result obtained was expressed in (µmol) MDA formed per gramme net tissue MDA.

The mean lethal concentration (LC₅₀) for 24 hours, 48 hours, 72 hours and 96 hours were computed using probit while the water quality parameters and enzymes concentrations in the tissues were analysed using analysis of variance (ANOVA). The *post hoc* comparison of means was carried out using Duncan’s multiple range test (Frank and Althoen, 1995; Hewlett and Plackett, 1979; Ayotunde, 2006; Jiraungkoorskul, 2002; 2003; Ayoola, 2008; Shallangwa and Auta, 2008) [16, 19, 9, 21, 22, 8, 31].

3. Results

The pH values were between 6.91 and 7.6 as shown in Figure 1. There were significant differences in hydrogen ion concentrations between treatments. The conductivity expressed in Figure 2 also showed that at high concentration of 15.0 mg/L there were lower conductivity, while Figure 3 expresses the dissolve oxygen concentration which was going down with increasing toxicant concentration.

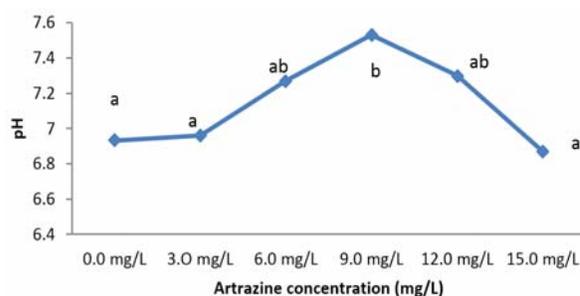


Fig 1: shows the hydrogen ion concentrations of the different treatments. Those means with the same alphabet were statistically the same.

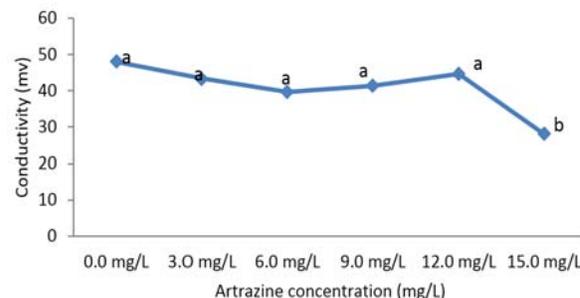


Fig 2: shows the conductivity (mv) of the different treatments. Those means with the same alphabet were statistically the same

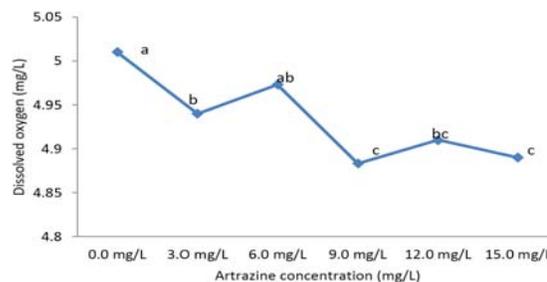


Fig 3: shows the dissolved oxygen concentrations of the different treatments. Those means with the same alphabet were statistically the same. The concentration was reducing with increase in concentration of toxicant.

Table 1: showing the behavioural and biological changes in sub-adult *Clarias gariepinus* exposed to Atrazine. N = no response of fish at that particular concentration while Y = yes there was response

	30 MINUTES						60 MINUTES						2 HOURS						6 HOURS						12 HOURS						24 HOURS											
	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/L	15.0 mg/L						
Loss of reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Molting	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Discoloration	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Air gulping	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Erratic swimming	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Haemorrhage	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	N	N	N	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Swimming upside down	N	N	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

At 12 hours upwards, there was loss of reflex, molting, discoloration, air gulping, erratic swimming, haemorrhage as well as swimming upside down. The LC50 FOR 24, 48, 72 and 96 hours are shown in Figure 4.

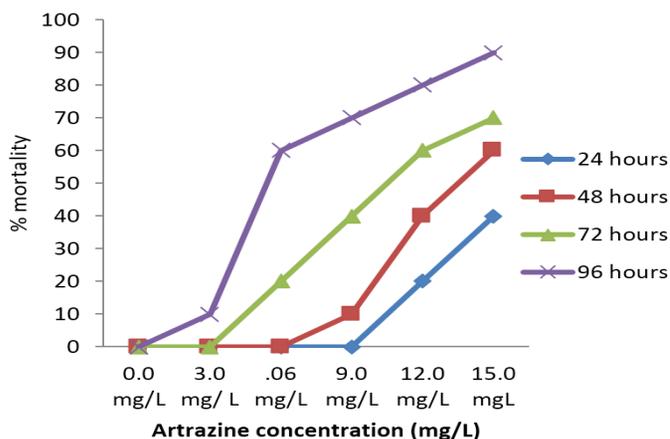


Fig 4: The LC50 24 hrs, 48 hrs, 72 hrs. and 96 hrs. of *Clarias gariepinus* sub adults exposed to Atrazine. No concentration applied was able to kill 50 % of the fish within 24 hour, 50 per cent of the fish were killed at a concentration of 15.0 mg/L of Atrazine. The LC50 72 hours was 11.0 mg/L while that of 96 hours was 6.0 mg/L

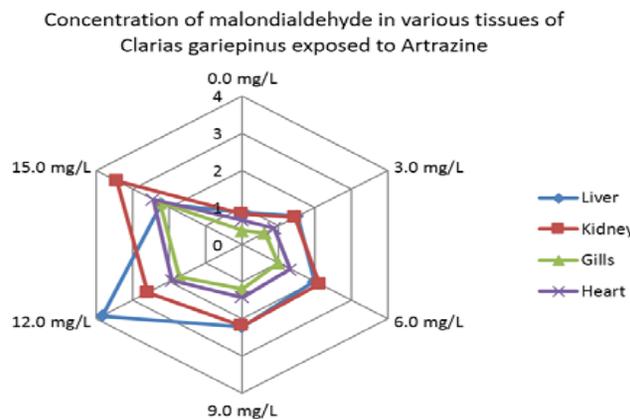


Fig 5: The concentration of Malondialdehyde in the various tissues of sub adult *Clarias gariepinus*. Statistical analysis revealed that the concentration was highest in the liver followed by kidneys and the heart in that order. The least concentration was in the gills. The concentration of this enzyme was also observed to be increasing with toxicant concentration.

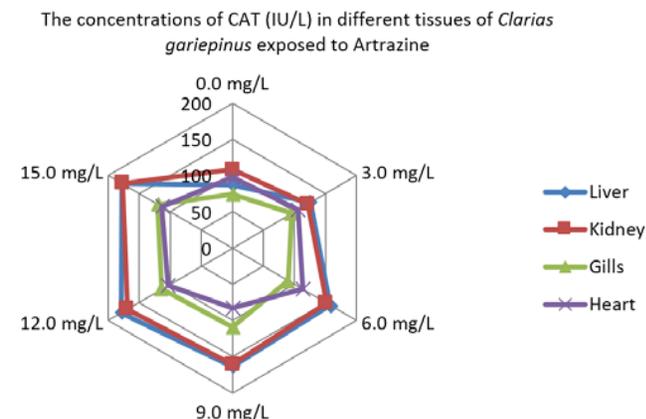


Fig 6: shows the concentrations of catalase enzymes (CAT) in the liver, kidney, gills and heart of sub adult *Clarias gariepinus* exposed to Atrazine. Enzymes activities were higher in the tissues of fishes exposed compare to control group and continue to increase with Atrazine concentration. The liver has the highest concentration followed by the kidney ($\alpha < 0.05$).

The concentrations of AST (IU/L) in different tissues of *Clarias gariepinus* exposed to Artrazine

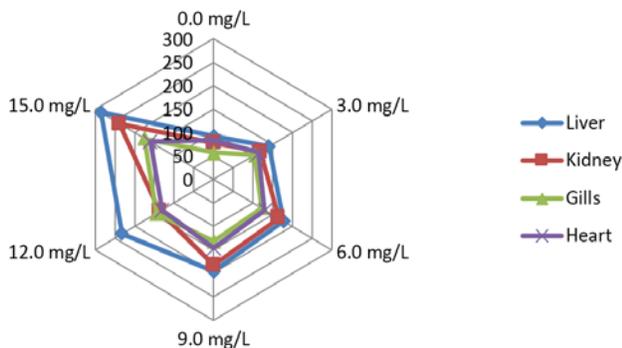


Fig 7: showing the concentrations of Aspartate aminotransferase (AST) in IU/L in the tissues: liver, kidney, gills and heart of *Clarias gariepinus* sub adult exposed to different concentrations of Artrazine herbicide. Observations showed that the concentration was higher in the liver, followed by the kidney, and then gills and heart. The concentration of this enzyme showed a positive relationship with toxicant concentration.

The concentrations of ALT (IU/L) in the various tissues of *Clarias gariepinus* sub adults exposed to Artrazine

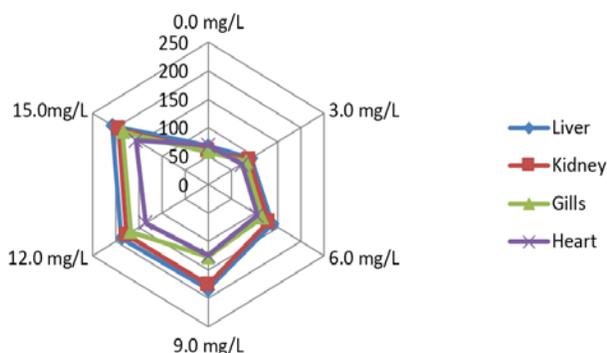


Fig 8: shows the concentrations of Alanine transaminase (ALT (IU/L)) in the various tissues. These were seen to be higher in the liver and kidney are these are detoxification and filtration centres. This enzyme concentration was observed to be increasing as the toxin (Atrazine) was increasing ($\alpha = 0.05$).

The concentration of ALP (IU/L) in the liver, kidneys, gills and heart of *Clarias gariepinus* sub adult exposed to Artrazine

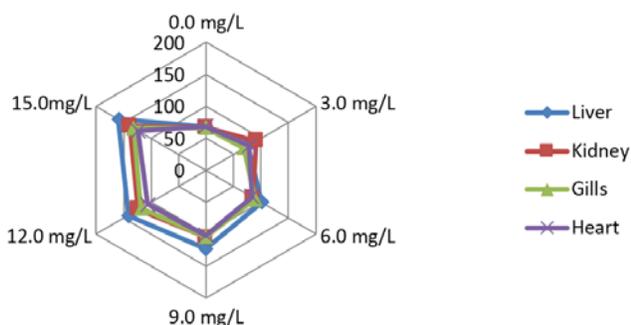


Fig 9: Is a graph showing the concentration of Alanine phosphatase (IU/L) in the tissues of sub-adult *Clarias gariepinus* as was influenced by the concentrations of Artrazine to which the fish was exposed. There were significant differences in the concentrations of the enzymes ($\alpha - 0.05$).

4. Discussion

There were differences in physicochemical parameters of the water as observed in Figures 1 to 3. The values were still within the tolerance limit for fish survival. Although slight changes in the values of these parameter can bring about great changes in the response of the fish to environments. These responses may not be easily observed physically but could go a long way to influence the physiological responses in fish. Atrazine has been shown to affect fishes in slowing down their reflexes, swimming activities and feeding. Hussein *et al.* (1996) [20] attributed these changes to decreased impulse transmitter enzyme (acetyl cholinesterase) activities. The LC_{50} was observed to decrease with time of exposure. This pattern was also observed by Chapadense *et al.* (2009) [13], who reported a LC_{50} 48 hours of 20 mg/l, while exposing *Colossoma macropomum* to Atrazine. This value is higher than the 7.9 mg/l recorded by Ada (2011) [3] while exposing *Oreochromis niloticus* to Artrazine. Ramesh *et al.* (2009) [30] observed that 18.5 ppm (18.5 mg/l) of Atrazine killed 50% of common carp within 24 hours, a value showing more potency than in this experiment where 50 per cent of *Clarias gariepinus* die at concentration of 15 mg/L (15 PPM). This may have been possible due to the hardy and extra breathing ability of this catfish compared with carp and tilapia. Weed Science Society of America (1993) records showed a range of 4 to 19.650 mg/l while exposing Atrazine to different fishes. According to Jaraungkoorskul *et al.* (2002), toxicity of any poison is species and environmental factors related. Death of fish as illustrated by Cengiz *et al.* (2001) [12] could be caused by inhibition of uptake of valuable nutrients from the gut.

Oxygen is an important element in the lives of organisms. It is required in almost all life processes including cellular respiration. Its break down products will remain reactive. Such reactive radicals of oxygen origin are described as free radicals or reactive oxygen species (ROS). Species such as superoxide anion radicals, hydroxyl radicals, perhydroxyl radicals as well as single oxygen do play important roles in several diseases (Kasperska-Zajac *et al.*, 2008) [23].

Malondialdehyde (MDA) was observed to be generally increasing with the concentration of Atrazine in the various tissues of *Clarias gariepinus* including the liver, kidney, gills and heart. MDA is a biomarker for the oxidant activities in organisms. The presence of higher concentrations of MDA with higher levels of Atrazine shows that there were higher levels of oxidative stress imposed on the fish. High oxidative stress is usually the result or the cause of certain diseases in organisms (Kasperska-Zajac *et al.*, 2008) [23].

Atrazine was reported to have increase heart and liver weight in rats. This resulted in reduced food intake and body weight gain at 7.5 mg/kg taken orally in rats. It reduced blood cell count in various organisms (U. S. EPA, 1988) [35]. It increased the frequency of chromatid break in bone marrow in rats. Atrazine has been suspected to cause endocrine system disruption, carcinogenicity, epidemiological disorder and low sperm count in man and has been implicated for low birth weight, birth defect, menstrual problems, retinal and muscular degeneration and mammary tumors (Ackermann, 2007).

Catalase is an enzyme that defends the body against oxidative stress in two ways, namely by peroxidative activity (the oxidation of hydrogen donors) and catalytic activity (decomposition of hydrogen peroxide to produce water and oxygen). The increase of catalase concentration in tissue of fish with higher concentration of Atrazine herbicide agrees with similar findings in rats by Al-Abrash *et al.* (2000) [6] that

reported higher catalytic activities with increase morbidity resulting from stress; emanating from cardiovascular diseases, diabetes, tumor, inflammation dermatological diseases and anemia. Because there is higher release of reactive oxygen species during stress in aerobic cells, the enzyme catalase is produced in higher quantity to help protect the cell against oxidative damage (Kisader *et al.*, 1997) [24].

Alanine aminotransferase (ALT) is a transaminase enzyme also known as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALAT). It is similar to aspartate transaminase (AST) because they are found in the liver and in various bodily tissues. However, ALT has a higher concentration in the liver compared to other tissue in normal health conditions McPhalen *et al.* (1992) [25]. AST is commonly measured clinically as a part of diagnostic liver function tests to determine liver health. AST has been found to be elevated in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute haemolytic infarction, severe burns, renal diseases musculoskeletal diseases, and trauma (Baker *et al.*, 2001) [10]. According to Cox and Nelson (2008) [15], Alanine aminotransferase and Aspartate aminotransferase leak out of injured cells, particularly of the liver and heart. The enzymes are then detected in concentrations which are related in quantity to the extent of the injury to the cells. It is therefore deducible that Atrazine caused different kinds of injuries to the cells of the tissues, which released the enzymes that were detected.

Alkaline phosphate is a hydrolase responsible for removing phosphates (dephosphorylation) from many molecules including nucleic acids, DNA in basic environments (Tamas *et al.*, 2002) [32]. Wikipedia (2014) [42] explained that the enzyme concentration could be elevated during biliary obstruction, osteoblastic bone tumors, hepatitis, sarcoidosis among other problems in mammals. The elevated concentrations in treated groups compared to control were as a result of stress imposed on the fish in the treated groups. Atrazine has been reported by various investigators to be responsible for a variety of damages to the cells. Its use in aquatic environment should be minimised if it could not be completely stopped.

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