AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society

Some biological and hematological responses of *Oreochromis niloticus* juveniles exposed to Atrazine herbicide

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Abstract. This research was aimed at finding the influence of Atrazine on a most widely farmed fish, *Oreochromis niloticus* (Linnaeus, 1758), in Nigeria. Specific areas of investigation were to find the LC_{50} after 24, 48, 72 and 96 hours of the chemical administration to the fish. The effects of the chemical on the behavioral and biological responses of *O. niloticus* were equally observed. The ten fish each were stocked in six different tanks containing 40 litres of water. These tanks contained graded concentrations of the chemical and the treatments were replicated three times. Dissolved oxygen content was reducing with increasing Atrazine concentration, while temperature and pH increased with increasing Atrazine concentration. The LC_{50} of Atrazine in 24, 48, 72 and 96 hours were 7.9, 7.6, 7.3 and 7.2 respectively. Behavioral and biological responses included loss of reflex, air gulping, erratic swimming, discoloration, hemorrhage and molting. The blood parameters observed showed that there were increases in packed cell volume (PCV) and red blood cell count (RBC) and decrease in hemoglobin (Hb), erythrocyte sedimentation rate (ESR) and mean cell hemoglobin (MCH), while there was no significant change in white blood cell count (WBC) and mean cell volume (MCV) values. The chemical was therefore observed to be lethal to juvenile *O. niloticus*.

Key Words: Atrazine, tilapia, Oreochromis niloticus.

Introduction. Tilapia has admirable feature as aquaculture candidate (Popma & Masser 1999; Ekanem & Okoronkwo 2003) and is an important fish all over the world as a fisheries resource. It can grow to large size rapidly and has good flavour (Popma & Masser 1999). It is the most widely cultured fish in the tropics and subtropics, and the second only to carp among the fresh water fish in the world (Offem et al 2010). It is commonly available and easy to manipulate both in capture and culture fisheries due to its qualities such as good taste, hardy nature, fast growth, resistance to diseases, ease with which to reproduce in captivity, switching of diet and tolerance to poor water quality such as low dissolved oxygen levels. Apart from being a good candidate for aquaculture, it serves as important source of high level of animal protein especially in the developing countries where there are high levels of animal protein deficiencies (Fagbenro & Adebayo 2002; Ogunji et al 2008; Uchida et al 2003). According to Holden & Reed (1972) tilapia forms 90% of fish by number in Sokoto River of Nigeria.

Although many newer biocide agents are produced yearly (Mironescu et al 2010), the old ones are still used, even though they are highly toxic for non-target organisms in long term application. Atrazine shows high degree of crop tolerance and soil activity due to its high solubility in water, though lower than other s-triazines. Its uptake is by plants roots as well as young leaves and it has high crop selectivity. Atrazine is scientifically called 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine and it is a photosynthesis inhibitor. It functions by binding to the plastoquinone-binding protein in photosystem II, which animals lack. As a result, plants die from starvation and oxidative damage caused by breakdown in electron transport process. The oxidative damage is quickened in high light intensity (Appleby et al 2002). It is used to stop the development of pre-emergence

and post-emergent broad leaf and grassy weeds in major crops. Because of its effectiveness and relatively low price, it is well suited in production systems with very narrow profit margins as in the production of maize where it was shown to increase corn yield by 5.7 bushels per acre (North Central Weed Science Society, 2008).

It could reach aquatic systems by direct application, spray drift, aerial spray, precipitation, erosion and run off from agricultural lands, by discharge from factories and in sewage and often by misuse (Shallangwa & Auta 2008; Asogwa & Dongo 2009). Because human population is more concentrated around the riparian areas, increase in human population has resulted in increased pollution of aquatic environment with herbicides.

Fish farming is a fast growing business in many parts of the world including Nigeria. Like aquaculture practices, the use of pesticides all over the world is not traditional and particularly in the tropics. Their uses have become particularly relevant only in the past five decades. Similarly, Asogwa & Dongo (2009) reported that the annual quantity of pesticides used in Nigeria is 125,000 - 130,000 metric tones, 31% of which are used in cocoa farm alone. Since herbicides, are able to destroy weeds on a large scale before or on emergence without interfering with the crops and without heavy dependence on human labour, their importance has been and will continue to be felt. Their rate of use is expected to increase in the tropics in the nearest future, especially as crop that are genetically modified to tolerate herbicides are continuing to emerge (Cox 2004; Asogwa & Dongo 2009; Antofie et al 2010). The residues of these chemicals including pesticides, heavy metals, soaps and detergents end up in the aquatic ecosystems (Ogundele et al 2004). Given that at too high concentrations, these chemicals act as poisons, they could therefore, directly or indirectly, influence the productivity of the aquatic ecosystem as well as the health of man himself. In the aquatic environment these chemicals may adversely affect many none target organisms such as fish or amphibians (Petrescu-Mag et al 2010). Even though knowledge about the effect of some pesticides in the environment is very poor, Cengiz et al (2001) stressed that organophosphate insecticides could be absorbed through gills and the digestive tract of the fish thereby resulting in histological alterations. This case is not singular, there is a long list of pesticides, pathways of access into organisms and negative effects reported (Fleseriu 2010; Al-Baggou et al 2011).

According to APHA (1981) and Jauncey & Ross (1982) the 48-96 hour LC₅₀ values are useful values of relative acute lethal toxicity to organisms under specific conditions. These values do not represent the safe concentration in the natural habitats because long term contact with much lower concentrations may be lethal to fish and may cause a lethal impairment of their functions (Al-Qutob & Nashashibi 2009; Al-Qutob et al 2011). Another longer term effect of herbicide is that of the endocrine or hormonal system disruption. By disrupting the hormonal system, a wide range of biological processes such as control of blood sugar, growth and function of reproductive system, regulation of metabolism, brain and nervous system development and development of an organism from conception to adulthood may become impossible (US EPA 2000). The possibility of synergism or antagonism and other multiple effects or multiple toxicants must be considered. Akobundu (1987) called these substances safeners for they are used to modify the selective actions of pesticides. Jones (1983) and Brown & Sadler (1989) as well as other workers cited in Kori-Siakpere & Ubogu (2008) reported similar association of heavy metal toxicity.

Among the numerous studies carried out on the effects of agrotoxicants on fish are those of Oloruntuyi et al (1992) Jiraungkoorskul et al (2002), Abd El Gowad (1999), Kovinznych & Ubancikova (1998), Visoottiviseth et al (1999), Babatunde et al (2001) Agbon et al (2002), Kori-Siakpere et al (2007), Ayotunde (2006), Ayotunde et al (2010ab) and Ada et al (2011). The present study was initiated to investigate the influence of Atrazine on the hemopathology of *O. niloticus* which is a widely cultured species in Cross River State and many parts of Nigeria.

Rice farming, which makes use of large quantities of herbicides, is practiced in the Central and Northern Senatorial districts of Cross River and the adjoining states, such as Benue, Ebonyi and Abia, requires the use of these chemicals in high quantities. The use of these chemicals, if not controlled, is likely to hamper fish production. Chemical method is cheaper than hand weeding, this method has gained ground in recent times and thus results in the introduction of more of these herbicides into the natural aquatic system (US EPA 1988; Extoxnet 2010; Min 1986; Offem et al 2010).

The toxins can be carried from one place to another or one organism to another along a food chain (Shallangwa & Auta 2008). Omitoyin et al (2006) emphasized that the gradual degradation of the aquatic ecosystem can not be ignored. The choice of Atrazine in this experiment was because it is among the most frequently used chemicals in many parts of Nigeria including Cross River State for the control of weeds. Any human activity, which seems to be a threat to this promising species that is likely to reduce the present lag (Daramola et al 2007) of about nine million metric tones between supply and demand of fish and animal protein must be given watchful eyes. It is therefore pertinent to investigate the tolerance limit to fish and other aquatic organisms, because this chemical is beneficial to the farmer and their use can not be prevented.

Material and Method. Specimens collection and acclimation: tilapia juveniles were obtained between the hours of 7.00 and 9.00 from the Faculty of Agriculture and Forestry fish farm, Obubra campus of CRUTECH, Cross River State in January to March, 2012 and transported in plastic buckets to the laboratory of the same campus. The weight and length of fish for the experiments at stocking are displayed in Table 1.

The fish were batch weighed using a spring balance (Arca) to the nearest mg (to reduce stress), while the length was measured using a measuring board to the nearest mm (Thomas et al 2003). The fish were fed at 6% (with 1.8 - 2 mm of Coppens feeds) of their body weight, split into two times (Gilbert 1996; Ajani et al 2007). Feeding at two rations per day has higher digestibility coefficient and higher feed conversion efficiency (Mills 1986) Feeding was discontinued 48 hours prior to the commencement of the experiment and throughout the experiment (Omitoyin et al 2006; Mills 1986; Beitlich et al 1995).

Table 1

Trait	Control	Experiment						
		Range finding test	Definitive test					
Weight	9.70 <u>+</u> 0.47 g	9.97 <u>+</u> 1.61	9.53 <u>+</u> 1.65					
Length	7.07 <u>+</u> 0.98 cm	6.57 <u>+</u> 0.82	6.87 <u>+</u> 0.82					

Initial weight and total length of *O. niloticus* dosed with Atrazine

Experimental design to find the range of chemicals that could kill all the fish in 24 hours: plastic aquaria of 52 cm length, 38 cm width and 30 cm height were filled with stream water up to 20.3 cm level giving a volume of 40 litres of water per tank. These were subjected to five different concentrations of Atrazine as described by Beitlich et al (1995) and Martins et al (2008). Ten fish specimens were selected randomly and stocked in each aquarium (APHA 1981; Cengiz et al 2001; Adeyemo 2005; Ayoola 2008). These experiments were replicated three times (Ayoola 2008) for each concentration and for each chemical treatment.

The procedures for range finding test were repeated for definitive test. But the highest concentration in definitive test in the experiment was derived from the concentration, which killed 100% of tilapia juveniles within 24 hours in the range finding experiment. Six concentrations were also prepared and labeled T1, T2, T3, T4, T5 and T6 for the graded concentrations. The control in the range finding test also served as control for definitive test (Ayoola 2008).

Biological and physicochemical parameters: the fish were observed every 30 minutes for abnormal behaviours. Length of interval of observation increased to every one hour after 12 hours for the remaining period of the experiment. Organs of dead fish were immediately removed and preserved in 10% formaldehyde (Ayoola 2008). Solid tissues were preserved in 10% formalin for histological processing. Temperature was

measured daily using mercury in glass thermometer and electronically by WTW OXI 196 to the nearest degree Celsius. Oxygen and pH were also measured electronically using WTW OXI 196 and WTW PH 90 respectively.

Hematological analysis: a heparinised syringe was used to remove blood from the dorsal blood vessel lying below the vertebral column (Lewbert 2001). About 3-5 mL of blood was taken from fish in each tank for hematological analysis. Fish from the various treatments were analysed for the following hematological parameters: packed cell volume (PCV), erythrocyte sedimentation rate (ESR), hemoglobin (Hb), mean cell hemohlobin (MCH), mean cell hemoglobin concentration (MCHC), erythrocyte count, white blood cell count and mean corpuscle volume (MCV) Ada et al (2011, 2012), Ayotunde et al (2011).

The hematological parameters were analysed using ANOVA at 0.05% alpha level by SPSS, version 13.0. The post hoc comparison of means was carried out using Duncan's multiple range tests (Frank & Althoen 1995). The mean lethal concentration (LC_{50}) for 24 hours, 48 hours, 72 hours and 96 hours were computed using probit and logit (Hewlett & Plackett 1979; Ayotunde 2006; Jiraungkoorskul 2002; Ayoola 2008; Shallangwa & Auta 2008).

Results. Behavioural and biological responses of *O. niloticus* to Atrazine are displayed on Table 2 for definitive test. There was loss of reflex at concentration of 1.9 mg/L. Molting occurred at the 48th hour, at concentration 11.4 mg/L, while colour faded in 72 hours when exposed to 11.4 mg/L. There was air gulping in all the concentrations within 24 hours. There was hemorrage in 48 hours. In 72 hours, there was loss of scales at concentration of 11.4 mg/L while scale loss occurred in 96 hours at concentration of 9.5 mg/L. The mortality as a response to the concentration of Atrazine is shown on Figure 1. The LC₅₀ 24 hours, 48 hours, 72 hours and 96 hours for *O. niloticus* juveniles exposed to Atrazine was observed to be 7.9 mg/L, 7.6 mg/L, 7.3 mg/L and 7.2 mg/L respectively.

The water physical and chemical properties exposed to Atrazine showed significant variation in acid pH. Temperature and dissolved oxygen: oxygen concentration was reducing with increase in concentration of toxicant while temperature was observed to be rising with concentration of Atrazine (see Table 3). Hemogobin, mean cell hemoglobin and mean cell hemoglobin concentration were observed to be falling with herbicide strength as seen in Table 4. The effect of Atrazine on packed cell volume was parabolic. The value dropped with the concentration and continued to rise with Atrazine concentration. Hemoglobin and erythrocyte sedimentation rate values in exposed groups showed significant reduction in values compared to the control. The red blood cell count increased significantly in the exposed groups especially at higher concentrations (9.5 to 11.4 mg/L). White blood cell count and mean cell volume did not show significant difference between control and treated groups. Mean cell volume did not show a significant difference between the exposed and the unexposed groups.

Table 2

Exp /	12	hrs	16 hrs					hrs			20 hrs							24 hrs						
Beh																								
%	1.9	3.8	5.7	7.6	9.5	11.4	1.9	3.8	5.7	7.6	9.5	11.4	1.9	3.8	5.7	7.6	9.5	11.4	1.9	3.8	5.7	7.6	9.5	11.4
L.r	Ν	Ν	Ν	Υ	Υ	Y	Ν	Ν	Υ	Υ	Υ	Y	Ν	Ν	Υ	Υ	Υ	Υ	Ν	Ν	Υ	Y	Υ	Y
Μ	Ν	Ν	Ν	Ν	Ν	N	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Υ	Y	Υ	Y	Y	Y	Y	Y	Υ
D	Ν	Ν	Ν	N	N	Ν	Ν	Ν	N	Ν	Ν	Ν	N	Ν	N	Ν	Ν	Ν	Ν	Ν	Ν	Y	Υ	Υ
A.g	Ν	Y	Υ	Υ	Υ	Y	Y	Υ	Υ	Y	Y	Υ	Ν	Υ	Υ	Y	Y	Υ	Y	Y	Y	Y	Y	Υ
E.s	Ν	Ν	Ν	Ν	Ν	N	Ν	Ν	Ν	Y	Y	Υ	Υ	Υ	Υ	Y	Y	Y	Y	Y	Y	Y	Y	Υ
Н	Ν	Ν	Ν	Ν	Ν	N	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Y	Υ
L.s	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Υ	Ν	Ν	Ν	Ν	Y	Υ

Behavioral changes and biological responses in *O. niloticus* juveniles exposed to different concentrations of Atrazine herbicide (Definitive test)

N - No change in behavior found, Y - Yes, change in behavior found; % - Concentration mg/L; L.r.-Loss of reflex; M – Molting; D – Discoloration; A.g. - Air gulping; E.s. - Erratic swimming; H-Hemorrhage; L.s. - Loss of scale; Exp – exposure time; Beh – behavior.



Figure 1. The LC₅₀ 24hrs, 48hrs, 72hrs and 96hrs of *O. niloticus* juveniles exposed to different concentrations of Atrazine using probit analysis (Definitive test).

Table 3

The physicochemical properties of water exposed to different concentrations of Atrazine during range finding and definitive tests

Dose (mg/L)	Dissolved oxygen	Acid pH	Temperature
1.9	7.7 <u>+</u> 0.90 ^a	6.46 <u>+</u> 1.92 ^b	26.79 <u>+</u> 0.69 ^b
3.8	7.20 <u>+</u> 0.29 ^{ab}	6.86 <u>+</u> 0.61 ^{ab}	26.79 <u>+</u> 0.58 ^b
5.7	7.10 <u>+</u> 0.44 ^{bc}	7.11 <u>+</u> 0.15 ^{ab}	26.82 <u>+</u> 0.52 ^b
7.6	6.99 <u>+</u> 0.42 ^{bc}	7.10 <u>+</u> 0.19 ^{ab}	27.06 <u>+</u> 0.70 ^{ab}
9.5	6.92 <u>+</u> 0.49 ^{bc}	7.34 <u>+</u> 0.34 ^{ab}	27.21 <u>+</u> 0.62 ^{ab}
11.4	6.69 <u>+</u> 0.56 ^c	7.00 <u>+</u> 0.42 ^a	27.43 <u>+</u> 0.62 ^a

Same superscripts show statistically similar means while those that are carrying different superscript show statistically different means.

Table 4

Hematological parameters of O.	niloticus juveniles exposed
to different concentrations	of Atrazine herbicide

Conc.	PCV	Hb	ESR	WBC	RBC	МСН	MCHC	MCV
(mg/L)	(%)	(g/L)	(mm/h)	(103/mm³)	(106/mm ³)	(pg)	(g/dL)	(fL)
0.0	13.00 ^{ab}	5.33 ^a	12.30 ^a	2.77 ^a	1.73 ^b	30.77 ^{ab}	0.41 ^{ab}	7.62 ^a
	<u>+</u> 1.00	<u>+</u> 0.65	<u>+</u> 0.58	<u>+</u> 0.06	<u>+</u> 0.21	<u>+</u> 0.30	<u>+</u> 0.08	<u>+</u> 1.37
1.9	10.33 ^b	3.70 ^b	10.67 ^{ab}	3.20 ^a	1.50 ^b	25.75 ^b	0.36 ^b	7.37 ^a
	<u>+</u> 1.53	<u>+</u> 0.10	<u>+</u> 1.15	<u>+</u> 0.80	<u>+</u> 0.36	<u>+</u> 6.89	<u>+</u> 0.05	<u>+</u> 3.07
3.8	9.17 ^c	4.70 ^a	14.00 ^a	2.87 ^a	1.33 ^b	35.50 ^a	0.52 ^a	6.99 ^a
	<u>+</u> 1.61	<u>+</u> 0.20	<u>+</u> 0.10	<u>+</u> 0.83	<u>+</u> 0.15	<u>+</u> 3.62	<u>+</u> 0.10	<u>+</u> 1.90
5.7	9.40 ^c	4.90 ^a	12.33 ^a	2.80 ^a	1.73 ^b	28.39 ^b	0.52 ^a	5.44 ^a
	<u>+</u> 0.53	<u>+</u> 0.30	<u>+</u> 0.58	<u>+</u> 0.10	<u>+</u> 0.11	<u>+</u> 0.52	<u>+</u> 0.06	<u>+</u> 0.39
7.6	9.57 ^c	3.60 ^b	10.03 ^{ab}	2.70 ^a	1.43 ^b	26.20 ^b	0.39 ^{ab}	6.72 ^a
	<u>+</u> 0.81	<u>+</u> 0.17	<u>+</u> 2.31	<u>+</u> 0.10	<u>+</u> 0.15	<u>+</u> 2.64	<u>+</u> 0.05	<u>+</u> 0.89
9.5	11.50 ^{bc}	3.50 ^b	11.00 ^a	3.13 ^a	2.30 ^a	15.20 ^c	0.31 ^c	4.98 ^a
	<u>+</u> 2.29	<u>+</u> 0.30	<u>+</u> 4.36	<u>+</u> 0.72	<u>+</u> 0.10	<u>+</u> 0.64	<u>+</u> 0.04	<u>+</u> 0.77
11.4	15.33 ^a	3.23 ^b	7.00 ^b	3.20 ^a	2.23 ^a	14.44 ^c	0.23 ^c	0.03 ^a
	+0.06	+0.7	+1.00	+0.40	+0.31	+2.18	+0.09	+2.1

Same superscripts show statistically similar means while those that are carrying different superscript show statistically different means.

Discussion. The LC₅₀ was observed to decrease with time of exposure. This pattern was also observed by Chapadense et al (2009), who reported a LC₅₀ 48 hours of 20 mg/L, while exposing *Colossoma macropomum* to Atrazine. This value is higher than the 7.9 mg/L recorded in this experiment for *O. niloticus*. Ramesh et al (2009) observed that 18.5 ppm (18.5 mg/L) of Atrazine killed 50% of common carps within 24 hours, a value showing more potency than in this experiment while Weed Science Society of America (1993) records showed a range of 4 to 19.650 mg/L while exposing different fishes to Atrazine. 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) could be caused by inhibition of uptake of valuable nutrients from the gut.

A parameter like temperature can influence metabolic rate in the bodies of organisms (Abdullah et al 2009; Petrescu-Mag & Petrescu-Mag 2010), affect density of ambient water of the organism and even food availability (Sverdrup et al 2006). In the present experiment, temperatures varied between 26 and 28°C which are within tolerance limits for the survival of fish (Ayoola 2008). The pH of 6.5-7.5 was also within the tolerance limit of fish survival, it could not have affected the fish, though slight changes in pH could grossly affect ammonium nitrogen toxicity (Ferguson et al 1977). Significant variations in oxygen concentrations were observed, though within tolerance limit, reduced levels as observed with increased Atrazine concentration could have led to stress according to Crane (1973) and Sverdrup et al (2006).

The behavioural patterns observed in the fish such as erratic swimming, air gulping, loss of balance and reflex were attributed to the effect of Atrazine. Jumping to the water surface to gulp air could be traced to two possible causes namely: oxygen depletion as a result of herbicide concentration and irritation caused by dermal contact. This causes irritation of gills as well as hampering gaseous exchange. Hemorrhage was observed in *Colossoma macropomum* exposed to Atrazine from concentrations of 20–25 mg/L by Chapadense et al (2009). In the present study, 9.5 mg/L caused hemorrhage in juvenile tilapia within 96 hours. The lower concentration was able to cause hemorrhage in this fish possibly due to the tender nature of the juvenile fish. This herbicide may have affected the fish nervous coordination as well as the cardiovascular system as was suggested by Ramesh et al (2009). Atrazine has been shown to affect fishes in slowing down their reflexes, swimming activities and feeding. Hussein et al (1996) attributed these changes to decreased impulse transmitter enzyme (acetyl cholinesterase) activities.

Erythrocyte micronuclei increased in the fish, *Colossoma macropomum*, exposed to Atrazine from concentration 10–30 mg/L by Chapadense et al (2009), the herbicide is therefore said to be capable of increasing micronuclei which result in cell damage. The slight but non statistically significant reduction in white blood cells count could likely be due to the damage made to the cells nuclei. The multiplication of blood cells is an adaptation to fight oxygen deficiency and other environmental stressors. Increase in red blood cell number may be an attempt to make more surface area available for oxygen binding. The high cell number and lower weight per cell due to increase cell number may have led to a significantly lower erythrocyte sedimentation rate in the exposed groups as shown at the concentration of 11.4mg/L compared to the control. This probably may be the reason why the concentration of hemoglobin, was observed to be falling with increase in concentration of Atrazine. Reduced hemoglobin means reduced ability to carry oxygen. This was coupled with less dissolved oxygen concentration in the stock solution. The combined effects of these factors provide less oxygen to the tissues for normal metabolism. Such anoxic conditions are capable of morbidity and mortality.

Unexpectedly, the white blood cell count did not increase with poison concentration. Ajani et al (2007) made similar observations in *Clarias gariepinus* exposed to nitrite. The poison may have eliminated them suddenly without the chance to produce young ones to replace the older cells, which was contrary to the findings of Prasad et al (1991), Rusia & Sood (1992), Ayoola (2008), Kori-Siakpere & Ubogu (2008) and Ramesh et al (2009).

The mean cell hemoglobin recorded lower concentration with increasing number of red blood cells per mm³ as well as packed cell volume. The factors which affect the packed cell volume include the size of individual cell as well as the total number of the

cells. Though cells in the control were significantly fewer, they carried significantly higher hemoglobin per cell compared to the exposed groups. The reduced hemoglobin is capable of stressing fish tissues which could at extreme levels result to mortality. Though there was reduction in mean cell volume in the exposed groups, statistical analysis showed that these changes were not significant. Physiological stress may not however be concluded from one parameter only. This can pose chronic poisoning or reduction in cell volume in a long term exposure as earlier suggested by Hardell et al (2002).

Vajayan et al (2001) observed estradiol to significantly lower the key liver enzymes as well as gill lactate enzymes (dehydrgenase and malate dehydrogenase) activities over a 24-hour period in *Oreochromis mossambicus*. They suggested that estradiol impair ion exchange in tilapia mediated by the liver and decrease metabolic capacity of the gills and liver. The decreased tissue metabolic capacity is likely due to estradiol induced energy partitioning process that are geared towards vitelogine synthesis at the expense of other energy demanding pathways. Such effects of xenobiotics as lowering the key liver enzymes, or increasing the specific activity of other enzymes, are frequent reported in many cases of exposure of aquatic animals to individual or synergic pesticides (Belden & Lydy 2000).

Summary and Conclusions. This work on the effects of Atrazine on tilapia juveniles was necessitated by the fact that there has been increased use of pesticides by our farmers in the recent times. These practices have however increase food production due to less labour input in weed management. But for land availability, continual increase in agricultural output would have been expected due to expansion in farm sizes, because many farmers would be able to cultivate more land due to reduction in farming expenditure emanating from reduction in cost of weed control.

The Nile tilapia, which is the highest cultured species in Nigeria and the third most cultured fish species all over the world, could be affected by these chemicals. What is observed on the surface as tolerance may have underlying physiological and histological consequences, hence, the reason for investigating into how these chemicals affect fish behaviours and other biological responses, mortality and hematology. Mortalities observed may have got combinative influence acting on the various organs of the fish.

The chemical Atrazine was observed to change the behavioural and biological responses of *O. niloticus* juveniles. The LC_{50} 96 hours of Atrazine administered to *O. niloticus* in water was 7.2 mg/L. Such stressors may prevent the fish from growing to its maximum size (B ∞), which is expected to reach in a conducive and stress free environment. The chemical is therefore capable of reducing the fish's reproductive capacity as well as embryonic survival rate resulting from altered brood physiology. When these poisons are accumulated in the fish tissue, they could be consumed by piscivores, and directly or indirectly by other animals, including man (situated over the top of the trophic pyramid).

References

- Abd El-Gogwad A. M., 1999 Histopathological studies on the liver and gills of Tilapia nilotica (*Oreochromis nilticus*) exposed to different concentrations of Lead acetate & Zinc sulphate. Journal of Egypts Society of Zoology 30:13-22.
- Abdullah K., Awan A. U., Khattak M. K., Yasmin S., 2009 Biological de-activation of granular formulation of a carbamate insecticide Cartap in water under laboratory conditions. AACL Bioflux 2(2):89-95.
- Ada F. B., Ekpenyong E. E., Ndome C. B., 2012 Haematological, behavioural and biological changes in *Oreochromis niloticus* juveniles exposed to Glyphosate herbicide. Biological Environmental Sciences Journal for the Tropics 9(2):147-154.
- Ada F. B., Ndome C. B., Bayim P. B., 2011 Some haematological changes in *Oreochomis niloticus* exposed to Butachlor. Journal of Agriculture and Food Technology (JAFT) 1(6):73-80.
- Adeyemo O. K., 2005 Haematological and histological effects of cassava mill effluent in *Clarias gariepinus*. African Journal of Biomedical Research 8:175–183.

- Agbon A. O., Ofojekwu P. C., Ezenwaka I. S., Alegbeleye W. O., 2002 Acute toxicity of diazonon to rotifers, cyclops, mosquito larvae and fish. Journal of Applied Science and Environmental Management 6(1):18-21.
- Ajani F., Olukunle O. A., Agbede S. A., 2007 Hormonal and haematological responses of *Clarias gariepinus* (Burchell, 1822) to nitrate toxicity. Journal of Fisheries International 2(1):48–53.
- Akobundu I. O., 1987 Weed Science in the Tropics: Principles & Practices. John Wily and Sons Ltd, New York. 522pp.
- Al-Baggou B. K., Naser A. S., Mohammad F. K., 2011 Hydrogen peroxide potentiates organophosphate toxicosis in chicks. HVM Bioflux 3(2):142-149.
- Al-Qutob M. A., Al-Hirsch I., Nashashibi T. S., 2011 The effects of COX2-inhibitors (etoricoxib and etodolac) on growth rate and mortality in Nile tilapia (*Oreochromis niloticus*). AACL Bioflux 4(5):691-703.
- Al-Qutob M., Nashashibi T., 2009 The effects of COX-Inhibitors (Diclofenac and Ibuprofen) on growth rate, mortality and sex reversal in Nile tilapia (*Oreochromis niloticus*). AACL Bioflux 2(4): 381-390.
- Antofie M.-M., Sand C., Brezeanu A., Doroftei E., 2010 Key elements related to GMOs and novel food. Ann Rom Soc Cell Biol 15(1):148-154.
- APHA, 1981 Standard Methods for the Examination & Waste Water, 15th Edn. APHA AWNA WPCF.
- Appleby A. P., Muller F., Carpy S., 2002 Weed Control in Ullmann's Encyclopedia of Industrial Chemistry, Wiley VCH.
- Asogwa E. U., Dongo L. N., 2009 Problems associated with pesticide usage and application in Nigeria cocoa production; a review. African Journal of Agricultural Research 4(8):675-683.
- Ayoola S. O., 2008 Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. African Journal of Agricultural Research 3(12):825-834.
- Ayotunde E. O., 2006 Toxicity of drumstick, *Moringa oleifera* to Nile tilapia *Oreochromis niloticus* (Linne, 1757), and African catfish, *Clarias gariepinus* (Burchell, 1822). A PhD Thesis submitted to school of post Graduate Studies, the Federal University of Techonology, Akure, Nigeria.
- Ayotunde E. O., Fagbero O. A., Adebayo O., 2010a Toxicity of aequeous extrct of *Moringa oleifera* seed powder to nile tilapia *Oreochromis niloticus* (Linne, 1779) fingerlings. International Research Journal of Agricultural Science 1(4):142-150.
- Ayotunde O. E., Offem B. O., Ada F. B., 2010b Evaluation of the acute and chronic toxicity of the seeds of (*Carica papaya* Linn.) its haematological and piscicidal effects on a freshwater catfish (*Clarias gariepinus*) adults. Journal of Fisheries and Aquatic Science 6(3):291-308.
- Ayotunde E. O., Offem B. O., Ada F. B., 2011 Toxicity of *Carica papaya* Linn.; Haematological and piscicidal effects on adult catfish (*Clarias gariepinus*). Journal of Fisheries and Aquatic Science 6(3):291-308.
- Babatunde M. M., Oludimiji A. A., Balogun J. K., 2001 Acute toxicity of Gamaxone to *Oreochromis niloticus* (Treweva) in Nigeria. Water Air Soil Pollution 13(1-4):1-10.
- Beitlich J. H., Jenning B. H., Lasse B. A., Nelson R. C., Ott J. J., Whitney J. M., Wooly S. K., 1995 Introduction to Fish Health Management. B. A. Lasse 2nd edn. Onalaska, Wisconsin, USA.
- Belden J. B., Lydy M. J., 2000 Impact of Atrazine on organophosphate insecticide toxicity. Environmental Toxicology and Chemistry 19(9):2266–2274.
- Brown D. J. A., Sadler K., 1989 Fish Survival in Acid Water. In: Acid Toxicity and Aquatic Animals, R. Morris, E. N. Taylor, D. J. A. Brown, J. A. Brown (eds.), Society for Experimental Biology. Seminar Series 34. Cambridge. Pp.31–44.
- Cengiz E. I., Unlu E., Balci K., 2001 The histopathological effects of thiodin on the liver and gut of mosquito fish, *Gambusia affinis*. Journal of Environmental Science and Health 36(1):75–85.
- Chapadense P. F. G., Castro F. J., Almeida J. A., Moron S. E., 2009 Toxicity of Atrazine herbicide in *Colossoma macropomum*. Rev Bras Saude Prod An 10(2): 398-405.
- Cox C., 2004 Pesticides factsheet: Glyphosate. Journal of Pesticides Reform 24:10-15.

- Crane J. M., 1973 Introduction to Marine Biology: A Laboratory Test. A Bell Howell Company, Ohio, USA.
- Daramola J. A., Osofero S. A., Kester C. T., Gbadamosi O. K., 2007 Overview of status of aquaculture in Nigeria with reference to Ekiti State. Agricultural Journal 2:447-452.
- Ekanem S. B., Okoronkwo T. E., 2003 Pawpaw seeds as antifertility control agent on male Nile tilapia. NAGA: World Fish Center Quarterly 26:8-10.
- Extoxnet, 1993 Extension Toxicology Network. http://extoxnet.orst.edu/
- Fagbenro O. A., Adebayo O. T., 2002 An Overview of the Animal Feed Husbandry in Nigeria. In: Live Stock and Fish Feeds in Subsahara Africa, Compiled by Tom Hecht. FAO Fisheries Technical Paper.
- Ferguson W. S., Koch W. C., Webster L. B., Gould J. R., 1977 Human physiological response and adaptation to ammonia. Journal of Occupational Medicine 19(5): 319-326.
- Fleșeriu A., 2010 Endocrine disrupting pesticides and their impact on wildlife and human health. HVM Bioflux 2(1):1-4.
- Frank H., Althoen S. C., 1995 Statistics: Concepts and Applications. Cambridge low perice edition, Cambridge University Press, Cambeidge.
- Gilbert P., 1996 Breeding and Propagation of tilapia (*Oreochromis niloticus*) in a floating hatchery, Gabon. NAGA: ICLARM Quarterly 19(4): 26-33.
- Hardell L., Eriksson M., Nordstrom M., 2002 Exposure to pesticides as risk factors for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case control study. Leukemia and Lymphoma 43:1043-1049.
- Hewlett P. S., Plackett R. L., 1979 The interpretation of quantal responses in Biology. Edward Arnold (Publishers) Limited, London.
- Holden M., Reed W., 1972 West African Nature Fresh Water Fishes: West African Nature Handbooks, London.
- Hussein S. Y., El-Nasser M. A., Ahmed S. M., 1996 Comparative studies on the effect of herbicide Atrazine on the freshwater fish *Oreochromis niloticus* and *Chrysichthys auratus*. Egyptian Bulletin of Environmental Contamination and Toxicology 57: 503–570.
- Jiraungkoorskul W., Upatham E. S., Kruatrachue M., Sahaphong S., Vichasri-Gram S., Pokethitiyook P., 2002 Histopathologocal effects of Roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). ScienceAsia 28:121-127.
- Jones J. R. E., 1983 The active toxicity of lead, zinc & copper to stickle back (*Gasterostus aculeatus*) and the effect of calcium on the toxicity of lead and zinc salts. Journal of Experimental Biology 15: 394-407.
- Jauncey K., Ross B., 1982 A Guide to Tilapia Feeds Feeding. Institute of Aquaculture, University of Stirling, Scotland, UK. 111PP.
- Kori-Siakpere D., Ubogu E., 2008 Sub lethal haematological effects of Zinc on the fresh water fish, *Heteroclarias sp.* (Osteichthyes: Claridae). African Journal of Biotechnology 7(12):2068-2073.
- Kori-Siakpere D., Adamu K. M., Madukelum I. T., 2007 Acute haematological effect of sub lethal levels of Paraquat on the African catfish, *Clarias gariepinus* (Ostechthyes: Claridae). Journal of Research of Environmental Sciences 1(6): 335– 331.
- Kovinznych J. A., Ubancikova M., 1998 Acute toxicity of Acetachlor pollution for zebra fish (*Danio rerio*) and guppy (*Poecilia reticulata*) Aquculture Environment Quality 17(4):449-456.
- Lewbert G. A., 2001 Diagnostic techniques for fish. In: Proceedings of the Atlantic Coast Veterinary Conference.
- Martins M. L., Mouriño J. L. P., Amaral G. V., Vieira F. N., Dotta G., Jatoba A. M. B., Pedrotti F. S., Jerônimo G. T., Buglione-Neto C. C., Periera Jr. G., 2008 Haemathological changes in Nile tilapia experimentally infested with *Enterococcus* species. Braz J Biol 68(3):657-661.
- Mills D., 1986 You and your Aquarium. Dorling, London, 288pp.

Min L. K., 1986 A Review of Rice – Fish Culture in China. Chinese Academy of Fisheries Science, Wuxi, China.

Mironescu M., Mironescu I. D., Georgescu C., 2010 Microstructural changes induced by five new biocidal formulations on moulds. Ann Rom Soc Cell Biol 15(2):162-167.

North Central Weed Science Society, 2008 Fawcett, twenty years of university corn yield data: with and without Atrazine. Proceedings of North Central Weed Science Society. http://www.ncwss.org/proceed/2008/abstract/137.pdf.30/9/2010.

Offem B. O., Ikpi G. U., Ada F. B., 2010 Fish culture technologies in South-Eastern Nigeria. African Journal of Agricultural Research 5(18):2521-2528.

Ogundele O., Ihuahi J. A., Omojowo F. S., Bitrus P., 2004 Toxicity of linear alkynbenzene sulphonate (LAS) detergent to *Clarias gariepinus* fingerlings. In: Proceedings of the 19th Annual Conference of the Fisheries Society of Nigeria. 29th November – 3rd December, 2004, held in Ilorin: 273 – 276.

Ogunji J., Toor R. S., Schulz C., Kloas W., 2008 Growth performance, nutrient utilization of Nile tilapia, *Oreochromis niloticus* fed housefly maggots (magmeal) diets. Turkish Journal of Fisheries & Aquatic Sciences 8:141–147.

Oloruntuyi O. O., Mulero O., Odukale B., 1992 The effects of two pesticides on *Clarias gariepinus*. Proceeding of the 10th Annual Conference of the Fisheries Society of Nigeria held in Abeokuta, 16 – 20th November, 1992.

Omitoyin B. O., Ajani E. K., Fajimi O. E., 2006 Toxicity of Gramoxone (Paraquat) to juvenile African catfish, *Clarias gariepinus* (Buchell, 1822). American Euroasian Journal of Agricultural and Environmental Sciences 1(1):26–30.

Petrescu-Mag I. V., Păsărin B., Todoran C. F., 2010 Metallurgical, agricultural and other industrial related chemical pollutants: biomonitoring and best model organisms used. Metalurgia International 15(Sp.Issue 9):38-48.

Petrescu-Mag I. V., Petrescu-Mag R. M., 2010 Heavy metal and thermal stress in fishes: The implications of HSP in adapting and acclimation to different environments. Metalurgia International 15(10):107-117.

Popma T., Masser M., 1999 Tilapia: Life History and Biology. Southern Regional Agricultural Center, SRAC, Publication No. 283.

Prasad T. A. V., Srinivas T., Rafi G. M., Reddy D. C., 1991 In Vivo effect of Atrazine on haematology and O₂ consumption in fish *Tilapia mossambica*. Biochemistry International 23:157-161.

Ramesh M., Srinivasan R., Saravanan M., 2009 Effect of Atrazine (herbicide) on blood parameters of common carp *Cyprinus carpio.* African Journal of Environmental Science and Technology 3(12): 453–458.

Rusia V., Sood S. K., 1992 Routine haematological tests. In: Medical laboratory technology, Kanai (Ed., Mukerjee, L.). Volume 1. Tata McGraw Hill Publishing company Limited, New Delhi. Pp 252-258.

Shallangwa S. M., Auta J., 2008 Sub lethal effect of 2, 4 – dichlorophenoxy acetic acid on growth and food utilization of the African cat fish. Bulletin of Fisheries International 3(3):65–67.

Sverdrup K. A., Duxbury A. B., Duxbury A. C., 2006 Fundamentals of oceanography. Fifth edition. Mc Graw Hill Higher Education, New York.

Thomas J., Venu S., Kurup B. M., 2003 Length–weight relationship of some deep sea fish inhabiting the continental slope beyond 250m deep along the West Coast of India. NAGA, World Fish Center Quarterly 26(2):17-21.

Uchida K., Kajimura S., Riley L. G., Hirano T., Aida K., Grau E. G., 2003 Effects of fasting on growth factor 1 axis in tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 134(2):429-439.

US EPA, 1988 Atrazine: Health Advisory. Office of Drinking Water, US EPA, Washington DC.

US EPA, 2000 Endocrine Disruptor Screening Program. Report to Congress. http://www.epa.gov/oscpont/oscpendo/reporttocongress0800.pdf.

Vijayan M. M., Takemura A., Mommsen T. P., 2001 Estradiol impairs hyposmoregulatory capacity in the euryhaline tilapia, *Oreochromis niloticus*. American Journal of Physiology 281(4):1161–1168.

- Visoottiviseth P., Thamamaruitkum T., Sahaphong S., Reingroipitak M., Kruatrachua 1999. Histological effects of triphemyltin hydroxide on liver, kidney and gill of Nile tilapia (*Oreochromis niloticus*). Applied Organometallic Chemistry 13(10):749-763.
- Weed Science Society of America, 1983 Herbicide Handbook of Weed Science Society of America. 5th edn. 515pp.

Received: 10 September 2012. Accepted: 05 October 2012. Published online: 02 November 2012. Authors:

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How to cite this article:

Ada F. B., Ayotunde E. O., Bayim P.-R. B., 2012 Some biological and hematological responses of *Oreochromis niloticus* juveniles exposed to Atrazine herbicide. AACL Bioflux 5(5):369-379.