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Gonado-hepatosomatic Indexes of *Clarias gariepinus* Sub-adult Exposed to Artrazine, *Cocos nucifera* Water and *Phyllanthus muelarianus* Extract

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Abstract

Success in fish culture is influenced by several factors. Elucidation of influences of some of these factors has become necessary as there is a lingering need to narrow the gap between supply and demand in the fish market in Nigeria. The influence of Atrazine (a systemic herbicide), coconut water (*Cocos nucifera*: also called coconut milk due to its rich nutrients content) and *Phyllanthus muellerianus* extract (a medicinal plant) on the gonads and liver were investigated. Ten fish were randomly selected and cultured in each tank containing 40 L of water for a particular treatment with three replicates. The average weight 76.26 ± 0.92 g and standard length 22.50 ± 0.61 cm were used for Atrazine experiment. For coconut water, the fish of weight 62.86 ±1.52 g and standard length 19.68 ± 0.73 cm were used. In *Phyllanthus muellerianus* experiment, fish with average weight 65.99 g and standard length 21.72.72 ± 0.92 cm were used. Atrazine and coconut water were lethal to *Clarias gariepinus* and had the LC5096 hours as 6.0 mg/L and 250.0 mg/L respectively. *Phyllanthus muellerianus* did not kill any fish for the fourteen day period of culture. All the three factors did not change the gonadosomatic index nor the hepatosomatic index significantly compared to the control. This observation did necessarily mean lack of influence on these organs but could be attributed to short periods for which the experiment was conducted.

Keywords: Atrazine; *Cocos nucifera* water; *Phyllanthus muellerianus* extract; Sub adults; *Clarias gariepinus*; Gonadosomatic index; Hepato-somatic index

Introduction

Food provision the world over has always been lower than demand. Man from antiquity keeps making effort to bridge the gap between food provision and need. However, some foods could be provided in excess. Some vital foods like animal protein are poorly consumed in tropical Africa due to their inadequacy. Being aware of the dwindling natural resources, man continues to shift his emphasis from food capture to culture in order to be able to use a small area to produce high quantity of the food. Culturing aims at manipulating food chains to favour the desired species. The quality of feeds for a proper culture medium should contain the varying nutrients in the proper proportion. Environment (internal or external) of the fish is very important in its health and growth. External environment include the quality and quantity of food available, the presence or absence of xenobiotics and pollutants. *Clarias* are important food fishes because of their large size, good flavour and rapid growth [1]. They are cosmopolitan and are widely cultured fish in the tropics and subtropics, and the third only to carp and tilapia, widely farmed fresh water fish in the world [2]. Presently, weed management has become a more serious problem because land fallowing, which was a method for controlling weeds in the tropics is no more tenable. It is observed that weed infestation is a limiting factor in farm size expansion in sub-Saharan Africa). Having realized that 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 now and in feature (U. S. EPA, 1993).

The indiscriminate use of pesticides, careless handling, accidental spillage and discharge of untreated effluents into natural water bodies have harmful effects on fish population and other forms of aquatic life and may also have long–term adverse effects on the environment. Apart from finding the hindrances of fish health, other substances, which man uses as food or medicines, are incorporatable in the fish culture environment to enhance their growth or investigate their influences.

In the natural environment, abnormalities in gonads were investigated in natural habitat of whitefish by Bernet et al. [3] in Lake Thun. Some abnormalities recorded include adhesions/fusions to the peritoneal wall and the lateral trunk musculature, asymmetry, atrophy, compartmentations, constrictions and hermaphroditism. Stentiford et al. [4] investigated the histopathological alterations in selected organs and tissues of three species of estuarine fish (*Platichthys flesus, Pomatoschistus minutus and Zoarces viviparus*), captured from four British estuaries (the Tyne, Tees, Mersey and Alde) differently impacted by contaminants. Sampson et al. opined that flatfish (*Pleuronectes platessa*) are in close contact with sediments and are thus exposed to xenobiotics stored in the sediments and were observed to alter the histology of their liver, kidney and gonads in Mersey Estuary. Other studies carried out on the effects of agrotoxicants on fish are those of Oloruntuyi et al. [5] who worked on the toxicity of Glyphosate and Gramoxone to *Clarias giriepinus*; Agbon et al. [6] carried out a renewable static experiment on the toxicity of Diaxonon on rotifers, Cyclops, mosquito larvae and fish; Kori-Siakpere et al. [7] investigated the effects of sublethal Paraquat on blood plasma and organic constituents of African catfish. Also, Ayotunde [8] determined the toxicity of *Moringa oleifera* seeds on the juvenile and adults of Nile tilapia and African catfish and Ayotunde et al. [9] exposed adult *Clarias gariepinus* to pawpaw seed extracts to observe their

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haematological changes. Ada et al. [10,11] investigated the influence of several herbicides (Glyphosate, Paraquat, Artrazine and Butachlor) on the hapatosomatic index of Nile Tilapia (*Oreochromis niloticus*) and the influence of *Aloe barbadensis* on white blood cell counts of adult *Heterobranchus bidorsalis*.

Atrazine is one of the two most widely used herbicides, and Atrazine has been suspected to be associated with hormonal system disruption in vertebrates (U. S. EPA, 2000) [12]. Some herbicides can be transferred from one location to the other through water flow or by bioaccumulation along food chains.

The genus *Phyllanthus* is said to be the largest of the Angiosperms and has a large number of medically important species for man. Some species are said to have potential effects against hepatitis B and antiviral activity against human immunodeficiency virus [13]. *Phyllanthus muellerianus* extracts are antimicrobial [14,15]. It is used in the treatment of various liver disorders in India. In Thailand, *Phyllanthus amarus* (Schum. & Thonn) has also been widely used as an antipyretic, a diuretic, to treat liver diseases and viral infections [16].

Its medicinal importance is due to the presence of the phytochemical compositions. Members of the Genus Phyllanthus have been observed to contain the following active ingredients: alkaloids, coumarins, flavoids, phenols, steroids, saponins, tritaponoids, glucosides and dihydrochalcones [17].

It is the desire of the researcher to investigate into the influence of *Phyllanthus muelarianus* extract, *Cocos nucifera* water and the herbicide Artrazine on the gonado-hapatosomatic indexes of *Clarias gariepinus,* as important organs of this fish, and to determine the effects of different concentrations of substances on the biological behaviours such as air gulping, erratic swimming, operculation, discolouration, haemorhage, loss of reflex on the African catfish (*Clarias gariepinus*).

Methods

The fish were weighed using a digital balance Model EK-5350 to the nearest mg, while length was measured using a measuring board to the nearest mm. The liver was removed and weighed using a digital balance (Scout-pro SPU402). The hepatosomatic index was obtained by dividing the weight of the liver by the total weight of the fish [18] expressed as a percentage. The gonadosomatic index was estimated in the same way [11,19]. After exposure, five fish were measured in each tank and their averages taken. Temperature, conductivity and pH were measured using digital meter mettle Toledo 320.

To each 40 L of stream water drawn from Department of Fisheries and Aquatic Science, CRUTECH, fish farm, ten fish selected randomly from a parent population were cultured. The concentration of Atrazine in the definitive experiment were 0.0 mg/L, 3.0 mg/L, 6.0 mg/L, 9.0 mg/L, 12.0 mg/L and 15 mg/L. that of coconut water was 0.0 mg/L, 250.0 mg/L, 350 .0 mg/L, 450.0 mg/L, 550.0 mg/L, 650.0 mg/L and 750.0 mg/L while that of *Phyllanthus muellerianus* extract were 0.0 mg/L, 16.0 mg/L, 32.0 mg/L, 65.0 mg/L, 130.0 mg/L and 260.0 mg/L. Acute toxicity experiments were conducted for four days in tests involving Atrazine and coconut water. In the test involving *Phyllanthus muellarianus,* the experiment lasted for 15 days to provide enough time for any observable physiological change. Fish were fed 6 % using industrially made pelleted feed (COUPENS), unlike in experiment involving coconut water and Atrazine.

Results

Phyllanthus muelarianus did not kill any of the exposed fish. Atrazine and coco nut water killed fish at various concentrations as expressed in Table 1 and Figures 1-12. It also did not change dissolved oxygen concentration and pH outside the normal range for fish survival in Figures 11 and 12.

Discussion

Death of organisms in any environment could result from several factors. Toxins introduced into the tanks could attack the organisms in

S/N	Time(hrs)	*Phyllanthus muellarianus* extract	*Cocos nucifera* juice	Atrazine herbicide
1	20	Nil	750	20.5
2	24	Nil	650	17.1
3	36	Nil	550	15.0
4	48	Nil	450	12.7
5	72	Nil	350	11.0
6	96	Nil	250	6.0

Table 1: The LC_{50} values (mg/L) for *Phyllanthus muellarianus* extract, *Cocos nucifera* juice and Atrazine herbicide.

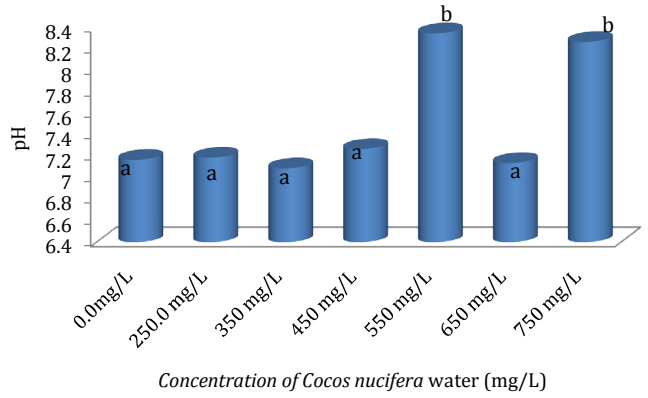

Figure 1: pH of water in aquaria exposed to different concentrations of coconut water. Mean pH (the bars) carrying the same letters are statistically the same while those carrying different letters are different (α=0.05).

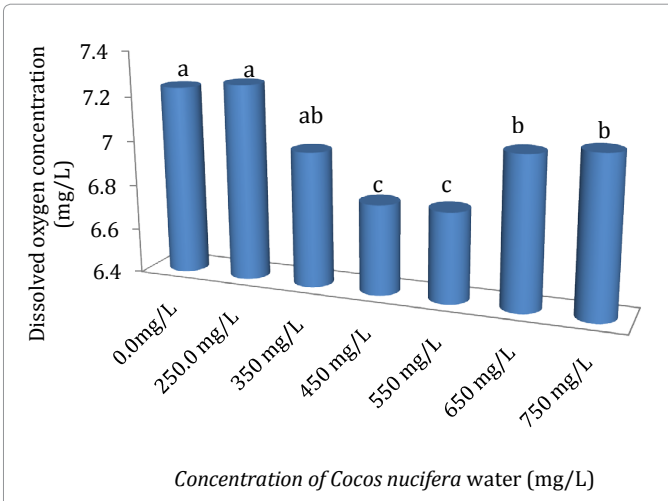

Figure 2: Dissolved oxygen concentration in water containing different concentrations of coconut water. ANOVA (α=0.05) revealed that dissolved oxygen concentrations differ among treatments. Means with the same letters are the same statistically.

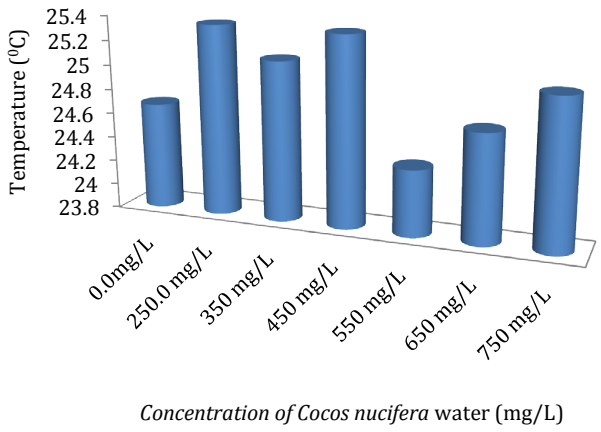

Figure 3: Temperature variation in water exposed in different aquaria exposed to different concentrations of coconut water. The temperature did not differ significantly among the aquaria.

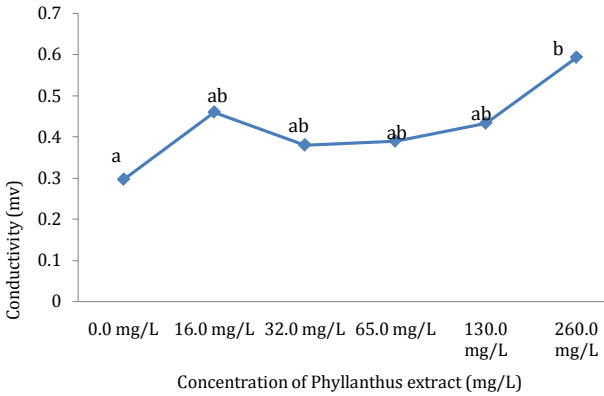

Figure 4: Increase in conductivity of water when treated with *Phyllanthusmuelarianus* extract. This was observed to be higher with increase extract concentration. Means with same letters are statistically the same while those with different letters are different (α=0.05).

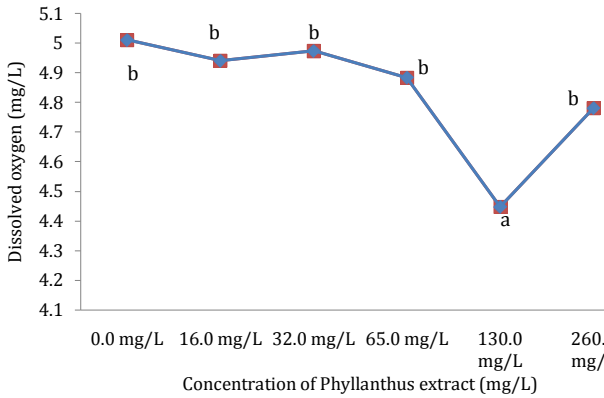

Figure 5: Dissolved oxygen was not significantly different in treatment groups compared to control except in treatment 5 with extract concentration of 130 mg/L. Means with the same letter are statistically the same (α=0.05).

their physiological systems. For instance, it was observed that coconut water did not change the temperature in the cultured tanks irrespective of the concentration. But it influenced the pH of the water in the tanks. Coconut water reduced dissolved oxygen concentration. Insufficient dissolved oxygen in water is capable of causing malfunction in many metabolic processes. Coconut water also raised the pH of the water above the optimum range of 6.5 to 8.5 [20-22]. WHO'S classification of Atrazine is in class II. Coconut water has thus far not been classified. It killed the organisms at very high concentrations unlike Atrazine. *Phyllanthus* did not cause any mortality. It is rather a health booster. It

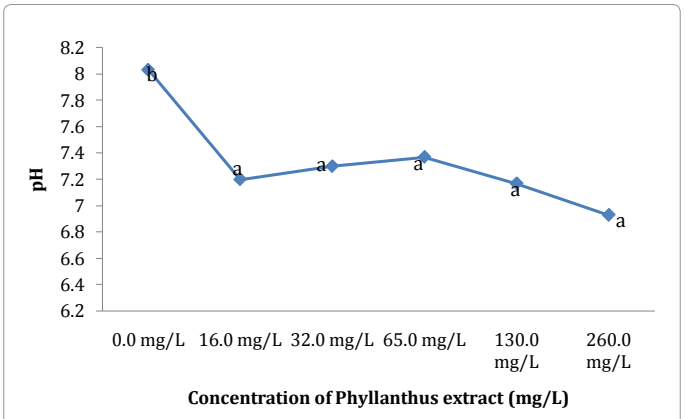

Figure 6: Hydrogen ion concentration as influenced by extract concentration, which was lower in treatment groups compared to control. All means with the same letter are statistically the same while the control group alone has a different letter showing that it was different from others (α=0.05).

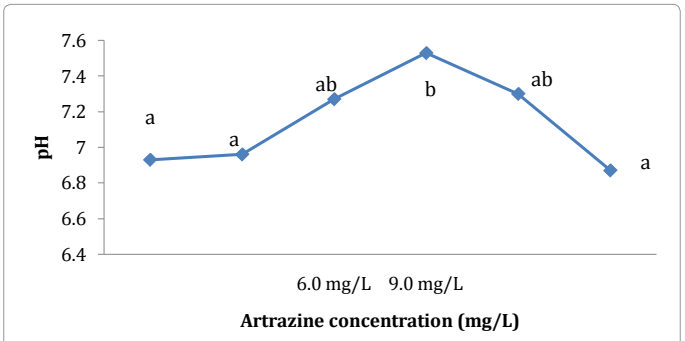

Figure 7: Effects of Atrazine on the pH of culturing water. There was significant difference among treatments (α = 0.05). Means carrying the same latters are statistically the same while those with different letters are different.

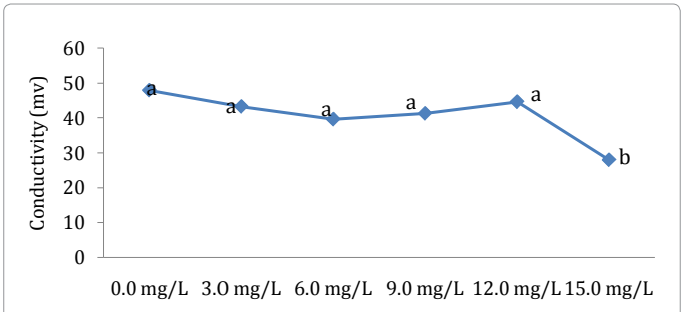

Figure 8: Significant difference in conductivity of water when treated with Atrazine. Means with same letters are statistically the same while those with different letters are different (α=0.05).

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was not seen to change neither the pH nor the dissolved oxygen outside the normal range for fish optimal survival.

Conclusion

Both liver and gonads were observed not to be affected by both factors (test substances) described as life enhancing and threatening respectively. This lack of observation was not enough to conclude that these substances did not cause harm to the organisms. Jauncey and Ross [23] pointed that apart from the mortality of adult fish, xenobiotics affect egg hatchability and fingerling survival and growth. According to APHA [24] 'the 48-96 hour LC_{50} 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. Langiano and Martinez [25] observed increase in plasma glucose in fish

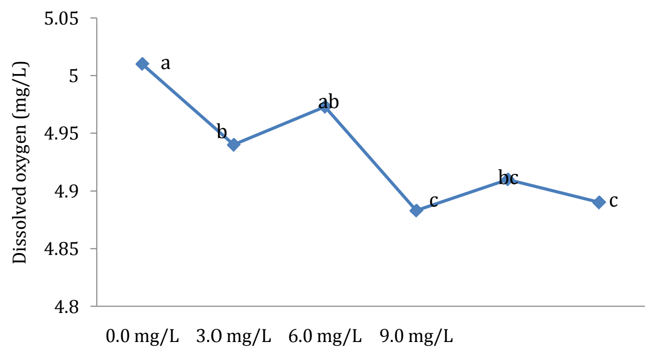

Figure 9: Efects of Atrazine on the dissolved oxygen concentration of culturing water. There was significant difference among treatments (α = 0.05). Means carrying the same letters are statistically the same while those with different letters are different.

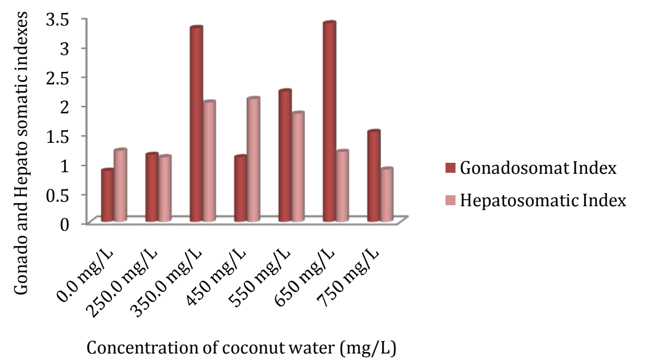

Figure 11: Gonado-hepatosomatic indexes of sub adult *Clariasgariepinus* exposed to *Cocusnucifera* water. Statistical analysis showed no significant difference among treatments (α = 0.05)

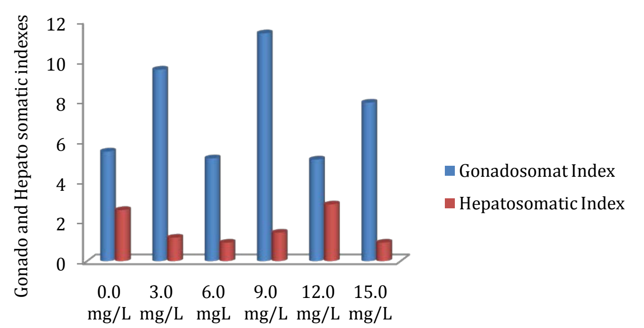

Figure 10: Gonado-hepatosomatic indexes of sub adult *Clariasgariepinus* exposed to Atrazine. Statistical analysis showed no significant difference among treatments (α=0.05).

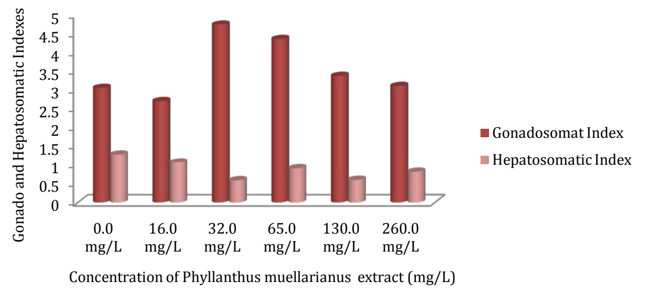

Figure 12: Gonado-hepatosomatic indexes of sub adult *Clariasgariepinus* exposed to *Phyllanthus muellarianus* extract. Statistical analysis showed no significant difference among treatments (α = 0.05)

	12 Hours						24 Hours						36 Hours							48 Hours					72 Hours						96 Hours					
	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/	0.0 mg/L	3.0 mg/L	6.0mg/L	9.0 mg/L	12.0 mg/	15.0 mg/
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
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
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
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
Erratic swimming	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	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	N	Y	Y	Y	Y	N	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

Table 2: 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.

	12 HOURS						24 HOURS						36 HOURS						48 HOURS					72 HOURS						96 HOURS					
	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L	250 mg/L	350 mg/L	450 mg/L	5500 mg/	650 mg/	750 mg/L
Air gulping	N	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Eratic swimming	N	N	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	N
Molting	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Discolouration	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	Y	Y	Y
Barbal deformation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	Y	Y	N	N	N	Y	Y	Y
Excessive mocus secretion	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	Y	Y	N	N
Tall beat frequency	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	N	N	N	N	Y	Y
Operculum movement	N	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N	Y	Y	N	N	N	Y	Y	Y	N	N	Y	Y	Y	Y
Loss of reflex	N	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	Y	Y	Y	N	N	N	Y	Y	Y	N	N	N	Y	Y	Y	N	N	Y	Y	Y	Y
Fin movement	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	N	N	Y	Y	Y	N	N	N	Y	Y	N	N	N	N

Table 3: Behavioural and biological observations that occurred in sub-adult *Clarias gariepinus* exposed to *Cocos nucifera* water. N = no response of fish at that particular concentration while Y = yes there was response.

exposed to 10 mg/L of Glyphosate, indicating a typical stress response. There was also increase in catalase liver activity in fish exposed to 10 mg/L of the herbicide, suggesting the activation of antioxidant defenses after Roundup exposure. The herbicide also induced several liver histological alterations that might impair normal organ functioning. Similar results were noticed by Ada et al., [26].

Biological and behavioural changes as reported in Tables 1-3 are immediate. Gross morphological changes has to be cumulative and do take a longer time to manifest. 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 [12]. The possibility of synergism or antagonism and other multiple effects or multiple toxicants must be considered. Jones [27] and Brown and Sadler [28] as well as other workers cited in Kori - Siakpere and Ubogu [29] reported association of heavy metal toxicity.

These substances may cause changes in the liver which is a detoxification centre [30]. The liver has to carry out defensive mechanism to be able to detoxify poisons and since these substances are highly attracted to the organ, the liver. It is therefore an organ that has to be highly protected.

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