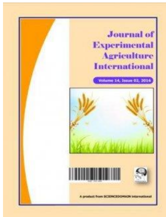

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Growth and Haematological Changes in African Catfish *Clarias gariepinus* Juveniles Exposed to Mercuric Chloride

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to assess the effect of Mercuric chloride on the growth and haematological parameters in the freshwater catfish, *Clarias gariepinus*. A total of 30 fishes were used for each concentration as well as in the control. *Clarias gariepinus* was exposed to 0.02, 0.04, 0.06, 0.08 and 0.10 mg/l of $HgCl_2$ for 56 days. The treatment with mercuric chloride was found to inflict a drastic reduction in the total count of RBC's. The reduction was time dependent; as concentration of mercuric chloride increased, the RBC levels declined. Exposed fishes showed a significant decrease in WBC count when compared to the control. The morphological indices MCV, MCH and MCHC fluctuate as the test concentration increased. The chronic exposure to sublethal

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concentration of mercuric chloride to the studied fish showed a significant decrease in final body weight in comparison to control group. Also, Growth parameters such as specific growth rate (SGR), food conversion efficiency (FCE), protein efficiency ratio (PER), food conversion rate (FCR) decreased with increased concentration of mercuric chloride. The mercuric chloride caused a significant decrease in the survival rate (P < 0.05).

Keywords: Clarias gariepinus; mercuric chloride; haematological parameters; growth parameters.

1. INTRODUCTION

Many metals are natural components of the freshwater environments. Some of them are beneficial or even necessary for life but many are toxic to aquatic life. The concentrations, at which the role of metals may be considered significant, vary; as some are essential at low concentration levels while others show toxicity at higher concentrations [1]. Heavy metal contamination usually causes depletion in food utilization in fish and such disturbance may result in reduced fish metabolic rate and hence cause reduction in their growth [2]. Growth is a sensitive and reliable endpoint in chronic toxicological investigations [3].

Mercury is a black list element by environmentalist and is released into the environment by several sources ranging from mining, sewage disposal, research laboratories, agriculture, fungicides and industrial operation, electrical equipment, paints and disinfectants [4,5]. Mercury a hazardous metal that may cause adverse health effects including neurological, renal, respiratory, immunological, dermatological, reproductive, developmental sequels in wild life [6]. The toxicity of mercury depends greatly on the forms of the mercury compound, elemental, inorganic and organic. Mercuric chloride was chosen in this experiment because of its lower toxicity compared to the other forms. It is used in agriculture as fungicide, pesticides, tanning and wood preservation, in medicine as a tropical antiseptic, disinfectant, and it is used in electroplating and as intensifier in photography.

2. MATERIALS AND METHODS

2.1 Experimental Fish

Juveniles of *Clarias gariepinus* of a mean weight of 31.07±1.23 g and mean length of 19.50 ± 0.50 cm were obtained from Finite fish farm, Makurdi and acclimatized for 14 days in the fish hatchery Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fish were feed twice daily at 0800 and 1600 hours at 5% of their body weight.

2.2 Experimental Site

The study was carried out in the Laboratory of Fisheries Department in the University of Agriculture Makurdi, Benue State.

2.3 Experimental Design

A complete randomized design was used for the experiment in triplicates. A total of one hundred and eighty (180) juveniles of *Clarias gariepinus* were randomly distributed into the plastic containers at a stocking rate of 10 fish. The eighteen (18) tanks were assigned to 5 treatments with (control inclusive).

2.4 Haematological Anaysis

Blood samples were taken by randomly selecting fish from the various treatments and injecting in a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation and analyzed at Tosema Specialist Diagnostic Laboratory in Makurdi. Haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC) was determined by Blaxhall and Daisley's [7] methods. Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) was determined by Svobodova et al. (2001). Platelets count was determined by Zinkl, [8].

2.5 Growth Parameters Evaluated

The weight of the test fish in treated and untreated (control) test media were recorded at the commencement and after 8 weeks of the sub-lethal test. Fish were weighed every week using--weighing balance, following growth parameters were computed.

Mean Weight Gain (MWG) = (Mean final weight – mean initial weight / Mean initial weight)

% Mean Weight Gain = (Mean Final weight-mean Initial weight / Mean Initial weight) x100

Specific Growth Rate = (In mean final weight -In mean initial weight) / Duration of the experiment) x 100

Food Conversion Ratio (FCR) = (Weight of feed fed / Mean final weight-mean initial weight)

Food Conversion Efficiency (FCE) = (Gain in wet weight of fish/ Feed Fed) x 100

Protein Efficiency Ratio (PER) = Gain in body mass / protein in intake

2.6 Water Quality Parameters

Temperature, pH total dissolved solids and electrical conductivity were measured using Hanna II 9813 GRO multi parameter meter while dissolved oxygen was measured using (APHA,2005) method.

2.7 Statistical Analysis

One- Way Analysis of Variance (ANOVA) was used to analyse the data followed by LSD.

3. RESULTS

3.1 Haematological Observations of *Clarias gariepinus*

Table 1 presents haematological parameters in the blood of *Clarias gariepinus* exposed to sub-acute concentrations of mercuric chloride for 56 days. The amounts of hematocrit (Hct), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC). MCH, MCV and MCHC values fluctuated as the test concentration increased. ($P<0.05$) as a result of chronic mercuric chloride exposure.

Other blood factors, such as monocytes, eosinophils, and myelocytes, were not observed in the examined blood samples.

3.2 Growth Parameters

The growth performance of *Clarias gariepinus* exposed to sub-lethal concentration of mercuric chloride is shown on Table 2. There was no significant difference ($P>0.05$) in the mean initial weight of all fish used in the experiment. However, there was loss of weight in the all treatments and this was manifested in the mean weight gain. The control had the highest Mean Weight Gain (MWG) of 2.47 g while treatment 5 had the least Mean Weight Loss of (1.63 g). The control had the highest Feed Conversion Ratio, Protein Efficiency Ratio and Specific Growth Rate while Treatment 5 in each of the mentioned parameters was the lowest. The control, treatments 1 and 2 had 100% survival rates while treatments 3, 4 and 5 had 99%, 97% and 97% survival rates respectively.

From Fig. 1, growth was slow between week 1 and week 2, week 3 and 4. The control gave the best Mean Weight Gain, Feed Conversion Ratio, Protein Efficiency Ratio and Specific Growth Rate, all of which were significantly higher ($P<0.05$) than what was obtained in fish exposed to the mercuric chloride.

4. DISCUSSION

Haematological variables have been used as a tool to determine the effect of sublethal concentrations of pollutants on animals [9]. The results of the present investigation exhibit that mercuric chloride reduces the RBC's count drastically and the reduction in number is dosage dependent. Panigrahi and Misra [10] observed reduction in haemoglobin percentage and RBC count of the fish *Anabas scandens* treated with mercuric chloride. Decrease in haemoglobin, Red Blood Cell (RBC) count and haematocrit were observed in fish *Tinca tinca* exposed to mercuric chloride [11]. Decline in red blood cell values and anemia were reported in fishes such as *Salvalinus fontinalis* [12] *Salmo gairdneri* [13], *Colisa Fusciatus* [14] and *Barbus conchonius* [15] which were exposed to heavy metals. The decline in RBC count in the current study might have resulted from inhibition of RBC manufactured by mercuric chloride. Likewise, Li et al. [16] report a reduction of total content of RBC in the blood of rainbow trout (*Oncorhynchus mykiss*) exposed to verapamil (VPR), a cardiovascular medicine. *Clarias gariepinus* exposed to mercuric chloride exhibited a decrease in haemoglobin which indicates that mercury caused anaemia. This may be due to the decreased rate of production of RBC or an increased loss of these cells. Kumar et al. [17] observed decline in haemoglobin of *Heteropneus fossilis* after 30 days exposure to deltamethrin. Morsy and Protasowicki, [18] demonstrated cadmium bioaccumulation resulting in reduction of erythrocytes count, haemoglobin contents and haematocrit in compassion to the control. In the present investigation, leucocytes showed greater and quite different pattern of change with the effect of mercuric chloride when compared with erythrocyte level of the control fishes. Blood of all experimental groups contained lower concentration of leucocytes than those of the control. Allen, [19] observed increased WBC count in *Oreochromis aureus* after mercury exposure. The decrease in number of WBC observed in the present study may be attributed to the stimulation of immune system in response to tissue damage caused by mercuric chloride. Dhanekar et al. [20] reported the increase in large lymphocytes, reduction in small lymphocyte and thrombocytes populations as also elevation in monocytes, neutrophils and eosinophils cells in *Heteropneustes fossilis, Channa punctatus* and *Mastaemebalus puncalus* on long exposure to least effective concentration of mercuric chloride. The increase in mean corpuscular volume (MCV) in high concentrations can result from an expansion of unripe RBC [21]. In the present study the increase in MCV in high concentration might be caused by the above reason. The expansion of MCV studied in individuals of *H. malabaricus* exposed to methyl mercury (MeHg) is illustrated by the existence of a larger amount of older or larger red blood cells as reported by [22]. Also, MCV was reduced in stripped bass exposed to mercury [23] and in tilapia *Oreochromis aureus* treated with 0.10ppm mercury after 1 week [24,19]. The fluctuation in Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) values found in this study was 32.5 pg and 33.5% respectively. Annune and Ahuma, [25] found there was an increase in MCH and MCHC observed when *Orechoromis niloticus* were exposed to zinc. But different with the finding of Kori-siakpere et al. [26] who reported that, potassium permanganates can adversely affects haematology of fish at 19.25 and 13.60mg/l for *Clarias gariepinus* exposed to sub-lethal concentration. The fluctuations in the MCH and MCHC in the present study, indicates that the concentration of the haemoglobin in the RBC were much lower in the exposed fish than in the control fish as reported by Bhagwart and Bhikajee [27].

Table 1. Effects of Sub-lethal concentrations of Mercuric chloride on haematological parameters of *Clarias gariepinus*

Haematological parameters	Treatments (mg/l)						
	(Control)	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	LSD
PCV (%)	37.50±0.50^{e}	21.50±0.50^{d}	16.50±0.50^{c}	13.50±0.50^{b}	12.00±0.00^{b}	9.50±0.50^{a}	1.579
HB (g/dL)	12.20±0.10^{f}	7.35±0.15^{e}	5.85±0.05^{d}	4.65±0.05^{c}	4.20±0.10^{b}	3.40±0.10^{a}	0.339
WBC (x10^{9}/L)	11.20±0.10^{e}	2.90±0.10^{d}	2.80±0.10^{d}	2.25±0.15^{c}	1.70±0.10^{b}	1.25±0.05^{a}	0.339
RBC (x10^{12}/L)	5.15±0.15^{f}	2.45±0.05^{e}	2.05±0.05^{d}	1.80±0.10^{c}	1.30±0.00^{b}	0.95±0.05^{a}	0.234
MCV (fL)	75.50±0.50^{b}	92.30±0.70^{c}	84.50±0.50^{b}	73.80±0.20^{a}	93.15±0.85^{c}	101.00±1.0^{d}	2.390
MCH (pg)	24.25±0.05^{b}	29.50±0.50^{c}	28.50±0.50^{bc}	23.60±0.60^{a}	30.75±0.75^{c}	32.50±0.50^{d}	1.828
PLATELET (x10^{9}/L)	153.50±0.50^{a}	88.50±0.50^{b}	88.00±1.00^{b}	89.50±0.50^{b}	98.00±0.00^{c}	101.50±0.0^{d}	2.343
MCHC (%)	31.85±0.05^{a}	33.25±0.55^{a}	35.00±0.90^{b}	33.20±0.40^{a}	34.55±0.45^{ab}	33.55±0.55^{b}	1.966

Means on the same row with different superscript are statistically different (P<0.05).
Keys: Control = 0.00, Trt 1 = 0.02mg/l, Trt 2 = 0.04mg/l, Trt 3 = 0.06mg/l , Trt 4 =0.08mg/l, Trt 5 = 0.10mg/l

Table 2. Growth, nutrient utilization of *Clarias gareipinus* juveniles exposed to sub- lethal concentrations of Mercuric chloride for eight weeks

Growth Parameters (g)	Treatments (mg/l)						
	(Control)	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	LSD
MIW	29.91±1.65	29.29±1.72	29.96±1.49	28.50±1.03	27.92±1.62	29.58±0.68	-
MFW	32.38±1.91	28.03±1.69^{c}	28.35±1.48^{b}	26.64±0.95^{a}	26.00±1.69^{a}	27.95±0.64^{ab}	4.01
MWG	2.47±0.68^{b}	-1.26±0.09^{a}	-1.61±0.04^{a}	-1.86±0.16^{a}	-1.92±0.20^{a}	-1.63±0.25^{a}	0.91
FCR	0.95±0.20^{c}	-1.16±0.10^{b}	-0.88±0.04^{ab}	-0.77±0.08^{a}	-0.73±0.08^{a}	-0.30±0.49^{a}	0.62
PER	3.26±0.70^{b}	-2.04±0.17^{a}	-2.58±0.15^{a}	-3.08±0.25^{a}	-3.52±0.57^{a}	-2.61±0.40^{a}	1.26
SGR	0.061±0.01^{b}	-0.059±0.00^{a}	-0.059±0.00^{a}	-0.058±0.00^{a}	-0.058±0.00^{a}	-0.059±0.00^{a}	0.03
Survival Rate	100.00	100.00	100.00	99.00	97.00	97.00	-

Means on the same row with different superscript are statistically different (p<0.05).
Keys: Control = 0.00 , Trt 1 = 0.02mg/l, Trt 2 = 0.04mg/l, Trt 3 = 0.06mg/l, Trt 4 = 0.08mg/l, Trt 5 = 0.10mg/l
MIW=Mean initial weight, MFW=Mean final weight, MWG=Mean weight gain, SGR=Specific growth rate, FCR=Food conversion rate, FCE=Food conversion efficiency, PER= Protein Efficiency Ratio

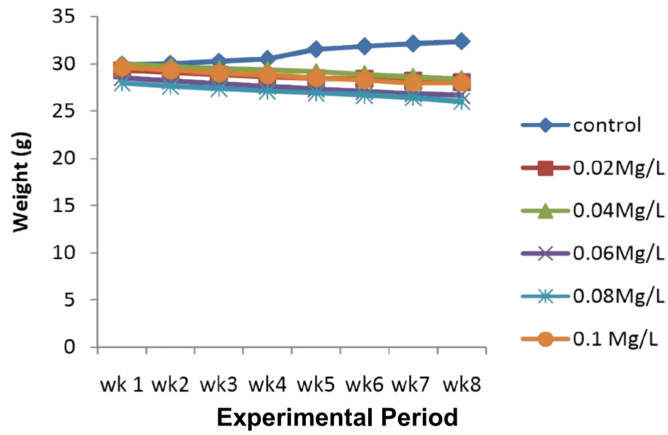

Fig. 1. Mean weight gain of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Mercuric chloride for 8 weeks

Nanda and Behera [28] have studied the significant reduction of red blood cell counts in *Anabas testudineous* and *Heteropneustes fossilis* exposed to cadmium and nickel respectively and they have suggested that reduction in RBC count might be due to disturbance in the metabolism of the haemopoietic organs.

Decline in Haemogloblin and haematocrit was observed in *Channa punctatus* exposed to mercury [29]. Kumar et al. [17] observed decline in haemoglobin of *H. fossilis* after 30 days exposure to deltamethrin. Morsy and Protasowicki [18] demonstrated cadmium bioaccumulation resulting in reduction of erythrocyte count, haemoglobin content and haematocrit in compassion to the control. White blood cells play major role in the defence mechanism of fish. They consist of granulocytes, monocytes, lymphocytes and thrombocytes.

4.1 Growth Parameters

Growth parameters showed an increase in the control group and significant decrease in the exposed ones. Gbem et al. [30] reported that *Clarias gariepinus* growth was reduced as concentration of toxicant increased. The exposure of *Oreochromis niloticus* to Thiobencarp herbicide for 8 weeks revealed that, fish showed a reduction in body weight gain compared to the control group [31]. Growth parameters such as specific growth rate (SGR), food conversion efficiency (FCE), protein efficiency ratio (PER), food conversion rate (FCR) decreased with increased concentration of mercuric chloride. Decrease growth rate as observed by Toussain et al. [32] and Onusiriuka, [33] that exposed Japanese Medaka fish and *Clarias gariepinus* to sub-lethal concentrations of chloroform and formalin respectively. Better growths were reported in control group of certain fish than those exposed to toxicants as observed in this study. This might be due to the fact that fish in control were able to utilize the feed.

5. CONCLUSION

i. Haematological indices of fish, caused by mercuric chloride toxicity to *Clarias gariepinus*, can be secondary responses to toxicants, including exposure to low concentrations of heavy metals, which reflect the launch of stress reaction in the affected fish.
ii. Growth parameters such as specific growth rate (SGR), food conversion efficiency (FCE), protein efficiency ratio (PER), food conversion rate (FCR) decreased with increased concentration of mercuric chloride.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Javed M. Relationship among water, sediments and plankton for the uptake and accumulation of heavy metals in the river Ravi. International Journal of Pure and Applied Science. 2005;2:326-331.
2. Javed M, Abdullah S, Yaqub S. Studies on tissue specific metal bioaccumulation patterns and differences insensitivity of fish to waterborne and dietary metals. First

Annual Research Report (HEC Project # R & D 803). 2008;1-32.
3. Boeck De, Vlaeminck GA, Blust R. Effect of sub-lethal copper exposure on copper accumulation, food consumption, growth, energy stores and nucleic acid content in common carp. Architectural Environmental Contamination Toxicology. 1997;33:415-22.
4. Dieter MP, Boorman GA, Jameson CW, Fustis SL. Development of renal toxicity in F34 rats gavaged with mercuric chloride for 2 weeks or 2, 4, 6, 15 and 24 months. J. Toxicol. Environ. Health. 1992;36:319-340.
5. Khangrot BS. Mercuric induced morphological changes in the respiratory surface of an Asia freshwater catfish *Saccobranchus fossilis*. Bul. Env. Cont. Toxicology. 2003;70:705-712.
6. Risher JF, Amler T, Sherlita N. Mercury exposure: Evaluation and intervention, the inappropriate use of chelating agents in diagnosis and treatment of putative mercury poisoning. Neurotoxicology. 2005;26(4):691–699.
7. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. Journal of Fish Biology. 1973;5:771-781.
8. Zinkl JG. Avian haematology. Schalms Veterinary Haematology, Jain, N. C (Ed). 4th Edn. Philadelpia: Lea and Febiyer. 1986;256-273.
9. Wedemeyer GA, Nelson NC. Statistical methods for estimating normal blood chemistry ranges and variance in rainbow trout (*Salmo gairdneri*), Shasta strain. Journal of Fisheries Research Board of Canada. 1975;32:551-554.
10. Panigrahi AK, Misra BN. Toxicological effects of mercury on a freshwater fish, Anabas scandens, CUV. & VAL. and their ecological implications. Environmental Pollution. 1978;16:31-39.
11. Shah SL, Altindag A. Haematological parameters of tench (*Tinca tinca* L) after acute and chronic exposure to lethal and sublethal mercury treatments. Bulletin Environmental Contamination Toxicology. 2004;73:911-918.
12. Holcombe GW, Benoit DA, Leonard EN, McKim JM. Long term effects of lead exposure on three generations of brook trout, *Salvalinus fontinalis*. Journal of Fish Research Board Canada. 1976;33:1731-1741.
13. Johansson-Sjobeck ML, Larsson A. Effect of inorganic lead on deltaaminolevulinic acid dehydratase activity and haematological variables in the rainbow trout, *Salmo gairdneri*. Architectural Environmental; 1979.
14. Srivastava AK, Mishra S. Blood dyscrasia in a teleost, *Colisa fasciatus* after acute exposure to sublethal concentrations of lead. Journal of Fish Biology. 1979;24: 199-203.
15. Tewari H, Gill TS. Impact of chronic lead poisoning on the haematological and biochemical profiles of fish, *Barbus conchonius*. Bulletin Environmental Contamination Toxicology. 1987;38:748-752.
16. Li C, Xu H, Xu J, Chun X, Ni D. Effects of aluminium on ultrastructure and antioxidant activity in leaves of tea plant. Acta Physiology of Plant. 2011;33:973–978.
17. Kumar S. S. Lata, Gopal K. Deltamethrin induced physiological changes in freshwater catfish *Heteropneustes fossilis*. Bulletin Environmental Contamination and Toxicology. 1999;62:254-258.
18. Morsy MG, Protasowicki M. Cadmium bioaccumulation and its effects on some hematological and histological aspects in carp, *Cyprinus carpio* (L) at selected temperature. Acta Ichthyologica et Piscatoria: Fasc. 1990;1(20):105-115.
19. Allen P. Changes in the hematological profiles of the cichlid *Oreochromis aureus* (Steindachner) during acute inorganic mercury intoxication. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endo-crinology. 1994;108(1):117-21.
20. Dhanekar S, Srivastava S, Rao KS, Pandya SS. Studies on toxic effects of least effective concentration of mercury in fish: A haematological study. Matsya. 1985;11:75-78.
21. Carvalho CS, Fernandes MN. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. Aquaculture. 2006;251:109–17.
22. Mazon AF, Pinheiro GHD, Fernandes MN. Haematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Brazilian Journal of Biology. 2002;62(4A): 621-631.

23. Dawson VK. A rapid high-performance liquid chromatography method for simultaneously determining the concentrations of TFM and Bayer 73 in water during lampricide treatments. Canadian Journal of Fisheries and Aquatic Sciences. 1982;39:778–782.
24. Stephan EC. Increasing the Usefulness of acute toxicity test. In Aquatic Toxicity and Hazard Assessment. Proceedings of the Fifth Annual Symposium on Aquatic Toxicology: Ed. J.G. Pearson, R.B. Foster and W.E., Bishop. ASTM Special Technical Publication. 1982;766:69-81.
25. Annune PA, Ahuma H. Haematological change in mudfish *Clarias gariepinus* exposed to sub-lethal concentrations of copper and lead. Journal of Aquatic Science. 1993;13:33-36
26. Kori-Siakpere D, Adamu KM, Madukelum IT. Acute haematological effect of sub-lethal levels of paraquat on the African catfish, *Clarias gariepinus* (Ostechthyes: Claridae). Journal of Research of environmental Sciences. 2007;1(6):335-331.
27. Bhagwart S, Bhikajee M. Induction of hypo-chromic macrocytic anaemia in Oreochromis hybrid (Cichlidae) exposed to 100 mg/L sublethal dose of aluminium. Journal of Science and Technology. 2002;5:9-16.
28. Nanda M, Behera MK. Nickel induced changes in some heamoto biochemical parameters of a cat fish *Heteropneustes fossils* (Bloch). Environmental Ecology. 1996;14:82-85.
29. Sastry KV, Sharma K. Mercury induced haematological and biochemical anomalies in Ophiocephalus (*Channa punctatus*). Toxicology Letters. 1980;5: 245-249.
30. Gbem TT, Balogun JK, Lawal FA, Annune PA. Trace metal accumulation in *Clarias gariepinus* exposed to sublethal levels of tannery effluent. Science Toxicology and Environment. 2001;271:1-9.
31. Hossam HA, Mohammed MA, Iman, MKA, Ashraf AEB. Studies on the effect of thiobencarb herbicide on some biological, physiological, biochemical, histological and genetic aspects of Nile tilapia; *Oreochromis niloticus*. Environmental Pollution, Egypt. 2007;56-79.
32. Toussain MW, Rosencrance AB, Brenna LM, Beaman JR, Wolfe MJ, Hoffmann FJ, Gardner HS. Chronic toxicity of chloroform to Japanese Medaka fish. Environmental Health Perspective. 2001;109(1):35-40.
33. Onusiriuka BC. Effects of sub-lethal concentrations of formalin on weight gain in African catfish, *Clarais gariepinus* (Tougals). Journal of Aquatic Sciences. 2002;17:66-68.

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