



GROWTH AND HAEMATOLOGICAL CHANGES IN AFRICAN CATFISH *CLARIAS GARIEPINUS* JUVENILES EXPOSED TO MERCURIC CHLORIDE

¹Isiyaku, M. S., ¹Usman B. I., ²Annune, P. A., ²Tiamiyu, L. O. and ³Aladi, S. L.

¹Department of Fisheries and Aquaculture, Bayero University, Kano

²Department of Fisheries and Aquaculture, University of Agriculture, Makurdi

³Department of Fisheries and Aquaculture, Kogi State University, Anyigba

Corresponding Author's Email: imsani.faq@buk.edu.ng **Tel.:** 08035337548

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 0.10mg/l of HgCl₂ 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 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 ($P < 0.05$). The study recommends timely fish fed since time was a strong dependent variable in all the treatment, so as increase concentration of mercuric chloride increased.

Keywords: *Clarias gariepinus*, Growth parameters, Haematological parameters, Mercuric chloride.

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 (Javed, 2004).

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 (Javed *et al.*, 2008). Growth is a sensitive and reliable endpoint in chronic toxicological investigations (De Boeck *et al.*, 1997).

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 (Dieter *et al.*, 1992 and Khangrot, 2003).

Mercury a hazardous metal that may cause adverse health effects including neurological, renal, respiratory, immunological, dermatological, reproductive, developmental sequels in wild life (Risher *et al.*, 2005). The toxicity of mercuric chloride 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.

MATERIALS AND METHODS

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.

Experimental site

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

Experimental design

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

Haematological analysis

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 (1973) 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 (1986).

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.

$$\text{Mean weight gain (MWG)} = \frac{\text{Mean final weight} - \text{mean initial weight}}{\text{Mean initial weight}} \quad \dots (1)$$

$$\% \text{ Mean weight gain} = \frac{\text{mean Final weight} - \text{mean Initial weight}}{\text{Mean initial weight}} \times 100 \quad \dots (2)$$

$$\text{Specific growth rate} = \frac{(\ln \text{ mean final weight} - \ln \text{ mean initial weight})}{\text{Duration of the experiment}} \times 100 \quad \dots (3)$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Weight of feed fed}}{\text{Mean final initial weight} - \text{mean initial weight}} \quad \dots (4)$$

$$\text{Food conversion Efficiency (FCE)} = \frac{\text{Gain in weight of fish}}{\text{Feed Fed}} \quad \dots (5)$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain}}{\text{Protein fed} - \text{fed Quantity of feed} \times \text{Crude protein of the diet}} \quad \dots (6)$$



Statistical Analysis

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

RESULTS AND DISCUSSION

Haematological observations of *Clarias gariepinus*

Table 1 presents haematological parameters in the blood of *Clarias gariepinus* exposed to sub-lethal concentrations of mercuric chloride for 56 days. The amounts of haematocrit (Hct), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC). MCH, MCV and MCHC values fluctuated as the test concentration increased.

Table 1: Growth, nutrient utilization of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Mercuric chloride for eight weeks

Growth Parameters (g)	Treatments (mg/l)						LSD
	(Control)	Treatment	Treatment	Treatment	Treatment	Treatment	
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	0.01

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

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 2.47g while treatment 5 had the least Mean Weight Loss of (1.63g). 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.

Haematological variables have been used as a tool to determine the effect of sublethal concentrations of pollutants on animals (Wedemeyer and Nelson, 1975). 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 (1987) 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 (Shah and Altindag, 2004).

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

Growth Parameters (g)	Treatments (mg/l)						LSD
	(Control)	Treatment	Treatment	Treatment	Treatment	Treatment	
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	0.01

Means on the same row with different superscript are statistically different ($p < 0.05$).

Key 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.

From Figure 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. Decline in red blood cell values and anemia were reported in fishes such as *Salvalinus fontinalis* (Holcombe *et al.* (1979) *Salmo gairdneri* (Johansson-Sjoberg and Larson, 1979); *Colisa Fuscatus* (Srivastava and Mishra, 1979); *Barbus conchoni* (Tewari and Gill, 1987) 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.* (2011) 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.* (1999) observed decline in haemoglobin of *Heteropneus fossilis* after 30 days exposure to deltamethrin.

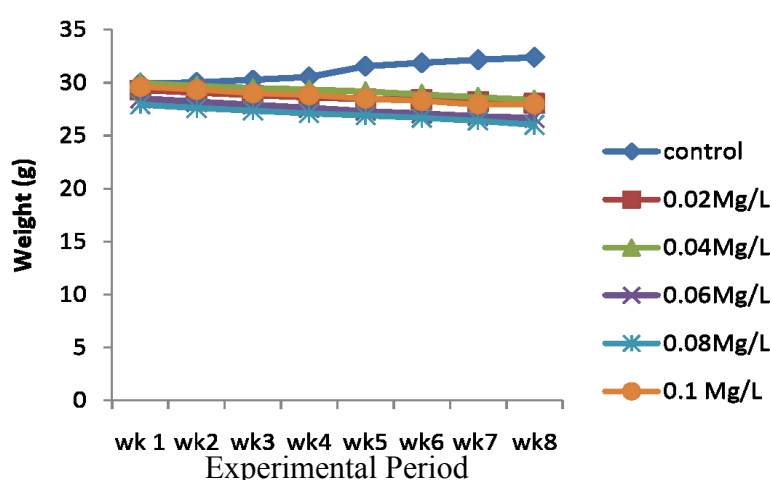


Figure 1: Mean weight gain of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Mercuric chloride for 8 weeks.



Morsy and Protasowicki (1990) demonstrated cadmium bioaccumulation resulting in reduction of erythrocytes count, haemoglobin contents and haematocrit in comparison to the control. In the present investigation as revealed in Table 3, 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.

Table 3: Physico-chemical parameters of the test solution obtained during the exposure of juveniles of *Clarias gariepinus* to sub-lethal concentrations of mercuric chloride.

Treatment (mg/l)	Water Quality Parameters				
	Total Dissolved Solids (mg/l)	Electrical Conductivity (μ S/cm)	pH	Temperature ($^{\circ}$ C)	Dissolved Oxygen (mg/l)
Control	461.50 $\pm$ 1.50 ^a	927.00 $\pm$ 1.00 ^a	9.30 $\pm$ 0.01 ^{ab}	26.20 $\pm$ 0.10 ^{ns}	5.65 $\pm$ 0.05 ^d
Treatment 1	487.00 $\pm$ 2.00 ^b	974.50 $\pm$ 3.50 ^b	9.29 $\pm$ 0.02 ^a	26.35 $\pm$ 0.15 ^{ns}	4.25 $\pm$ 0.05 ^c
Treatment 2	559.00 $\pm$ 3.00 ^c	1032.50 $\pm$ 3.00 ^c	9.34 $\pm$ 0.01 ^b	26.20 $\pm$ 0.10 ^{ns}	4.05 $\pm$ 0.05 ^c
Treatment 3	572.00 $\pm$ 2.00 ^d	1079.50 $\pm$ 1.50 ^d	9.35 $\pm$ 0.01 ^c	26.30 $\pm$ 0.10 ^{ns}	3.45 $\pm$ 0.05 ^b
Treatment 4	584.00 $\pm$ 3.00 ^e	1103.50 $\pm$ 2.50 ^e	9.35 $\pm$ 0.02 ^c	26.25 $\pm$ 0.15 ^{ns}	2.95 $\pm$ 0.05 ^a
Treatment 5	602.00 $\pm$ 2.00 ^f	1120.00 $\pm$ 1.00 ^f	9.36 $\pm$ 0.01 ^c	26.20 $\pm$ 0.10 ^{ns}	2.90 $\pm$ 0.00 ^a
LSD	8.023	7.960	0.044	0.412	0.158

Means on the same column with different superscript are statistically different at $p < 0.05$.

Key: Control = 0.00, Treatment 1 = 0.02mg/l, Treatment t 2 = 0.04mg/l, Treatment 3 = 0.06mg/l, Treatment 4 = 0.08mg/l, Treatment = 0.10mg/l

Allen (1994) 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.* (1985) 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 *Mastomys natalensis* 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 (Cavalho and Fernandes, 2006). 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 (Mazon *et al.*, 2002). Also, MCV was reduced in striped bass exposed to mercury (Dawson, 1982) and in tilapia *Oreochromis aureus* treated with 0.10ppm mercury after 1 week (Stephan, 1982; Allen, 1994). The fluctuation in Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) values found in this study was 32.5pg and 33.5%, respectively.

Annune and Ahuma, (1993) found there was an increase in MCH and MCHC observed when *Oreochromis niloticus* were exposed to zinc. But different with the finding of Koriakpere *et al.* (2007) 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 (2002).

Nanda and Behera (1996) have studied the significant reduction of red blood cell counts in *Anabas testudineus* 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 Haemoglobin and haematocrit was observed in *Channa punctatus* exposed to mercury (Sastry and Sharma, 1980). Kumar *et al.* (1999) observed decline in haemoglobin of *H. fossilis* after 30 days exposure to deltamethrin. Morsy and Protasowicki (1990) demonstrated cadmium bioaccumulation resulting in reduction of erythrocyte count, haemoglobin content and haematocrit in comparison to the control. White blood cells play major role in the defence mechanism of fish. They consist of granulocytes, monocytes, lymphocytes and thrombocytes.

Growth parameters showed an increase in the control group and significant decrease in the exposed ones. Gbem *et al.* (2001) 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 (Hossain *et al.*, 2007). 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.* (2001) and Onusiriuka (2002) 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.

CONCLUSION AND RECOMMENDATIONS

The study concludes as follows:

- 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.
- iii. The physico-chemical parameters of water such as Temperature, dissolved oxygen, pH, electrical conductivity and total dissolved solids were normal and within acceptable limits.

Based on the findings, the study recommends timely fish feed since time was a strong dependent variable in all the treatment, so as increase concentration of mercuric chloride increased.

REFERENCES

- Allen, P. (1994) 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 Endocrinology, **108**(1):117-21.



- Annune, P. A. and Ahuma, H. (1993). Haematological Change in Mudfish *Clarias gariepinus* exposed to Sub-lethal Concentrations of Copper and Lead. *Journal of Aquatic Science*, **13**:33-36.
- Bhagwart, S. and Bhikajee, M. (2002). Induction of hypo-chromic macrocytic anaemia in *Oreochromis* hybrid (Cichlidae) exposed to 100mg/L sublethal dose of aluminium. *Journal of Science and Technology*, **5**: 9-16.
- Blaxhall, P. C. and Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, **5**: 771-781.
- Carvalho, C. S. and Fernandes, M. N. (2006). Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture*, **251**: 109–17.
- Dawson, V. K. (1982). 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*, **39**: 778–782.
- De Boeck, G., Vlaeminck, A. and Blust, R. (1997). 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*, **33**: 415-22.
- Dhanekar, S., Srivastava, S., Rao, K. S. and Pandya, S. S. (1985). Studies on toxic effects of least effective concentration of mercury in fish: a haematological study. *Matsya*, **11**: 75-78.
- Dieter, M. P., Boorman, G. A., Jameson, C. W. and Fustis, S. L. (1992): 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*, **36**: 319- 340.
- Gbem, T. T., Balogun, J. K. Lawal, F. A. and Annune, P. A. (2001). Trace metal accumulation in *Clarias gariepinus* exposed to sublethal levels of tannery effluent. *Science Toxicology and Environment*, **271**: 1-9.
- Holcombe, G. W., Benoit, D. A., Leonard, E. N. and McKim, J. M. (1976). Long term effects of lead exposure on three generations of brook trout, *Salvelinus fontinalis*. *Journal of Fish Research Board Canada* **33**: 1731-1741.
- Hossam, H. A, Mohammed, M. A, Iman, M. K. A. and Ashraf, A. E. (2007). *Studies on the effect of Thiobencarb herbicide on some biological, physiological, biochemical, histological and genetic aspects of Nile tilapia. Oreochromis niloticus*. *Environmental pollution, Egypt*. Pp 56-79.
- Javed, M. (2005). 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*, **2**: 326-331.
- Javed, M., Abdullah, S. and S. Yaqub (2008). *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). Pp. 1-32.
- Johansson-Sjoberg, M. L. and Larsson, A. (1979). Effect of inorganic lead on deltaaminolevulinic acid dehydratase activity and haematological variables in the rainbow trout, *Salmo gairdneri*. *Architectural Environmental Contamination Toxicology* **8**: 419-43.
- Khangrot, B.S. (2003): Mercuric induced morphological changes in the Respiratory surface of an Asia freshwater catfish *Saccobranchus fossilis*. *Bul.Env.Cont. Toxicology*, **70**: 705-712.
- Kori-Siakpere, D., Adamu, K. M. and Madukelum, I. T. (2007). Acute haematological effect of sub-lethal levels of Paraquat on the African catfish, *Clarias gariepinus* (*Osteichthyes: Claridae*). *Journal of Research of environmental Sciences*, **1**(6): 335-331.
- Kumar, S. S. Lata and K. Gopal (1999). Deltamethrin induced physiological changes in freshwater catfish *Heteropneustes fossilis*. *Bulletin Environmental Contamination and Toxicology*, **62**: 254-258.



- Li, C., Xu, H., Xu, J., Chun, X. and Ni, V. (2011). Effects of aluminium on ultrastructure and antioxidant activity in leaves of tea plant. *Acta Physiology of Plant*, **33**:973– 978.
- Mazon, A. F., Pinheiro, G. H. D. and Fernandes, M. N. (2002). Haematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Brazilian Journal of Biology*, **62**(4A): 621-631.
- Morsy, M. G and Protasowicki, M. (1990). Cadmium Bioaccumulation and Its Effects on Some Hematological and Histological Aspects in Carp, *Cyprinus carpio* (L) at Selected Temperature. *Acta Ichthyologica et Piscatoria*: Fasc, **1**(20) 105 - 115.
- Nanda, M. and Behera, M. K. (1996). Nickel induced changes in some heamotobiochemical parameters of a cat fish *Heteropneustes fossilis* (Bloch). *Environmental Ecology*, **14**: 82-85.
- Onusiriuka, B. C. (2002). Effects of sub-lethal concentrations of formalin on weight gain in African catfish, *Clarias gariepinus* (Toungals). *Journal of Aquatic Sciences*, **17**: 66-68.
- Panigrahi, A. K and Misra, B. N. (1978). Toxicological effects of mercury on a freshwater fish, *Anabas scandens*, CUV. and VAL. and their ecological implications. *Environmental Pollution* **16**: 31-39.
- Risher, J. F., Amler, T. and Sherlita, N. (2005). Mercury exposure: evaluation and intervention, the inappropriate use of chelating agents in diagnosis and treatment of putative mercury poisoning. *Neurotoxicology*, **26** (4): 691–699.
- Sastry, K. V and Sharma, K. (1980). Mercury induced haematological and biochemical anomalies in *Ophiocephalus* (*Channa punctatus*), *Toxicology Letters*, **5**: 245-249.
- Shah, S. L. and Altindag, A. (2004). Haematological parameters of tench (*Tinca tinca* L) after acute and chronic exposure to lethal and sublethal mercury treatments. *Bulletin Environmental Contamination Toxicology*, **73**: 911-918.
- Srivastava, A.K and Mishra, S. (1979). Blood dyscrasia in a teleost, *Colisa fasciatus* after acute exposure to sublethal concentrations of lead. *Journal of Fish Biology*, **24**: 199-203.
- Stephan, E. C. (1982). 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*, **766**: 69-81.
- Svoboda, M., luskova, V., Drastichova, J. and Zlablek, V. (2001). The effect of diazoinion on hematological indices of common carp (*Cyprinus carpio* L.). *Acta Veterinary Brno*, **70**: 457-465.
- Tewari, H. and Gill, T.S. (1987). Impact of chronic lead poisoning on the haematological and biochemical profiles of fish, *Barbus conchoniis*. *Bulletin Environmental Contamination Toxicology*, **38**: 748-752.
- Toussain, M. W.; Rosencrance, A. B.; Brenna, L.M.; Beaman, J. R.; Wolfe, M. J.; Hoffmann, F. J. and Gardner, H. S. (2001). Chronic Toxicity of Chloroform to Japanese Medaka Fish. *Environment*.
- Wedemeyer, G. A. and Nelson, N. C. (1975). Statistical methods for estimating normal blood chemistry ranges and variance in rainbow trout (*Salmo gairdneri*), Shasta strain. *Journal of Fisheries Research Board of Canada*, **32**: 551-554.
- Zinkl, J. G. (1986). Avian Haematology. Schalm's Veterinary haematology, Jain, N. C (Ed). 4th Edn. Philadelphia: Lea and Febiyer, **4**(1): 256-273.