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Impact of Copper Sulphate on Hematological and Some Biochemical Parameters of Common Carp (*Cyprinus carpio* L., 1758) In Different pH

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Abstract: The sensitivity of the neotropical freshwater fish *Cyprinus carpio* to copper was evaluated at low and high water pH. Juvenile fish were acclimated at 27±2°C and exposed to copper sulphate in water with pH 5.0 and 9.0. No copper was added to controls groups in water pH 5.0, 9.0 and 7.5. The obtained results in copper-exposed group with alkaline water showed a decrease in RBC, Hct and Hb than those kept in copper-exposed group in acidic water. WBC showed an increase in copper- exposed group in acidic water than those kept in control pH 7.5. Lymphocite in copper-exposed fish in water pH 9.0 and 5.0 showed a decrease in relation to those kept in control pH 7.5. Glucose in control pH 9.0 group, decrease compared to control pH 7.5. Total Protein in control pH 9.0 and in copper-exposed fish in acidic water and alkaline water decreased compared to control pH 7.5. It was concluded that *Cyprinus carpio* is affected by the change of water pH and heavy metal in alkaline water which in turn affect the hematological and some biochemical parameters of fish.

Key words: Common Carp · Alkaline · Toxicity of Copper Sulphate

INTRODUCTION

Copper is considered to be an necessary trace element for plants and animals. It is a unit of many metalloenzymes and respiratory pigments [1]. Copper also attends to, as the oxygen connecting site in haemocyanin and the respiratory blood pigment in many molluscs and certain other invertebrates [2]. However, copper becomes toxic to aquatic biota when biological necessity are exceeded. Many studies indicate that copper in the water is held in solution essentially by complexation with naturally happening organic ligands [3]. In freshwater, organic ligands are more important in obligatory copper than inorganic ligands containing sulfur, phosphorus, chloride, nitrogen. These latter complexes are more important in seawater because of higher concentrations of these ligands [4]. Dissolved organic-copper include complexes with amino acids, carboxylic acids, humic acids and various copper compound [5]. The closeness of copper for humic acids is greater than for fulvic acids [6]. The shock of copper on the aquatic environment is complicate and depends on the physicochemical characteristics of water [7] Alkalinity, hardness and pH forcefully influence copper speciation in water and, so, its bioavailability for absorption by fish [8] Copper speciation is directly affected by water pH and the free cupric ion concentration is higher in water with low pH, while copper hydroxide complexes prevails in water with high pH [8, 9]. Copper toxicity in aquatic animals is lower when chelating factors such as EDTA and NTA, humic acids and suspended solids are present in water and is known to be higher in soft water than in hard water [10]. However, little is known about the concomitant effects of temperature and pH on copper toxicity.

Heavy metals are absorbed through gills and/or gastrointestinal track by fish organisms, free of the uptake way they are chiefly accumulated in metabolically active tissues such as liver and kidney. Gills are indirect contact with the outer environment therefore with the pollutants. Liver acts in the transformation of basic nutrients and in detoxification on storage of toxic materials. The interference in the gas exchange,

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nitrogenous waste excretion, acid-base and ionic stability due to the change in water pH cause stress in fish affecting its body physiology and growth [11, 12]. Though majority of the fish farmers in Iran take safeguard to hinder abrupt changes in water pH in aquaculture operations, such situations may happen due to the excessive inputs of supplementary feed, manures and inorganic fertilizers to get higher production per unit area. the present study aimed to evaluate the hematological and biochemical parameters of common carp (*Cyprinus carpio*) exposed to the toxicity of copper at low and high water pH, Common carp is a potential species for fish culture and is highly tolerant to changes in water pH and is so sensitive to copper sulphate. Also a comparative study of these responses in the common carp exposed to $CuSo_4$ in different environmental pH would further help in identifying better species for culture. Moreover, the hematological and biochemical variables were measured since they are frequently used to evaluate the state of fish.

MATERIALS AND METHODS

C. carpio [W=64.5±1.6 g and L=17.6± 0.1 cm] were obtained from the nearest of fish farms in Sari and transported to the Caspian Sea Ecology Research Institute. The fish were kept at 27±2°C and pH ranging from pH 7.1 to 7.5 in tanks supplied with a successive flow of aerated dechlorinated water which was checked up once a day [water characteristics range (hardness=150-165 mg L^{-1} as $CaCO_3$, Alkalinity=145-159 mg L^{-1} as $CaCO_3$, Dissolved oxygen 7/36±1/12 mg L^{-1}). After which the fish were kept at this temperature for a minimum of 14 days previous to the experiments. The natural light cycle of laboratory was 12D:12L and the fish were fed with balanced fish food (pellet food, Chineh Co., Tehran, Iran). Feeding was postponed 24 h previous to the experiments. Toxicity tests with 3 replications (30 fish in each copper concentration/pH and controls in each pH) were performed in static systems (200-L glass aquaria) with successive aeration, fixed temperature (27± 2°C) and pH (5.0 or 9.0) for 2 weeks. The water used in the toxicity tests was the same as the acclimation period and no copper was added to the tanks containing the control fish. The fish exposed to sublethal concentration of copper sulphate 2 mg/l [4]. The pH was fitted using ultra pure HCl and/or NaOH and was ceaselessly observed during the toxicity test. Exchange of water from the experimental tank was on a daily basis to remove the excreta and uneaten feed and maintain the pH. Prior to water exchange, required stock solution of acid and alkaline were added to stored tap water kept in separate containers to create the desired pHs of 5.0 and 9.0 with fitted as required. After copper was added to the water, the dead fish in each aquarium were removed and counted every 24 h. The control and copper groups were placed in 200-L glass aquaria as described above and The Stock solutions for acid and alkaline water was prepared in separate tanks to be used as needed.

No copper sulphate was added to the aquaria of the control groups. 3 additional control groups (water with pH 7.5 at 27±2°C) were used in order to verify the effect of pH on the hematological parameters. Survivors of copper sulfate exposure and fish from each control group were sampled after 2 week of experiment.

Blood Sampling: The fish were removed and anaesthetized with 0.01% benzocaine and blood was taken from the caudal vein. Blood samples were used to measure hematocrit (Hct), hemoglobin (Hb) concentration and red blood cells count (RBCs), which was done immediately. Hct was determined by spinning the blood sample contained in heparinized capillary tubes in a microhematocrit centrifuge the Hb concentration was determined using the cianmethemoglobin method and RBCs and WBCs count was carried out in a modified Neubauer chamber after saline (0.9% NaCl solution) dilution of the blood differential white cell counts were done on blood films stained with Giemsa.

The blood indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were then calculated using the blood measurements above [13].

HCT/RBC)*100)= MCV)) Hb/RBC)*10)= (MCH
(Hb/HC)*100)=(MCHC)

Plasma biochemical parameters of plasma glucose, total plasma protein, Albumin and ALT and AST were all determined colorimetrically using commercial diagnostic kits (Randox Ltd, UK) following the manufacturer's instruction; using a spectrophotometer (Spectrumlab 21A, Lenjguang Tech, China). Serum protein and blood glucose content were measured spectrophotometrically following the diagnostic protocol of Boerhinger Manheim GmbH (Mannheim, Germany) and using the diagnostic kits.

Albumin concentration was assayed spectrophotometrically at 630 nm used the standard kit (Felicit-Diagnosis, Ukraine). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using kits supplied by Diamond Diagnostics.

Statistical Analysis: All replicates were used for calculation of mean values. Statistics were performed with the SPSS 10.1 computer program. Differences in hematological parameters between different pH and exposure to copper sulphate were processed statistically by means of the analysis of variance (One-way ANOVA). The hematological parameters were expressed as means ± standard deviation. Differences were considered significant at 0.05 probability.

RESULTS

Erratic swimming movement was observed in Common carp when exposed to control pH 5.0 and 9.0 and copper exposure groups.

No control fish died during the experimental period. Fish mortality occurred only in the copper-exposed groups. In general fish did not die before at least 24 h of copper exposure, regardless of the temperature and pH in which they were kept, the only exception being the fish kept in water with pH 9.0 at 27±2°C which began to die between 10-24 h.

Table 1 and 2 shows the blood parameters data and the interactions of pH and copper on these parameter.

The Hct, RBCs, Hb concentrations were lower in water pH 9.0 with copper sulfate than those kept in water pH 5.0 with copper sulphate. Hb in conrol pH 5.0 increase than those kept in water pH 9.0 with copper. MCHC increase control pH 5.0 compared control pH 7.5 and copper exposure in water pH 9.0.WBCs in water pH 9.0 with copper sulphate decrease than in water pH 5.0 with copper sulphate and increase in water pH 5.0 with copper sulfate than those kept in water pH 7.5. LYM in water pH 9.0 and 5.0 with copper sulfate decreased in relation to those kept in water pH 7.5. NUT in copper exposed group in water pH 5.0 was increased compared control pH 9.0.

ALT in copper- exposed fish in water pH 5.0 and 9.0 decreased comparing to control pH 7.5 and in control pH 9.0. and AST in copper-exposed group in water pH 9.0 and 5.0 respectively, showed an increase compared to control pH 7.5. GLU in water pH 9.0 and 5.0 respectively, showed a decrease compared to control pH 7.5 and in copper-exposed fish in water pH 9.0 increased compared to control pH 7.5. Tpro in water pH 9.0 and in copper-exposed group in water pH 5.0 and 9.0, respectively, decreased compared to control pH 7.5.

DISCUSSION

The present result approve that copper toxicity in *C. carpio* is dependent on water pH, as already reported by Radhakrishnaiah *et al*. [14] and Stouthart *et al*. [11]. The disturbances in the gas trade, nitrogenous waste excretion, acid-base and ionic balance due to the change, in water pH cause stress in fish affecting its body physiology and growth [12-14]. The effect of water pH on the metal activity is complex, since the pH affects either the solubility and/or the speciation of many metals [15]. The dominant copper species in water pH lower than 6.0 is the free cation form (Cu^{2+}) and the solubility of metal increases. The $Cu(OH)_2$ is the dominant species in water pH 9.0 and higher [9]. The effect of pH on copper speciation is especially important in soft and low alkalinity waters such as most of Iran's continental waters. In hard water, metal activity is low due to copper complexes with Ca^{2+} or CO_3^{2-} (8) while, in soft water, which is low in calcium and has low buffering capacity, copper activity is expected to be higher and more toxic to fish, especially at low pH.

However, in *C. carpio* (this study), as in *Oncorhynchus mykiss* [16] and *Prochilodus scrofa* [10] copper toxicity was lower at low water pH. The gill is the primary aim organ for the toxic action of copper. deterioration of the respiratory and the ionoregulatory functions may happen due to the structural changes and an increased the ion penetrability of the gill epithelia [17, 18]. Such toxic effects may result in biochemical and physiological changes in fish blood [19]. The rivalry of H^+ and Cu^{+2} ions for the similarly binding -uptake sites on the gill epithelium [20] may describe the low toxicity of copper in low water pH in *C. carpio*. The measurement of biochemical and hematological changes in blood of fish exposed to the toxicant may be used to predict the toxic effects of toxicant. Copper and pH stress are known to induce changes in the blood parameters of fish [21]. Most changes in blood cells at low water pH express interference in the ionic status and fluid volume. In the case of *C. carpio,* blood changes to be affected by the water pH. decrease of the Hct, RBCs, Hb concentrations in copper exposure in water pH 5.0 compared to in copper exposure in water pH 9.0, may demonstrate hemodilution and the dynamic imbalance between the extracellular (ECFV) and intracellular (ICFV) fluid volume caused by ion loss and the shift of fluid from extracellular to intracellular compartments resulted and decrease of the MCHC [10]. Haemodilution resulting from the damaged osmoregulation across the gill epithelium also might have caused to such reduction in Hb content in copper exposure in water pH 9.0 [12-22]. The increase of hemoglobin content in fish kept in control water pH 5.0 than those kept in copper exposure in water pH 9.0 also indicate that there was a possibility of respiratory stress

Table 1: Hematological data from *C. carpio* of the control groups (water pH 7.5, 5.0 and 9.0 free of copper) and groups exposed to copper (2 mg/l) in water pH 5.0 and 9.0

	Control			Copper exposure (2 mg/l)	
	pH			pH	
	7.5	5.0	9.0	5.0	9.0
Hematocrit (%)	26.50±1.87	32.33±2.30	27.33±1.66	26.33±1.30^{b}	22.66±2.22^{a}
RBCs (*10^6/mm^3)	1.59±1.27	1.71±1.55	1.61±1.28	1.90±9.56^{b}	1.36±1.72^{a}
Hemoglobin (g/dl)	5.91±0.53	7.54±0.75^{b}	6.68±0.61	6.78±0.75^{b}	5.19±0.50^{a}
MCV (µm^3)	1.82±0.80^{a}	1.61±6.51^{a}	1.70±11.25^{a}	1.70±6.84^{a}	1.70±10.09^{a}
MCH (pg)	40.18±1.41^{a}	44.06±2.73^{a}	41.78±1.56^{a}	40.62±2.20^{a}	39.05±1.88^{a}
MCHC (%)	22.18±0.74^{a}	27.39±1.84^{b}	25.14±1.38	23.93±1.03	23.13±0.91^{a}
WBCs (*10^3/ml)	5.53±6.44^{a}	7.83±1.06	11.16±1.08	14.33±1.49^{c}	8.03±1.25^{ab}
NUT (%)	2.16±0.79	3.33±0.80	1.33±0.33^{a}	6.00±2.01^{b}	3.33±1.54
MONO (%)	0.00±0.00^{a}	0.33±0.33^{a}	1.33±0.33^{a}	0.00±0.00	0.00±0.00
LYM (%)	97.66±0.76^{b}	94.50±1.91	98.00±0.57^{b}	89.66±2.70^{a}	91.16±3.01^{a}

Values are the means±SE. Letter with same superscript in the same row are not significant (p>0.05)

Table 2: Biochemical data from *Cyprinus carpio* of the controls groups (water pH 7.0,5.0 and 9.0 free of copper) and groups exposed to copper (2 mg/l) in water pH 5.0 and 9.0

	Control			Copper exposure (2 mg/l)	
	pH			pH	
	7.5	5.0	9.0	5.0	9.0
Tpro (g/dl)	3.46±1.53^{b}	1.90±0.75^{ab}	0.76±0.14^{a}	0.76±0.12^{a}	0.93±0.12^{a}
GLU (mg/dl)	150.00±11.9^{b}	70.00±18.43^{a}	57±9.62^{a}	130.65± 21.49^{b}	232.80± 22.61^{c}
ALB(g/dl)	3.23±1.53^{a}	3.26±0.75^{a}	3.73±0.12^{a}	4.66±0.14^{a}	4.73±0.12^{a}
ALT(g/dl)	51.93±6.72^{a}	51.20±6.12^{a}	57.46±26.42^{a}	27.10±32. 33^{b}	11.10±13.02^{a}
AST(U/L)	2.85±0.4^{a}	4.53±0.32	3.46±0.52	271.40±32.37^{c}	111.80±13.02^{bc}

Values are the means±Std Error. Letter with same superscript in the same row are not significant (p>0.05)

in carp. In this study there were no significant changes in MCV and MCH in any groups. As we know the leukocytes are involved in regulation of immunological function in the organism [23]. The increase of WBCs in copper exposure in water pH 5.0 than those kept in control water pH 7.5 showed a mobilization of cell defenses and decrease of LYM in water pH 9.0 and 5.0 with copper sulphate than those kept in water pH 7.5 suggest secondary effect of copper [23]. Also Neutrophils and monocytes are important white blood cells to defend the body, through their exalted phagocytic activity, against bacterial infection in damaged tissue. The percentage of these cell types generally decreases during acute exposure to copper [24], but in this research the neutrophil percentage has been reported to increase in exposure copper in water pH 5.0. In *C. carpio* the monocyte did not change in none of groups but neutrophil percentage increased significantly which may also be related to gill tissue damage [25]. The higher serum protein reduction in control pH 9.0 and copper exposure in water pH 5.0 and 9.0 may be attributed to the protein catabolism, the process converting blood and structural protein to energy, to meet higher energy demand during the prevailing stress and it can indicates cirrhosis or significant liver damage [26]. In copper exposure in water pH 9.0 serum glucose level increased compared to control water pH 7.5, This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (27) and also indicate stress levels in carp [28]. The progressive agglomeration of blood glucose in copper exposure in water 9.0 suggest a predominance hyperglycaemia. The significant reduction in Lym in copper exposure in water pH 5.0 and 9.0 and greater elevation in blood glucose, particularly at higher water pH, could also be attributed to the additional stress due to possible accumulation of ammonia in fish body [18] An alanine aminotransferase (ALT) and An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. ALT is found mainly in the liver but

also in smaller amounts in the kidneys, heart, muscles and pancreas and AST in liver and red blood cells. Most increases in ALT and AST levels are caused by liver damage. both of ALT and AST in copper exposure in water pH 5.0 and 9.0 respectively increased compared to control pH 7.5 and control pH 9.0 indicate the incorporation of amino acids by way of amino transferases activities of these enzymes, into krebs cycle to overcome the stress of the exposed fish and also This may be due to hepatic cells injury or increased synthesis of the enzymes by the liver [24]. Alb had not significant differences among groups.

CONCLUSION

In conclusion, the blood parameters of *C. carpio* exposed to copper sulfate showed ionoregulatory interference, but also compensatory responses to allow fish to endure and showed that a changed in water pH near to the acidic or alkaline boundaries significantly affected hematology of *C. carpio* and pH instability should be prevent when using aquaculture inputs such as feed and fertilizer to guarantee optimum growth of this species. also for control of fish parasites and disease in ponds we should accent the sensitivity of the fishes to copper and principally the water pH and hardness of water of aquaculture ponds.

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REFERENCES

1. Demayo, A. and M.C. Taylor. 1981. Guidelines for Surface Water Quality. Vol. 1 Inorganic Chemical Substances. Copper. Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa.
2. Birge, W.J. and J.A. Black, 1979. Effects of Copper on Embryonic and Juvenile Stages of Aquatic Animals. pp: 373-399. In: J.O. Nriagu, (ed.). Copper in the Environment. Part ll. Health Effects.
3. Spear, P.A. and R.C. Pierce, 1979. Copper in the Aquatic Environment: Chemistry, Distribution and Toxicology. NRCC Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, pp: 227.
4. Fish Disease, Diagnosis and Treatment by Edward J. Noga, 2010. 2nd edition. New York, Wiley-Blackwell Publisher.(Jun 29, 2010), pp: 367.
5. Nelson, H., D. Benoit, R. Erickson, V. Mattson and J. Lindberg, 1986. The Effects of Variable Hardness, pH, Alkalinity, Suspended Clay and Humics on the Chemical Speciation and Aquatic Toxicity of Copper. EPA/600/3-86/023. PB86-1714444, pp: 132.
6. Huang, C. and Y.L. Yang, 1995. Adsorption characteristics of Cu (II) on humus-kaolin complexes. Water Res., 29: 2455-2460.
7. Lauren, D.J. and D.G. McDonald, 1986. Influence of water hardness, pH and alkalinity on the mechanism of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci., 43: 1488-1496.
8. Playle, R.C., R.W. Gensemer and D.G. Dixon, 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill micro-environment. Environ. Toxicol. Chem., 11: 381-391.
9. Tao, S., Y. Wen, A. Long, R. Dawson, J. Cao and F. Xu, 2001. Simulation of acid–base condition and copper speciation in fish gill microenvironment. Comp. Chem., 25: 215-222.
10. Carvalho, C.S. and M.N. Fernandes, 2006. Effect of temperature on copper toxicity and hematological responses in the neo tropical fish *Prochilodus scrofa* at low and high pH. Aquaculture, 251: 109-117.
11. Xander, J.H.X., Stouthar, Jeroen, L.M. Hanns, Robert, A.C. Lock and Sjoerd E. Wendelarbonga, 1996. Effect of water pH on copper toxicity to early life stage of the common carp (*Cyprinus carpio*). Environmental Toxicology and Chemistry, 15(3): 376-383.
12. Jeney, Z.S., J. Nemcsok, G. Jeney and J. Olah, 1992. Acute effect of sublethal ammonia concentrations on common carp (*Cyprinus carpio*): I. Effect of ammonia on adrenaline and noradernaline levels in different organ. Aquaculture, 104: 139-148.
13. Houston, A.H., 1990. Blood and circulation. In: C.B. Schreck and P.B. Moyle, (Eds.), Methods for Fish Biology. American Fish. Sc., Bethesda, pp: 273-334.
14. Sreenivasa Reddy, A., M. Venkata Reddy and K. Radharkishnaiah, 2008. Impact of copper on the oxidative metabolism of the fry of common carp at different pH, Journal of Environmental Biology, 29(5): 721-724.

15. McDonald, D.G., J.P. Reader and T.R.K. Dalziel, 1989. The combined effects of pH and trace metals on fish ionoregulation. In: R. Morris, E.W. Taylor, D.J.A. Brown and J.A. Brown, (Eds.), Acid Toxicity and Aquatic Animals. Cambridge University Press, Cambridge, pp: 221-242.
16. Cusimano, R.F., D.F. Brakke and G. Chapman, 1986. Effectof pH on the toxicities of cadmium, copper and zinc to steelhead trout (*Salmo gairdneri*). Can J. Fish. Aquat. Sci., 43: 1497-1503.
17. Lauren, D.J. and D.G. McDonald, 1985. Effect of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richard-son. J. Comp. Physiol., 155(B): 635-644.
18. Wilson, R. and E.W. Taylor, 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. J. Comp. Physiol., 163(B): 38-47.
19. Nussey, G., J.H.J. Van Vuren and H.H. Du Preez, 1995. Effect of copper on the hematology and osmoregulation of the mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). Comp. Biochem. Physiol., 111C: 369-380.
20. Lauren, D.J. and D.G. McDonald, 1986. Influence of water hardness, pH and alkalinity on the mechanism of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci., 43: 1488-1496.
21. Wang, T., P.K. Knudsen, C.J. Brauer, M. Busk, M.M. Vijayan and F.B. Jensen, 1998. Copper exposure impairs intra-and extracellular acid-base regulation during hypercapnia in the fresh water rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol., 168 B: 591-59.
22. Wedemeyer, G.A., D.J. McLeay and C.P. Goodyear, 1984. Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. In: W.V. Cairns and P.V. Hodson and J.O. Nriagu, (Eds.), Contaminant Effects on Fisheries. John Wiley and Sons Inc, New York, pp: 164-195.
23. Santhakumar, M., M. Balaji and K. Ramudu, 1999. Effect of sublethal concentration of monocrotophos on erythropoietic activity and certain hematological parameters of fish *Anabus testudineus* (Bloch). Bull. Environ. Contam. Toxxicol., 63: 379-384.
24. Svobodova, Z., B. Vykusova and J. Machova, 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. In: R. Müller and R. Lloyd, (eds.), Sublethal and Chronic Effects of Pollutants on Freshwater Fish. Fishing New Books, London.
25. Dick, P.T. and D.G. Dixon, 1985. Changes in circulating blood cell levels of rainbow trout, *Salmo gairdneri* Richardson, following acute and chronic exposure to copper. J. Fish Biol., 26: 475-484.
26. Das, P.C., S. Ayyappan and J.K. Jena, 2006. Hematological changes in the three Indian major carps *Catla Catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to acidic and alkaline water pH. Aquaculture, 256: 80-87.
27. Almeida, J., F. Novelli, M. Dal Pai Silva and R. Junior, 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. Environ. Pollut., 114: 169-175.
28. Mazeud, M.M. and I. Mazeud, 1981. Adrenergic response to stress in fish. In: A.D. Pickering, (Ed.), stress and fish. Academic Press, London, pp: 49-75.