http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (10): 1655-1660, 2007
ISSN 1028-8880

Effects of Copper Sulfate ($CuSO_4$) on the Levels of Glucose and Cortisol in Common Carp, *Cyprinus carpio*

[1]A. Afaghi, [1]S. Zare, [1]R. Heidari, [2]Y. Asadpoor and [2]R. Malekzadeh Viayeh
[1]Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
[2]Iranian Artemia Research Center, Urmia, Iran

Abstract: The objective of this study was to evaluate the possible effects of $CuSO_4$ exposure on variations of glucose and cortisol levels in *Cyprinus carpio*. Three replicates of 6 fish were subjected to two sub-lethal concentrations of $CuSO_4$ (0.16 and 0.53 mg L^{-1}) for 14 and 21 days. Blood samples were isolated from the fish following the exposure, to measure the levels of cortisol and glucose compared to the control group. The results showed significant increases ($p<0.05$) in cortisol levels for both fish groups after 14 days of exposure, whereas, the levels of blood cortisol in both groups did not differ from that of control when the fish subjected to copper sulfate for 21 days. We found significant increases ($p<0.05$) in the levels of blood glucose of two groups of fish after 14 days of exposure to two doses of $CuSO_4$, as well as significant decrease in the blood glucose of both groups exposed for 21 days. In the later treatment, the rate of decrease in group II (exposed to 0.53 mg L^{-1} $CuSO_4$) was higher than that of group I (exposed to 0.16 mg L^{-1} $CuSO_4$) ($p = 0.001$ compared to $p = 0.032$). Our findings attest that exposing to waterborne copper would affect the levels of both cortisol and glucose, as indicators of stress response in *Cyprinus carpio*.

Key words: Copper sulfate, cortisol, *Cyprinus carpio*, glucose

INTRODUCTION

Heavy metals are considered as the main causes of pollution in aquatic ecosystem and are expected to be so in the future, having the highest environmental stress index, often in excess of the recommend threshold limit values (Abdelmeguid *et al*., 2002). The toxic effects may result from bioconcentration of the metals and their consequent binding with biological active constituents of the body such as lipids, amino acids, enzymes and proteins (Vutukuru *et al*., 2005). Copper is one of the most abundant transition metals in nature and an essential constitute of all living tissues, however, when present at high concentrations, it becomes toxic to living organisms, including fish (Monteiro *et al*., 2005). Despite the critical role of copper in a number of vital processes including cellular respiration, the metal has the potential to exert adverse toxicological effects (Grosell *et al*., 2003).

The plasma corticosteroid level is generally used as an indicator to determine the magnitude of stress response in vertebrates including fish (Wu *et al*., 2002). Among with the primary adrenergic and cortisol response, secondary and tertiary responses such as elevated blood glucose concentration, suppressed levels of thyroid hormones, growth hormone and electrolytes and altered behavior are evident reactions in fish encountered to environmental stress (Flodmark *et al*., 2002). Heavy metals stimulate the Hypothalamus-Pituitary-Interrenal (HPI) axis and cause an elevation of cortisol in blood of fish (Handy, 2003). Chronic exposure to metals blunted the normal cortisol stress response and perturbed carbohydrate metabolism in perch (Levesque *et al*., 2002). Significant increases were reported in the cortisol levels of rainbow trout exposed to cadmium (Gill *et al*., 1993) and copper (Dethloff *et al*., 1999b). The release of corticosteroid hormones in sockeye salmon, *Oncorhynchus nerka*, was recorded following the treatment with copper (Shah, 2002).

Glucose is one of the most sensitive indices of the stress state of an organism and its high as well as lowered concentrations in blood has been considered as indicator of stress in fish (Vosyliene, 1999). The varying levels of blood glucose were noticed as to be indicative of abnormal carbohydrate metabolism and possibly the result of impaired hormonal control (Shah, 2002). The depletion of liver glycogen and the rise in blood glucose levels were observed in carp, *Cyprinus carpio*, after exposure to sub-lethal concentrations of different pollutants (Abdelmeguid *et al*., 2002). Increased and decreased glucose levels were recorded in rainbow trout following acute and long-term exposure to copper, respectively (Vosyliene, 1996a, b).

Fish are largely used in evaluation of aquatic systems quality and some of their physiologic changes can be

Corresponding Author: Azam Afaghi, Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
Tel: +989141701436

considered as biologic markers of environmental pollution (Dautremepuits *et al*., 2004). It has a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru *et al*., 2005). Common carp is a widely used species in aquaculture for food supply in Iran (Salehi, 2006). It provides a good model to study responses and possible adaptations of local fish populations exposed to diffuse pollution originated from various sources. In present study, we hypothesized that changes in the levels of blood cortisol and glucose of the carp exposed to sub-lethal concentrations of copper sulfate could be as bio-indices of pollution in a water source.

MATERIALS AND METHODS

Animals and experimental conditions: This study was carried out in the Laboratory of Fish Pathology of Iranian Artemia Research Center (Urmia, Iran), during the months June and August 2006. Common carp (200±50 g and 18.3±5 cm) were purchased from a local hatchery and were maintained in 200 L tanks containing aerated tap water. The other physicochemical elements of the water and experimental condition were kept quite instant during the course of study (Water temperature: 17±1°C; Ca^{2+}: 145.09 mg L^{-1}; Mg^{2+}: 398.7 mg L^{-1}; salinity: 1.7 g L^{-1}; dissolved oxygen: 7.25±3 mg L^{-1}; photoperiod: 12L/12D). Prior to the onset of treatment, the fish were acclimatized to the laboratory conditions for 14 days. They were fed once daily with commercial trout pellets (Chineh Co., Karaj, Iran), comprising the following ingredients: protein 36, lipid 14, ash 16, fiber 3.5, phosphorus 1, wet 11, carbohydrate 22.5 and fish meal 50%. Examined fish were divided into two groups exposed to two sub-lethal concentrations of copper (0.16 and 0.53 mg L^{-1} for group I and II, respectively). Copper stock-solutions were made from hydrated copper sulfate ($CuSO_4.5H_2O$), added subsequently to dechlorinated tap water in the tanks to obtain test concentrations. Control groups were the carps maintained in normal, aerated tap water. The water in each tank was replenished daily to keep the metal concentrations unchanged. From each of the exposed as well as control groups, three replicates of six fish were anesthetized by stroking on their headand their blood samples were taken through puncturing the caudal vessel.

Measurement of the levels of glucose and cortisol: Blood was taken from the tail vein of the fish with non-heparinized syringe, collected in plastic Eppendorf tubes. After centrifugation, blood plasma was removed and the samples were then analyzed for measuring the levels of glucose by Spectrophotometer Enzymatic Methods using a commercial glucose kit (Enzymatic, GOD Trinder). Cortisol levels in the blood were assessed by Radioimmunoassay (RIA), using LKB-Wallac apparatus and a cortisol kit made by Kavoshyar Co. (Iran).

Statistics: The data of glucose and cortisol measures were analyzed and compared with those of the controls by two methods of Analysis of Variance (ANOVA) and t-test using SPSS (version. 12) and at a significant level of $p<0.05$.

RESULTS

The mean values (±SEM) of cortisol and glucose levels for exposed and control groups of the fish are shown in Table 1 and 2. Statistical analyses have given evidence of significant differences between exposed and the control groups.

Exposure to both concentrations of $CuSO_4$ (0.16 and 0.53 mg L^{-1}) for 14 days, increased plasma cortisol levels of the examined fish significantly ($p<0.05$). While the control group had cortisol levels averaged 193.6±10.2 μg L^{-1}, the levels of cortisol in the fish exposed to the lower and higher doses of cupper sulfate reached to 291.3±26.9 and 379±10 μg L^{-1}, respectively. The minimum and maximum cortisol levels measured in the 14 day control group were 174 and 208 μg L^{-1}, respectively. Whereas, the maximum levels of cortisol grew up to 342 and 399 μg L^{-1} in the fish exposed to the lower and higher concentrations of copper sulfate, respectively. Nevertheless, the fish subjected to a twenty-one-day treatment with two different concentrations of copper sulfate had cortisol measures near or below the control values. So that, the difference between the cortisol levels of each of the latter groups and that of the control

Table 1: The levels of cortisol (μg L^{-1}) in blood serum of two groups of common carp exposed to two doses of $CuSO_4$ (0.16 and 0.53 mg L^{-1} for groups I and II, respectively) for 14 and 21 days

		Exposure time	
Groups	No.	14 days	21 days
Control	18	193.6±10.2	209.3±3
I	18	291.3±26.9[a]	257.0±28.5
II	18	379.0±10[a]	176.3±14

Data shown as mean±SEM; A: symbolizes having significant difference ($p<0.05$) from the control

Table 2: Concentrations of glucose (mg L^{-1}) in blood serum of two groups of common carp exposed to two doses of $CuSO_4$ (0.16 and 0.53 mg L^{-1} for groups I and II, respectively) for 14 and 21 days

		Exposure time	
Groups	No.	14 days	21 days
Control	18	240.0±11.5	245±8.6
I	18	720.0±11.5[a]	180±11.5[a]
II	18	747.5±18.7[a]	140±11.5[a]

Data shown as mean±SEM; A: symbolizes having significant difference ($p<0.05$) from the control

was not significant (Table 1). Plasma cortisol in the 21-day control fish had a range between 203 and 210 μg L^{-1}, while its maximum levels quantified to be 302 and 203 μg L^{-1} in the fish exposed to the lower and higher doses of copper sulfate, respectively. Changes in concentrations of blood glucose of the treated fish are presented in Table 2. Exposure to both concentrations of copper sulfate for 14 days has dramatically elevated plasma glucose levels of the fish comparing to the control ($p<0.05$). While mean glucose levels was measured to be 240±11.5 mg L^{-1} in the control group, it rose to 720±11.5 mg L^{-1} and 747.5±18.7 mg L^{-1} in the fish exposed to the lower and higher concentrations of copper sulfate, respectively. The lowest and highest glucose levels in the 14 day control group were 210 and 250 mg L^{-1}, respectively. However, they maximized to 740 and 780 mg L^{-1} in the fish groups treated with the lower and higher doses of copper sulfate, respectively. After 21 days of exposure to the two concentrations of $CuSO_4$, the blood glucose levels of the studied fish dropped to levels lower than that for the control group, recording as 180±11.5 and 140±11.5 mg L^{-1} for the groups exposed to the lower and higher copper-sulfate doses, respectively (Table 2). These decreases were significant as tested statistically, however, the magnitude of decrease in glucose level was higher for the group exposed to higher (0.53 mg L^{-1}) concentration of copper sulfate ($p = 0.001$ against $p = 0.03$). The control group for twenty-one day of treatment had glucose levels ranged from 230 to 260 mg L^{-1}, while the highest glucose levels, measured for the fish exposed to the lower and higher concentrations of copper sulfate, valued 200 and 160 mg L^{-1}, respectively.

DISCUSSION

In fish, copper is a classical limiting factor as it is both essential and toxic. As a micronutrient, it is necessary for hemoglobin synthesis and for being as a component of cytochrome oxidase (Benneth *et al*., 1995). Copper ions are quit toxic to fish at various functional levels when environmental concentrations are increased (Vutukuru *et al*., 2005). The toxic effects of copper are related to its capacity for catalyzing oxidative reactions that leads to the production of Reactive Oxygen Species (ROS) (Lopes *et al*., 2001). These highly reactive compounds may also induce tissue alterations and change some physiologic responses of fish, thus leading to oxidative stress (Sies, 1991; Paris-Palacios *et al*., 2000; Varanka *et al*., 2001). Elevated levels of copper become actually and chronically toxic to aquatic lives. While acute effects may be death, chronic effects could be reduced growth, shorter lifespan, reproductive problems, reduced fertility and behavioral changes (Olaifa *et al*., 2004). Furthermore, copper can affect metabolic activity at the biochemical levels (Valarmathi and Azariah, 2003).

Concentrations of cortisol and glucose are considered among the most important stress indicators in fish (Yang and Chen, 2002). Serum concentrations of glucose are regulated by complex interaction of such hormones as glucagons and cortisol. However, environmental stress can also cause marked elevation in plasma glucose levels (Martin and Black, 1998).

In this study, the levels of two biochemical components of blood, i.e., cortisol and glucose, have been considered as indices to display the degree of stress-induced responses in the carp subjected to two sub-lethal doses of copper sulfate. Plasma cortisol levels indicated significant increases when it exposed to two sub-lethal concentrations of copper sulfate (0.16 and 0.53 mg L^{-1}) for 14 days, while they reduced to the levels similar to those of the control in the fish exposed for 21 days (Table 1). Table 2 illustrates the varying concentrations of blood glucose of the treated fish. As can be seen in the table, exposing to both concentrations (0.16 and 0.53 mg L^{-1}) of copper sulfate for a period of 14 days caused significant elevations ($p<0.05$) in the levels of glucose in the fish plasma. Furthermore, there were significant decreases in the levels of glucose of the two fish groups following 21 days of exposure, however, the group exposed to higher concentration of copper sulfate (0.53 mg L^{-1}) revealed greater decrease, as indicated by a lower p-value. Monitoring of the other consequences of copper exposure than blood cortisol and glucose levels was not the focus of this study.

The increased cortisol and glucose levels observed in the fish following their exposure to copper sulfate was similar to the results obtained in a previous study on Nile tilapia, *Oreochromis nilotiscus* (Monteiro *et al*., 2005). A periodic treatment to copper sulfate caused significant elevation in glucose levels of tilapia (Chen *et al*., 2004). Cortisol is not stored in the interrenal tissue, but is synthesized on demand (Sumpter, 1997). Thus, elevation of cortisol levels in blood of fish must be a function of *de novo* stimulation of HPI axis (Handy, 2003). Cortisol release via the HPI axis was found to be stimulated by copper exposure (Pelgrom *et al*., 1995; Dethloff *et al*., 1999a). Cortisol affects carbohydrate metabolismand a rise in its levels is frequently followed by hyperglycemia in fish (Wendelaar Bonga, 1997). Although the mechanisms involved remain unclear, the rapid rise in plasma glucose concentration following an acute stressor has been associated with the activation of the Hypothalamus-Symphatic-Chromaffin cell (HSC) axis (McDonald and Milligan, 1997), rather than with the

cortisol rise mediated by the Hypothalamus-Pituitary-Interrenal (HPI) axis (Arends *et al*., 1999). Hyperglycemic response illustrated in this study is an indication of a disrupt carbohydrate metabolism, possibly due to enhanced breakdown of liver glycogen (glycogenesis) and the synthesis of glucose from extra-hepatic tissue proteins and amino acids (Larsson *et al*., 1985; Gill *et al*., 1993). Glucose may also be released into circulation in heavy metal-induced hypoxia, which enhances the mobilization of catecholamine (Žikiě *et al*., 2001) and processes of glycogenolysis by cortisol (Van-Raaij *et al*., 1996). Cortisol reduces uptake, phosphorylation and consumption of glucose by the extra-hepatic tissues and increases the liver glycogenolysis, resulting in the elevation of glucose levels in blood of fish (Shahbazi and Maleknia, 1999).

The fish subjected to lower concentration of $CuSO_4$ (0.16 mg L^{-1}) for 21 days, showed a slight cortisol and glucose decrease, whereas, those treated with the higher metal value (0.53 mg L^{-1}) revealed a larger glucose and cortisol decrease. This might be because the fish could provide an adaptation to the low copper values, while that for the higher concentration seem to characterize exhaustion. Adaptation implies changes in several related physiological processes, permitting homeostasis return (Martinez *et al*., 2004). It is, however, known, that the hypothalamus-hypophysis-interrenal coupling is one of those system responsible for the adaptive potential in fish (Flodmark *et al*., 2002). Stimulation of the system leads to intensified or reduced secretion of numerous hormones (Donaldson *et al*., 1990). Exhaustion occurs when the Hypothalamus-Pituitary-Interrenal (HPI) axis has been stimulated to a degree where the chronically elevated cortisol titer leads to a down-regulation of the system through negative feed back. This disables the fish to react appropriately to other eventual stressors. HPI axis exhaustion involves atrophy of the interrenal tissue, which is a slow process that cause decreased cortisol levels (Flodmark *et al*., 2002). Lowered or absent of cortisol responses in exhaustion status, observed in fish exposed to toxicants for long periods of time (Friedmann *et al*., 1996; Hontela *et al*., 1997). Glucose is one of the most sensitive indices of the stress state of an organism: its high concentrations in blood indicate that the fish is in stress and it is intensively using its energy reserves, i.e., glycogen, in liver and muscles. Mean while, a decreased concentration of glucose indicates the exhaustion of energy (glycogen) resources and subsequently, the worsening of an organism status. Namely, a decrease in glucose in the blood of fish was observed during long-term exposure to heavy metals (Vosyliene, 1996b). Similarly, a significant decrease in glucose levels was recorded in rainbow trout, *Oncorhynchus mykiss*, after a chronic dietary exposure to copper (Handy *et al*., 1999). As a result, the declined glucose levels in the fish treated for 21 days in this study, suggest that the fish might have been in a worsening state or were experiencing liver failure (Kram and Keller, 2001). Prolonged exposure to stressors in high concentration makes it difficult for a fish to adapt to and creates weakness characterized by decrease in serum cortisol and glucose levels. This, subsequently, creates a series of alterations in the metabolism and shortens the life span of organisms (Cicik and Engin, 2003).

To conclude, this study denotes that the fish responded to the copper exposure as represented by the changes in its blood cortisol and glucose levels and that the response was dose and period-dependent.

ACKNOWLEDGMENT

Thanks are due to the colleagues in Iranian Artemia Research Center for their assistance.

REFERENCES

Abdelmeguid, N., A.M. Kheirallah, Abou-Shaban, K. Adham and A. Abdel-Moneim, 2002. Histolochemical and biochemical changes in liver of *Tilapia zilli* G. as a consequence of water pollution. J. Biol. Sci., 2: 224-229.

Arends, R.J., J.M. Mancera, J.L. Munoz, S.E. Wendelaar Bonga and G. Flik, 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. J. Endocrinol., 163: 149-157.

Benneth, W.A., A. Sosa and T.L. Beitinger, 1995. Oxygen tolerance of fathead. J. Environ. Contam. Toxicol., 55: 517-524.

Chen, C.Y., G.A. Wooster and P.R. Bowster, 2004. Comparative blood chemistry and histopatology of tilapia infected with *Vibrio vulnificus* or *Streptococusiniae* or exposed to carbon tetrachloride, gentamicin or copper sulfate. J. Aquaculture, 239: 421-443.

Cicik, B. and K. Engin, 2003. The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L. 1785). Turk. J. Vet. Anim. Sci., 29: 113-117.

Dautremepuits, C., S. Betoulle, S. Paris-Palacios and G. Vernet, 2004. Immunology-related perturbations induced by copper and a chitson in carp (*Cyprinus carpio*). Arch. Environ. Contam. Toxicol., 47: 370-378.

Dethloff, G.M., D. Schlenk, S. Khan and H.C. Bailey, 1999a. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol., 36: 415-423

Dethloff, G.M., S. Daniel, T.H. Jonathan and C.B. Howard, 1999b. Alterations in physiological parameters of rainbow trout (*Onchorynchus mykiss*) with exposure to copper and copper/zinc mixtures. J. Ecotox. Environ. Safe, 42: 253-264.

Donaldson, E.M., V.H.M. Fragerlund and J.R. McBride, 1990. Aspects of the Endocrine Stress Response to Pollutants in Salmonids. In: Contaminant Effects on Fisheries. Cairns, V.W., P.V. 1Hodson, J. Widey and J.O. Nriagu (Eds.). New York, Publisher, pp: 213-220.

Flodmark, L.E.W., H.A. Urke, J.H. Halleraker and J.V. Arenekleiv *et al*., 2002. Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. J. Fish. Biol., 60: 238-248.

Friedman, A.S., M.C. Watzin, T. Brinck-Johnsen and T.C. Leiter, 1996. Low levels of diet. methyl mercury inhibit growth and gonad development in juvenile walleye (*Stizostedion vitreum*). J. Aquat. Toxic. Ams, 35: 265-278.

Gill, T.S., G. Leitner, S. Porta and A. Epple, 1993. Response of plasma cortisol to environmental cadmium in the eel, *Anguilla rostratalesueur*. J. Comp. Biochem. Physiol., 104: 489-495.

Grosell, M., M.W. Chris and J.W. Patrick, 2003. Copper homeostasis and toxicity in the elasmobranch *R. erinacea* and the teleost *M. octodecemspinosus* during exposure to elevated water-born copper. J. Com. Biol. Physiol., 135: 179-190.

Handy, R.D., D.W. Sims, A. Giles, H.A. Campbell and M.M. Masonda, 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic diet. exposure to copper. J. Aquat. Toxicol., 47: 23-41.

Handy, R.D., 2003. Chronic effects of copper exposure versus endocrine toxicity: Two sides of the toxicol. Process (review). J. Comp. Biochem. Physiol., 135: 25-38.

Hontela, A., C. Daniel and J.B. Rasmussen, 1997. Structural and functional impairment of the hypotalamo-pituitary-interrenal axes in fish exposed to bleached craft mill effluent in the St Maurice River, Quebec. J. Ecotoxicol., 6: 1-12.

Kram, J. and D.K.A. Keller, 2001. Toxicol. Testing Handbook. Marceldekker, New York.

Larsson, A., C. Haux and M. Sjóbeck, 1985. Fish physiology and metal pollution. Results and experiences from laboratory and field studies. J. Ecotoxicol. Environ. Safe, 9: 250-281.

Levesque, H.M., T.W. Moon, P.G.C. Campbell and A. Hontela, 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*P. flavescens)* chronically exposed to metals in the field. J. Aquat. Toxicol., 60: 257-267.

Lopes, P.A., T. Pinheiro, M.C. Santos, M. Mathias, M.J. Collares-Perira and A.M. Viegas-Crespo, 2001. Response of antioxidant enzymes in fresh water fish populations (*Lenciscus alburnoides*) complex to inorganic pollutants exposure. J. Sci. Total Environ., 280: 153-163.

Martin, L.K. and M.C. Black, 1998. Biomarker assessment of the effects of coal-strip mine contamination on channel catfish. J. Ecotoxicol. Environ. Safe, 41: 307-320.

Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Acute morphological and physiological effects of lead in the neotropical fish *P. lineatus*. Braz. J. Biol., 64: 797-807.

Mc Donald, G. and L. Milligan,1997. Ionic, Osmotic and Acid-Base Regulation in Stress. In: Fish Stress and Health in Aquaculture. Iwama, G.K., A.D. Pickering, J.P. Sumpter and C.B.S. Chreck (Eds.), pp: 119-144.

Monteiro, S.M., M.M. Juan, A. Fontinhas-Fernandes and M. Sousa, 2005. Copper induced alterations of biochemical parameters in the gill and plasma of *Orechromis niloticus*. J. Com. Biochem. Physiol., 141: 375-383.

Olaifa, F.E., A.K. Olaifa and T.E. Onwude, 2004. Lethal and sub lethal effects of copper to the African catfish (*Clarias gariepinus*) Juveniles. Afr. J. Biochem. Res., 7: 65-70.

Paris-Palacios, S., S. Biagianti-Ribourg and G. Vernet, 2000. Biochemical and (ultra) structural hepatic perturbation of *Brachydanio rerio* (teleostei, cyprinidae) exposed to two sub-lethal concentrations of copper sulfate. J. Aquat. Toxicol., 50: 109-124.

Pelgrom, S.M.G.J., R.A.C. Lock, P.H.M. Balm and S.E. Wendelaar Bonga, 1995. Effects of combined water-born Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, *Oreochromis mossambicus*. J. Comp. Biochem. Physiol., 111: 227-235.

Salehi, H., 2006. An analysis of the consumer market for carp products in Iran. Iran J. Fish. Sci., 47: 370-378.

Shah, S., 2002. Behavioral abnormalities of *Cyprinus wastoni* on exposure to copper and zinc. Turk. J. Zool., 26: 137-140.

Shahbazi, P. and N. Maleknia, 1999. General Biochemistry Tehran University Press, pp: 465- 488.

Sies, H., 1991. Oxidative Stress, Oxidants and Antioxidants. Academic, San Diego, pp: 1-15.

Sumpter, J.P., 1997. In: The Endocrinology Stress. Iwama, G.K., A.D. Rickering, J.P. Sumpter and C.B. Schreck (Eds.). Cambridge University Press, Cambridge, pp: 95-118.

Valarmathi, S. and J. Azariah, 2003. Effect of copper chloride on the enzyme activities of the crab *Sesarma quadratum* (Fabricius). Turk. J. Zool., 27: 235-256.

Van-Raaij, M.T.M., T. Van-Den, G.E.E.J.M. Llart, G.J. Vianen, D.S.S. Pit, P.H.M. Balm and A.B. Steffens, 1996. Substructure mobilization and hormonal changes in rainbow trout (*Onchorhynchus mykiss*, L.) and common carp (*Cyprinus carpio*, L.) during deep hypoxia and subsequent recovery. J. Comp. Physiol., 166: 443-452.

Varanka, Z., I. Rojik, I. Varanka, J. Nemcsok and M. Abrahám, 2001. Biochemical and morphological changes in carp (*Cyprinus carpio* L.) liver following exposure to copper sulfate and tannic acid. J. Comp. Biochem. Physiol., 128: 467-478.

Vosyliene, M.Z., 1996a. The effect of long-term exposure to copper on physiological parameters of rainbow trout (*Oncorhynchus mykiss*). 2. Studies of haematol parameters. J. Ekologija, 1: 3-6.

Vosyliene, M.Z., 1996b. Haematological parameters of rainbow trout (*Oncorhynchus mykiss*) during short-term exposure to copper. J. Ekologijia, 3: 12-18.

Vosyliene, M.Z., 1999. The effect of heavy metals on haematological indices of fish. J. Acta. Zool. Litu. Hydrobio., 9: 76-82.

Vutukuru, S.S., Ch. Suma, K. Radha Madhavi, Juveria, J. Smitha Pauleena, J. Venkateswara Rao and Y. Anjaneyulu, 2005. Studies on the development of potential biomarkers for rapid assessment of copper toxicity to freshwater fish using *Esomus danricus* as model. Int. J. Environ. Res. Public Health, 2: 63-73.

Wendelaar Bonga, S.E., 1997. The stress response in fish. J. Physiol. Rev., 77: 591-625.

Wu, S.M., Y.Y. Chou and A.N. Deng, 2002. Effects of exogenous cortisol and tolerance to waterborne cadmium in Tilapia (*Oreochromis mossambicus*). J. Zool. Study, 41: 11-118.

Yang, J. and H. Chen, 2002. Toxic effects of semiconductor metal gallium on common carp, *Cyprinus carpio*. Taiwan. J. Zool., 13: 63-72.

Žikiě, R.A., A.Š. Štajn, S.Z. Pavilovic, B.J. Ognjanovic, Z.S. Paviloviě, B.J. Organjanoviě and Z.S. Saiěiě, 2001. Activities of super oxide-dismutase and catalase in erythrocytes and plasma transaminases of gold fish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. J. Physiol. Res., 50: 105-111.