

Serum Biochemical Change Induced by *In vitro* Sub Chronic Mercury Chloride in Yellowfin Sea Bream (*Acanthopagrus latus*)

¹Aliakbar Hedayati, ²Alireza Safahieh, ²Ahmad Savari,
²Abdolali Movahedinia, ¹Parviz Zare and ¹Tahere Bagheri

¹Department of Fishery, Faculty of Fisheries and Environment,
Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

²Department of Marine Biology, Faculty of Marine Science,
University of Marine science and Technology Khorramshahr, Iran

(Received: February 18, 2011; Accepted: June 2, 2011)

Abstract: Biochemical parameters are indicators measured in a biological system which can be related to exposure to, or effects of, a contaminant compound. In current study some serum biochemical parameters were measured in order to investigate patterns of response and change caused by the mercury contamination in yellowfin sea bream. According to the LC₅₀, five mercury treatment was selected. Serum glucose was measured photometrically based on the quantification of NADH after a glucose oxidation catalyzed by glucose dehydrogenase. Serum total protein levels were determined with bovine serum albumin serving as standard. Values recorded for activity of total protein show high significance depletion (P<0.001) with mercury exposed. Although glucose was increase in different treatment, but there was not find significant variation. Results of the present investigation indicated that the sub- acute mercury concentrations tested is a toxic substance in yellowfin sea bream and may cause several changes in the serum biochemical parameters.

Key words: Serum biochemical % Mercury % Yellowfin sea bream

INTRODUCTION

Fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants. Fish are used as test organisms in aquatic toxicology because of their top-position in the trophic chain and their role as food for humans [1].

Biochemical markers are indicators measured in a biological system which can be related to exposure to, or effects of, a contaminant compound. They are measures of the rates of chemical reactions or the amounts of biochemical products in cellular or subcellular systems, sublethal biological effects, or histological measures [2].

Serum glucose concentration depends on intestinal absorption, hepatic production and tissue uptake of glucose. The balance between hepatic production and tissue uptake is influenced by a variety of hormones. Corticosteroids, catecholamines and growth hormone are called insulin antagonists because they interfere with

insulin's action on cells. Further more, glucagons and glucocorticoids stimulate hepatic gluconeogenesis and glucagons and catecholamines glycogenolysis [3].

These actions tend to increase serum glucose concentration. The practice of fasting animals prior to blood collection decreases the variability that accompanies postprandial intestinal absorption of glucose. Another procedural consideration for glucose analysis is prompt separation of the serum from clotted blood. Also, erythrocyte and to a lesser degree leukocyte, glycolysis will reduce serum glucose concentration by approximately 7 to 10 mg/dl every hour that the blood cells remain in contact with the serum at room temperature [3]. Blood Glucose level in fish is known to be very useful as a criterion for diagnosis of liver and muscle tissue functions [4].

Total serum protein concentration is a measure of all of the different proteins in plasma with the exception of those that are consumed in clot formation such as fibrinogen and the clotting factors. For this reason,

plasma protein concentration is generally about 0.3 to 0.5 g/dl higher than serum protein concentration [3].

Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning [5].

As we know proteins are a major constituent in the metabolism of animals and heavy metals may be involved in the normal working of these molecules, therefore it is important to detection of alterations in protein metabolism induced by metal exposure for further information. Alterations that may occur are the increased synthesis or breakdown of proteins and the inhibition or activation of certain enzymes. These changes can be observed in the total protein content, free amino acid (FAA) concentration and the activity of proteases and transaminases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [6, 7].

In current study some serum biochemical parameters were measured in order to investigate patterns of response and change caused by the mercury compounds, so the aim of current study was to determine the *in vitro* effects of mercury on biochemical characteristics of yellowfin sea bream.

MATERIALS AND METHODS

***In vitro* Design:** Total fish were obtained from Mahshahr creeks with hooks in a Upon capture, (only healthy fish, as indicated by their activity and external appearance, were used in the experiments), the fish were maintained alive on board in a fiberglass tank and on return to shore transferred to a 300-L aerated vat filled with sea water for transport back to the nearby laboratory. In laboratory Fish maintained in seawater re-circulatory system (300-L tanks) equipped with physical/chemical filters and with aeration.

Fish were randomly divided into five equal groups and allocated to a 15 static cylindrical polyethylene tank filled with the appropriate concentration of an aqueous solution of Hg in dechlorinated tap water. The Yellowfin sea bream were exposed to nominal mercury concentrations of 0 µg l (tank 1), 10 µg l (tank 2), 20 µg l (tank 3), 40 µg l (tank 4), 80 µg l (tank 5) respectively and maintained for three weeks with aeration. These sub-lethal doses were chosen on the basis of preliminary toxicity tests and determinations of LC_{50} 96h for this species, suggestive of inducing toxic effects but not lethally so [8]. Conditions within each experimental tank were monitored daily with the temperature $25C \pm 1$, pH 7.8 ± 0.1 and salinity 46 ± 1 ppt under a natural photoperiod (12hL:12hD) in controlled room. Water was oxygen saturated through constant aeration in a static system.

Fish Sampling: At sampling time (3 weeks post-exposure), nine fish from control and any confinement tanks were netted into a bucket containing clove, Fish were anesthetic and weighed, total lengths were recorded and an external examination for any signs of abnormalities or infestation was conducted [9].

Blood Collecting: To obtain blood samples, fish were quickly taken out from the water and held firmly on a bench with a cloth covering the head and blood was withdrawn from caudal vessels by a vacuum heparinized syringes and needle containing 0.1mL of anticoagulant (after filling up and expelling about 1.0 mL), to the Eppendorf tubes, whole blood withdrawal process took less than 1 min per fish. Handling time was less than 1min to minimize stress effects.

Extracted blood (1.5 mL), was immediately placed in tubes for biochemical analyses. Blood were placed in non-heparinized tubes and left to clot at 4°C for 15 min, Afterwards, tubes were centrifuged at 3000 rpm (20000-g) using an Eppendorf centrifuge for 10 min to obtain serum. The serum was separated into aliquots and was frozen and stored at -80°C until biochemical analyses. All samples were immediately immersed in liquid nitrogen and then transferred to a -80°C freezer until analysis.

Glucose Analysis: The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37 °C by the glucose oxidase method according to Trinder [10]. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay Mean \pm SD were 64.2 ± 1.12 and 92.5 ± 1.10 mg/dl respectively. Glucose was measured photometrically according to a method modified from Banauch *et al.*, [11] based on the quantification of NADH after a glucose oxidation catalyzed by glucose dehydrogenase. The quantity of NADH formed is proportional to the glucose concentration.

Protein Analysis: Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard by the method of Lowry *et al.*, [12] at 546 nm and 37C. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay coefficients of variation were of 0.91% and 1.06% respectively. Intra-assay and Inter-assay Mean \pm SD were 5.27 ± 0.05 and 5.24 ± 0.06 g/dl respectively.

Statistical Procedure: Significant differences were determined with one-way analysis of variance Anova with Duncan Post Hoc was used. To investigate associations between bioaccumulation and its effects, Pearson correlation coefficients (r) were calculated between mercury concentrations and enzymatic parameters. Multiple regressions were used to determine the relationship between mercury concentration and blood parameters. The differences between means were analyzed at the 5% probability level (p value of less than 0.05 was considered as statistically significant). Data are reported as means \pm standard deviation.

RESULTS

Results of *in vitro* biochemical analysis are presented in table 1. Values recorded for activity of total protein show high significance depletion ($P < 0.001$) with mercury exposed. Although glucose was increase in different treatment, but there was not find significant variation ($P < 0.05$).

The correlation between mercury with biochemical parameters were statistically tested by analyzing the data obtained during the mercury exposed. Both glucose and protein parameters show significant correlation ($P < 0.05$)

Table 1: *In vitro* biochemical activities of yellowfin sea bream exposed to mercury chloride.

	Control	10 μg	120 μg	140 μg	180 μg l
Glucose (mg/dl)	66.83 \pm 25.89 ^a	67.83 \pm 3.81 ^a	77.33 \pm 11.60 ^a	92.66 \pm 31.72 ^a	88.33 \pm 29.10 ^a
Total protein (mg/dl)	7.32 \pm 0.90 ^a	7.40 \pm 1.20 ^a	6.29 \pm 0.62 ^a	5.10 \pm 0.78 ^b	4.99 \pm 1.13 ^b

Table 2: *In vitro* correlation of enzyme activities of yellowfin sea bream with mercury chloride

	Glucose	Total protein
Pearson correlation (r)	0.36 [*]	0.67 ^{**}
sig (p)	0.04	0.00

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 3: *In vitro* curve fit linear regression of biochemical activities of yellowfin sea bream with mercury

	Glucose	Total protein
R square (r^2)	0.13 [*]	0.45 ^{**}
F	4.2	23.7
sig (p)	0.04	0.00

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

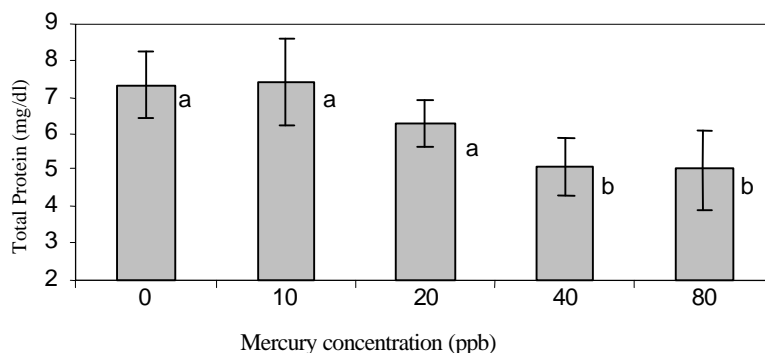


Fig. 1: Total protein response in the yellowfin sea bream during *in vitro* exposed to different concentration of mercury chloride

with mercury exposed, that also both correlations were negative (Table 2).

Curve estimation regressions data were used to determine the relationship between mercury concentration

and total protein and glucose content. Both biochemical parameters show significant linear regression ($P < 0.05$) with mercury (Table 3). Regressions model $Y = a \pm bX$ of significant parameter are in Fig. 2.

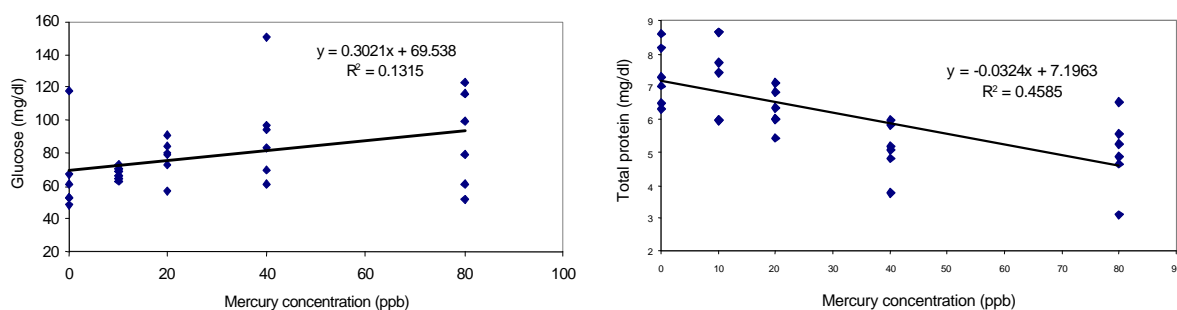


Fig. 2: Regressions model ($Y = a \pm bX$) of Glucose and Total protein of the yellowfin sea bream during *in vitro* exposed to different concentration of mercury chloride.

DISCUSSION

Levels of glucose were measured as conventional stress markers to assess the reliability of stress response triggered under our experimental conditions. Serum glucose levels in control fish did not significantly vary, however amount of glucose had increased regularly with increase of mercury dose that means hyperglycemia.

In animals, the most frequently encountered causes of hyperglycemia are failure to fast an animal and catecholamine release secondary to excitement or fear. Animals that become moribund occasionally develop hyperglycemia. Less frequently encountered causes, especially in toxicology studies, include insufficient insulin (diabetes mellitus and pancreatitis) and increased glucocorticoids (hyper adrenocorticism and steroid therapy) [3].

Some irregularities have been reported in glucose response when anesthetics are used before sampling. The ideal role of anesthetics is to minimize fish stress response, prevent any negative impact on performance and thus measure real values of glucose or other blood components [13]. Velisek *et al.*, [14] reported a significant increase in rainbow trout glucose when anesthetized with clove oil. Also clove oil and MS_{222} blocked cortisol secretion but increased glucose in another experiment with rainbow trout [15]. Those controversial results suggest that perhaps anesthetic efficacy in halting glucose increase is species dependant and also previous tests may be required.

Levels of glucose often increase during the first phase of the stress response due to an elevated breakdown of glycogen [16]. Smet and Blust [17] observed no significant changes in the levels of serum glucose at cadmium exposure.

Significant increases in glucose were observed as a result of toxic effect of Cu [18]. These results may be attributed to the hepatocellular damage. The plasma

glucose concentration of gilthead sea bream exposed to acute confinement was increased [19]. From aspect of increase, our observations show no disagreement with the literature values. West *et al.*, [20] argued that during peak activity glucose use can increase by almost 30- fold. A weak or no change in plasma glucose may be attributed to a high energy demand so that glucose cannot be accumulated (acute experiments) or the organism be habituated (chronic experiments).

Some authors reported a weak rise of glucose [21], others found no change [22] and even a decrease [23]. Sometimes no significant changes in plasma glucose may be observed, because under stress the fish is rapidly consuming the energetic substrates generated (glucose) since the main function of the central nervous system (CNS) is to maintain homeostasis.

Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy [24]. In suboptimum or stressful conditions (internal or external) the chromaffin cells release catecholamine hormones, adrenaline and noradrenaline toward blood circulation [25]. Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through gluconeogenesis and glycogenolysis pathways [26] to cope with the energy demand produced by the stressor for the "fight of flight" reaction. Glucose is then released (from liver and muscle) toward blood circulation and enters into cells through the insulin action [27].

In current study, serum total protein levels in exposed fish had significance difference which amount of protein had depletion regularly with increase of mercury dose that means hypoproteinemia. This results from either decreased production or increased loss of protein. In dietary toxicity studies, decreased protein production can result from effects on food consumption, digestion, or absorption. Because of the reserve capacity of the liver,

hepatic injury must be fairly severe before protein synthesis is notably diminished. However, in large studies, small differences between the control and treated groups might be apparent with mild to moderate hepatotoxicity.

Loss of protein, both albumin and globulin, occurs with hemorrhage and exudative lesions such as burns. Albumin is the principle protein lost as a result of enteropathies and glomerulopathies. The half-life of albumin is shorter in smaller species, at approximately 2 days for mice and approximately 8 days for dogs [2]. Theoretically, impaired albumin synthesis or albumin loss can be detected earlier in the smaller species. Hydration status of the animal is always an important factor for proper interpretation of changes in serum protein concentrations. Hypoproteinemia, like anemia,

can be masked by dehydration. A small, statistically significant decrease in serum albumin concentration is one of the most frequent findings in toxicology studies. The exact mechanism is usually not apparent but a combination of factors, similar to those causing mildly lower glucose, are probably responsible [3].

Sancho *et al.*, [28] observed protein depletion under propanil stress in eel liver and muscle. Some studies indicate a decrease in total protein content during heavy metal exposure. Such decreases were, for example, found in the edible crab *Scylla serrata* exposed to cadmium or in the freshwater fish *Sarotherodon mossambicus* and the common carp exposed to mercury [7, 6]. Depletion in the protein content of the *Catla catla* exposed to mercury chloride sub-lethal concentrations were estimated [29]. Total protein contents of the liver of *Clarias gariepinus* showed a noticeable decrease after exposure to fenvalerate [30]. These results agree with those of Tripathi and Verma [31] who reported that exposing fish *Clarias batrachus* to fenvalerate induced a highly significant decrease of protein content. Reddy and Bhagyalakshmi, [6] found that total, structural and soluble proteins were decreased, in fenvalerate exposed fish *Cyprinus carpio*. Total protein content significantly decreased after treating freshwater fish *Labeo rohita* with chlordane [32]. Sancho *et al.*, [28], reported that total proteins had decreased in the European eel, *Anguilla anguilla* after fenitrothion exposure.

The rapid decrease in total protein content was associated with active degradation of proteins under stress. This fact is correlated to the development of resistance toward to toxic stress. Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism. Catabolism of

proteins makes a major contribution to the total energy production in fishes. Under stress situations may constitute a physiological mechanism with an important role in providing energy to cope with the stress situation. Therefore, depletion of total protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery [33]. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted [13]. The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period [4]. Reduction in protein content in liver of exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress [30].

Results of the present investigation indicated that the sub-acute mercury concentrations tested is a toxic substance in yellowfin sea bream and may cause several changes in the serum biochemical parameters.

ACKNOWLEDGEMENT

The authors are thankful to the Iranian National Science Foundation (INSF) with grant number 88000678 for providing financial support and necessary facilities.

REFERENCES

1. Besten, P.J. and M. Munawar, 2005. Ecotoxicological Testing of Marine and Freshwater Ecosystems. Taylor and Francis, pp: 293.
2. Kaneko, J.J., 1997. Clinical biochemistry of domestic animals (5th ed.). San Diego, CA: Academic Press.
3. Shayne, C.G., 2007. Animal Models in Toxicology. Taylor and Francis, pp: 950.
4. Shakoori, A.R., A.L. Mughal and M.J. Iqbal, 1996. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella*. Bull. Environ. Contam. Toxicol., 57: 487-494.
5. Jacobs, J.M., N. Carmicheal and J.B. Cavanagh, 1977. Ultra structural changes in the nervous system of rabbits poisoned with methyl mercury. Toxicol. Appl. Pharmacol., 39: 249-261.
6. Reddy, P.S. and A. Bhagyalakshmi, 1994. Changes in oxidative metabolism in selected tissues of the crab (*Scylla serrata*) in response to cadmium toxicity. Ecotoxicol. Environ. Saf., 29: 255-264.

7. Canli, M., 1996. Effects of mercury, chromium and nickel on glycogen reserves and protein levels in tissues of *Cyprinus carpio*. J. Zool., 20: 161-168.
8. Hedayati, A., A. Safahieh, A. Savar and J. Ghofleh Marammazi, 2010. Detection of mercury chloride acute toxicity in Yellowfin sea bream (*Acanthopagrus latus*). World J. Fish and Marine Sci., 2(1): 86-92.
9. Hodson, P.V., P.J. Klopffer-Sams, K.R. Munkittrick, W.L. Lockhart, D.A. Metner, P.I. Luxon, I.R. Smith, M.M. Gagnon, M. Servos and J.F. Payne, 1991. Protocols for measuring mixed function oxygenases of fishliver. Canadian Technical Report on Fisheries and Aquatic Science, 1829: 51.
10. Trinder, P., 1969. Determination of glucose concentration in the blood. Ann. Clin. Biochem., 6: 24.
11. Banauch, D., W. Brummer, W. Ebeling, H. Metz, H. Rindfrey and H. Lang, 1975. A glucose dehydrogenase for the determination of glucose concentrations in body fluids. Z. Klin. Chem. Klin. Biochem., 13: 101-7.
12. Lowry, O.H., N.J. Rosenborough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.
13. Pickering, A.D., 1998. Stress responses of farmed fish. In: K.D. Black, A.D. Pickering, (eds). Biology of Farmed Fish. Sheffield Academic Press, Sheffield, pp: 222-255.
14. Velisek, J., Z. Svobodová and V. Pia..ková, 2005. Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno, 74: 139-146.
15. Wagner, G.N., T.D. Singer and R.S. McKinley, 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Res., 34: 1139-1146.
16. Wendelaar-Bonga, S.E., 1997. The stress response in fish. Physiol. Rev., 77: 591-625.
17. Smet, H. And R. Blust, 2001. Stress Responses and Changes in Protein Metabolism in Carp *Cyprinus carpio* during Cadmium Exposure, Ecotoxicol. Environ. Safety, 48: 255-262.
18. Abdel-Tawwab, M., M. Mousa and F. Abbass, 2007. Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. Aquaculture, 272: 335-345.
19. Saera-Vila, A., J. Caldach-Giner, P. Prunet and J. Pérez-Sánchez, 2009. Dynamics of liver GH/IGF axis and selected stress markers in juvenile gilthead sea bream (*Sparus aurata*) exposed to acute confinement Differential stress response of growth hormone receptors Comparative Biochem. Physiol., Part A., 154: 197-203.
20. West, T.G., P.G. Arthur, R.K. Suarez, C.J. Doll and P.W. Hochachka, 1993. *In vivo* utilization of glucose by heart and locomotory muscles of exercising rainbow trout (*Oncorhynchus mykiss*). J. Experimental Biol., 177: 63-79.
21. Davis, K.B. and M.E. McEntire, 2006. Comparison of the cortisol and glucose stress response to acute confinement and resting insulin-like growth factor-I concentrations among white bass, striped bass and sunshine bass. Aquaculture America Book of Abstracts, pp: 79.
22. Jentoft, S., A.H. Aastveit, P.A. Torjesen and Ø. Andersen, 2005. Effects of stress on growth, cortisol and glucose levels in nondomesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comparative Biochem. Physiol. Part A., 141: 353-358.
23. Wood, C.M., P.J. Walsh, S. Thomas and S.F. Perry, 1990. Control of red blood cell metabolism in rainbow trout after exhaustive exercise. J. Experimental Biol., 154: 491- 507.
24. Lucas, A., 1996. Physical concepts of bioenergetics. In: Lucas, A. (ed). Bioenergetics of aquatic animals. English edition, Taylor and Francis, France.
25. Reid, S.G., N.J. Bernier and S.F. Perry, 1998. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochem. Physiol. Part C, 120: 1-27.
26. Iwama, G.K., M.M. Vijayan, R.B. Forsyth and P.A. Ackerman, 1999. Heat shock proteins and physiological stress in fish. American Zoologist, 39: 901-909.
27. Nelson, D.L. and M.M. Cox, 2005. Lehninger Principles of Biochemistry. 4th ed.; WH Freeman and Co. New York, pp: 1013.
28. Sancho, E., M.D. Ferrando, C. Fernandez and E. Andreu, 1998. Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion. Ecotoxicol. Environm. Saf., 41(2): 168-175.

29. Prasath, M. And S. Arivoli, 2008. Biochemical Study of Freshwater Fish *Catla catla* with Reference to Mercury Chloride. *Iran. J. Environ. Health. Sci. Eng.*, 5(2): 109-116.
30. Sakr, S.A. and J.S.M. Al lail, 2005. Fenvalerate Induced Histopathological and Histochemical Changes in the Liver of the Catfish *Clarias Gariepinus*. *J. Appl. Sci. Res.*, 1(3): 263-267.
31. Tripathi, G. And P. Verma, 2004. Fenvalerate-induced changes in a catfish, *Clarias batrachus*: metabolic enzymes, RNA and protein. *Comp. Biochem. Physiol.*, 138(1): 75-79.
32. Bansal, S.K., S.R. Verma, A.K. Gupta and R.C. Dalela, 1979. Physiological dysfunction of the haemopoietic system in a fresh water teleost *Labeo rohita* following chronic chlordane exposure. Part II. Alterations in certain organic components and serum electrolytes. *Bull. Environ. Contam. Toxicol.*, 22: 674-680.
33. David, M., S.B. Mushigeri, R. Shivakumar and G.H. Philip, 2004. Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein metabolism profiles. *Chemosphere*, 56: 347-352.