

Biochemical and Hematological Profiles of Common Carp (*Cyprinus Carpio*) under Sublethal Effects of Trivalent Chromium

Zeynab Abedi¹, Mohammad Kazem Khalesi^{1*}, Sohrab Kohestan Eskandari¹

Received: 23.10.2012

Accepted: 28.11.2012

ABSTRACT

Background: In natural waters and/or aquaculture facilities, fish are often exposed to chromium waste and demonstrate cumulative deleterious effects. To our knowledge, there are no studies concerning the effects of trivalent Cr on *C. carpio* hematology. This study presents hematological and some biochemical parameters of common carp, *Cyprinus carpio*, affected by sublethal concentration of trivalent chromium.

Methods: The fish in the experimental aquaria (three replicates each) were exposed to a sublethal chromium chloride concentration of 2 mg L⁻¹, which was prepared as stock solution and added depending on the volume of the aquaria to obtain the required concentration. After a period of 28 days, parameters such as hematocrit (Hct), hemoglobin (Hb), lymphocytes (Lym), neutrophils (Neu), total protein (TP), albumin, immunoglobulin M (IgM), glucose, red and white blood cells (RBC and WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were examined.

Results: Chromium exposure for 28 days significantly ($P < 0.05$) reduced the amounts of Hct, Hb, RBC, WBC, MCH, and MCHC, whereas albumin and glucose significantly ($P < 0.05$) increased in the examined fish as opposed to the control. The levels of Lym, Neu, MCV, IgM, and TP were not significantly different ($P > 0.05$) between the Cr-exposed fish and the control.

Conclusion: Hematological indices of fish, caused by chromium toxicity to *C. carpio*, 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.

Keywords: Biochemistry, Chromium, Common Carp, Hematology.

IJT 2013; 782-792

INTRODUCTION

The contamination of fresh waters with a wide range of heavy metals released from domestic, industrial and other man-made activities has become a matter of concern over the last few decades (1-6), which may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (7,8). The heavy metal, chromium, exists primarily in Cr (III) and Cr (VI) oxidation states; the later,

hexavalent species, is considered as more toxic in the environment due to its higher solubility and mobility (1,9). Chromium is a compound of biological interest, probably having a role in glucose and lipid metabolism as an essential nutrient (10). Among the heavy metals, chromium is an important pollutant from industrial effluents and induces deleterious effects on non-target aquatic organisms resulting in imbalance of an ecosystem (11). The toxic effects of hexavalent chromium have been

1- Department of Fisheries, Faculty of Animal Sciences and Fisheries, Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran.

*Corresponding Author: E-mail: m.khalesi@sanru.ac.ir

demonstrated on survival and physiology of fishes (12-16), gill of roach (17), inhibition of various metabolic processes (18), osmoregulatory ability and respiration in fish (19), and various physiological processes of mudskipper *B. detanus* (20). In addition, biochemical profiles in fish and other aquatic organisms under heavy metal stress serve as important bio-indicators for monitoring of aquatic environment (21-24). Hematological indices are very important parameters for the evaluation of fish physiological status, the changes of which depend on fish species, age, cycle of sexual maturity of spawners, and diseases (25-27). Previous research on the resultant carp hematology following chromium exposure have either examined the consequences of mixed Cr with other heavy metals (28) or hexavalent Cr (29). Hence, studies concerning the effects of trivalent Cr alone on *C. carpio* hematology are absent.

In natural waters and/or aquaculture facilities, fish are often exposed to Cr waste and demonstrate cumulative deleterious effects as a function of time. In addition, both lethal and sublethal concentrations of the metal determine the sensitivity of individual organisms across species (30). Thus, fish serve as an excellent model to understand the mechanistic aspects of metal toxicity (31). Accordingly, the objective of this study was to investigate the effects of sublethal concentrations of chromium on hematological and serum biochemical parameters as sensitive indices for the evaluation of fish physiology under metallic stress in the economically important common carp, *Cyprinus carpio*, following 28 days of Cr (III) exposure.

MATERIALS AND METHODS

The common carp, *Cyprinus carpio*, (11.68 ±1.92 cm and 25.92±6.3 g) were sampled from Nasr Fish Culture Pond (Sari, Iran). Prior to toxicity testing, the fish were acclimatized for one week under laboratory conditions (25±1° C, 12 hours

light/12 hours dark). Water quality parameters (TDS= 600 ppm, pH= 6.75, EC= 1 ds/m) were measured during the experiment. During acclimation and toxicity test, the fish were not fed. The heavy metal chromium in the form of chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, APLICAM) was used in the present study.

Fish were divided into two groups, with the first group serving as control and the other as experimental group each with three replicates located at glass aquaria (average volume 100 L). The fish in the experimental aquaria were exposed to a sublethal Cr concentration of 2 mg L⁻¹, which was prepared as stock solution and added depending on the volume of each aquarium to obtain the required concentration, for a period of 28 days. This concentration was taken from our earlier determination of Cr LC₅₀-96 h value for this species (31). After the exposure period, blood samples (1 ml) were immediately taken from the caudal vein of 10 fish at each aquarium using both heparinized and non-heparinized syringes (for hematological and biochemical analyses, respectively) and then poured into heparinized plastic tubes for determination of blood factors. Red and white blood cells (RBC and WBC) were counted by Neubauer hemocytometer using a special kit (Pars Azmoon Co., Iran) by diluting fluid (3 g sodium citrate, 99 ml distilled water and 1 ml formalin), counting the four corners and the center fields. Hemoglobin (Hb) was determined spectrophotometrically at 540 nm absorbance (cyanmethemoglobin method) by the use of Pars Azmoon kit. Hematocrit (Hct) was determined by standard microhematocrit method and expressed in percentages. Erythrocyte indices [mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV)] were calculated according to the method applied by (33). Differential leukocyte counting was performed with blood smears stained

with May-Grunwald/Giemsa solution. The smears (two slides per fish) were examined by light microscopy under oil immersion at 100 X magnification.

In order to separate serum, the blood samples (1 ml) simultaneously collected from the caudal vein by a non-heparinized, 2-ml syringe were placed in 1-ml vials treated with heparin to prevent clotting. The samples were left to coagulate for 15–20 min at 4 °C prior to centrifugation (3000 rpm, 20 minutes). The fresh serum was then subjected to biochemical analysis. Serum biochemical analysis [total glucose and protein (TP), albumin, and Immunoglobulin M IgM] were determined by the use of Pars-Azmoon Kit with a EUROLYSER plus auto-analyzer. Comparison of the control and experimental groups was statistically analyzed by Student's t-test, and the results were taken as significant when $P < 0.05$.

RESULTS

Table 1 presents hematological and biochemical parameters in the blood of *C.*

carpio exposed to sub-acute concentrations of chromium for 28 days. The amounts of Hct, Hb, RBC, WBC, MCH, and MCHC significantly decreased ($P < 0.05$) (Figures 1 and 2) as a result of chronic chromium exposure, whereas blood albumin and glucose significantly ($P < 0.05$, Figures. 2&3) increased in the examined fish as opposed to the control. No significant differences ($P > 0.05$) were found between the Cr-exposed fish and the control in the levels of Lym, Neu, MCV, IgM, and TP (Figures 1-3). Other blood factors, such as monocytes, eosinophils, and myelocytes, were not observed in the examined blood samples.

Chronic chromium contamination increased glucose, neutrophils, and albumin concentrations over 1.56, 1.52, and 4.4 times the control levels. Immunoglobulin M (IgM) also insignificantly elevated approx. 2.3 times in the Cr-treated fish with large inter-individual variations compared to the control (Figure 3).

Table 1. Hematological and biochemical parameters in the blood of *C. carpio* exposed to sub-acute concentrations of trivalent chromium for 28 days.

Blood parameter	Chromium	Control	P-value
Hct (%)	24.89±1.36	32.8±2.16	$P < 0.05$
Hb (g/dl)	5.96±.57	8.76±0.68	$P < 0.05$
RBC (10^3 mm^3)	1.10±0.08	1.42±0.05	$P < 0.05$
WBC ($10^6. \mu\text{l}$)	4955.56±1542	6700±1063	$P < 0.05$
Lym (%)	98.78±1.48	99.20±0.83	$P > 0.05$
Neu (%)	1.22±1.48	0.80±0.83	$P > 0.05$
MCV (fl)	198.457±15.39	230.860±8.77	$P > 0.05$
MCH (pg)	54.34±4.74	61.7±4.63	$P < 0.05$
MCHC (g/dl)	23.933±1.38	26.780±2.33	$P < 0.05$
IgM (mg/dl)	151.43±55.69	66.3±2.82	$P > 0.05$
Alb (g/dl)	1.1±0.0.26	0.25±0.07	$P < 0.05$
TP (g/dl)	1.66±0.7	2.0	$P > 0.05$
Glu (mg/dl)	188.2±10.69	120.5±16.54	$P < 0.05$

Hct: hematocrit; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular haemoglobin; Lym: lymphocyte; Neu: neutrophyles; Alb: albumin; TP: total protein; RBC: red blood

cells; Hb: hemoglobin ; MCV: mean corpuscular volume; IgM: immunoglobulin M; Glu: glucose; MCV: mean corpuscular volume; IgM: immunoglobulin M; Glu: glucose; White blood cell.

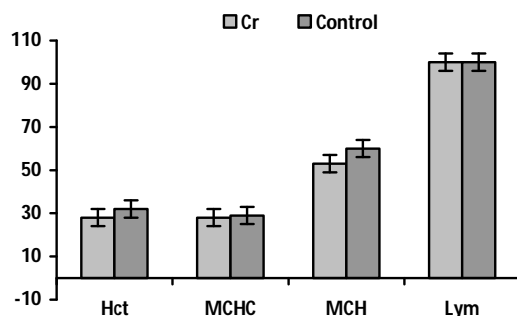


Figure 1. Blood factors of *C. carpio* exposed to sub-acute concentration of trivalent chromium for 28 days. Hct: hematocrit (%); MCHC: mean corpuscular hemoglobin concentration (%); MCH: mean corpuscular haemoglobin (pg); Lym: lymphocyte (%).

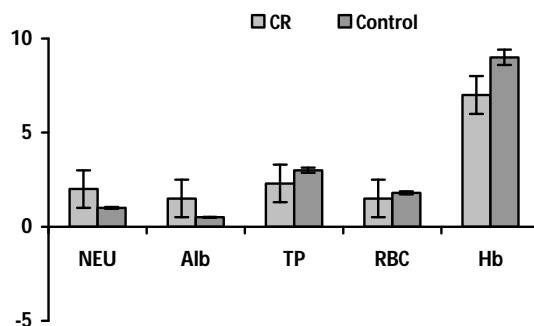


Figure 2. Hematology and blood biochemistry of *C. carpio* exposed to sub-acute concentration of trivalent chromium for 28 days. Neu: neutrophyles (%); Alb: albumin (g/dl); TP: total protein (g/dl); RBC: red blood cells (10^3 mm^3); Hb: hemoglobin (g/dl).

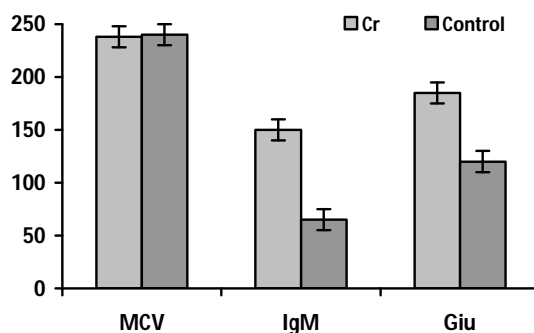


Figure 3. Hematology and blood biochemistry of *C. carpio* exposed to sub-acute concentration of trivalent chromium for 28 days. MCV: mean corpuscular volume (fl); IgM: immunoglobulin M (mg/dl); Glu: glucose (mg/dl).

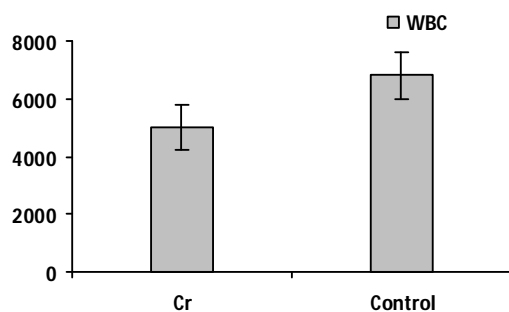


Figure 4. White blood cell (WBC) of *C. carpio* exposed to sub-acute concentration of trivalent chromium for 28 days.

DISCUSSION

The present study demonstrated that common carp, *Cyprinus carpio*, exposed to sublethal concentration of trivalent chromium for 28 days displayed a significant elevation in the level of blood glucose (i.e. hyperglycemia). Such an observation was also reported previously in the blood of common carp exposed to a mixture of heavy metals including chromium (1.2 mg L^{-1}) for 32 days (28). Similarly, Velma *et al.*, by using hexavalent Cr, showed increased level of serum glucose in *C. carpio* following 8, 16, 24, and 32 days (29). Hyperglycemic events were also reported in fish and rats treated with cadmium (34-36). Additionally, significant hyperglycemia were detected in catfishes, *Heteropneustes fossilis* and *Saccobranchus fossilis*, exposed to nickel and chromium (18, 37), *Labeo rohita* and *Clarias gariepinus* subjected to copper (38, 39), and tilapia, *Oreochromis mossambicus*, exposed to lead (40). Increased blood glucose content as a result of heavy metals has been attributed to intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids (41) as well as the involvement of Cr in glucose metabolism as an insulin co-factor (42). Sublethal (60 mg L^{-1}) administration of hexavalent Cr in the freshwater fish, *Colisa fasciatus*, caused hyperglycemic conditions in blood and depleted glycogen levels in

liver compared with control groups (43). One of the earliest studies on glucose uptake by epithelial cells in the intestine of rainbow trout showed a reduced rate of glucose absorption leading to the conclusion that chromium can alter the glucose transport rate in epithelial cells of the intestine (44). On the other hand, the teleost *Channa punctatus* exposed to 2.6 mg L^{-1} of Cr (as in this study) for 60 days exhibited no significant alterations in blood glucose or muscle glycogen content but showed hypoglycemia after 120 days of Cr toxicity (45). It, therefore, appears that the duration of heavy metal exposure should also be taken into account when extrapolating the respected consequences observed in organisms.

The concentration of hemoglobin decreased considerably in the blood of common carp exposed to chromium in the present study. Similarly, chronic exposures of *Tilapia sparrmanii* and the catfish, *S. fossilis*, (after 28 days) to 0.098 and 3.2 mg L^{-1} of hexavalent Cr, respectively, led to significant decreases in hemoglobin concentrations (37, 46). Likewise, blood hemoglobin decreased in the Indian carps, *L. rohita* and *Catla catla*, treated by sub-acute Cr and Cd exposures, respectively, within 96 h and for 25 days (1, 47). Vutukuru concluded that Hb decline reflects the anemic state of the fish which can be due to iron deficiency and its consequent decreased utilization for Hb synthesis (1). Furthermore, such

hematological changes under the effect of chromium toxicity might result in impairment of energy requiring vital processes, and, hence, give an idea about the health status of the fish population (29). The decreased hematocrit of common carp in the current study is in line with those found in *T. sparrmanii* and the catfish, *S. fossilis*, chronically examined by hexavalent Cr (37, 46). Our findings also agree with the fact that long-term exposure of fish to sublethal concentrations of heavy metals usually decreases the above-mentioned indices (48).

The reported fluctuations in these blood indices, in addition to differences in species and milieu, may also be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis, and are also indicative of the toxic effects of Cr on both metabolic and hemopoietic activities of *C. carpio* (28). On the other hand, long-term exposure (30 days) to low concentrations of chromium (1.9 and 2.9 mg L⁻¹) increased the erythrocyte count, hemoglobin concentration, and hematocrit percentage in the blood of freshwater barbus (*Barbus conchoni* Ham: 49) and rainbow trout (*Salmo gairdneri*: 50).

The reported fluctuations in these blood indices, in addition to differences in species and milieu, may also be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis, and are also indicative of the toxic effects of Cr on both metabolic and hemopoietic activities of *C. carpio* (31). Our findings agree with the fact that long-term exposure of fish to sublethal concentrations of heavy metals usually decreases such indices as Hb and Hct (3).

The Cr-treated common carp in this study had reduced numbers of RBC and WBC, while discernible augmentation of WBC and RBC numbers were observed in the Indian carp *Catla catla* and common carp subjected to Cd and Cr, respectively, (47, 28). This is similar to Cd-contaminated (0.5 mg dm³) *C. carpio* for 24 h (51), *Tinca tinca* (4.5 mg of Cd dm⁻³) during 96

h (52), and *S. fossilis* (3.2 mg of Cr) after 28 days (37) that displayed lowered WBC counts. Comparable to our RBC result is that of the Indian carp, *L. rohita*, exposed to hexavalent Cr (39.4 mg L⁻¹) which revealed a significant decrease in total erythrocyte count at the end of both 24 h and 96 h (1). Interestingly, the tilapia, *T. sparrmanii*, chronically exposed to 0.098 mg L⁻¹ of Cr showed no significant changes in RBC count (46). A decrease in the erythrocyte count or in the percentage of hematocrit indicates the worsening of an organism state and its developing anaemia (48). Increases in these indices have been reported in the blood of various fish species after 15 and 21 days of exposure to 10 and 2 mg L⁻¹ of hexavalent chromium (53). Possible explanation for the rise and/or fall of blood attributes in the above investigations and this study can be either because of using mixed heavy metals (as in 28), or hexavalent versus trivalent chromium in the majority of cited literature; the former being more toxic to the animal due to its higher solubility and oxidizing potential as well as easy penetration in biological membranes (9, 54). Moreover, hexavalent Cr alters lymphocyte functions reflecting decreased resistance to pathogens observed in fishes under chronic Cr challenge (30). This study, however, found no alteration in lymphocyte densities probably due to the use of trivalent Cr, which has low toxicity owing to poor membrane permeability and noncorrosivity (54). Nonetheless, reduction in the percentage of blood lymphocytes was observed in *O. mossambicus* following the administration of both forms of chromium (11). Further, chromium toxicity and accumulation in fish has been proven to be minimal at low and high pH/alkalinity as well as water hardness (55), with pH/alkalinity having a tremendous influence on determining the bioavailability of the metal to the fish and its associated toxic effects (30). Accordingly, the discrepancies observed in related literature and this study can be

attributed to the contrasting water acidity/alkalinity as well.

Decrease in serum total protein level (hypoproteinemia) of common carp detected in this 28-day investigation was insignificant. However, significant protein drops were recorded in *C. carpio* treated with hexavalent Cr (1.01 mg dm^{-3}) for 38 days (56), *Catla catla* treated with sub-acute Cd density for 25 days (47) and *C. carpio* intoxicated by hexavalent Cr (0.1 of LC_{50} -96 h) during 32 days (29), all of which reflect differences in dosage, duration, and metal species and form. Decreases in serum total protein (hypoproteinemia) may be due to destruction of protein-synthesizing subcellular structures inhibition of hepatic synthesis of blood protein (57), and loss of protein from the damaged kidney (58).

The level of serum albumin in *C. carpio* rose significantly as a result of Cr contamination. Albumin is one of the most abundant proteins present in plasma samples containing a number of metal binding sites so that albumin would be exposed to the metals prior to any potential metabolism that could take place (59). This makes it a quantitatively important antioxidant in blood and extracellular fluids (60); this plasma molecule has been shown to be the major plasma protein target of oxidative stress under chronic conditions (61).

After all, the observed fluctuations in the blood factors, excluding serum protein levels, of *C. carpio* imposed by sublethal effects of Cr (III) in the present study corroborate the findings of Mekkawy *et al.*, who pointed out that sublethal effects of chromium intoxication include altered blood parameters such as hematocrit, and blood glucose and albumin (62). In the present study, MCH and MCHC significantly decreased due to chromium toxicity to *C. carpio* while MCV remained statistically unaffected. Similarly, Köprücü found significant decreases in MCH and MCHC values of *Oreochromis niloticus* after exposure to cadmium for 15 and 45

days (63). Similar findings were recorded under the influence of heavy metals and pesticide stress in different fish species (64-66). These chemical-derived alterations in MCV, MCH, and MCHC have been attributed to direct or feedback responses of structural damage to RBC membranes resulting in hemolysis and impairment in hemoglobin synthesis, stress-related release of RBCs from the spleen, and hypoxia (67, 68). The concentration of immunoglobulin M (IgM) in the serum of *C. carpio* in this study was not statistically different between the Cr-treated and control groups, however, the fish exposed to chromium displayed large variations in IgM levels. IgM is the first antibody to appear in response to initial exposure to antigen that reappears after further exposure (69). Several fish species were surveyed for IgM and some also demonstrated considerable individual variations in serum IgM levels amongst fish. This may be related to size/age (70-72), the environmental conditions (73, 74), or the disease status (75).

Overall, chromium toxicity to aquatic biota is significantly influenced by abiotic variables, such as water hardness, pH, and metal form, and biological factors such as species, life stage, and potential differences in sensitivities of local populations. Our findings mostly agree with the general report that the toxic mechanism of action differs for hexavalent versus trivalent chromium (54). Besides, sensitivity to chromium varies widely, even among closely related species. Moreover, freshwater fish can regulate chromium over a wide range of ambient concentrations. It has even been stated that freshwater fish seem to be relatively tolerant of chromium. Adverse effects of chromium on sensitive species have been documented at 30.0 ug L^{-1} of trivalent Cr in freshwater (76).

CONCLUSION

Water born metals may alter the physiological and biochemical parameters

in fish blood and tissues. The reaction and survival of aquatic animals depend not only on the biological state of the animals but also on the toxicity, type, and time of exposure to the toxicant (77). Hematological indices of fish, as emanated from chromium toxicity to *C. carpio*, can be secondary responses to irritants, including exposure to low concentrations of heavy metals that mostly induce fluctuations in these parameters reflecting the launch of stress reaction in the affected fish. These findings can play an important role in monitoring fish health and risk assessment during periods of fluctuating levels of pollutants in both natural and farm environments.

ACKNOWLEDGEMENTS

The authors would like to thank the involved personnel Sari Agricultural Sciences and Natural Resources University for supporting this study.

REFERENCES

1. Vutukuru S. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health*. 2005;2(3):456-62.
2. Dirilgen N. Accumulation of heavy metals in freshwater organisms: Assessment of toxic interactions. *Turkish Journal of Chemistry*. 2001;25(2):173-80.
3. Voegborlo R, El-Methnani A, Abedin M. Mercury, cadmium and lead content of canned tuna fish. *Food Chemistry*. 1999;67(4):341-5.
4. Canli M, Ay O, Kalay M. Levels of Heavy Metals (Cd, Pb, Cu, Cr and Ni) in tissue of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* from the Seyhan River, Turkey. *Turkish Journal of Zoology*. 1998;22:149-58.
5. Velez D, Montoro R. Arsenic speciation in manufactured seafood products. *Journal of Food Protection*. 1998;61(9):1240-5.
6. Conacher H, Page B, Ryan J. Industrial chemical contamination of foods. *Food Additives & Contaminants*. 1993;10(1):129-43.
7. Farombi E, Adelowo O, Ajimoko Y. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*. 2007;4(2):158-65.
8. Vosylienė MZ, Jankaitė A. Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologija*. 2006;4:12-7.
9. Reynolds M, Peterson E, Quievryn G, Zhitkovich A. Human nucleotide excision repair efficiently removes chromium-DNA phosphate adducts and protects cells against chromate toxicity. *Journal of Biological Chemistry*. 2004; 279(29): 30419-24.
10. Langård S, Norseth T. Chromium. In: Friberg L (Ed). *Handbook on the toxicology of metals*. 2 ed. Amsterdam: Elsevier/North-Holland Biomedical; 1979.
11. Arunkumar RI, Rajasekaran P, Michael RD. Differential effect of chromium compounds on the immune response of the African mouth breeder *Oreochromis mossambicus* (Peters). *Fish & Shellfish Immunology*. 2000;10(8):667-76.
12. Arillo A, Melodia F. Effects of hexavalent chromium on trout mitochondria. *Toxicology Letters*. 1988;44(1):71-6.
13. Sornaraj R, Baskaran P, Thanalakshmi S. Effects of heavy metals on some physiological responses of air-breathing fish *Channa punctatus* (Bloch). *Environment and ecology Kalyani*. 1995;13(1):202-7.
14. Abbasi S, Kunahmed T. Influence of the acidity on chromium toxicity –A study with the teleost *Nuria dannicus* as model. *Pollution Research*. 1995;14:317-23.
15. Sastry K, Sunitha K. Chronic toxic effects of chromium in *Channa punctatus*: Biochemical studies. *Journal of Environmental Biology*. 1984; 5(1):53-6.
16. Singh M. Haematological responses in a freshwater teleost *Channa punctatus* to experimental copper and chromium poisoning. *Journal of Environmental Biology*. 1995;16(4):339-41.

17. Strike J., Delongh H. Toxicity of chromium VI in fish, with special reference to organ weights, liver and plasma enzyme activities, blood parameters and histological alterations. In: Koeman J, Strike J(Ed). Sublethal effects of toxic chemicals on aquatic animals, Elsevier, New York, 1975;31-41.
18. Nath K, Kumar N. Hyperglycemic response of *Heteropneustes fossilis* exposed to nickel. *Acta Hydrochimica et Hydrobiologica*. 1988;16(3):333-6.
19. Kundu R, Lakshmi R, Mansuri A, editors. The entry of mercury through the membrane: an enzymological study using a tolerant fish *Boleophthalmus dentatus*. *Proc Acad Environ Biol*; 1992;1:1-6.
20. Abbas H, Mahmoud H. Hematological and biochemical changes in *Oreochromis aureus* and *Clarias gariepinus* exposed to mixture of copper and lead salts. *Egyptian Journal of Basic and Applied Physiology*. 2004;3:89-106.
21. Shalaby A, Abbas H. The effect of sublethal doses of cadmium on the disposition of some trace elements and liver function in common carp *Cyprinus carpio* L. *Egyptian Journal of Basic and Applied Physiology*. 2005;4:383-95.
22. Abbas HH. Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. *Pakistan Journal of Biological Sciences*. 2006;9(12):2215-21.
23. Abbas H, Mahmoud H, Miller J. Vitamin C and cadmium toxicity in fish *Oreochromis niloticus*. *Online Journal of Veterinary Research*. 2007;11:54-74.
24. Lusková V. Annual cycles and normal values of hematological parameters in fishes. *Acta Scientiarum Naturalium Acad Scientiarum Bohemicae Brno NS*. 1997;31(5):31-70.
25. Golovina N, Trombicky I. Haematology of pond fish. *Kishinev, Shtiinca*. 1989:158.
26. Golovina N. Morpho-Functional Characteristics of the blood of fish as objects of aquaculture. Doctorial thesis Moscow. 1996:53.
27. Vinodhini R, Narayanan M. The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus Carpio* L.). *Iranian Journal of Environmental Health, Science & Engineering*. 2009;6(1):23-8.
28. Parvathi K, Sivakumar P, Ramesh M. Sublethal effects of chromium on some biochemical profiles of the fresh water teleost *Cyprinus carpio*. *International Journal of Applied Biology Tech*. 2011;2:295-300.
29. Velma V, Vutukuru S, Tchounwou PB. Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. *Reviews on Environmental Health*. 2009;24(2):129-46.
30. Velma V, Tchounwou PB. Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *Carassius auratus*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2010;698(1):43-51.
31. Abedi Z, Khalesi MK, Kohestan Eskandari S, Rahmani H. Comparison of Lethal Concentrations (LC50-96 H) of CdCl₂, CrCl₃, and Pb (NO₃)₂ in Common Carp (*Cyprinus carpio*) and Sutchi Catfish (*Pangasius Hypophthalmus*). *Iranian Journal of Toxicology*. 2012;6(18):672-80.
32. Dacie J, Lewis S. *Practical Haematology*. 8th. Ed Churchill Livingstone, London. 1995.
33. Baer KN, Benson WH. Influence of chemical and environmental stressors on acute cadmium toxicity. *Journal of Toxicology and Environmental Health, Part A Current Issues*. 1987;22(1):35-44.
34. Pratap H, Bonga S. Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*. 1990;95(2):313-7.
35. Hontela A, Daniel C, Ricard AC. Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquatic toxicology*. 1996;35(3):171-82.
36. Khangarot B, Rathore R, Tripathi D. Effects of chromium on humoral and cell-mediated immune responses and host resistance to disease in a freshwater catfish, *Saccobranchus fossilis* (Bloch). *Ecotoxicology and Environmental Safety*. 1999;43(1):11-20.

37. Radhakrishaniah K, Venkataramana P. Effect of lethal and sublethal concentrations of copper on glycolysis in the liver of the fresh water teleost, *Labeo rohita* (Ham). *Journal of Environmental Biology*. 1992;13:63-8.
38. Van Vuren J, Van der Merwe M, Du Preez H. The effect of copper on the blood chemistry of *Clarias gariepinus* (clariidae). *Ecotoxicology and Environmental Safety*. 1994;29(2):187-99.
39. James R, Sampath K, Alagurathinam S. Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost *Oreochromis mossambicus* (Peters) during accumulation and depuration. *Asian Fisheries Science Metro Manila*. 1996;9(2):87-100.
40. Almeida J, Novelli E, Dal Pai Silva M, Alves Junior R. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environmental Pollution*. 2001; 114(2):169-75.
41. Galvin M. Occurrence of metals in waters: An overview. *Water S A*. 1996;22(1):7-18.
42. Nath K, Kumar N. Hexavalent chromium: Toxicity and its impact on certain aspects of carbohydrate metabolism of the freshwater teleost, *Colisa fasciatus*. *Science of the Total Environment*. 1988;72:175-81.
43. Stokes R, Fromm P. Effect of chromate on glucose transport by the gut of rainbow trout. *Physiological Zoology*. 1965; 38(3):202-5.
44. Sastry K, Sunita K. Enzymological and biochemical changes produced by chronic chromium exposure in a teleost fish, *Channa punctatus*. *Toxicology Letters*. 1983;16(1):9-15.
45. Wepener V, Van Vuren J, Du Preez H. The effect of hexavalent chromium at different pH values on the haematology of *Tilapia sparrmanii* (Cichlidae). *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*. 1992; 101(2):375-81.
46. Remya SR, Ramesh M, Sajwan KS, Senthil Kumar K. Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish *Catla catla*. *Fish Physiology and Biochemistry*. 2008;34(2):169-74.
47. Vosylienė MZ. The effect of heavy metals on haematological indices of fish (survey). *Acta Zoologica Lituanica*. 1999;9(2):76-82.
48. Gill TS, Pant JC. Erythrocytic and leukocytic responses to cadmium poisoning in a freshwater fish, *Puntius conchonus* Ham. *Environmental Research*. 1985;36(2):327-37.
49. Schiffman RH, Fromm PO. Chromium-induced changes in the blood of rainbow trout, *Salmo gairdnerii*. *Sewage and Industrial Wastes*. 1959;31(2):205-11.
50. Morsy M, Protasowicki M. Cadmium bioaccumulation and its effects on some hematological and histological aspects in carp, *Cyprinus carpio* L., at selected temperature. *Acta Ichthyologica et Piscatoria*. 1990;20:105-16.
51. Witeska M, Jezierska B, Wolnicki J. Respiratory and hematological response of tench, *Tinca tinca* (L.) to a short-term cadmium exposure. *Aquaculture International*. 2006;14(1):141-52.
52. NRCC. National Research Council Canada: Effects of chromium in the Canadian Environment; 1976. p.15017-17.
53. Adhikari S, Ghosh L, Ayyappan S. Combined effects of water pH and alkalinity on the accumulation of lead, cadmium and chromium to *Labeo rohita* (Hamilton). *International Journal of Environmental and Scientific Technology*. 2006;3(3):289-96.
54. O'Neill J. The humoral immune response of *Salmo trutta* L. and *Cyprinus carpio* L. exposed to heavy metals. *Journal of Fish Biology*. 2006;19(3):297-306.
55. Fontana L, Moreira E, Torres MI, Fernández I, Ríos A, de Medina FS, et al. Dietary nucleotides correct plasma and liver microsomal fatty acid alterations in rats with liver cirrhosis induced by oral intake of thioacetamide. *Journal of Hepatology*. 1998;28(4):662-9.
56. Gad N. Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of *Tilapia zillii*. *Journal of the Egyptian German Society of Zoology*. 2005; 48(A):279.
57. Carney Almroth B. Oxidative Damage in Fish Used as Biomarkers in Field and Laboratory Studies: Department of Marine

- Ecology; Institutionen för marin ekologi; 2008.
58. Halliwell B. Albumin-an important extracellular antioxidant? *Biochemical Pharmacology*. 1988;37(4):569-71.
 59. Himmelfarb J, McMonagle E. Albumin is the major plasma protein target of oxidant stress in uremia. *Kidney International*. 2001;60(1):358-63.
 60. Holdway DA. The toxicity of chromium to fish. In: Nriagu JO, Nieboer E (Ed). *Chromium in the Natural and Human Environments*. John Wiley and Sons, New York; 1988.
 61. Shalaby AME. Effect of EDTA on reduction of cadmium toxicity on growth, some hematological and biochemical profiles of Nile tilapia (*Oreochromis niloticus*). *Journal of Fisheries and Aquatic Science*. 2007;2:100-9.
 62. Mekkawy I, Hussein S, Abdel-Nasser M, Ahmed S. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthys auratus* at Assiut, Egypt. *Journal of the Egyptian German Society of Zoology*. 1996;19:283-320.
 63. Köprücü SŞ, Köprücü K, Ural MŞ, İspir Ü, Pala M. Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochemistry and Physiology*. 2006;86(2):99-105.
 64. Kudirat Adeyemo O. Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. *Turkish Journal of Fisheries and Aquatic Sciences*. 2007;7:163-9.
 65. Marie M, Haggag A, El-Badawy A. Physiological and biochemical responses of the common carp, *Cyprinus carpio* to an organophosphorus insecticide Profenofos. *Egyptian Journal of Zoology*. 1998;13:279-302.
 66. Shah SL. Hematological parameters in tench *Tinca tinca* after short term exposure to lead. *Journal of Applied Toxicology*. 2006;26(3):223-8.
 67. Wiersma EJ, Collins C, Fazel S, Shulman MJ. Structural and functional analysis of J chain-deficient IgM. *The Journal of Immunology*. 1998;160(12):5979-89.
 68. Matsubara A, Mihara S, Kusuda R. Quantitation of yellowtail serum antibody specific for *P. piscicida* LPS using the ELISA technique. *Bulletin of the Japanese Society of Scientific Fisheries*. 1985;51(6):927-32.
 69. Klesius P. Effect of size and temperature on the quantity of immunoglobulin in channel catfish, *Ictalurus punctatus*. *Veterinary Immunology and Immunopathology*. 1990;24(2):187-95.
 70. Magnadóttir B, Jónsdóttir H, Helgason S, Björnsson B, Solem ST, Pilsröm L. Immune parameters of immunised cod (*Gadus morhua* L.). *Fish & Shellfish Immunology*. 2001;11(1):75-89.
 71. Olesen NJ, Jørgensen PEV. Quantification of serum immunoglobulin in rainbow trout *Salmo gairdneri* under various environmental conditions. *Diseases of Aquatic Organisms*. 1986;1:183-9.
 72. Magnadóttir B, Jónsdóttir H, Helgason S, Björnsson B, Jørgensen TØ, Pilsröm L. Humoral immune parameters in Atlantic cod (*Gadus morhua* L): I. The effects of environmental temperature. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. 1999;122(2):173-80.
 73. Magnadóttir B, Gudmundsdóttir S, Gudmundsdóttir B. Study of the humoral response of Atlantic salmon, naturally infected with *Aeromonas salmonicida* spp. a chromogenes. *Veterinary Immunology and Immunopathology*. 1995;49(1):127-42.
 74. Eisler R. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review, United States Fish and Wildlife Service. 1986.
 75. Brungs W, McCormick J, Neiheisel T, Spehar R, Stephan C, Stokes G. Effects of pollution on freshwater fish. *Journal (Water Pollution Control Federation)*. 1977;49(6):1425-9.