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RESEARCH ARTICLE

Alterations in the Hematological and Biochemical Parameters and Plasma Ion Concentrations of Common Carp, (*Cyprinus carpio* L., 1758) After Short Term Exposure to Sub-lethal Concentrations of Lead

Mehmet Borga ERGÖNÜL * Sibel ATASAĞUN * Kübra KOCATÜRK *

* Ankara University, Faculty of Science, Department of Biology, TR-06100 Tandoğan, Ankara - TURKEY

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Summary

In this study the 96 h acute toxicity (LC_{50}) of lead (Pb) for common carp (*Cyprinus carpio* L., 1758) was investigated and the fish was exposed to sub-lethal concentration of lead for 96 h. The hematological and biochemical parameters (red blood cell count, hemoglobin, hematocrit, white blood cell count and leucocrit) and plasma glucose, lactate and total protein levels and plasma ion concentrations of fish were investigated and compared to control fish. It was found that lead had significant effects on red blood cell count, hemoglobin levels, plasma glucose and lactate levels and plasma ions, while hematocrit, white blood cell count, leuocrit and plasma total protein levels remained unchanged.

Keywords: *Lead, Lactate, Acute toxicity, LC_{50}, Plasma ions, Probit*

Kurşunun Sub-lethal Dozlarına Maruz Bırakılan Sazan Balıklarında (*Cyprinus carpio* L., 1758) Hematolojik ve Biyokimyasal Parametreler ve Plazma İyon Konsantrasyonlarındaki Değişimler

Özet

Bu çalışmada kurşunun (Pb) sazan balıkları (*Cyprinus carpio* L., 1758) için için 96 saatlik LC_{50} değeri belirlenmiş ve balıklar 96 saat süreyle kurşunun sub-lethal konsantrasyonuna maruz bırakılmıştır. Çalışmada balıkların alyuvar sayısı, hemoglobin ve hematokrit seviyesi, akyuvar sayısı ve lökokrit gibi hematolojik parametrelerinin yanısıra plazma glukoz, laktat ve total protein seviyesi gibi biyokimyasal parametreleri ile birlikte plazma iyon konsantrasyonları da incelenmiş ve kontrol grubu balıklar ile karşılaştırılmıştır. Kurşunun alyuvar sayısı, hemoglobin seviyesi, plazma glukoz ve laktat seviyesi ve plazma iyonları üzerinde önemli bir etkisi olduğu, akyuvar sayısı, lökokrit ve plazma total protein seviyesinin ise değişmediği bulunmuştur.

Anahtar sözcükler: *Kurşun, Laktat, Akut toksisite, LC_{50}, Plazma iyonları, Probit*

INTRODUCTION

The contamination of aquatic environments with a wide range of pollutants has become a matter of great concern over the last few decades. Among these pollutants, heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities [1,2], thus, constitute a considerable part of the aquatic pollution. Discharge of heavy metals into aquatic environment can change both aquatic species diversity and ecosystem, due to their toxicity and accumulation. In addition, although, physiologic roles of heavy metals such as iron, zinc, copper and to a lesser extent chromium are known, metals such as lead and cadmium are not believed to be essential for health even in trace amounts [3]. Chronic

İletişim (Correspondence)
+90 312 2126720
borga@science.ankara.edu.tr

low level exposure to heavy metals has adverse effects not only on aquatic animals but also on human beings via food chain, due to the fact that there is no effective mechanism for their elimination from the body [4]. Thus, accurate information on heavy metal toxicity on aquatic organisms is needed.

Among heavy metals, lead (Pb) is non-essential, non-beneficial and toxic to many organisms even at very low concentrations [5-8]. Lead toxicity may occur from chronic waterborne exposure at concentrations as low as 4 µg l^{-1} [8] which is in the range of concentrations reported from freshwater environments [1], thus creating a demand for further studies. Although, a number of studies focus on the toxicity of lead on various fish species including eel (*Anguilla anguilla* L., 1758) [5], Nile Tilapia (*Oreochromis niloticus* L., 1758) [9], rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) [7,10], streaked prochilod (*Prochilodus lineatus* Valenciennes, 1837) [11], tench (*Tinca tinca* L., 1758) [12], and carp (*Cyprinus carpio* L., 1758) [6,13], little is known on the physiological effects of lead on common carp.

Investigations on the toxic effects of heavy metals on fish may be accompanied by the analysis of changes in certain hematological and biochemical blood parameters. Hematological and biochemical profile in fish is proved to be a sensitive index for the evaluation of fish metabolism under metallic stress and can be used as an indicator for pollution. The hematological parameters of fish such as red blood cell count, hematocrit, hemoglobin, white blood cell count and leucocrit may be used to assess the functional status of the oxygen carrying capacity of the blood stream and as an indicator of metal pollution in aquatic environment [12,14]. Changes in carbohydrate metabolism measured as plasma glucose and lactate can also be used as general stress indicators in fish. Ions of body fluids have various functions such as contributing a majority of the osmotically active particles, to provide buffer systems and the mechanisms for the regulation acid-base balance [15].

The present study was performed on common carp (*C. carpio*) which has a widespread distribution in many Asian and European countries, since it is one of the most commercially important and widely cultivated freshwater fish. Thus, available data on carp biology provide a comprehensive database for further studies. Although, it has been the subject of many toxicological studies, there are no studies demonstrating the changes in the hematological and biochemical parameters of common carp exposed to Pb. Hence the aim of this study was to determine the acute toxicity (96 h LC_{50}) of Pb for carp with a static test system and to investigate the possible effects of sub-lethal lead exposure on carp by determining certain hematological and biochemical parameters such as red blood cell count, hemoglobin, hematocrit, white blood cell count and leucocrit, plasma glucose, lactate and total protein levels and plasma concentrations of ions such as sodium (Na^+), potassium (K^+) and chloride (Cl^-).

MATERIAL and METHODS

Test fish, common carp (*C. carpio*), were captured with gill nets from Lake Mogan (Ankara, Turkey). Fish were brought to laboratory and placed in 800 l tanks and allowed for at least 2 weeks for acclimatization to the laboratory conditions. Mean weight and length of fish was 210.2±8.9 g and 20.2±3.3 cm, respectively. Fish were maintained in aerated dechlorinated tap water at 17.7±1.47°C and fed with commercial feed (45% crude protein) at a rate of 2% body weight. Fish were not fed for 48 h prior to and during the experiments. The physico-chemical properties of the water were as follows; pH 7.71±0.49, electrical conductivity (EC) 217.47±16.95 µS cm^{-1}, dissolved oxygen 6.39±0.45 mg l^{-1}, total hardness and total alkalinity 77.5 mg l^{-1} and 80 mg l^{-1} as $CaCO_3$, respectively.

After acclimatization period, randomly selected 10 fish were transferred to 100 l aquaria for the determination of 96 h LC_{50} of lead. Lead was added to the water as lead nitrate $Pb(NO_3)_2$ (Sigma-Aldrich). Ten concentrations were tested to estimate lethal concentration of lead *(Table 1)*.

Table 1. *Tested lead concentrations and mortality rates of fish*
Tablo 1. *Test edilen kurşun konsantrasyonları ve balıklardaki mortalite oranı*

Exposure Concentration (mg Pb^{2+} l^{-1})	Number Exposed	Number Responded	Mortality Rate	Expected Mortality Rate	Calculated Mortality Rate
1	10	0	0.00	0.00	0
5	10	0	0.00	0.00	0.0005
10	10	1	0.10	0.10	0.0448
15	10	2	0.20	0.20	0.2212
20	10	4	0.40	0.40	0.4568
25	10	6	0.60	0.60	0.6566
30	10	7	0.70	0.70	0.6890
35	10	8	0.80	0.80	0.8799
40	10	10	1.00	1.00	0.9307
45	10	10	1.00	1.00	0.9600

Half of the water in the aquaria was replaced once (at 48 h intervals) during bioassays and lead nitrate solution was added to water to keep the required concentration constant. Dead fish were counted at 12 h intervals and removed from aquaria. After bioassay, probit analysis was performed with EPA [16] probit analysis program (v 1.5).

After calculation of the 96 h LC_{50} of lead, fish were exposed to sub-lethal concentration (10% of lethal concentration = 2 mg Pb^{2+} l^{-1}) of lead for 96 h. After exposure, blood samples were taken from the caudal vein from fish from both exposure (N=10) and control (N=10) groups with heparinized syringes. Control fish were handled in the same way. Hemoglobin (Hb) levels were determined with a commercial kit (Roche) according to the instructions supplied with. Hematocrit (Hct) and leucocrit (Lct) measurements were made according to Blaxhall and Daisley [17] and McLeay and Gordon [18], respectively. Red blood cell (RBC) and white blood cell (WBC) counts were made with Improved Neubauer hemocytometer, using Natt-Herrick as the diluent and stain [19]. Blood plasma was obtained by centrifugation at 14.000 rpm for 10 min and stored in eppendorf tubes at -35°C until analysis. Plasma sodium (Na^+), potassium (K^+), chloride (Cl^-), glucose, total protein levels (Teco Diagnostics) and plasma lactate levels (Randox) were determined by using biochemical kits according to the instructions supplied with.

Data are presented as mean±standart deviation (SD) and were compared with One-Way ANOVA using the statistical packet program (SPPS v 9.0) at a significance level of $P<0.05$.

RESULTS

The data obtained from the toxicity tests of lead nitrate on *C. carpio* were evaluated by using EPA probit Analysis Program [14] and the 96 h LC_{50} value was found as 20.97 mg Pb^{2+} l^{-1} (95% confidence limits; 17.43-24.29) *(Table 2)*. No mortality was observed during 96 h at lead concentration of 1.00 and 5.00 mg l^{-1} and mortality rate was 100% at 40 and 45 mg l^{-1}. No mortality was observed for control fish.

Table 2. *Estimated LC values for carp exposed to lead*

Tablo 2. *Kurşuna maruz bırakılan sazan balıkları için hesaplanan LC değerleri*

Point	Exposure Concentration (mg l^{-1})	95% Confidence Limits
$LC_{1.00}$	7.60	3.75 - 10.61
$LC_{5.00}$	10.23	5.99 - 13.27
$LC_{10.00}$	11.99	7.67 - 14.99
$LC_{15.00}$	13.34	9.05 - 16.31
$LC_{50.00}$	**20.97**	**17.43 - 24.29**
$LC_{85.00}$	32.95	28.04 - 43.30
$LC_{90.00}$	36.67	30.69 - 50.76
$LC_{95.00}$	42.96	34.86 - 64.65
$LC_{99.00}$	57.84	43.84 - 102.78

Parameters related to red blood cell system showed marked changes. Red blood cell count and hemoglobin levels were elevated by 47.47% and 25.42% respectively *(Fig. 1a, 1b)*. Hematocrit levels did not show a marked difference compared to control *(Fig. 1c)* ($P>0.05$). No significant differences were observed between exposure and control groups in respect to white blood cell counts and leucocrit levels *(Fig. 2a, 2b)*.

The changes in plasma ion concentrations for carp exposed to lead at a concentration of 2 mg l^{-1} (10% of the 96 h lethal concentration) for 96 h are shown in *Fig. 3a-3c.* Plasma Na^+ and Cl^- levels decreased by 23.18% and 17.12%, respectively, compared to control fish. Plasma K^+ levels increased by 53.43% compared to control fish. Plasma glucose levels of fish exposed to lead increased by 60.39% *(Fig. 4a)*. Plasma lactate levels also showed an increase (47.42%) *(Fig. 4b)*. Total protein levels of fish exposed to lead showed no significant alterations *(Fig. 4c)*.

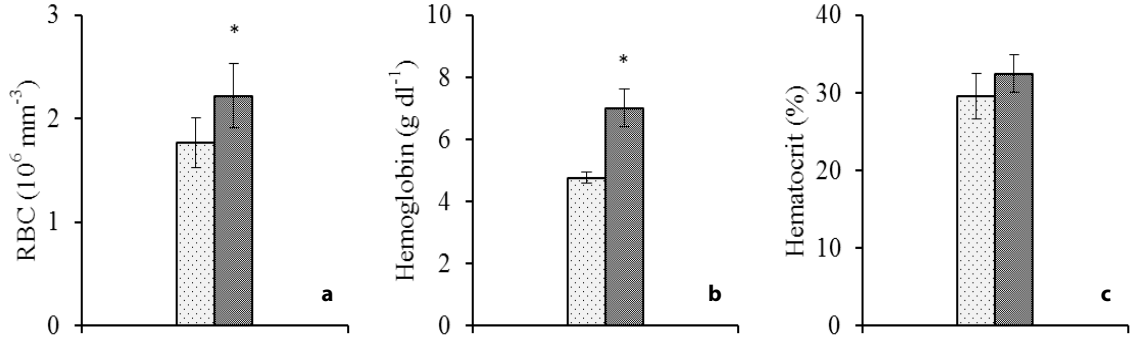

Fig 1. Red blood cell (RBC) counts (a), hemoglobin (b) and hematocrit levels (c) of fish. Dark bars represent exposure group, light bars represent control group, * $P<0.05$

Şekil 1. Balıklarda alyuvar sayısı (a), hemoglobin (b) ve hematokrit seviyesi (c). Koyu sütunlar deneme grubunu, açık sütunlar kontrol grubunu temsil etmektedir, * $P<0.05$

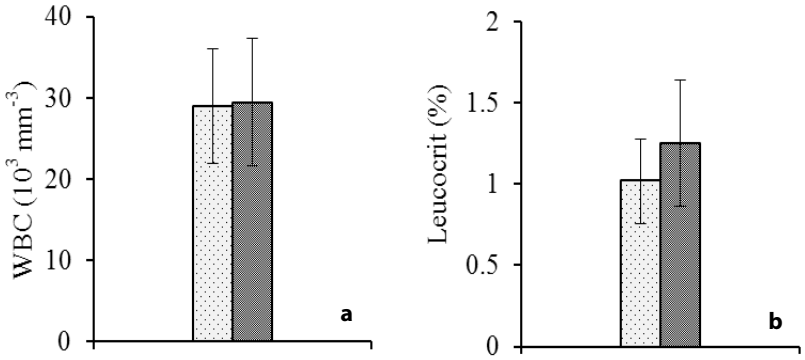

Fig 2. White blood cell (WBC) counts (a) and leucocrit levels (b) of fish. Dark bars represent exposure group, light bars represent control group

Şekil 2. Balıklarda akyuvar sayısı (a) ve lökokrit seviyesi (b). Koyu sütunlar deneme grubunu, açık sütunlar kontrol grubunu temsil etmek-tedir

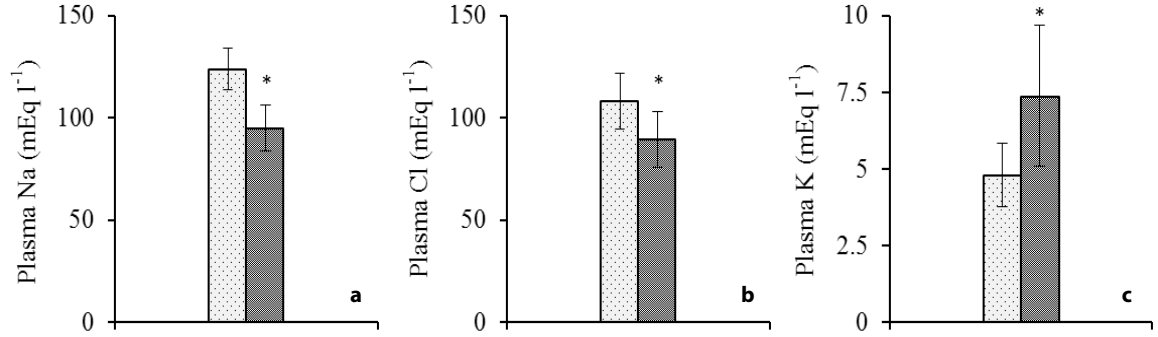

Fig 3. Plasma Na^+ (a), Cl^- (b) and K^+ (c) concentrations of fish. Dark bars represent exposure group, light bars represent control group, * P<0.05

Şekil 3. Balıklarda plazma Na^+ (a), Cl^- (b) ve K^+ (c) konsantrasyonları. Koyu sütunlar deneme grubunu, açık sütunlar kontrol grubunu temsil etmektedir, * P<0.05

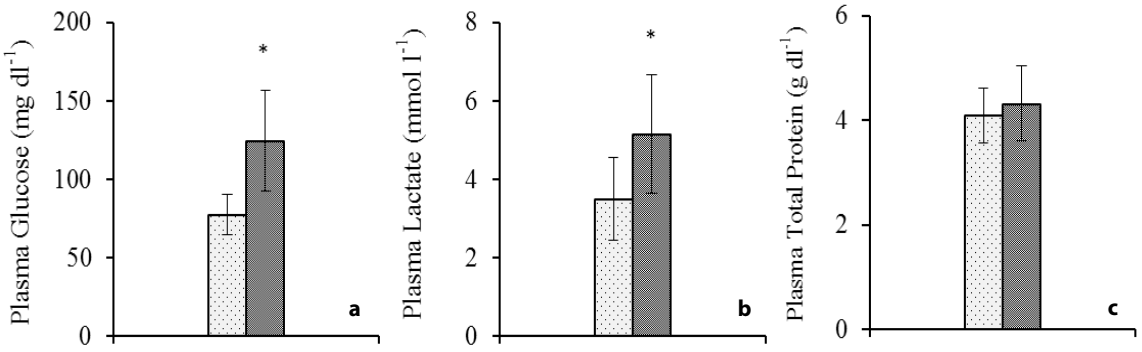

Fig 4. Plasma glucose (a), lactate (b) and total protein (c) levels of fish. Dark bars represent exposure group, light bars represent control group. * P<0.05

Şekil 4. Balıklarda plazma glukoz (a), laktat (b) ve total protein (c) seviyesi. Koyu sütunlar deneme grubunu, açık sütunlar kontrol grubunu temsil etmektedir, * P<0.05

DISCUSSION

In the present study, the 96 h LC_{50} value of lead for carp was found as 20.97 mg Pb^{2+} l^{-1}. Martinez et al.[11] found that 96 h LC_{50} for *P. lineatus* was 95 mg Pb^{2+} l^{-1}. Al-Akel and Shamsi [9] reported the 96 h LC_{50} as 12.45 and 22.65 mg Pb^{2+} l^{-1} for *O. niloticus* and *Clarias gariepinus* (Burchell, 1822), respectively. Hodson et al.[10] stated that 21 day LC_{50} was 2.4 mg Pb^{2+} l^{-1} for *O. mykiss*. Rogers et al.[7] showed that for same fish species 96 h LC_{50} was 1.0 mg Pb^{2+} l^{-1}. Shah [12] found that 96 h LC_{50} for *T. tinca* was 300 mg Pb^{2+} l^{-1}. The 96 h LC_{50} for *C. carpio* was reported as 0.44-0.80 mg Pb^{2+} l^{-1} and 8.2-1291 mg Pb^{2+} l^{-1} by Alam and Maughan [13] and Datta and Das [6], respectively. The values obtained for lead by toxicity tests by different authors show a high degree of variation, even for the same species. It is known that such variation might be due to several factors influencing toxicity, such as temperature, pH, dissolved oxygen as well as the fish species itself [6,7]. Furthermore, it is also known that in static bioassays, lead content may vary depending on the absorption, adsorption and precipitation [11].

Since blood is a pathophysiological reflector of the whole body, hematological parameters may provide a reliable data for the health status of fish [14] and may be used as indicator of pollution [12,20]. It was observed that sub-lethal lead exposure for 96 h caused a marked increase in red blood cell count and hemoglobin levels of carp. However, it was found that hematocrit levels remained unchanged. Elevated numbers of red blood cells and hemoglobin levels without a marked increase in hematocrit levels, may indicate the increase of newly formed immature red blood cell population and shortening the life span of mature red blood cells, which may give rise to the pattern observed in this study; a shift in red blood cell population from mature red cells to newly formed immature cells. Such a pattern may also give acceleration to release of new red blood cells from the erythropoetic organs and synthesis of hemoglobin to compensate for the loss in oxygen carrying capacity and compensating for red blood cell loss of fish exposed to lead [9-11]. Besides, the increase in hemoglobin levels may be a response to replace abnormal hemoglobin which might have been oxidized or denatured by the metal that enter the red blood cells, which in turn stimulate erythropoietic tissues [21].

It was found that lead exposure had no effect on the white blood cell count and leucorit levels of carp in this study. However, Shah and Altındağ [20] found that lead exposure had significant effects on white blood cell counts and leucocrit levels; with both decreases and increases depending on the exposure time and concentration. Al-Akel and Shamsi [9] reported that lead exposure had no effect on white blood cell count of *O.niloticus*. Santos and Hall [5] reported that thrombocyte counts remained unchanged. They also reported that lymphocyte counts increased while neutrophil counts decreased. According to our results it is reasonable to conclude that sub-lethal lead exposure did not cause an immunological impairment in carp.

Ions of body fluids in fish have various functions such as contributing a majority of the osmotically active particles and providing buffer systems for the regulation acid-base balance. In freshwater fishes ions such as Na^+, Cl^- and K^+ have a significant role in keeping the body fluids hyperosmotic [15] and it is suggested that plasma ion levels may be employed for quantifying toxic effects of metals [22]. In the present study it was found that plasma Na^+ and Cl^- levels were decreased and plasma K^+ levels were increased. The pattern observed for plasma ions in the present study was also reported for common carp exposed to diazinon [23], cypermethrin [14] and acidic pH [24]. Rogers et al.[7] found that plasma K^+ levels did not show any significant differences while a decrease was observed for plasma Na^+ and Cl^- in rainbow trout exposed to lead. Santos and Hall [5] found no differences in the plasma Na^+ and K^+ levels of eel exposed to lead (Cl^- levels were not mentioned). In freshwater fish, it is known that Na^+ and Cl^- ions are taken up by active transport across osmoregulatory surfaces, especially by gills. The decrease in both plasma Na^+ and Cl^- ions during lead exposure in this study may be attributed to the increased permeability of gill epithelium which lead to elevation in the efflux of Na^+ and Cl^- from branchial epithelium and increased water uptake by the gills [15,25], or a decrease and/or inhibition of Na^+/K^+-ATPase activity [7]. It is possible that the decrease in plasma Cl^- levels might be due to a shift of Cl^- into red blood cells due to the effect of lowered plasma pH and/or a penetration of Cl^- into the intracellular compartment to balance the efflux of lactate to intercellular compartment that leads to decrease blood pH. The increase in the plasma K^+ levels may be the result of an efflux from intracellular compartment. Potassium is the dominant intracellular cation and plasma ionic dilution would favor efflux of K^+ into extracellular fluid. On the other hand, it is also possible that the alterations observed on the plasma ions may simply result from a non-specific stress response of fish.

Plasma or serum glucose level has been used a sensitive indicator of stress in fish. In the present study plasma glucose levels showed an increase when compared to control fish. Lactate is a closely related parameter to glucose metabolism and has been used as an indicator of anaerobic metabolism. In the present study, lactate levels also significantly increased. Although there are a few studies that show lead has no effect on plasma lactate levels in fish [7], several authors reported an increase both in glucose and lactate levels in fish [5,26,27]. It is known that plasma lactate levels increase typically in stressed fish, particularly if any aspect of the stressor results in a decrease in oxygen availability [28]. Thus it is acceptable to conclude that lead exposure may lead to a shift from aerobic to anaerobic metabolism in carp. There were no significant differences between the total plasma protein levels of exposure and control groups. A similar pattern was observed for eel exposed to lead [5].

In conclusion, in the present study it is found that lead at sub-lethal concentrations had marked effects on red blood cell system, plasma ion concentrations and plasma glucose and lactate levels. However, we did not find any marked alterations in the white blood cell count and leucocrit levels. Taking the whole picture into account, it seems that sub-lethal lead exposure effects mainly the respiratory system of carp by altering the function of gills via ion balance alteration and/or enzyme activity in gills [7] and/or simply by physical blockage of gill surface by excess mucus [12] caused by lead exposure which in turn stimulated erythropoietic tissues to compensate for the decrease in oxygen levels which is also proved by the increase in lactate levels. It is also reasonable to speculate that lead also had deleterious effects on mature red blood cell life span which might also has given rise to the release of immature red blood cells.

REFERENCES

1. WHO (World Health Organization): Environmental Health Criteria 165. International Programme on Chemical Safety. Geneva, 1995.

2. USEPA (United States Environmental Protection Agency): National Recommended Water Quality Criteria. Office of Water, Washington, DC, 2006.

3. Canlı M, Atlı G: The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ Pollut*, 121, 129-136, 2003

4. Bahemuka TE, Mubofu EB: Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dares Salaam, Tanzania. *Food Chem*, 66, 63-66, 1999.

5. Santos MA, Hall A: Influence of organic lead on the biochemical blood composition of the eel (*Anguilla anguilla*). *Ecotoxicol Environ Saf*, 20, 7-9, 1990.

6. Datta S, Das RC: Influence of some abiotic environmental factors on acute toxicity of inorganic lead to *Cyprinus carpio* var *communis* and *Catla catla* in simulated toxic aquatic environment. *Toxicol Environ Chem*, 85, 203-219, 2003.

7. Rogers JT, Richards JG, Wood CM: Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout. *Aquat Toxicol*, 64, 215-234, 2003.

8. Grosell M, Gerdes RM, Brix KV: Chronic toxicity of lead to three freshwater invertebrates - *Brachinous calyciflorus*, *Chironomus tentans* and *Lymnea stagnalis*. *Environ Toxicol*, 25, 97-104, 2006.

9. Al-Akel AS, Shamsi MJK: A comparative study of the toxicity of lead and its impact on the carbohydrate metabolism and some hematological parameters of cichlid fish *Oreochromis niloticus* and catfish *Clarius gariepinus* from Saudi Arabia. *Toxicol Environ Chem*, 74, 19-28, 2000.

10. Hodson PV, Blunt BR, Spry DJ: Chronic toxicity of water borne and dietary lead to rainbow trout in lake Ontario water. *Water Res*, 12, 869-878, 1978.

11. Martinez CBR, Nagae MY, Zaia CTB, Zaia DAM: Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus*. *Braz J Biol*, 64, 797-807, 2004.

12. Shah SL: Hematological parameters in tench *Tinca tinca* after short term exposure to lead. *J Appl Toxicol*, 26, 223-228, 2006.

13. Alam MK, Maughan OE: Acute toxicity of heavy metals to common carp (*Cyprinus carpio*). *J Environ Sci Health*, 30, 1807-1816, 1995.

14. Suvetha L, Ramesh M, Saravanan M: Influence of cypermethrin toxicity on ionic regulation and gill Na/k-ATPase activity of a freshwater teleost *Cyprinus carpio*. *Environ Toxicol Pharm*, 29, 44-49, 2010.

15. Wendelaar Bonga, SE, Lock RAC: Toxicants and Osmoregulation in fish. *Neth J Zool*, 42, 478-493, 1992.

16. EPA (Environmental Protection Agency): LC_{50} software programme, version 1.5. Center for Exposure Assessment Modelling (CEAM). Distribution Center, 1999.

17. Blaxhall PC, Daisley KW: Routine haematological methods for use with fish blood. *J Fish Biol*, 5, 771-781, 1973.

18. McLeay DJ, Gordon MR: Leucocrit: A simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulp mill effluent. *J Fish Res Board Can*, 34, 2164-2175, 1977.

19. Stoskopf MK: Fish Medicine. Saunders, Philadelphia, 1993.

20. Shah SL, Altındağ A: Alterations in the immunological parameters of tench (*Tinca tinca*) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. *Turk J Vet Anim Sci,* 29, 1163-1168, 2005.

21. Cyriac PJ, Antony A, Nambisan PNK: Hemoglobin and hematocrit levels in the fish *Oreochromis mossambicus* after short term exposure to copper and mercury. *Bull Environ Contam Toxicol*, 43, 315-320, 1989.

22. Mayer FL, Versteeg DJ, Mckee MJ, Formoar LC, Graney RL, Mccume DC, Rattner BA: Physilogical and non-specific biomarkers. **In**, Huggest RJ, Kimerle RA, Mehrle-Jr PM, Bergman HL (Eds): Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. pp. 5-86, Lewis Press, Boca Raton, 1992.

23. Luskova V, Svoboda M, Kolarova J: The effect of dianizon on blood plasma biochemistry in carp (*Cyprinus carpio*). *Acta Vet Brno*, 71, 117-123, 2002.

24. Mathan R, Kurunthachalam S, Priya M: Alterations in plasma electrolyte levels of s freshwater fish *Cyprinus carpio* exposed to acidic pH. *Toxicol Environ Chem*, 92, 149-157, 2010.

25. Singh NN, Das VK, Srivastava AK: Insecticides And Ionic Regulation In Teleosts: A Review. *Zoologica Poloniae*, 47, 21-36, 2002.

26. Kennedy CJ, Sweeting RM, Johansen JA, Farrell AP, McKeown BA: Acute effects of chlorinated resin exposure on juvenile rainbow trout. *Environ Toxicol Chem*, 14, 977-982, 1995.

27. Santos MA, Pacheco M: *Anguilla anguilla* stress biomarkers recovery in clean water and secondary treated pulp mill effluent. *Ecotoxicol Environ Saf*, 35, 96-100,1996.

28. Thomas PM, Pankhurst NW, Bremner HA: The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *J Fish Biol*, 54, 1177-1196, 1999.