Impacts of copper sulfate on hematological parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792)

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Abstract

Copper sulfate has been used for many years as an algaecide and parasite treatment. The usage of copper has certain issues as there is a thin line that separates effective treatment levels from overdoses. Copper sulfate can be extremely toxic to fish under certain conditions. This study is focused on the effects of copper sulfate on some blood indices in *Oncorhynchus mykiss* during a 24 hour period. Our research used one procedure 40 *Oncorhynchus mykiss* with the average weight of 220 ±10 g. The experimental group had 20 fish that were treated with the blue vitriol (dose of 0,012 g/40l), during a 24 hour period. The control group had 20 fish. The presence of copper in the water leads to the significant increase of erythrocyte, hemoglobin, MCHC and leukocyte levels while the MCV levels were noted to be considerably low. Monocytes, unsegmented and segmented granylocites were significantly increased in the experimental group of fish. Lymphocyte count was considerably reduced in the same group of fish.

Keywords: Copper Sulphate, Rainbow trout, Toxic, Hematological values

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Introduction

Fish are one of the most widely distributed organisms in the aquatic environment and considered as one of the main protein sources for human (Rashed, 2001). Freshwater fish are similar to the sea water fish which plays an important role in determining resident's diet (Ahmad *et al.*, 2010). Fish has been found to be excellent indicator for the heavy metal contamination level in aquatic system because it occupies different food chain levels (Karadede-Akin and Unlu, 2007). Fish are often seen at the top of the aquatic food chain and may accumulate large amount of heavy metals from its environment (Mansour and Sidky, 2002). In addition, fish are one of the most indicative aspects in freshwater ambience, for the evaluation of heavy metals pollution and health risk potential of human consumption (Papagiannis *et al.*, 2004). The fish respond and adapt differently to environmental changes (Kirin, 2002). Poorwater quality may significantly affect health and haematological status of the fish. Water pollutants may affect the blood count and haematological status in fish (Durand *et al.*, 2000; Guerriero, 2007). The early detection of water pollutants can be determined on a molecular level, tissue or organ abnormality and thus provide researchers timely actions that will prevent further degradation of the organism and the environmental habitat (Małgorzata, 2005). Christensen *et al.* (1977) have researched the impact of mercury and cadmium on brook trout (*Salvelinus fontinalis*) during the period of six to eight weeks. Significant increase of sodium and chlorine was detected in the blood plasma while the hemoglobin and glutamate were reduced. Cadmium caused an increase of chloride in the blood plasma while the mercury salts led to an increase of hemoglobin in the blood plasma. It has been determined that the pollutants that effect haematological status, may not necessarily effect the general health and survival. The answer is in the homeostatic mechanisms that enable organisms to maintain physical and physiological functions (Richards *et al.*, 1996; Unver *et al.*, 1999). The organisms maintain its functions when the pollutant level is small, thanks to the homeostatic mechanisms. The fish can manage elevated levels of poluted water without the significant metabolic damages. However, the higher levels of pollutants lead to an ireparable biochemical and physiological damages and sometimes death (Yilmaz *et al.*, 2005).

Toxicological research often exploresthe effect of certain pollutants on the respiratory system of the fish. Due to the well developed techniques, it is possible to control the oxygen level, metabolite and pollutants in the blood system of fish (Kirin, 2000). Zink reduces transport of oxygen through the gill membranes, reducing its concentration in the blood, which leads to the hypoxia and death. However, if the zink is directly injected into the

blood stream of fish, death and hypoxia will not occur (Walter *et al*., 2005). The pollutants may also effect osmoregulation. Different amonia concentrations effect urine formation in the trout. Elevated amonia levels in the water lead to the elevated urine formation. Fish will die if amonia levels are 20 mg/L. Sublethal zink concentration leads to a decrease of protein, globulin and lysozyme in the carp. The haematological profile can reliably indicate presence of toxinsin in the water (Alakel and Shamsi, 1996). The phenol, pesticide and metal in the water decrease leukocyte levels. They also lead to changes in differential blood count, increase of granulocyte and a significant decrease of lymphocytes. Decrease of lymphocytes causes reduction of globulin transfer and respectively, a reduced antibody production (Dušek *et al*., 2005). There are matters in the water that originate from various human activities, different chemicals as well as heavy metals like cadmium and nickel. The concentration of these metals in the poluted water may reach critical levels and thus affect development of aquatic animals. These effects may lead to physiological and biochemical changes in organisms. The haematological monitoring provides early detection of these conditions (Svodoba *et al.*, 2001; Seker *et al*., 2005). The haematological monitoring provides early detection of ilness in fish. The haematological system is attacked fast, before the behavioral changes occur. The heavy metal toxicity attacks organisms very fast as the blood distributes metals to all parts of the body (Fijan, 2006). The haematological effects of cadmium and nickel on the carp, have been measured in the experiment.

The intraperitoneal, sublethal dose of cadmium had been administered (10 μg Cd/kg) and nickel (60 μg Ni/kg).

We monitored haematological levels as well as detoxification potential after the transfer of fish to the safe zone. The individual entry of the sublethal dose has caused disintegration of erythrocytes in the carp, damages of the metabolism and hematopoiesis. The leukocytes and lymphocytes levels were on the wane in comparison to those of the control group (measured 6 hours after the ingestion). The nickel ingestion had caused reduction of erythrocytes in comparison to those of the control group and the group that has been treated by cadmium. The hemoglobin content was reduced after the cadmium exposure and decreased after the nickel exposure.

The aim of this study is to evaluate the role of the blue vitriol on some heamatological parameters in rainbow trout.

Materials and methods

Animals

This study includes 40 fish *Oncorhynchus mykiss* from river Rakitnica, which is the main tributary of the first section of the Neretva River. This research includes two groups of fish: control (n=20) and experimental group (n=20). Fish was placed in the aquariums of 40 litar volume. After the

adaptation, experimental group of fish was treated with the blue vitriol ($CuSO_4 \cdot 5H_2O$) for 24 hours, (dose of 0,012 g/40l). No feeding was done for fish during the experiment. Handled animals were treated in accordance with the "Declaration on the Rights of Animals" (UNESCO, 1978), "Universal Declaration on Animal Welfare" (WSPA, 2000) and Animal Protection and Welfare Law of Bosnia and Herzegovina ("Official Gazette" 25/09).

Experimental Design

Treated animals were with the weight of approximately 250 and 350 g. For bleeding, the caudal vein was punctured by using 2 mm heparinized the small gauge needle (21G; Semikem, Sarajevo, BiH). During the blood draw, the place of the puncture was disinfected and all other sterile rules were applied. The native blood, without the added antianticoagulants, was used for the further research.

Heamatological Techniques

The following heamatological parameters were analyzed: erythrocytes count, RBC (via Hayem solution, Semikem, Bosnia and Herzegovina), leukocyte count, WBC (via Türk reagens, Semikem, Bosnia and Herzegovina), hemoglobin concentration, HB by Drabkin's hemoglobin cyanide method (Blaxhall and Daisley, 1973), hematocrit, HCT (centrifuging blood samples via micro hematocrit method, 5 min, 16 000 rpm by Hettich Haematokrit 24 zentri-fugen, Germany). Erythrocyte and leukocyte count was determined by hemocytometer chamber (Neuber's chamber). Differential count of leukocytes was done by providing a blood smear and was examined with light microscope. The hematological indices: Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined from the following equations (Campbell 2004). Fresh smears were stained by May-Grünwald and Giemsa stains (Semikem, Sarajevo, BiH) for procentual analysis of leukocyte levels in the peripheral blood. Cell identification and procentual count of blood cells (were performed on Olympus BX41 light microscope).

Morphometric Characteristics

All fish were analized for body weight, total and standard body length and Fulton's Coefficient (Vuković, 1982). The total and standard length was measured by the Fishmeter while the body weight was measured with the precise scale.

Statistical

Results were evaluated by the IBM SPSS (Version 20.0, SPSS, Inc., Chicago, IL, USA). The collected data was analyzed through the methods of the descriptive statististics (mean, standard deviation and range). Variances between the groups were analized via One Way ANOVA method. P values lower than 0,05 were considered significant.

Table 1: Morphometric Characteristics of Controlled and Experimental fish.

Parameters	Control	Experiment	*p*-values
Total length (cm)	27,45±2,04	27,05±1,74	0,286
Standard length (cm)	23,26±2,21	24,55±1,87	0,048
Mass (g)	225,26±50,70	237,41±41,58	0,239

Results

Table 1 presents middle value, standard deviations, and p value (Student's t test) for morphometric parameters: total length, standard length and body mass.

We were not able to determine significant differences in body length and body mass between the controlled and experimental fish. The experimental group has higher values of almost all parameters. The chematocrit and MCV count are the only parameters without the significant cross group differences (Table 2).

Table 3 presents Spearman's Test of Correlation between erythrocytes and HCT, Hb, MCV, MCH and MCHC. R value shows positive or a negative correlation, p values and the level of significance.

There is a negative correlation between erythrocytes and other heamatological parameters in the blood of controlled fish. There is a positive correlation between erythrocytes and hemoglobin concentration in the blood of experimental fish, as well as the negative correlation for HCT and MCHC, and MCV and MCH. Both groups have significantly different erythrocyte, MCV and MCH values. The experimental group has significantly increased levels of monocytes, unsegmented and segmented granulocytes as well as considerably lower lymphocyte count (Table 4).

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Table 2: Haemathological Parameters Values and Between Differences.

Haemathological	Control group		Experimental group		Sig.
Parameters	Mean	Range	Mean	Range	
RBC	1.21±0.21	1.02-1.74	1.55±0.12	1.34-1.75	0.00*
HCT	0.41±0.09	0.237-0.51	0.392±0.06	0.29-0.48	0.267
HB (g/L)	56.29±20.85	31.95-98.55	76.22±4.67	66.66-82.45	0.00*
MCV	367.72±119.27	208.04-591.91	281.67±49.23	153.57-338.99	0.02*
MCH	47.63±18.34	24.13-72.03	49.16±3.96	43.55-58.04	0.394
MCHC (g/L)	152.19±36.89	105.79-235.20	198.517±30.84	159.68-260.99	0.00*
WBC (109/L)	3.34±1.04	1.75-5.11	4.14±0.28	3.55-4.56	0.01*

* Statistical significant values at 0.05.

Table 3: Spearman's Test of Correlation

Parameters	Erythrocytes			
	Experiment		Control	
	r	p	r	p
HCT	-0,213	0,446	-0,364	0,182
Hb	+0,127	0,652	-0,405	0,135
MCV	-0,947	0,000**	-0,848	0,000**
MCH	-0,892	0,000**	-0,800	0,000**
MCHC	-0,236	0,397	-0,023	0,934

Table 4: Blood leukocytes values and cross group differences

Blood leukocytes	Control group		Experimental group		Sig.
	Mean	Range	Mean	Range	
Limfociti	81.27±3.22	77-87	71.85±3.03	66-76	0.00*
Monociti	1.77±0.83	1-3	2.50±0.76	1-4	0.02*
Eozinofili	1.23±0.42	1-2	1.33±0.51	1-2	0.08
Nesegmentirani	10. ±3.03	6-15	15.35±3.24	11-22	0.00*
Segmentirani	6.72±1.79	3-9	9.71±1.73	7-13	0.00*

Discussion

Fish is one of the most expensive sources of food in the water and are sensitive to the environmental pollution. It is possible to detect toxic symptom of herbicides by studying cytological and serological indices in fish. Fish are directly exposed to the environmental changes. Sensitivity to different toxic materials varies among fish. This is the reason why we used different types of fish in our study (Naji *et al.*, 2019).

Toxic metals in fish may have negative impact on the health of humans (kidney failure, liver damage) (Al-Busaidi, 2011). Toxic materials may be absorbed through the gills or through the digestive system. The absorbed toxic metals effect health of fish. Testing of hematological parameters enables early detection of health in fish. Early detection of water polutants can be determined on a molecular level, through the tissue or organ abnormality. The researchers can thus implement timely measures that will prevent further degradation of habitat (Małgorzata, 2005)**.** Heamatological profile is a reliable indicator of toxic pollutants in the water (Alakel and Shamsi, 1996). Physiological diagnostics of fish understands that both erythrocytes and leukocytes have a high diagnostic value (Bond, 1979; Kekić *et al.,* 1985).

Copper is the main component of multicellular organisms. Hemocyanins are copper containing respiratory pigments. Copper deficiency lowers erythrocyte count. Daily copper needs for an adult humans are 1 to 1,5 mg (Mercer, 1998). Copper plays an important role in the overall blood count: iron absorption, hemoglobin synthesis and erytrhrocyte production, colagen and elastin synthesis, bone formation. Copper is also an integral part of many enzymes. Copper deficency has an impact on enzyme processes. The copper

absorption is influenced by the vitamin C, phytates, sulfides etc. Copper binds with albumin and is thus transported into the liver. Metallothionen is crucial for the safe copper storage (Cvjetko *et al*., 2010). Concentration of copper in the water significantly increases erythrocyte and hematocrit count while it reduces leukocyte and hemaglobin count and hematological parameters. There are various reasons that lead to these changes. Intraparitoneal injection of cadmium and nickel causes destruction and lowered erythrocyte count, particularily the lymphocyte count in carp (Demirak *et al.,* 2006). Lowered leukocyte count was recorded by Dušek *et al.* (2005). Concentration of nickel in the water leads to hypoxia and death (Walter *et al.*, 2005). Our reserach also records lowered leukocyte count. Increased erythrocyte count prevents hematopoiesis of blood cells in both directions (erythropoiesis and leukopoiesis), due to the short term acute effects of copper. On the other hand, considerably high erythrocyte count may lead to hypoxia. Copper protein is proved to be non toxic and thus enables temporary storage of copper in the liver or it can act as a donor for ceruloplasmin synthesis. Copper protein is transported from the liver into the blood stream and it enters erythroyctes in the form of erythrocuorein. Ceruloplasmin is a safe, non toxic component in mammals that enters the blood from the digestive system. This process is different for fish. Therefore the copper is directly available to erythrocytes. Excessive amounts of copper are excreted mostly through the bile (Stalović *et al.,* 2013). This process is different in fish as the copper is directly available to erythrocytes. $CuSO_4$ is a copper sulphate that effects osmoregulation and pH of blood in fish. Copper sulphate effects ion state of protein, respectively hemoglobin in erythrocytes, which prevents oxygenation. This mechanism causes hypoxia that stimulates erythropoiesis and increased erythrocyte count while the volume (MCV) is low. Hemoglobin, that is non functional due to the copper binding, does not perform its transporting role. That leads to a lowered hemoglobin concentration and other heamatological parameters. Heavy metals are distributed differently in fish. The highest concentration of Cu, Zn and Fe is recorded in the liver, Pb and Mn in the gills, while the muscles have the lowest concentration of metals. The concentration of metal is influenced by the diet, for example Herbivor S. Gibbus has the lowest Zn concentration in all of its organs (El-Moselhy *et al*., 2014). Gills are the main absorbants of metal but its large surface rapidly difuses toxic metals (Dhaneesh *et al*., 2012). This facilitates blood difusion. Depending on the type of metal and its concentration, toxic effects are directly manifested in erythrocytes. Various studies have recorded heavy metal concentration in the gills (Quadir and Malik, 2011; Shriadah, 2004). Concentration of metal varies in different organs, fish species, dietary processes and genetic properties (El-Moselhy, 2014). Concentration and

transport of these metals can cause major changes in the blood count, plasma protein functions, changes in pH and ultimately severe respiratory functions.

Conclusion

The effects of toxic metals depend on the type of tissue affected and transporting routes. The absorption of copper through the gills directly impacts pH, ion state of plasma and the process of oxygenation in erythrocytes. An acute and short term accumulation of erythrocuprein in erythrocytes changes ion shape of hemoglobin. This process causes hypoxia, considerable increase in the red blood cells and reduced hemoglobin concentration. On the other hand, leukopoiesis is decreased which weakens the immunity.

The presence of copper in the water leads to the significant increase of erythrocyte, hemoglobin, MCHC and leukocyte levels while the MCV levels were noted to be considerably low. Monocytes, unsegmented and segmented granylocites were significantly increased in the experimental group of fish. Lymphocyte count was considerably reduced in the same group of fish.

Refferences

Ahmad, A.K. and Shuhaimi-Othman, M., 2010. Heavy metal concentrations in sediments and fishes from Lake Chini, Pahang, Malaysia. *Journal of Biological Sciences*, 10, 93-100.

Alakel, A.S., Shamsi, M.J.K., 1996. Hexavalent chromium-toxicity and impact on carbohydrate-metabolism and hematological parameters of carp (*Cyprinus carpio* L) from Saudi-Arabia. *Aquatic Sciences*, 58, 1, 24-30.

Al-Busaidi, M., Yesudhason, P., Al-Mughairi, S., Al-Rahbi, WA., Al-Harthy, KS., Al-Mazrooei, NA., Al-Habsi, SH., 2011. Toxic metals in commercial marine fish in Oman with reference to national and international standards. *Chemosphere Epub*, 85(1):67-73.

Blaxhall, P.C. and Daisley, K., 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Bond, C.E., 1979. Biology of Fishes. Saunders College Publishing, Philadelphia, USA., 514.

Campbell, T.W., 2004. Hematology of lower vertebrates – In: Proc. of the 55th Annual Meeting of the American College of Veterinary Pathologists (ACVPC) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP). ACVP and ASVCP, USA.

Christensen, G., Hunt, E. and Fiandt, J., 1977. The effect of methylmercuric chloride, cadmium chloride, and lead nitrate on six biochemical factors of the brook trout (*Salvelinus fontinalis*). *Toxicology and Applied Pharmacology*, 42, 3, 523–530.

Cvjetko, P., Tolić, S., Šikić, S., Pavlica, M., 2010. Effect of Copper on the Toxicity and Genotoxicity of Cadmium in Duckweed (*Lemna Minor* L.). *Archives of Industrial Hygiene and Toxicology*. 61(3):287-96

Demirak, A., Yilmaz, F., Tuna, A.L. and Ozdemir, N., 2006. Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey; *Chemosphere Epub*, 63, 1451-1458.

Dhaneesh, K., Ganeshamurthy, R., Gopi, M., Thipramalai, T.P.A., 2012. Bio-accumulation of metals on reef associated organisms of Lakshadweep Archipelago. Food Chemistry 131(3):985–991

Durand, J.D., Unlu, E., Doadrio, I., Pipoyan, S. and Templeton, A.R., 2000. Origin, radiation, dispersion and allopatrichybridization in the chub *Leuciscus cephalus. Proceedings of the Royal Society B: Biological Sciences*, 22, 267(1453), 1687-97.

Dušek, U., Frank, G.P., Helas, G., Iinuma, Y., Zeromskiene, K., Gwaze, P., Hennig, T., Massling, A., Schmid, O., Herrmann, H., Wiedensohler, A. and Andreae, M.O., 2005. Missing cloud condensation nuclei in peat smoke. *Geophysical Research Letters*, 32 (11), CiteID L11802.

El-Moselhy, K.M., Othman, A.I., Abd El-Azem, H., El-Metwally M.E.A., 2014. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian Journal of Basic and Applied Sciences*. 1, 297-105.

Fijan, N., 2006. Zaštita zdravlja riba, Poljoprivredni fakultet u Osijeku; Impressum Osijek

Guerriero, G., 2007. Seasonal steroids variations and maturity stages in the female chub, *Leuciscus cephalus* L. (Pisces, Cyprinidae). *Italian Journal of Zoology*, 74, 317-324.

Karadede-Akin, H. and E. Unlu, 2007. Heavy metal concentrations in water, sediment, fish and some benthic organisms from Tigris River, Turkey. *Environmental Monitoring and Assessment*, 131, 323-337.

Kekić, H., Pavlović, V., Gvozdenović, O., Ivanc, A., Mijatović, N. and Pejić, K., 1985. Differential blood count in brown trout (*Salmo trutta m. fario*) under natural conditions of habitat in various seasons. *Iugoslavica Physiologica et Pharmacologica Aacta*, 21, Suppl. 4, 121.

Kirin, D., 2000. Ecologophaunistical study of the helminthological communities of *Leuciscus cephalus* L. from River Maritsa. - Research reports of the Union of Scientists of Bulgaria, 1, 405-408.

Kirin, D., 2002. Ecological Study of the Intestinal Helminth Communities of *Leuciscus cephalus* (L., 1758) and Appraisal of the Conditions of the Studied Freshwater Ecosystems from the Chepelarska River, Bulgaria. *Acta Zoologica Bulgarica*, 54(2), 73-85.

Małgorzata, W., 2005. Stress in fish-hematological and immunological effects of heavy metals. *Electronic Journal of Ichthyology*, 1, 35-4135.

Mansour, S.A. and Sidky, M.M., 2002. Ecotoxicological studies. 3. Heavy metals contaminating water and fish from fayoum Governorate, Egypt. *Food Chemistry*, 78, 15-22.

Mercer, J.F., 1998. Menkes syndrome and animal models. Am. J. Clin. Nutr. 67(5 Suppl.):1022S–1028S.

Naji, M., Yousefi Jourdehi, Y. and Hosseinzadeh Sahafi, H., 2019. Impacts of Atrazine on some blood and biochemical indices in farmed *Acipenser nudiventris*. *Journal of Survey in Fisheries Sciences*, 5(2) 19-27

Papagiannis, I., Kagalo, I., Leonardos, J., Petridis, D. and Kalfakaou, V., 2004. Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environ. Int.*, 30: 357-362.

Qadir, A. and Malik, RN., 2011. Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. *Biological Trace Element Research*. 143:1524-1540.

Rashed, M.N., 2001. Egypt monitoring of environmental heavy metals in fish from Nasser Lake. *Environment International*, 27, 27-33.

Richards, D.T., Hoole, D., Lewis, J.W., Evans, E. and Arme, C., 1996. Adherence of carp leucocytes to addults and cercariae of the blood fluke *Sanguinicola inermis*. *Journal of Helminthology*, 70, 1, 63-67.

Seker, Y., Karats, M. and Sezar, M., 2005. Determination of Biochemical Parameter Values of Chub (*Leuciscus cephalus)* Population in Almus Dam Lake, Turkey. *Juornal of Animal Andveterinary Advances*, 4 (11), 927-929.

Shriadah, **M.A., Okbah**, **M.**, **El-Deek**, **M.S., 2004**. Trace Metals in the Water Columns of the Red Sea and the Gulf of Aqaba, Egypt. *Water Air and Soil Pollution*. 153(1):115-124 ·

Shriadah, **M.A., Okbah**, **M.**, **El-Deek**, **M.S., 2004**. Trace Metals in the Water Columns of the Red Sea and the Gulf of Aqaba, Egypt. *Water Air and Soil Pollution*. 153(1):115-124 ·

Stalović, B. and Đorđević, S., 2013. Optical emission spectrometry and inductively coupled plasma (ICP/OES) like an analytical technique for determination of heavy metals in biological samples. Med Data Rev. 5; 1:053-057.

Svodoba, M., Kouril, J., Hamačkova, J., Kalab, P., Savina, L., Svodobova, Z. and Vykusova, B., 2001. Biochemical profile of blood plasma of tench (*Tinca tinca*) during pre- and postspawning period. *Acta Veterinaria Brunensis*, 70, 259-268.

Unver, B. and Tanyolac, J., 1999. Growth Properties of Chub (*Leuciscus cephalus* L., 1758) in Lake Todurge (Zara/Sivas). *Turkish Journal of Zoology*, 23, 271-278.

Vuković, T., 1982. Sistematika riba. Slatkovodno ribarstvo;

Jugoslovenska medicinska naklada, Zagreb, 99-168.

Walter, I., Tschunek, W., Schabus, M., Miller, I. and Grilitsch, B., 2005. Structure of the Seminal Pathway in the European Chub (*Leuciscus cephalus*); *Anatomia, Histologia, Embriologia,* 34, pp. 56-56 (1)

Yilmaz, F., Ozdemir, N., Demirak, A. and Levent, A., 2005. Heavy matal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus. Chemosphere Epub*, 63(9):1451-8.