African Journal of Biotechnology Vol. 10(16), pp. 3204-3209, 18 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2505
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Acute effects of copper and lead on some blood parameters on Coruh trout (*Salmo coruhensis*)

Ramazan Serezli[1]*, Süleyman Akhan[2] and Fatma Delihasan-Sonay[1]

[1]Faculty of Fisheries, Rize University, 53100, Rize, Turkey.
[2]Faculty of Fisheries, Akdeniz University, 07059, Antalya, Turkey.

Accepted 18 March, 2011

The present study was to evaluate whether short-term exposures (3 h) to high concentrations of heavy metals may induce blood cells in Coruh trout (*Salmo coruhensis*). It was investigated that copper and lead have effects on haematocrit, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic and pyruvic transaminase (SGPT), total protein, blood cell numbers and erythrocyte morphology of *S. coruhensis* exposed to two copper and lead concentrations for 3 h. Copper and lead concentrations tested in the experiments were 5 and 10 mg Cu/L; 5 and 10 mg Pb/L, respectively. Blood was sampled immediately after the end of exposures and then 24, 48 and 72 h later post exposures. Treatments were not inducing micronuclei in the erythrocyte nuclei.

Key words: Copper, lead, heavy metal, *Salmo coruhensis*, micronuclei, blood cells.

INTRODUCTION

Water pollution has become a global problem. Heavy metals have long been recognized as serious pollutants of the aquatic environment. They can enter a water body by industrial and consumer waste or even from acidic rain, thereby breaking down soils and releasing heavy metals into streams, lakes, rivers and ground waters (Obasohan et al., 2008).

Although, trace metals are essential for normal physiological processes, abnormally, high concentrations can be toxic to aquatic organisms. Due to the insidious nature of metal bio concentration, it would be too late to apply preventive measures to reduce the pollution effects by the time the chronic effects become visible (Wepener et al., 2001). Living organisms require varying amounts of some heavy metals for their metabolic function, for instance; copper (Cu) is an essential trace nutrient for plants and animals. In humans, it helps in the production of blood hemoglobin, the oxygen carrying compound in red blood cells (Heath, 1995). Copper can be both good and bad for living organisms. It is necessary for life at very low concentrations. But, when the concentration increases, it becomes toxic and interferes with cell metabolism and also, it is very toxic to fungi and algae (Hall et al., 1997; Kjoss et al., 2005). Unlike copper, lead (Pb) is not necessary for the biological functions of animals, even at low concentration. It is being discharged to aquatic systems, mainly from petroleum, chemistry, dye and mining industries, which has toxic effects and can cause mortality to aquatic animals (Sorensen, 1991; Heath, 1995; Ciftci-Soydemir et al., 2008).

The natural concentration of lead in surface water has been estimated at 0.02 µg l^{-1} and it rarely exceeds a few micrograms/L (Martinez et al., 2004). High levels of heavy metals cause environmental pollution, especially in water body. Water pollution with heavy metals, affects various physiological processes in fish, including blood cells. In fish, toxic substances taken up from the water enter the blood and therefore, blood cells are among the first targets of toxicity, immediately after the gill epithet-lium. Therefore, the effects of waterborne heavy metals on fish are related to their uptake from gills, resulting in interfere with blood circulating system and also blood cells (Vosyliene, 1999; Martinez et al., 2004).

Fish erythrocyte morphology is one of the most sensi-tive indicators of toxic impact of various environmental factors on fish. A significant number of data on the changes

Corresponding author. E mail: rserezli@gmail.com

Abbreviations: SGOT, serum glutamic oxaloacetic transaminase; **SGPT**, serum glutamic pyruvic transaminase, **TP,** total protein; **RBCL,** red blood cell length; **RBCW,** red blood cell width; **NucL,** nucleus length; **NucW,** nucleus width.

of hematological indices of salmonid fish, influence various environmental factors such as chemicals or heavy metals as well (Heath 1995; Wepener et al., 2001; Zeni et al., 2002; Jezierska et al., 2009). Many fish species are susceptible to the deleterious effects of heavy metals, as reflected in the blood changes, including anemia, eosinophilia, lymphocytosis, alterations in erythrocyte morphology and branchial and renal lesions (Gill and Pant, 1987).

However, information on the adverse effects of Cu and Pb on Coruh trout (*Salmo coruhensis,* described by Turan et al., 2009) which is the new culture species in Black Sea region of Turkey, is very scarce. This study was performed for hematological changes and erythrocyte-metric parameters of coruh trout, which were exposed to 5 and 10 mg/l Cu^{+2} ($CuSO_4.5H_2O$) and Pb^{+2} as Pb $(NO_3)_2$ for 3 h.

MATERIALS AND METHODS

Experimental fish

Two years old coruh trout (mean: 108.4±1.96 g) were obtained from the commercial trout farm in Macka, Trabzon, Turkey. They were held in a 100 L tank (20 fish per tank), with continuously aerated and flow-through well water (T= 13°C, pH= 7.1, hardness= 25 mg/l as $CaCO_3$), with a 10 /14 h light/dark cycle. Fish were adapted to the experimental condition for 30 days prior to experiments and they were fed ad libitum with pellet feed three times a day before the experiment, except during and on the day preceding the experiments.

Experimental design

Three hundred Coruh trout was used in this study. They were subjected to 3 h immersion in heavy metal solutions (5 and 10 mg/l Pb as Pb $(NO_3)_2$, 5 and 10 mg l^{-1} Cu as ($CuSO_4$ $5H_2O$)). Control fish were subjected to similar manipulations as the experimental ones. All experiments were performed in triplicate groups. Fish were anaesthetized with benzocaine and blood was sampled from fish by caudal cutting, immediately after the end of exposures (Cont0, Cu5.0, Cu10.0, Pb5.0, Pb10.0) and after 24 (Cont1, Cu5.1, Cu10.1, Pb5.1, Pb10.1) and 48 h (Cont2, Cu5.2, Cu10.2, Pb5.2, Pb10.2). Five fish were captured from each sample. Blood smears were prepared and fixed immediately, according to the standard treatment procedure (Blaxhall and Daisley, 1973) and were stained by Giemsa- May Grunwald stain in laboratory conditions. The long and short red blood cells (RBC) and their nuclei axis's were measured under the light microscope with immersion oil (×1000 magnification) in µm (30 cells per fish, 5 fish per group), with micrometric ocular and Leica (ICC 50) camera system. On the other hand, erythrocyte and leukocyte number were counted by Thoma chamber. Hematocrit (Hct) values were determined by blood centrifugation (5 min, 5000 g) in glass capillaries using a micro-hematocrit centrifuge. The blood haemoglobin concentration was measured with a spectrophotometer at 550 nm using a cyano-methemoglobin method as described by Smith and Hatting (1980). Blood samples were then centrifuged (10 min, 5.000 g) and plasma samples were taken and stored frozen (-20°C) until chemical analyses. All samples were analyzed in duplicate. Total protein (TP), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined with auto-analyzer.

The results were expressed as mean values ± SE for each group; then data were tested for statistical significance by a one-way analysis of variation (ANOVA). Duncan's multiple comparison tests were used to assess significant differences between samplings. Differences were considered statistically significant at the 0.05 probability level.

RESULTS

Effects of Cu and Pb on hematological parameters on Coruh trout was investigated in this study at concentration of 5 and 10 mg/l.

Effect of Cu and Pb on erythrocyte and leukocyte numbers

Erythrocyte and leukocyte numbers are summarized in Table 1. In the control group, general erythrocyte number was estimated as 498.631±26.051 cells/ml. In the Cu treatment groups, after exposure to copper, the erythrocyte numbers were suppressed at 5 and 10 mg/l Cu^{2+} and 48 h later the erythrocyte numbers increased to normal level. Unlike Cu treatments, in the Pb treatment groups, the erythrocyte numbers increased and 48 h later, suddenly decreased to very low levels (Table 1). Leukocyte numbers estimated about $20x10^3$ cells/ml in controls, but in the experimental groups, except in the 10 mg l^{-1} Pb group, this number increased about 30 $x10^3$ cells ml^{-1} and in the 10 mg/l Pb group, the leukocyte number decreased $15x10^3$ cells/ml (Table 1).

Effect of Cu and Pb on haemoglobin and haematocrit

Although, haematocrit value was determined as 45.7% in the control group, it was elevated in the copper groups but on the contrary, has no changed in the lead groups (Table 1).

Hemoglobin value was determined as 4.8 g/dl in the control group, while copper groups were determined to be statistically different from the control group. Hemoglobin values of fish exposed to lead were higher than the control, although, this difference is not statistically significant ($p > 0.05$).

Effect of Cu and Pb on TP, SGOT and SGPT

In this study, the total protein (TP) values in copper groups when compared with the control group were a little high, but this difference was not statistically significant. On the other hand, TP values of lead groups were similar to the control.

In addition, except for Cu 10 group, there were no differences in SGOT values between the groups statistically. The Cu 10 group were seen lower than other groups and this was found to have statistical significance.

Table 1. Blood parameters of coruh trout (*S. coruhensis*) exposed different Cu and Pb concentration.

Dose	Time	Erythrocyte	Leucocyte	Heamotocrit	Heamoglobin	SGOT	SGPT	TP
Control	0	465.920±29.263	24.000±1.746	45.7±0. 50	4.7±0.05	295.5±2.46	30.8±0.63	4.86±0.061
	24	513.707±23.697	18.667±1.687	49.4±0.57	4.8±0.08	295.2±4.11	28.5±0.84	4.86±0.043
	48	516.267±24.500	20.267±2.292	46.1±0.86	4.8±0.08	301.3±3.86	28.7±0.72	4.86±0.100
	72	-	-	46.7±0.87	4.8±0.09	297.5±3.04	29.9±0.76	4.86±0.054
	General	498.631±26.051	20.978±1.971	45.7±0.80	4.8±0.08	297.4±3.40	29.5±0.76	4.86±0.085
Copper 5 (mg/L)	0	389.120±26.610	27.200±1.797	48.3±0.41	5.6±0.25	292.4±5.99	19.5±0.65	5.49±0.050
	24	576.000±38.663	16.800±1.306	63.5±1.16	5.0±0.12	286.7±2.23	22.6±0.59	6.63±0.118
	48	-	-	60.1±1.83	-	293.4±2.70	31.1±0.56	6.46±0.075
	72	-	-	-	-	290.5±5.07	30.9±0.91	-
	General	482.560±40.812	22.000±2.061	57.3±2.11	5.3±0.02	290.8±4.24	26.0±1.48	6.20±0.156
Copper 10 (mg/L)	0	428.373±22.166	29.067±1.766	-	6.7±0.22	230.3±6.72	17.7±0.81	5.15±0.082
	24	740.693±36.501	30.133±1.985	-	6.6±0.19	237.5±5.65	25.1±0.61	6.10±0.090
	48	-	-	-	-	287.1±3.67	31.5±0.92	6.47±0.083
	72	-	-	-	-	294.9±5.86	31.9±0.71	-
	General	584.533±52.618	29.600±1.851	-	6.7±0.20	262.4±9.26	26.6±1.69	5.91±0.167
Lead 5 (mg/L)	0	546.133±17.473	26.133±1.696	47.9±0.31	5.2±0.12	271.3±4.62	26.9±1.41	4.69±0.044
	24	689.493±30.338	13.133±1.545	49.3±0.37	4.9±0.07	291.0±2.61	24.9±0.55	5.09±0.044
	48	302.080±8.361	17.600±2.095	48.3±0.81	-	280.4±2.44	30.7±0.76	5.13±0.064
	72	-	-	48.9±0.86	6.6±0.23	291.3±2.91	29.5±0.68	4.73±0.061
	General	512.569±46.435	30.133±2.236	57.3±0.64	5.5±0.14	283.5±1.92	28.0±0.53	4.91±0.037
Lead 10 (mg/L)	0	801.280±23.858	16.267±1.850	49.9±0.37	5.2±0.07	285.9±3.80	22.9±1.05	4.92±0.048
	24	610.987±15.971	28.267±2.389	45.1±0.37	4.5±0.15	269.7±2.97	26.6±0.70	5.21±0.044
	48	302.933±14.763. 933±14.763	15.467±2.305	48.5±0.37	-	275.9±3.67	30.3±0.83	4.77±0.057
	72	-	-	44.5±0.37	5.5±0.17	293.7±2.25	28.8±0.76	4.65±0.098
	General	571.733±56.624	20.000±2.633	47.3±0.37	5.1±0.18	281.3±3.95	27.1±1.10	4.89±0.085

When compared with the control group, there were no significant differences between the groups in SGPT values statistically ($p > 0.05$).

Effect of Cu and Pb on erythrocyte size and shape

Normal mature erythrocytes shape of coruh trout are oval with smooth acidophilic cytoplasm and centrally located oval nucleus showed almost homogenous chromatin. Erythrocyte morphology parameters are summarized in Table 2 and Figure 1. Except the control group, copper and lead groups erythrocyte shape was round and had less small nucleus (Figure 2). But, no nucleus deformity and micro nuclei were observed.

DISCUSSION

Coruh trout is more sensitive to Cu treatment than Pb at the same concentration. Although, there was no death in the Pb groups, fish death were observed in Cu groups at a concentration of 10 mg/l when the fish were exposed for over two hour. For this reason, 10 mg/l treatment was studied for 90 min. There was no death observed in the control and other groups.

Water quality, particularly hardness of water is important for heavy metals' toxic effects on organisms. Increased water hardness reduces lead toxicity to fish due to a significant inorganic complication process that controls lead availability to fish (Martinez et al., 2004). Pickering and Henderson (1966) showed that in soft water (20 mg $CaCO_3$/L), the 96 h LC_{50} for *Pimephales promelas* and *Lepomis macrochirus* was 5.6 and 23.8 mg Pb/L, whereas, in hard water (360 mg $CaCO_3$/L) 96 h-LC_{50} was 482 and 442 mg Pb/L, respectively. The short-term lethality test conducted in this study yielded 96 h-LC_{50} of 95 mg Pb/L for juveniles of *Prochilodus lineatus* in water of 82 mg/l hardness ($CaCO_3$).

Table 2. Erythrocyte and nuclei size (as micron).

Time	RBCL					RBCW					NucL					NucW				
	Control	Cu 5	Cu 10	Pb 5	Pb 10	Control	Cu 5	Cu 10	Pb 5	Pb 10	Control	Cu 5	Cu 10	Pb 5	Pb 10	Control	Cu 5	Cu 10	Pb 5	Pb 10
24	15.18^{a}	15.06^{a}	15.24^{a}	15.31^{a}	15.10^{a}	9.73^{b}	9.88^{b}	10.46^{c}	9.44^{a}	9.77^{b}	7.65^{e}	6.91^{b}	6.71^{a}	7.47^{d}	7.31^{c}	3.99^{ab}	4.10^{b}	3.96^{a}	3.98^{ab}	3.92^{a}
48	14.93^{b}	14.83^{ab}	14.53^{a}	14.57^{a}	15.07^{b}	9.33^{ab}	9.43^{ab}	10.85^{c}	9.50^{b}	9.22^{a}	6.98^{b}	6.89^{b}	6.62^{a}	7.19^{c}	7.40^{d}	4.06^{b}	3.78^{a}	4.30^{c}	4.25^{c}	4.09^{b}
72	15.48^{b}	15.18^{ab}	-	15.25^{ab}	15.08^{a}	9.33^{b}	10.29^{c}	-	9.22^{ab}	8.97^{a}	7.47^{a}	7.24^{a}	-	7.27^{a}	7.35^{a}	3.87^{a}	4.14^{b}	-	3.75^{a}	3.84^{a}

Different letter indicates significant difference from each other $p < 0.05$.

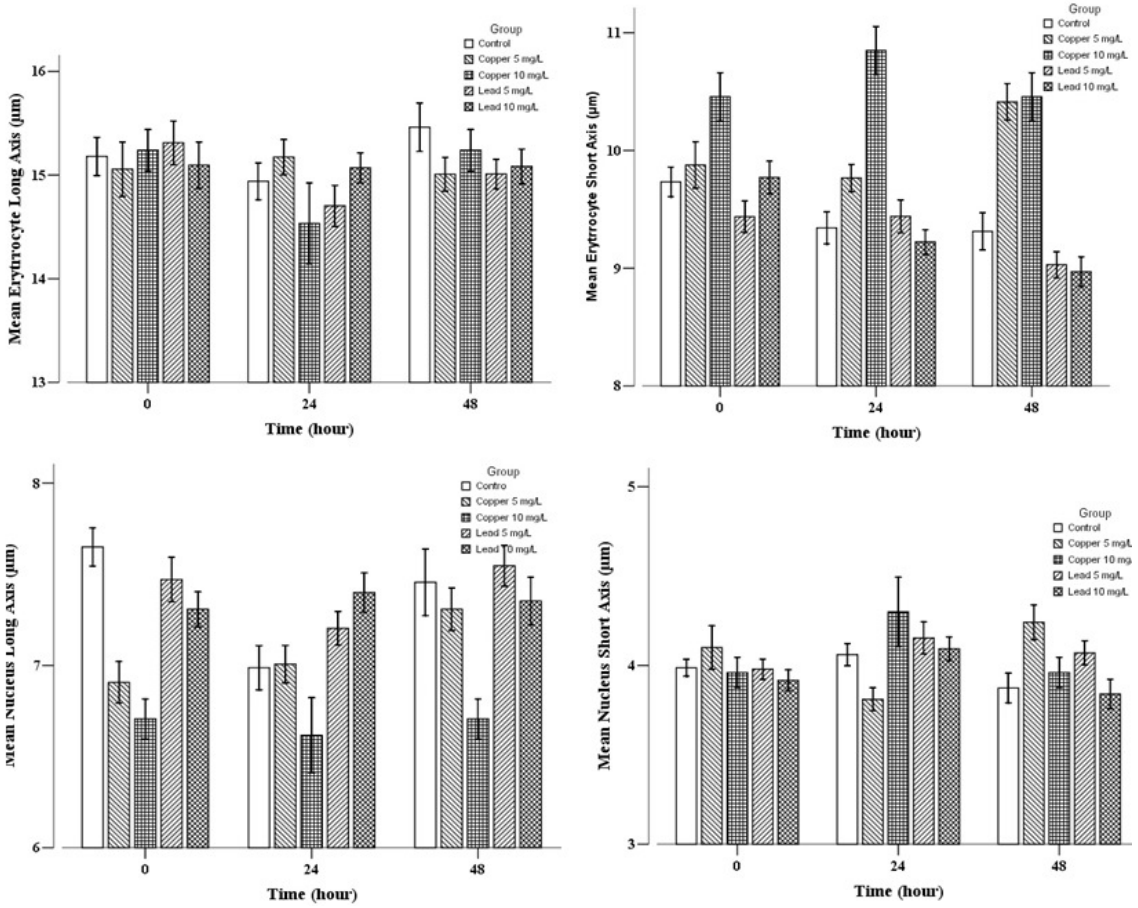

Figure 1. Erythrocyte and nuclei size changes in coruh trout.

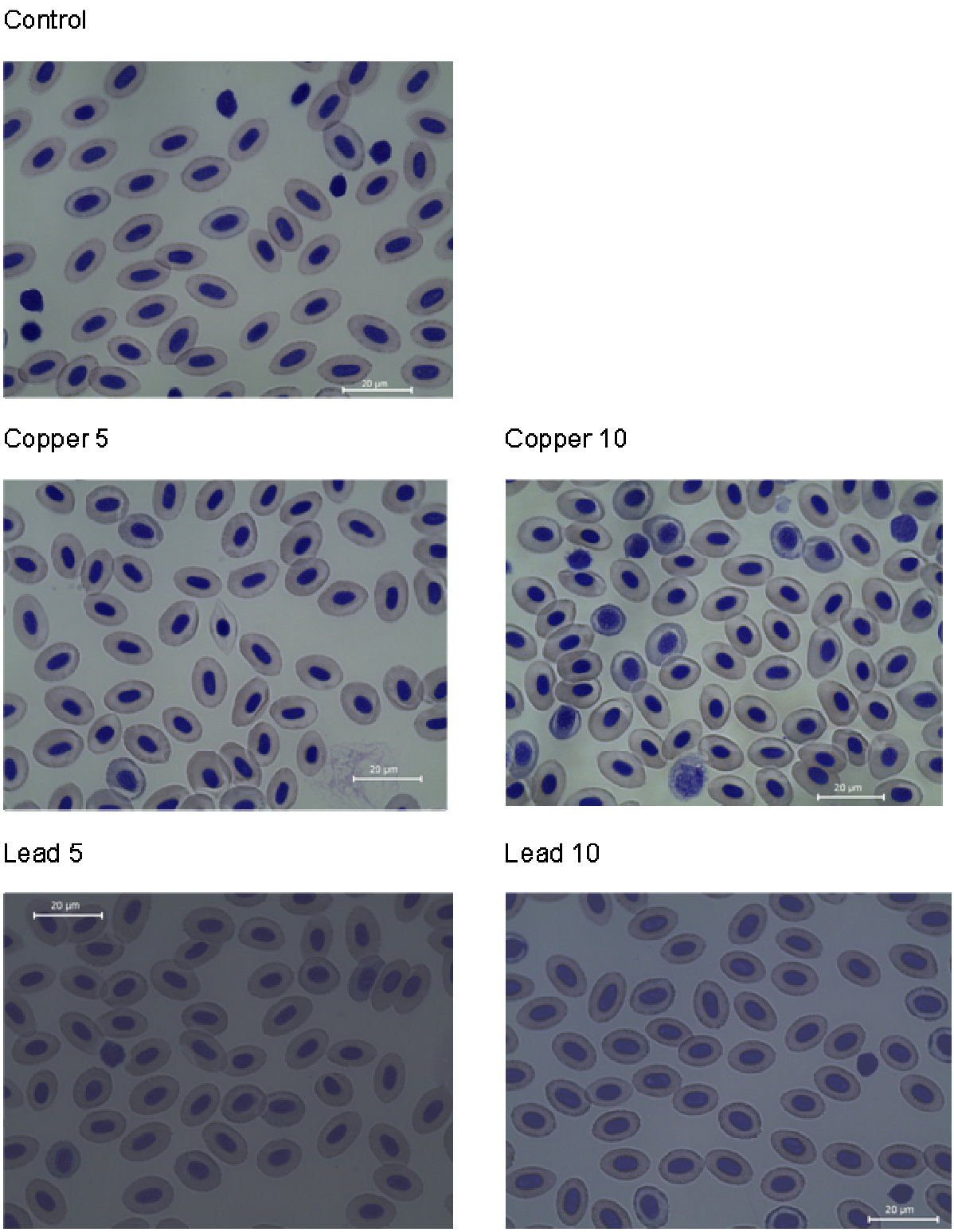

Figure 2. Erythrocyte shape and size of coruh trout (*S. coruhensis*) control.

Regarding the impact of lead on the hematological profile of coruh trout, polycythemias accompanied by elevated hemoglobin level and heamotocrit value were observed. Similar findings were observed for different fish by McKim et al. (1970), Hilmy et al. (1979), Taylor et al. (1985) and Zaki et al. (2008).

In this study, water hardness was determined as 25mg/l as $CaCO_3$. Over 5 mg dose of copper applications with short duration exposures causes death and blood cells hemolysis. Therefore, problems were experienced in 10 mg. 10 mg/L dose of copper resulted in hemolysis was found to cause fish mortality. However, higher doses of lead applications with short duration can be tested safely. Although, there were some deteriorations of erythrocyte morphology, no functional impairment and micronucleus were seen in fish exposed to heavy metals.

In conclusion, the blood based biochemical and hematological parameters measured responded to relatively high copper and lead concentrations. The results of the present study showed that, heavy metals induce morphological changes in coruh trout blood cells. Particularly, erythrocyte anomalies seem to be a good indicator of intoxicants.

ACKNOWLEDGEMENT

This research was funded by Rize University, Scientific Research Unit, project number 2008.10.02.2.

REFERENCES

Blaxhall PC, Daisley KW (1973). Routine Haematological Methods for Use with Fish Blood. J. Fish Biol. 5: 771-781.

Ciftci-Soydemir N, Cicik B, Erdem C, Ay O (2008). Effects of Lead Concentrations on Sera Parameters and Hematocrit Levels in *Anguilla anguilla* L, 1758. J. Fish Sci. Com. 2(4): 616-622.

Gill TS, Pant JC (1987). Hematological and pathological effects of chromium toxicosis in the freshwater fish, *Barbus conchonius*, Ham. Water Air Soil Pol. 35: 241-250.

Hall LW, Scott MC, Killen WD (1997). Ecological risk assessment of copper and cadmium in the surface waters of Chesapeake Bay watershed. Environ. Toxicol. Chem. 17(6): 1172-1189.

Heath AG (1995). Water pollution and fish physiology. CRC Press, Boca Raton, FL.

Hilmy M, Lemke A, Jaicb P (1979). Haematological changes in Kuwait mullet, liza maeralepis (smith induces by heavy metals). Ind. Mar. Sci. 8: 278-281.

Jezierska B, Ługowska K, Witeska M (2009). The effects of heavy metals on embryonic development of fish (a review). Fish Physiol. Biochem. 35: 625-640.

Kjoss VA, Grosell M, Wood CM (2005). The Influence of Dietary Na on Cu Accumulation in Juvenile Rainbow Trout Exposed to Combined Dietary and Waterborne Cu in Soft Water. Arch. Environ. Contam. Toxicol. 49: 520-527.

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

McKim J, Christensen G, Hunt E (1970). Changes in the blood of brook trout salvelinus after short term and long term exposure to copper. Fish Des. Board Can. 27: 1883-1889.

Obasohan EE, Oronsaye JAO, Eguavoen OI (2008). A Comparative Assessment of the Heavy Metal Loads in the Tissues of a Common Catfish (*Clarias gariepinus*) From Ikpoba and Ogba Rivers in Benin City. Nigeria. Afr. Sci. 9(1): 13-23.

Pickering QH, Henderson P (1966). The acute toxicity of some heavy metals to different species of warm water fishes. Int. J. Air Water Pol. 10: 453-463.

Smith GL, Hatting J (1980). Haematological assessment of generally used fresh water fish blood anticoagulant. J. Fish Biol. 17: 330-341.

Sorensen EM (1991). Metal poisoning in fish. CRC Press, Boca Raton, USA. p. 243.

Taylor D, Maddock BG, Mance G (1985). The acute toxicity of nine "grey list" metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: dab (*Limanda limanda*) and grey mullet (*Chelon labrosus*). Aquat. Toxicol. 7: 135-144.

Turan D, Kottelat M, Engin S (2009). Two new species of trouts, resident and migratory, sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae), Ichthyol. Explor. Freshwaters, 20(4): 333-364.

Vosyliene MZ (1999). The effect of heavy metals on haematological indices of fish (survey). Acta Zool. Lit. 9: 76-82.

Wepener W, Vuren van JHJ, Preez du HH (2001). Uptake and distribution of a copper, iron and zinc mixture in gill, live rand plasma of a freshwater teleost, *Tilapia sparrmanii*. Water SA. 27: 99-108.

Zaki MS, Moustafa S, Rashad H, Sharaf N (2008). Assessment of the Hazardous Effect of Lead Pollution on *Oreochromis niloticus*, Including Hematological, Biochemical and Immunological Parameters. Am. Euras. J. Agric. Environ. Sci. 3(1): 91-95.

Zeni C, Bovolenta MR, Stagni A (2002). Occurrence of echinocytosis in circulating RBC of black bullhead, Ictalurus melas (Rafinesque), following exposure to an anionic detergent at sublethal concentrations. Aquat. Toxicol. 57: 217-224.