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Araştırma Makalesi
Research Article

The Effects of Different Doses of Copper Sulphate Pentahydrate ($CuSO_4.5H_2O$) on Critical Swimming Speed and Haematology Parameters of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In this study were researched the effects of the two different dosage application of copper sulphate pentahydrate ($CuSO_4.5H_2O$) pesticide and rainbow trout (*Oncorhynchus mykiss*) was used as the material. As a result of the application period, control and application groups critical swimming speed and blood parameters were investigated. In the rainbow trout, exposed to different doses of the copper sulphate penta hydrate ($CuSO_4.5H_2O$) pesticide, critical swimming speed was investigated and the effects was found to be significant ($p<0.05$). Average critical swimming speed values was found to be 3.550±1.62 bl/sec in the control group, 3.746±2.03 bl/sec in the group applied 0.175 mg/lt and 5.060±0.34 bl/sec in the group applied 0.350 mg/lt. From the hematologic parameters, sedimentation (ESR), erythrocyte (RBC), leukocyte (WBC) and thrombocyte (Plt) values treatment x day interaction were not effected by $CuSO_4.5H_2O$ ($p>0.05$). The hemoglobin value was significantly effected by treatment x day interaction ($p<0.01$). While the effect of treatment and treatment x day interaction of erythrocyte sedimentation rate and erythrocyte number were not significant, the effect of day was found to significant ($p<0.05$). The number of white blood cell and thrombocyte treatment, day and treatment x day interaction effect was found to be insignificant ($p> 0.05$).

***Keywords*:** Copper sulphate pentahydrate ($CuSO_4.5H_2O$), rainbow trout, hematology,critical swimming speed.

Öz

Farklı Dozlarda Uygulanan Bakır Sülfat Pentahidrat'ın Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'in Kritik Yüzme Hızı ve Hematoloji Parametreleri Üzerine Etkilerinin Araştırılması

Bakır Sülfat Pentahidrat (Cu $SO_4.5H_2O$)'ın 2 farklı doz (0,175 mg/lt ve 0,350 mg/lt) uygulamasının 0, 14, 28. günetkilerinin araştırıldığı çalışmada, materyal olarak gökkuşağı alabalığı (Oncorhynchus mykiss) kullanılmıştır. Uygulama periyodu sonucunda kontrol ve uygulama gruplarında kritik yüzme hızı vekan parametreleri indeksleri incelenmiştir. Bakır sülfat pentahidrat'ın iki farklı dozuna maruz bırakılan gökkuşağı alabalığının kritik yüzme hızı incelenmiştir ve etkisi önemli bulunmuştur ($P<0,05$). Söz konusu parametre ile ilgili ortalama değerler kontrol grubu (K) için 3,550±1,62 bl/sn; 0,175 mg/lt uygulama yapılan grup (D1) 3,746±2,03 bl/sn ve 0,350 mg/lt uygulama yapılan grup (D2) için 5,060±0,34 bl/sn olarak tespit edilmiştir. Hematolojik parametrelerden sedimentasyon, eritrosit, lökosit ve trombosit sayısı değerlerinde muamele gün etkileşimi istatistik açıdan önemsiz bulunmuştur ($p>0,05$). Hemoglobin değerinin ise muamele x gün interaksiyonundan çok önemli ($p<0,01$) derecede etkilendiği belirlenmiştir. Eritrosit sedimentasyon oranı ve eritrosit sayısı kontrol grubuna göre muamele ve muamelexgün interaksiyonu önemsiz olurken gün ($p<0,05$) önemli bulunmuştur. Lökosit ve Trombosit sayısına ise muamele, gün ve muamele x tür interaksiyonunun etkisi önemsiz olarak tespit edilmiştir ($p>0,05$).

Anahtar Kelimeler: Bakır Sülfat Pentahidrat ($CuSO_4.5H_2O$), gökkuşağı alabalığı, hematoloji, yüzme performansı.

Introduction

Environmental pollution is a growing problem as a result of the increasing use of industrial, agricultural and commercial chemicals. There are millions of chemicals that pose danger to the environment and health. These chemicals, either natural or synthetic, are known to have toxic effects on both humans and other organisms. The most widely used among these chemicals, pesticides are of significant importance to the environment and health. Alongside with their persistence in the environment, pesticides are mutagenic, teratogenous and, more importantly, cancerogenic and their widespread use negatively affect the environment and organisms (Siemering *et al.,* 2005).

As an aquatic herbicide, copper is commercially associatedwith a chelator to prevent rapid loss (i.e., precipitation and / orcomplexation) of copper from the water and maintain thetoxicity of copper (in solution) over time (Elder and Horne,1978). Copper sulfate pentahydrate are aquatic pesticides and used in the control of wild aquatic plants, invertebrates and algae in irrigation and sewer systems, lakes and ponds and drainage channels (Davison, 1995).

Knowing the biological condition of the system is important for the sustainability of the aquatic ecosystem. Due to their place in the food chain and their importance as nutritional sources, fish are used as indicators of the pollution in aquatic systems. Thus, it is important for the future of the ecosystem to determine the possible physiological and biochemical effects of both trace and toxic pollutants on fish. Behaviour is an organismal level of all the above mentioned parameters including bio-chemical, physiological state of the animal under the influence of the environment. Fish usually respond to the effect of pollutants by changing their behaviors as well as showing metabolic and physiological reactions(Zarei *et al.,* 2013).

The swimming activity comprises various physiological processes and systems; therefore, the calculation of the swimming performance requires a specially created and controlled environment. The swimming performance is a sensitive index used in the determination of the health and stress condition of fish and varies depending on various factors such as fish species, fish size, their habitat and lifestyle, temperature, water parameters (e.g. salinity), water pollution, the current velocity of the waters, and dietary patterns and energy status of fish (Beaumont *et al.,* 1995).

Critical swimming speed, which is determined with the swimming performance assessment system, is one of the most important criteria in determination of the effects of pollutant toxicity on fish (Hammer,1995).

The studies have shown that exposure to the sublethal doses of organic and inorganic pollutants negatively affected the swimming performance of fish(Beamish, 1978; Nikl and Farrell 1993; Heath, 1995;Baltz et al., 2005; McKenzie et al., 2007).

As well as behavioral changes, determination of hematology parameters can also be used in the determination of the health condition of fish. Hematology is an indicator of the health condition of fish and fish blood is an effective indicator in the determination of the changes in the functional expression of the organisms. There is a growing interest in the hematological parameters and the structural properties of fish blood cells are regarded as important parameters in aquaculture. The simultaneous evaluation of the effects of environmental pollutants

on both the swimming performance and hematology indexes enables to determine the physiological condition of the organism from different aspects (Thangam *et al.*, 2016).

The investigation on the behavioral changes and hematologic parameters of fish species that hold an economical value has an important role in both reducing the disease rate and increasing the productivity of aquaculture and determining the metabolic and physiological condition of the organism under natural conditions and under the effect of various environmental factors. In the study to investigate the effect of two different doses (1.5 mg/l and 2.25 mg/l) of copper sulfate pentahydrate ($CuSO_4.5H_2O$) at the 0^{th}, 14^{th} and 28^{th} day, rainbow trout (*Oncorhynchus mykiss*) were used as the living material. The swimming performance and blood parameters were examined during the application period.

Materials and Methods

Experimental Design; The experiments were carried out in the Toxicology Experiment Unit in the Aquarium Fish Research and Application Center and the Laboratories of the Fisheries Faculty. The water temperature was 11.5±2.5°C during the research. The water quality parameters are given in Table 1.

Fiberglass tanks with a diameter of 1 m and a depth of 1 m and constructed with an inclined-tube system were used in the study (Atamanalp, 2000; Çiltaş, 2000). Tanks were culvert with draining pipes two times a day to remove feed and feces wastes. The rainbow trout (*O. mykiss*) weighing 165±25 g that were not exposed to infection or toxicity prior to the research and were obtained from the Inland Water Fish Research and Application Center of the Fisheries Faculty were taken into the experiment tanks.

Two of the tanks were determined to be the control tanks, while four of the tanks were the treatment tanks (Kocabatmaz and Ekingen, 1984; Atamanalp, 2000). The copper sulfate pentahydrate was obtained in Sigma. After 14 days of acclimation period, fish were exposed to the two different doses of the pesticide for 28 days.The application doses were received respectively (0.175 mg/L) (D1)and the dose (0.350 mg/L) (D2). Experiments were take in accordance with the procedure for experiments with renewed (Ünsal, 1998), the pesticide was

Table 1. Water quality parameters

	Control			**D1**			**D2**		
	0. day	14.day	28.day	0. day	14.day	28.day	0. day	14.day	28.day
Dissolved Oxygen (mg/L)	10.72	9.45	10.42	10.18	4.87	11.38	11.05	5.6	10.03
pH	8.94	8.02	8.95	8.79	7.97	8.92	9.05	7.23	8.99
Ca (mg/L)	38.4	39.2	35.2	36	37.2	34.8	31.6	36	37.6
Total Hardness(°FS)	15.5	18.5	13.5	13.5	14.5	15.5	14.7	15.5	16.3
Mg (mg/L)	14.337	21.141	11.421	10.935	12.636	16.524	16.524	15.795	17.01
Cu (mg/L)	6.4	2.944	3.84	3.265	5.312	6.432	4.032	3.328	8

fed to the tanks with pre-determined water volume once every 12-hours in concentrations which corresponds to this dose. Causing stress and damage to the fish was carefully avoided during all of the procedures from medium renewal to feeding and siphoning (Atamanalp, 2003).

Swimming performance procedure; Swimming performance was measured for all groups as critical swimming speed (U_{crit}) using 1200 swimming performance system of Akuamaks Company, Ankara, Turkey. The acrylic system was consisted of a flat bottomed tank of 36 cm depth with rounded edges with a perimeter of 14.65 m and flat sides. The flat sides were 400 cm in length and attached with four acrylic pedals connected to an engine set on one side and a swimming tunnel on the other side. A modified Brett-type cylindrical swimming tunnel (Brett, 1964) (100 cm length and 40 cm diameter) with semi-oval shaped transparent top (80 cm×30 cm) permitted to follow the movements of the fish (Figure 1).

The temperature of water in the swimming chamber was maintained at 10.0±0.5 °C and oxygen level at 13.0±0.5 ppm. The U_{crit} was calculated for each fish using the following equation (Brett, 1964):

$$\mathbf{U_{crit} = U_i + (T_i / T_{ii}) U_{ii}}$$

where, U_f (cm/s) is the highest speed at which the fish swam for the full time period and U_i (proportionally to the body length) is the water velocity increment. T_i (30 min) denotes the prescribed period of swimming at a given speed and T_{ii} (min) is the time duration for which the fish swam at the final speed. All absolute U_{crit} (cm/s) values were standardised for size by dividing the total length of the fish to obtain a value in body lengths per second, which denotes relative U_{crit} (BL/s) (Beaumont *et al.*, 1995).

Haematology analyzes; In accordance with the cyanmet hemoglobin method, 0.02 mL of blood sample was mixed with 5 mL of Drabkin's solution and the mixture was slowy

Figure 1. Swimming performance system.

turned upside down to obtain a homogenous solution. The mixture was rested for 10 minutes to obtain full hemoglobin conversion to cyanmet hemoglobin; then, the sediment at the bottom was removed with the help of toothpicks. The transmittance value (%T) was measured with a spectrophotometer at 540 nm and the hemoglobin amounts corresponding to the measured values were determined from the table and recorded in g/100 m^3units (Çiltaş, 2000; Uçar and Atamanalp, 2010).

Eritrosit sedimentation rate; To determine the erythrocyte settling rate, anticoagulant-containing blood samples were taken into hematocrit tubes with a 1.1-1.2 mm diameter and 7 cm length and after resting one hour in the vertical position (90°), the separated serum was measured with graph papers or rulers. The results were obtained in mm/h (Blaxhall and Daisley, 1973; Kocabatmaz and Ekingen, 1984; Atamanalp,2003).

Determination of the erythrocyte count; Blood sample was drawn up to the 0.5 line with an erythrocyte pipette and completed to the 101 line with the Dacie's solution and diluted to 1/200 with water. The thoroughly mixture was allowed to stain for 1-2 minutes. The first non-homogenized 4-5 drops were poured with a pipette and the rest were filled to the Thoma slide counting chamber. The samples were counted on the Thoma slide with a microscope for 1/5 mm^2 and the values were calculated in $10^6/mm^3$ (Blaxhall and Daisley, 1973; Atamanalp, 2000).

Determination of the leucocyte count; Following the application of the same method used in erythrocyte count, leukocytes were counted for 4 mm^2; if the amount was deemed insufficient, leukocytes were counted for 9 mm^3. The results were calculated in $10^3/mm^3$ (Blaxhall and Daisley, 1973).

Determination of the thrombocyte count; All squares were counted with the same method used in the determination of the erythrocyte count and the results were calculated in $10^3/mm^3$(Kocabatmaz and Ekingen, 1984; Satake *et al.*, 1986; Reddy and Bashamohideen, 1989).

Results

Critical swimming speed; The behavioural changes are the manifestation of motivational, biochemical, physiological and environmentally influenced state of the organism (Murthy, 1987).

For the rainbow trout exposed to the two different doses of copper sulfate pentahydrate, the critical swimming speed differences between the groups were significant ($p<0.05$). The average values for the C, D1 and D2 groups were 3.550±1.62 bl/s, 3.74±2.03 bl/s and 5.06±0.34 bl/s, respectively (Table 2). Figure 2 shows the linear regression equations for the relationship between critical swimming speed and length after different dose applications.

Table2. The effect of the different doses of copper sulfate pentahydrate on the critical swimming speed of *Oncorhynchus mykiss*

Group	Ucrit (bl/s)
C (Control)	3.550±1.62^b
D1 (Dose 1: 0.175 mg/lt)	3.746±2.03^b
D2 (Dose 2: 0.350 mg7lt)	5.060±0.34^a

*Significant ($p<0.05$), **Very significant ($p<0.01$)

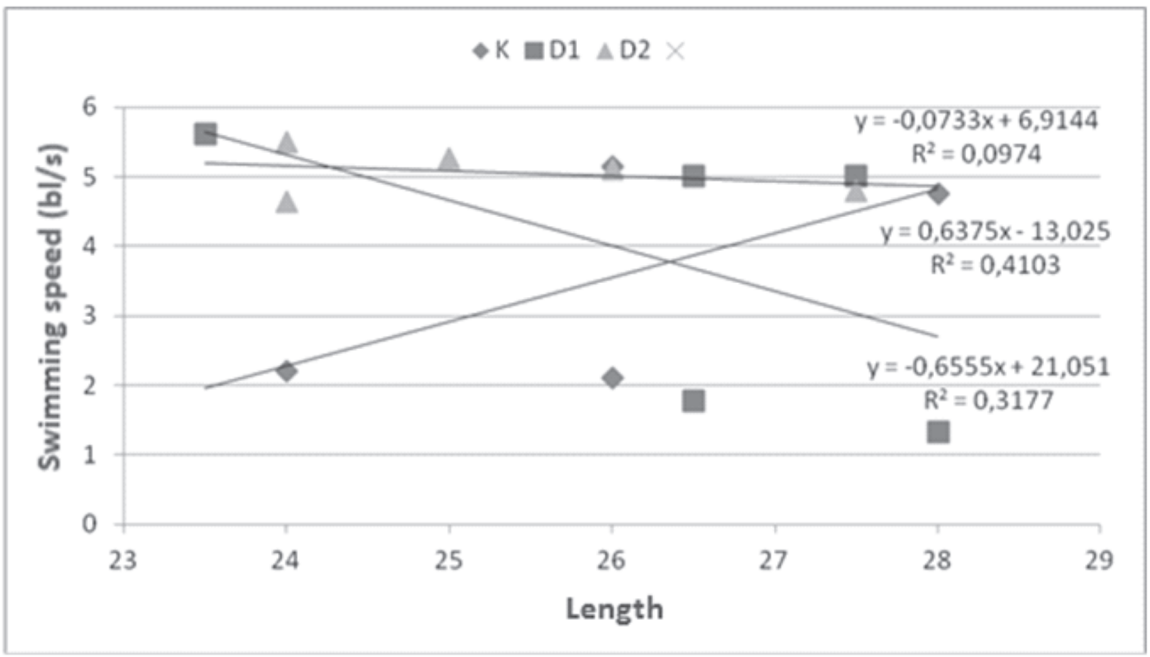

Figure 2. CSS data points, linear regression lines and equations for *O. mykiss* fish exposed to the different doses of copper sulfate pentahydrate.

Hematological Parameters; In the determination of the physiological changes due to stress in fish, hematological parameters are commonly used as indicators in measuring the physiological and biochemical changes resulting from a stress factor (Stoskopf, 1993; Cataldi *et al.,* 1998; Adeyemo *et al.,* 2003).

Discussion

Aquatic organisms initially respond to the stress factors in their habitat by showing behavioral anomalies. These reactions are the decrease in the swimming performance, the increase in operculum movements and the decrease in food conversion capacity. In this study, the application of the different doses of copper sulfate pentahydrate to rainbow trout caused similar behaviors (Khunyakari *et al.*, 2001; Levesque *et al.,* 2002).

This behavioral reaction of rainbow trout to copper sulfate pentahydrate can be attributed to the direct effect of the pollutant on the nervous system and the consequent slowing of the spontaneous muscle movements; along with the prevention of metal uptake, meeting the energy need for vital activities from the stock reserves instead of nutrients; the increase in the oxygen requirement due to the pollutant-induced stress conditions (Heat, 1995).

In the study, the swimming performance of rainbow trout significantly changed as a result of the effect of the pollutant. The effect of copper on the nervous system deteriorates the muscle coordination which in turn leads to slower swimming. This difference can be attributed to the dose and treatment duration. Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity and entails increased expenditure of energy (Murthy, 1987).

Peterson (1974), reported that critical swimming speed of *Salvelinus fontinalis* fish at 15°C'de was in the 4.63BL/s-4.86 BL/s range, whereas Brett and Glosal (1973) reported that

the critical swimming speed of Sockeye *salmon* (*Oncorhynchus nerka*) fish that have a 9-16 cm of body length and were kept at 10-15°C wasin the 3.3-4.4 BL/srange. Comparing to the other studies, this change may be attributed to the differences in body shape, gill shape, muscle function, swimming mode and fish size. Moreover, pH, oxygen, photoperiod, temperature, salinity and other pollutants were also observed to have an effect on swimming performance.

Pollutants reduce the swimming ability of fish and cause changes in its mechanism. As a result of the harmful effect of acute exposure to different substances on gills, respiration is hindered and oxygen uptake and, thus, the critical swimming speed decrease. In the case of chronic exposure, more metabolic activity is required to overcome detoxification and harm. Chronic exposure can lead to changes in nervous functions. The carbohydrate stock of pollutant-exposed fish will be emptied and, thus, the maximum swimming speed will substantially decrease.

Substances that affect the nervous system will not only affect natural activity but also affect all the energy metabolism including the critical swimming speed of the body (Heat, 1995; Esenbuğa, 2013).

Table 3. Mean values ± SD of Hematological parameters in rainbow trout

Parameters/Group	Days	Control	D1	D2
RBC (10^6/mm^3)	0 ^{a}	0.73±0.00 ^{a}	0.76±0.00 ^{a}	0.78±0.07 ^{a}
	14 ^{a}	0.84±0.55 ^{a}	1.98±0.09 ^{a}	1.19±0.85 ^{a}
	28 ^{a}	2.42±2.07 ^{a}	1.45±0.47 ^{a}	2.34±1.34 ^{a}
WBC (10^4/mm^3)	0 ^{a}	3.60±0.14^{a}	2.90±0.00 ^{a}	3.25±0.35 ^{a}
	14^{b}	1.39±0.74 ^{a}	2.14±0.22 ^{a}	1.35±0.77 ^{a}
	28^{ab}	1.11±0.38 ^{a}	2.25±0.71 ^{a}	3.93±2.04 ^{a}
PLT (10^4/mm^3)	0 ^{a}	1.35±0.07^{b}	1.30±0.00^{ab}	1.75±0.35 ^{a}
	14 ^{a}	0.86±0.48^{b}	1.38±0.18^{ab}	1.05±0.77 ^{a}
	28 ^{a}	0.78±0.25^{b}	1.69±0.69^{ab}	3.96±3.00 ^{a}
Hb (g/dl)	0^{a}	6.85±0.07^{a}	6.20±0.00^{b}	6.60±0.14^{c}
	14^{b}	4.50±0.70^{a}	4.80±0.14^{b}	3.00±0.28^{c}
	28^{b}	7.13±0.40^{a}	4.70±0.40^{b}	3.40±1.38^{c}
ESR (mm/h)	0 ^{a}	2.20±0.42^{a}	2.30±0.00 ^{a}	2.15±0.35 ^{a}
	14^{b}	0.17±0.15 ^{a}	0.70±0.47 ^{a}	0.42±0.40 ^{a}
	28^{b}	0.16±0.08 ^{a}	0.54±0.12 ^{a}	1.28±2.36 ^{a}

All data points are the average of n = 5 ± SD, Different superscript letters indicate statistically significant differences (P < 0.05)

The hematological parameters of fish including hemoglobin and hematocrit levels, erythrocyte and leukocyte counts and erythrocyte morphology change depending on the pollutant concentration in the environment, exposure time, and the physiochemical properties of water as well as the species, the development stage of the species, the reproductive cycle of the mature individuals and disease status (Levesque *et al.,* 2002; Şahin, 2009).

After the $CuSO_4.5H_2O$ application, hemoglobin values at the 0^{th}, 14^{th}, and 28^{th} day were lower than those of the control groups (Table 3). The statistical analyses showed that the treatment and the treatment x day interaction were significant ($p<0.05$), whereas day was very significant ($p<0.01$).

According to Christensen *et al.,*(1972) reported that the hemoglobin levels of *I. nebulosus* increased with short-term copper exposure. The researches attributed this initial increase to the catalyst effect of copper on iron binding to hemoglobin. The hemoglobin concentrations in fish that were exposed to 3 mg/L copper for 96 hours increased (Mishra and Srivatava, 1983). Similarly, the hemoglobin concentrations of *Indian catfish* increased with a 0.25 mg/L of copper concentration (Singh and Reidy, 1990). The Hct percentage, Hb value and RBC count of *Pseudopleuronectes americanus* and *Morone saxatilis* fish that were exposed to 10 µgL-1 of Cu decreased by 18-48% compared to those of the control groups (Calabrese *et al.,*1975; Dawson, 1979). The initial increase in the hemoglobin levels under copper exposure was attributed to the increase in red blood cells resulting from the catalyst effect of copper on iron-hemoglobin binding.

Fish were considered to be limited to supply sufficient amounts of oxygen to the tissues and consequently, their physical activity had decreased. The significant decrease in hemoglobin concentration was either due to the progressive decrease in hemoglobin or due to the decrease in hemoglobin synthesis. The decrease in blood cells and hemoglobin showed that the pollutant had a hemotoxic effect on fish. It was reported that the low levels of hemoglobin, which are indicators of the anemic condition of fish, were due to the hemolysis-induced stress and aerobic glycolysis limitation-interrupted hemoglobin synthesis. Low hemoglobin levels are the indicators of a deteriorating iron synthesis mechanism (Reddy and Bashanihideen, 1989).

After the $CuSO_4.5H_2O$ application, in comparison to the control group, changes in the sedimentation values were observed at the 0^{th}, 14^{th} and 28^{th} days; treatment and treatment x day interaction were not statistically significant, whereas day was significant ($p<0.05$).

The erythrocyte count is an important parameter to determine the oxygen-carrying capacity of blood as well as the functions of erythropoietic tissues (Witeska, 2005). Similarly, copper exposure increased the erythrocyte count in *Cyprinus carpio* and *Oreochromis mosambicus* (Cyriac *et al.,* 1989; Caldwell and Hinshaw, 1995; Houston *et al.,*1996). The sudden increase in the erythrocyte count can be attributed to the catecholamine-induced spleen contraction due to stress and new erythrocyte release into the circulation

It was determined that0.175 mg/L and 0.350 mg/L copper sulfate pentahydrate concentrations decreased the erythrocyte count in *O. mykiss* whereas the erythrocyte count reached the highest level at the 0^{th} day in D1 group.

The increase in the erythrocyte count can be attributed either to the deformations in the gill structure and the increase in the oxygen requirement of tissues as a result of the hypoxic

Conditions arising out of mucus-covered gill or to the stimulant effect of copper on erythrocyte formation in hematopoietic tissues (Wells and Weber, 1990).

Copper concentration and exposure time were determined to cause changes in the erythrocyte cells of fish. Acute tests showed that the copper concentrations close to the LC50 value caused increases in the erythrocyte count and hemoglobin and hematocrit values of carp and rainbow trout (Svobodova *et al.,* 1994). It was reported that, although the 0.125 mg/L of copper concentration was effective on rainbow trout, no changes was observed in the erythrocyte count during the 96-hours-long acute test period, while the 0.5 mg/L of copper concentration increased the erythrocyte count in various species (Vosylienë, 1996).

Çelik (2006), reported that hematological parameters showed different levels of sensitivity to different environmental factors and chemicals, and the decrease in the hematocrit percentage and RBC count was a result of progressive anemia and the deteriorating health condition of the organism. Fish are highly sensitive to the pollutant-induced stress and hematological parameters.

For the leucocyte values, although there were changes resulting from the different concentration and days, none of these changes were statistically significant. Thus, the different doses of copper sulfate did not have a substantial effect on the immune system of the fish during the 28- day-long experiment. Dhanapakiam and Ramasamy (2001) examined the toxic effects of copper and zinc mixture on some hematological and biochemical parameters of carp (*Cyprinus carpio*). At the end of a 30-day-long application, a significant decrease in the hemoglobin and erythrocyte count and a significant increase in the leukocyte count were observed. In the study that involved acute test applications, a significant decrease in the WBC count (especially in small fish) of the *O. mykiss* and *C. carpio* fish that were exposed to Cu concentrations close to the lethal dose were observed (Svobodova *et al.,*1994). In an acute experiment study, a significant decrease was observedin the WBC concentration of the *O. mykiss* that were exposed to a sublethal Cu concentration(0.301 mgl-1) (Dick and Dixon, 1985). In the study, although the changes in the leukocyte count were determined, they were not statistically significant. This difference was attributed to the different effects of pollutants on organisms.

The number of leucocyte cells is affected by physiological and environmental factors. The most common response of fish that were exposed to toxic substances is the decrease in lymphocyte percentage and the increase in heterophiles (Witeska, 2005).The suppression of both the activation and immune systems of fish, due to substantial variety between the time of exposure to pollutants, the dose of the toxic substance and the fish species, can be attributed to the aquatic contaminants (Cuesta *et al.,* 2011).

In comparison to the control group, the thrombocyte count decreased in the D1 group at the 14^{th} and 28^{th} day, whereas it increased in the D2 group. These changes among the groups were statistically significant ($p<0.05$).Under the stress conditions, the blood clotting system of fish becomes more active, and therefore thrombocyte count can markedly increase (Casillas and Smith, 1977).

The most recognized physiological role of thrombocytes /platelets is to initiate blood clotting in the process of hemostasis (Engelmann and Massberg, 2012). In fish, thrombocyte cells form protection walls, have phagocytic abilities and participate in the defense mechanism. These cells represent the connection between innate and acquired immunity and express intracellular and extracellular molecules that also involve immune functions (Tavares-Dıas *et al.,* 1999).

Thrombocytes play an important role in blood coagulation. Their clinical importance is yet to be discovered. In teleost fish, coagulation occurs in 5 minutes. In stress conditions, the blood coagulation system becomes more active and thus, can lead to an increase in the thrombocyte count. Thrombocytopenia can have a negative effect on fish because these cells are not only responsible for blood coagulation but also have a role in the control of blood flow from superficial wounds. The high levels of glucocorticoids can decrease the thrombocyte count and prolong coagulation (Campbell and Ellis, 2007).

Atamanalp (2000) reported that thrombocyte values were significantly affected by stress and the thrombocyte value of rainbow trout was $2.1x10^4/mm^3$prior to stress and reached $4.3x10^4/mm^3$ after stress. According to Satake *et al.* (1986) this value was 1.657 ± 0. 341 $x10^4/mm^3$ in armored catfish (*Hypostomus pulinus*). The thrombocytosis formation can be attributed tothe increase in the need for thrombocyte cells that play a role in blood coagulation due to disease-induced hemorrhage.

Haematology indexes can be viewed as a secondary response of organisms to pollutants and corrosive substances. Exposure to the low concentrations of heavy metals usually increases these hematological parameters. Chemicals cause all of the initial reflexes at the beginning of the reactions of fish to stress.

The stress reaction of fish causes osmotic imbalance and changes in the ionic change-regulating systems (the decrease in the blood pH, the increase in the erythrocyte volume and the subsequent increase in the hematocrit percentage). The stress-induced adrenalin causes spleen contraction and erythrocyte release from the spleen into the blood (Vosylienė, 1996).

Aquatic environments are the main receivers of domestic, industrial and agricultural wastes and therefore, aquatic organisms are directly affected by pollutants. Organic and inorganic pollutants cause accumulation in the tissues and organs of fish, mortality above their certain concentrations, stressand decrease in the number of eggs as well as changes in metabolic and physiological activities. Thus, the determination of the critical swimming speed as a behavioral change in aquatic organisms under the effects of pollutants and the determination of changes in metabolic and physiological activities and especially the changes in hematological parameters are of great importance in evaluating the pollution level of the environment.

Copper is a naturally-occurring compound in aquatic environments as a result of soil drainage and its concentration in the environment increases as a result of anthropogenic factors such as its use in the prevention of the decomposition of materials used in water and harmful organism growth on these materials and in the industry as food and raw silk preserver. The over-accumulation of copper in fish affects the development, ion balance, hematological parameters, protein levels, tissue permeability, membrane integrity and the endocrine system. Thus, copper sulfate, which is frequently used in aquaculture protection, treatment and disinfection, should be consciously used in facilities that necessitate the use of formaldehyde.

In the study, the application of the different doses of copper sulfate pentahydrate and application duration caused changes in the measured parameters. However, further studies should be carried out with different indicators, fish species and doses to better understand the toxicity metabolism of the same chemical.

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