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**EFFECTS OF CHROMIUM ON THE HEMATOCRIT LEVELS AND ERYTHROCYTE NUMBERS
OF *CYPRINUS CARPIO***

ABSTRACT

Changes in hematocrit levels and erythrocyte numbers of *Cyprinus carpio* were determined after exposing the animals to 0.5, 1.0 and 2.0 ppm chromium over 7, 15 and 30 days. Microhematocrit and hemocytometric methods were used for determining hematocrit levels and erythrocyte numbers respectively. No mortality was observed during the experiments. Hematocrit levels and erythrocyte numbers increased under the effect of metal on day 15. Hematocrit levels and erythrocyte numbers decreased by increasing exposure periods except at 0.5 ppm chromium.

Keywords: *Cyprinus carpio*, Chromium, Hematology, Hematocrit, Erythrocyte Number

***CYPRINUS CARPIO*'DA KROMUN HEMATOKRİT DÜZEYİ İLE ERİTROSİT SAYISI
ÜZERİNE ETKİLERİ**

ÖZET

Araştırmada bir tatlı su balığı olan *Cyprinus carpio*'da kromun 0.5, 1.0 ve 2.0 ppm'lik ortam derişimlerinin 7, 15 ve 30 gün sürelerle hematokrit düzeyi ve eritrosit sayısı üzerine etkilerinin belirlenmesi amaçlanmıştır. Hematokrit düzeyi ile eritrosit sayısının belirlenmesinde sırasıyla mikrohematokrit ve hemositometrik yöntemler kullanılmıştır. Kromun belirlenen süre ve derişimlerdeki etkisi balıklarda mortaliteye neden olmazken, 15. günde hematokrit düzeyi ile eritrosit sayısını derişime bağılı olarak arttırmış, 0.5 ppm dışında belirli bir derişimde etkide kalma süresindeki artış ise 7. güne oranla hematokrit düzeyi ile eritrosit sayısını azaltmıştır.

Anahtar Kelimeler: *Cyprinus carpio*, Krom, Hematoloji, Hematokrit, Eritrosit Sayısı

1. INTRODUCTION (GİRİŞ)

Anthropogenic factors, such as vast increase in human population, industrial development, acid rains caused by the usage of fossil fuels, global warming as a result of sera gases, enhanced usage of pesticides to increase agricultural production and sea accidents together with natural disasters, rapidly increases the circulation of heavy metals in nature.

Chromium is needed at low levels by the organisms to sustain their biological functions. While the lack of chromium causes metabolic and physiologic disturbances at structural and functional levels, the long term effect in excess amounts results in mortality [1].

Pure form of chromium is not present in nature, but its valance differs between -2 and +6 due to its readily reductive and oxidative capacity [2 and 3]. Among these chemical forms Cr (III) and the more toxic Cr (VI) are biologically active [4 and 5].

Chromium is widely used in metallurgy and chemistry industries such as metal and electrode plating, leather tanning, textile, phosphate fertilizers, stainless steel, ferrochromium and pigment production [5]. Disposal of chromium from these sources increase its concentration in nature which results changes in physiologic and biochemical disturbances due to tissue accumulation.

Studies carried out with different fish species showed that Cr (VI) at elevated concentrations causes inhibition at enzyme activity, changes in blood parameters, reduction of resistance against pathogenic organisms, changes in behavioral patterns, feeding disturbances and histopathologic changes in tissues [6, 7 8, 9, 10, 11, 12, 13 ve 14].

Hematological parameters such as hemoglobin and hematocrit levels and erythrocyte numbers in fish can be used to determine the physiological state of the organisms since they reflect the oxygen carrying capacity of the blood and as in hot blooded animals, they change very rapidly under the effect of injury, infection and pollutants [15,16].

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

Study of hematological parameters in fish is important in husbandry to increase the productivity, decrease diseases, and making transfers with minimum loss while in nature it is important to determine the physiological state of the organism under various environmental stress factors. Hence in the present study it was aimed to determine hematocrit levels and erythrocyte numbers of *C. carpio* exposed to 0.5, 1.0 and 2.0 ppm Cr (VI) over 7, 15 and 30 days.

3. EXPERIMENTAL METHOD (DENEYSSEL YÖNTEM)

C. carpio with the mean length 10,82±0,93 cm and mean weight 15,75 ± 3,85 g were used as study material. Fish were obtained from the cultivation pools of Çukurova University, Aquaculture Faculty, Adana. Experiments were conducted in research laboratory of MEÜ, Aquaculture Faculty, Basic Sciences Department under controlled conditions (24±0,1°C; 12 hour light/dark illumination regime).

Fish were adapted to laboratory conditions in ten glass aquaria 40x120x40 cm in size for 15 days.

Taking the concentrations into consideration, four glass aquaria were used in the experiments. 120 liters of the three mentioned chromium solutions were added into the first three aquaria and the same amount of chromium free tap water was added into the fourth one and used as control. Experiments were run in triplicate and 9 fish were placed in each aquarium.



Some physical and chemical properties of the experimental media were given in Table 1.

Table 1. Physical and chemical properties of the experimental media
 (Table 1. Deneysel medyanın fiziksel ve kimyasal özellikleri)

Temperature (°C)	24 ±1
Total Hardness (ppm CaCO ₃)	246,4 ± 3,28
Total Alkalinity (ppm CaCO ₃)	409 ± 0,63
pH	8,02 ± 0,06
Dissolved oxygen mg/L	7,42 ± 0,47

Aqueous solution of K₂Cr₂O₇ was used in the preparation of metal solutions. Tri sodium citrate was also added during the preparation of K₂Cr₂O₇ in order to prevent the precipitation of metal.

Fish were fed once a day with readymade fish feed at 2% amounts of total biomass. Central aeration was applied to all experimental tanks.

Time dependant changes in metal concentrations does occur due to evaporation, precipitation and absorption and hence experimental solutions were changed once in two days by serial dilution of the freshly prepared stock solution. Since the studied parameters show stress related changes, fish removed from aquaria at the end of each experimental period were anesthetized with Ethylene Glycol Mono Phenil Ether (=Phenoxyethanol, C₈H₁₀O₂; Merck). Metal residues on their body were washed by tap water, dried and prepared for blood sampling. Blood samples were taken by cutting the caudal peduncle of the fish vertically.

Blood samples to be used for determining erythrocyte numbers were transferred to EDTA (Ethylene Diamin Tetra Acetic Acid) containing tubes and those to be used in hematocrit analyses were directly heparinized capillary pipettes which were then blocked at one end.

Hematocrit pipettes were placed in a microhematocrit centrifuge (Nüve, NT 715/04-3272) and were centrifuged at 10.000 rpm for 5 minutes which divided the blood sample into blood cell and sera phases. The ratio of blood cell to sera in the hematocrit pipette was evaluated in hematocrit scale and the hematocrit levels were expressed as percentages.

Thoma slide was used in determining the erythrocyte numbers. Blood sample was drawn up to scale 1 followed by Dacia liquid up to scale 101 on the hematocrit pipette [17]. After disposing 2 drops of the 1/100 diluted blood sample, the sample was taken onto a Thoma slide and inspected under a light microscope at x40 magnification. Five squares, one from the four corners and one in the middle of Thoma slide, each containing 16 divisions were inspected totaling to 80 small divisions. After counting the erythrocyte numbers in each division, the total erythrocyte number in 1ml blood sample was calculated using the following formula [18,19];

$$\text{Erythrocyte Number} = \frac{\text{Erythrocyte Cell Number} \times \text{Dilution Ratio} \times 100}{\text{Number of Small Squares Counted}} \quad (1)$$

Dacia liquid was prepared by adding 10 ml formaldehyde, 31,3 g trisodium citrate and 1.0 g brilliant blue to a glass flask and the total volume was made to 1 L. by distilled water which was then filtered using a filter paper (Whatman, No:40). This solution was prepared freshly before each analysis and kept in dark glass bottles.



Arcsine transformation was applied to hematocrit level data before statistical analysis. All the experimental data were then analyzed by Student Newman Keul's Procedure (SNK) using SPSS statistical package.

4. FINDINGS AND DISCUSSIONS (BULGULAR VE TARTIŞMALAR)

Although the effect of heavy metals on fish depends on species, metal and environmental conditions the rate of mortality increases rapidly after a given concentration. Increasing concentrations of Cr(VI) increased mortality rate in *Labeo rohita* [20]. No mortality was observed in the present study in which *C. carpio* was exposed to 0.5, 1.0 and 2.0 ppm Cr(VI) over 7, 15 and 30 days which suggested the highest concentration tested was sublethal to this species during 30 days of exposure.

First response of fish to changes in environmental parameters caused by heavy metals is to change their behavioral patterns. Sudden movement, increase in mucus secretion, peeling of scales, change in color and movement towards water surface was observed in *L. rohita* at the beginning of exposure to Cr(VI), which returned to normal as the exposure period increased [20]. Similar observations were made in *C. carpio* exposed to Cr in the present study. These changes in behavior at the beginning of exposure to metals might be explained by stress caused by the metal firstly, and that metabolic and physiologic changes stimulated by detoxification mechanism might explain the later recovery [21].

Blood in fish functions in the exchange of respiration gases, transport of nutrients and wastes in the body and that it forms a target to toxic substances since the blood is in direct contact with the external medium via the gills [22]. Hence blood parameters change rapidly both under the effect of heavy metals and other environmental factors.

Erythrocyte numbers and hematocrit levels not only reflect the oxygen carrying capacity of the blood but also reflect the functioning of erythropoietic tissues [23]. Blood parameters such as hematocrit and hemoglobin levels and erythrocyte and leucocyte numbers change depending on the metal exposed, its concentration and physical and chemical properties of the water. These parameters also change depending on the species, developmental stage, reproduction cycle and disease [24].

Chromium significantly increased hematocrit level compared to control at all the concentrations and exposure periods except 15 day exposure to 0.5 ppm, while it decreased hematocrit level on day 30 compared to day 7 ($P < 0.05$) (Table 2).

Table 2. Effect of Chromium on the Hematocrit Level of *C. carpio*
 (Table 2. C.carpionun hematokrit düzeyine kromun etkisi)

Concentration (ppm Cr ⁺⁶)	Time (Days)		
	7	15	30
	$\bar{X} \pm S\bar{X}$ *	$\bar{X} \pm S\bar{X}$ *	$\bar{X} \pm S\bar{X}$ *
0.0	39,00 ± 1,00 as	39,50 ± 0,50 as	38,00 ± 2,00 as
0.5	48,50 ± 0,50 at	38,00 ± 0,00 bs	48,50 ± 0,50 at
1.0	59,00 ± 2,00 ax	55,50 ± 1,50 at	52,50 ± 0,50 at
2.0	63,50 ± 1,50 ax	56,00 ± 2,00 bt	48,00 ± 0,00 ct

*SNK; Letters a, b and c show differences among exposure times; s, t and x among Cr⁺⁶ concentrations. Data shown with different letters are significantly different at the $P < 0.05$ level.

$\bar{X} \pm S\bar{X}$: Mean ± Standard error.

Copper and zinc in *Mytilus vittatus* [25] and chromium in *C. fasciatus* [26] increased hematocrit levels significantly compared to controls. Chromium also increased hematocrit levels in *C. carpio* at the concentrations tested in the present study. The increase in hematocrit levels under the effect of chromium might be due to hemoconcentration resulted from metal related upset of gill osmoregulation. This increase in hematocrit levels might also be from stimulation of erythropoietic tissues such as kidneys and spleen and hence increasing the discharge of erythrocytes into the circulatory system.

The tested chromium concentrations increased the erythrocyte numbers significantly compared to controls on days 7 and 15 and decreased on day 30 ($P < 0.05$) (Table 3).

Table 3. Effect of Chromium on Erythrocyte Numbers (10^6 Cells/ mm^3)
(Table 3. Kromun eritrosit sayılarına (10^6 Cells/ mm^3) etkisi)

Concentration (ppm Cr^{+6})	Time (Days)					
	7		15		30	
	$\bar{X} \pm S\bar{X}$	*	$\bar{X} \pm S\bar{X}$	*	$\bar{X} \pm S\bar{X}$	*
0.0	2,44 \pm 0,28	as	2,34 \pm 0,50	as	2,64 \pm 0,96	as
0.5	3,56 \pm 0,12	ast	2,68 \pm 0,28	abs	2,00 \pm 0,16	bs
1.0	5,64 \pm 0,20	atx	4,78 \pm 0,00	bt	2,40 \pm 0,15	cs
2.0	7,74 \pm 1,06	ax	5,26 \pm 0,50	bt	0,49 \pm 0,00	ct

*SNK; Letters a, b and c show differences among exposure times; s, t and x among Cr^{+6} concentrations. Data shown with different letters are significantly different at the $P < 0.05$ level.

$\bar{X} \pm S\bar{X}$: Mean \pm Standard error.

Erythrocyte numbers of *C. fasciatus*, exposed to sublethal concentrations of chromium over 90 hours, increased compared to control fish [26], whereas in *L. rohita* exposure to 96 h LC_{50} concentration over 24 and 96 hours decreased the erythrocyte numbers [20]. Short term exposures to chromium increased whereas long term exposures decreased the erythrocyte numbers in *C. carpio*. The reason for the increase in erythrocyte numbers might be the same as explained for hematocrit levels whereas the decrease might result from hemodilution due to disturbances in osmoregulation or osmotic hemolysis resulted from the increase in permeability of erythrocyte membrane.

5. CONCLUSION (SONUÇ)

The results of this study revealed that Cr (VI) cause significant changes in erythrocyte numbers and hematocrit levels of *C. carpio* when the animals were exposed to 0.5, 1.0 and 2.0 ppm concentrations of the metal for 7, 15 and 30 days.

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