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RESEARCH ARTICLE

Changes in Hematological and Biochemical Parameters of European Chub (*Squalius cephalus* L.) in Unpolluted Reservoir and Polluted Creek [1]

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Summary

This study investigates the surface water quality parameters of the Atikhisar Reservoir and Sarıçay Creek, both of which are located in Çanakkale, the north-west part of Turkey. The conductivity, COD, BOD, TP, TN, and TSS content of water in Sarıçay Creek were higher than in Atikhisar Reservoir (P<0.05), however; water DO content in Sarıçay Creek was less than in Atikhisar Reservoir (P<0.05). Chl-a concentration, temperature, salinity and pH of water were not statistically significant in both of the resources (P>0.05). The quality of surface water samples of Atikhisar Reservoir and Sarıçay Creek were accepted first quality and third quality, respectively. The blood samples of European chub (*Squalius cephalus* L.) caught in the Atikhisar Reservoir (unpolluted) and Sarıçay Creek (polluted) provided the data to compare the hemato-chemical effects of pollution. The WBC, MCV, MCH, Ht, Hb, GLC, TP, and GLO levels of fish in Sarıçay Creek were significantly higher than those in Atikhisar Reservoir (P<0.05). The RBC, TBIL, CHOL, AST, and ALT levels of fish in Sarıçay were lower than the ones in Atikhisar Reservoir (P<0.05). There were no statistically significant differences in the MCHC, TG, ALB, Ca, and P values among the fish samples of Atikhisar Reservoir and Sarıçay (P>0.05). In conclusion, the data analyses infer that blood parameters can be used as an indicator of the fish health in general. On the other hand, the blood test can be used to determine fish stress due to water pollution.

Keywords: *European Chub, Pollution, Hematology, Blood, Biochemistry, Metabolite, Electrolyte*

Avrupa Tatlısu Kefali (*Squalius cephalus* L.)'nin Temiz Baraj ve Kirli Çay'daki Hematolojik ve Biyokimyasal Parametrelerindeki Değişimler

Özet

Bu çalışmada, Turkiye'nin batısında yeralan Marmara bölgesi'ndeki Atikhisar barajı ve Sarıçay'ın yüzey suyu kalite kriterleri 2006 Ağustos ayı boyunca incelenmiştir. Sarıçay'ın iletkenlik, COD, BOD, TP, TN ve TSS içeriği Atikhisar barajından yüksek bulunmuş (P<0.05), fakat; Sarçay'ın DO içeriği Atikhisar barajına göre daha düşük tespit edilmiştir (P<0.05). Chl-a konsantrasyonu, sıcaklık, tuzluluk ve pH ise her iki alanda farksız bulunmuştur (P>0.05). Yüzey su kalitesi Atikhisar ve Sarıçay'ın sırasıyla 1. kalite ve 3. kalite olarak kabul edilmiştir. Kirleticilerin hemato-kimyasal etkisinin karşılaştırılması için Atikhisar barajı (temiz) ve Sarıçay (kirli)'dan yakalanan Avrupa tatlısu kefali (*Squalius cephalus* L.)'nden alınan kan örnekleri incelenmiştir. WBC, MCV, MCH, Ht, Hb, GLC, TP ve GLO düzeyleri Sarıçay'dan yakalanan balıklarda Atikhisar barajına göre daha yüksek çıkmıştır (P<0.05). RBC, TBIL, CHOL, AST ve ALT miktarları Sarıçay'da yakalanan balıklarda Atikhisar barajından daha düşük bulunmuştur (P<0.05). Atikhisar barajı ve Sarıçay'dan yakalanan balıkların MCHC, TG, ALB, Ca and P değerleri arasında fark çıkmamıştır (P>0.05). Bu sonuçlar, kan parametrelerinin genel balık sağlığının indikatörü olarak kullanılabileceğini göstermektedir. Ayrıca, balıklarda su kirliliğinden dolayı oluşan stresin tespitinde kan parametreleri kullanılabilir.

Anahtar sözcükler: *Avrupa tatlısu kefali, Kirlilik, Hematoloji, Kan, Biyokimya, Metabolit, Elektrolit*

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INTRODUCTION

In a well-balanced aquatic ecosystem, the quality of water has a primary function between organisms and environment. It is also very important for the health of the ecosystem. Aquatic life is directly affected by ambient environment in rivers and reservoirs that are often contaminated by physical, chemical, biological and radioactive pollutants. For the habitat of fish, environmental factors are of particular importance. For example, freshwater fish are affected by a large number of environmental factors, such as temperature, salinity, conductivity, dissolved oxygen content, pH, photoperiod, chemical toxicants, nitrogen, phosphorus compounds, and other limiting factors [1].

Failing to meet the optimal environmental conditions for fish can cause some major physiological effects. One of the dysfunctional effects and adaptive responses of abnormal environmental factors is the negatively efects in their blood. Indeed, the blood parameters are regarded as pathophysiological indicators of the whole body, and therefore; they are important in diagnosing the structural and functional status of fish, exposed to deleterious factors such as diseases, radioactive effects, chemical toxicants, carcinogens, and other environmental stressors.

Atikhisar Reservoir and Sarıçay Creek supply drinking and irrigation waters for Çanakkale city. There are multiple factors threatening water quality of Sarıçay Creek such as sewage and agricultural wastewater [2]. The objectives of this study were in two-fold: (1) to investigate some water quality parameters of Atikhisar Reservoir and Sarıçay Creek, and (2) to determine the possible characteristic changes of hemato-chemical parameters of European chub (*Squalius cephalus* L.) in Atikhisar Reservoir and Sarıçay Creek.

MATERIAL and METHODS

The water quality parameters such as temperature (T), salinity (Sal), pH, dissolved oxygen (DO), conductivity (C), total nitrogen (TN), total phosphorus (TP), chlorophyll-a (Chl-a), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) were measured in August 2006. The samples were collected from Atikhisar Reservoir and Sarıçay Creek, which are located in Çanakkale, in the north-western part of Turkey. The sampling technique as outlined by the Standard Methods for the Examinations of Water and Wastewater [3] was used in the collection, preservation, and storage of the samples. One and two litter polyethylene bottles were used to determine the chemical properties of the water. The bottles were kept at 4°C and analyzed within 24 h upon their collection

Fishing expeditions were carried for European chub (*Squalius cephalus* L.) by using gillnet at a depth of 1.5 m to investigate the possible effects on hematological and biochemical indices. A total of 60 fish were caught as 30 European chub from each station of Atikhisar Reservoir and 30 from Sarıçay Creek. The body of each fish was weighed, and total length was measured. Their blood analyses were carried out to compare the blood parameters. About 4 mL of blood was drawn from each sample by caudal vein puncture and it was immediately placed in individual 2-mL silicone-coated Vacutainer Tubes (Becton Dickinson; not containing EDTA) and 2-mL tubes containing EDTA (ethylene diamine tetra acetic acid). The blood in coated Vacutainer Tubes was centrifuged promptly at 3.100 g for 10 min, and then its serum was removed with a disposable transfer pipette. The concentrations of phosphate (P), calcium (Ca), total protein (TP), albumin (ALB), globulin (GLO), total bilirubin (TBIL), glucose (GLC), cholesterol (CHOL), triglyceride (TG), aspartate aminotransferase (glutamate oxaloacetate transaminase; AST = GOT), and alanine aminotransferase (glutamate pyruvate transaminase; ALT = GPT) were determined spectrophotometrically (AWARNES-Stat Fax 1904) by using Spinreact kits.

The hematological analysis was conducted as by following the guidelines [4]. Count of leucocytes (WBC) and erythrocytes (RBC) in the blood samples were counted with Natt-Herrick solution on a Neubauer counting chamber. Hemoglobin (Hb) concentrations were determined with Sahli-Hellige method (by using Drabkin's reagent), and hematocrit (Ht) values were determined with microhematocrit method. The values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

The mean weight (WF), and length (LF) of the fish were calculated according to the methods [5] described and the statical comparison was conducted by using *t*-test analysis [6]. The data obtained from blood analyses of fish and water parameters were subjected to *t*-test by using the Minitab - User Guide program [6]. In these statistical comparisons, a value of $P<0.05$ (i.e. 95% Confidence Interval) was considered significant.

RESULTS

Conductivity, chemical oxygen demand (COD) and biochemical oxygen demand (BOD), total phosphorus, total nitrogen and total suspended solid (TSS) content of water in Sarıçay Creek were higher than in Atikhisar Reservoir however water dissolved oxygen content in Sarıçay Creek was less than in Atikhisar Reservoir ($P<0.05$) *(Table 1)*. Chlorophyll-a concentrations, temperature, salinity (two resources are freshwater) and pH of water were not statistically significant in two resources ($P<0.05$) *(Table 1)*.

Means of body weight and length of European chub

Table 1. *Physico-chemical parameters for surface waters of Atikhisar Reservoir and Sarıçay Creek in August 2006*
Tablo 1. *Atikhisar Barajı ve Sarıçay'ın Ağustos 2006'da yüzey suyu fiziko-kimyasal parametreleri*

Station	T	C	DO	COD	BOD	TP	TN	Sal	pH	Cl-a	TSS
Atikhisar Reservoir	27.70±0.15	296.50±13.21	8.50±0.85	1.00±0.05	0.10±0.02	0.05±0.01	8.71±0.89	0.28±0.06	7.76±0.13	18.83±0.95	0.004±0.001
Sarıçay Creek	26.40±0.26	561.00±10.50*	5.60±0.56*	16.00±1.28*	25.15±2.15*	0.14±0.05*	24.50±2.14*	0.35±0.09	7.99±0.16	15.14±1.05	0.014±0.002*

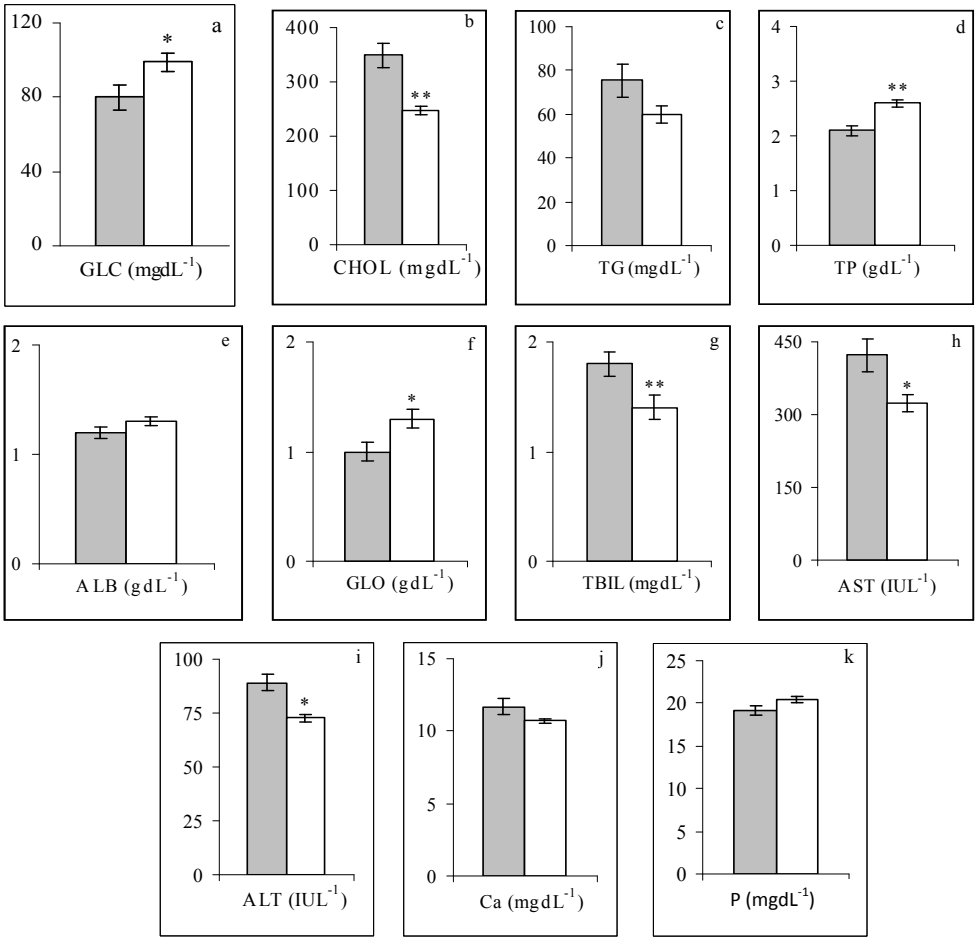
*n = 6, mean±S.E. Value along a column with asterisk was significantly different from other value in column; * P<0.05, Temperature = T (°C), Conductivity = C (µS/cm), Dissolved Oxygen = DO (mg/L), Chemical Oxygen Demand = COD (mg/L), Biochemical Oxygen Demand = BOD (mg/L), Total Phosphorus = TP (mg/L), Total Nitrogen = TN (mg/L), Salinity = Sal (ppt), pH (pH units), Chlorophyl-a = Chl-a (mg/L) and Total Suspended Solids = TSS (mg/L)*

Table 2. *Mean weight (±SE) (WF) and length (LF) data of European chub (Squalius cephalus L.) in polluted Sarıçay Creek and unpolluted Atikhisar Reservoir*
Tablo 2. *Kirli Sarıçay ve temiz Atikhisar Barajı'ndaki Avrupa Tatlısu Kefali (Squalius cephalus L.)'ın ortalama ağırlık (±SE) (WF) ve boy (LF) verileri*

Fish Size	Water Resource	n	Mean±SE
WF	Atikhisar Reservoir	30	227.10±18.24
	Sarıçay Creek	30	212.50±8.04
LF	Atikhisar Reservoir	30	27.10±0.64
	Sarıçay Creek	30	25.70±0.24

were not significantly different (P>0.05) in Atikhisar Reservoir and Sarıçay Creek *(Table 2)*.

The biochemical blood parameters are shown in *Fig. 1a-k* and the hematological parameters are shown in *Fig. 2a-g*. Serum-glucose, total protein and globulin concentrations of fish in Sarıçay Creek were significantly greater than those in Atikhisar Reservoir (P<0.05) *(Fig. 1a, d, f)*. However, the fish sample in Sarıçay showed lower serum total bilirubin, cholesterol, AST, and ALT levels as opposed to the fish sample of Atikhisar Reservoir (P<0.05) *(Fig. 1b, g, h, i)*. There were no significant differences in triglyceride, albumin, calcium and phosphate values between the fish samples of Atikhisar Reservoir and Sarıçay Creek

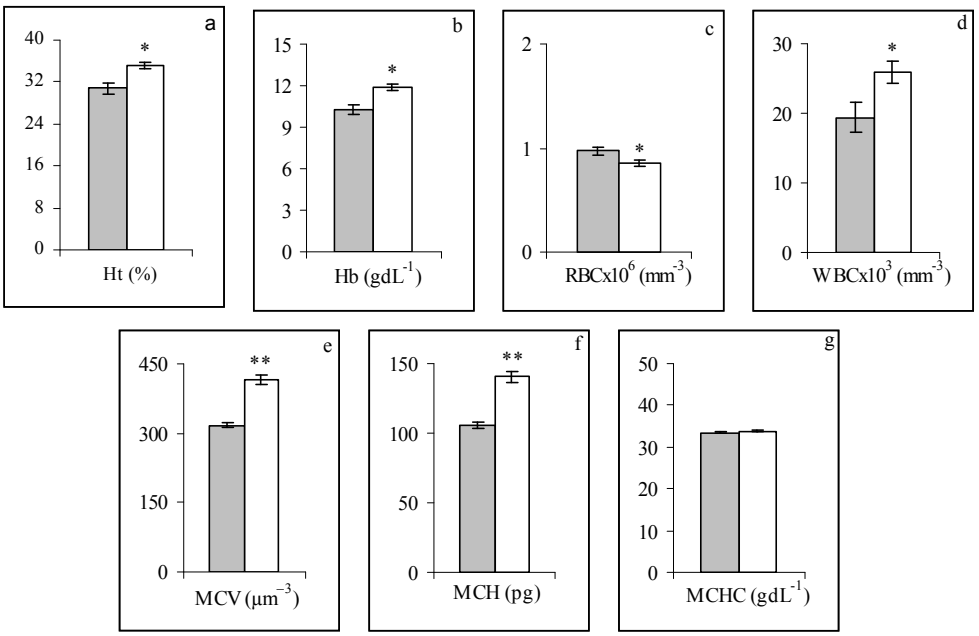


Value along a column with asterisk was significantly different from other value in column; * P<0.05, ** P<0.01, n = 30, mean±S.E.

Atikhisar Reservoir,
Sarıçay Creek

Fig 1. Serum biochemical parameters of European chub caught from unpolluted Atikhisar Reservoir and polluted Sarıçay Creek

Şekil 1. Kirli Sarıçay ve temiz Atikhisar Barajı'ndan yakalanan Avrupa Tatlısu Kefali'nin serum biyokimyasal parametreleri



Value along a column with asterisk was significantly different from other value in column; * P<0.05, ** P<0.01, n = 30, mean±S.E.

Atikhisar Reservoir,
Sarıçay Creek

Fig 2. Hematological parameters of European chub caught from unpolluted Atikhisar Reservoir and polluted Sarıçay Creek

Şekil 2. Kirli Sarıçay ve temiz Atikhisar Barajı'ndan yakalanan Avrupa Tatlısu Kefali'nin hematolojik parametreleri

(P>0.05) *(Fig. 1c, e, j, k)*. Leucocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hematocrit and hemoglobin concentrations in blood of the fish in Sarıçay Creek were statistically significantly higher than the fish sample of Atikhisar Reservoir (P<0.05) *(Fig. 2a, b, d, e, f)*. Blood erythrocyte count of the fish sample in Sarıçay Creek was significantly less than that the Atikhisar Reservoir's fish (P<0.05) *(Fig. 2c)*. No significant differences were observed mean corpuscular hemoglobin concentrations (MCHC) between both of the fish samples from the research sites (P>0.05) *(Fig. 2g)*.

DISCUSSION

The assessment of water quality is generally determined by employing the analysis of physico-chemical parameters. Conductivity, total suspended solids, chlorophyll-a, temperature and pH values of freshwater in both Atikhisar Reservoir and Sarıçay Creek were within the normal limits of quality standards indicated for surface waters adapted warm freshwater fish [1,7-9]. However, higher conductivity and total suspended solids in Sarıçay Creek was observed because of the pollution (i.e. domestic waste and agricultural runoff). Besides, the freshwater of Sarıçay Creek has been found to have higher values of BOD and COD in comparison with the levels in Atikhisar Reservoir, thus; it indicates the polluted water of Sarıçay. Hence, in the direction of pollution analysis marking, Sarıçay as the most oxygen demanding suggests the existence of pollution state. When BOD level is high, it means the dissolved oxygen level in water decreases because the oxygen is basically consumed by bacteria [10], and other microorganisms. In accordance with this, the decrease in dissolved oxygen value found in Sarıçay freshwater sample is inversely related and corroborate well with the BOD value of the present study. Atikhisar Reservoir was classified the first degree in terms of the dissolved oxygen, salinity, total nitrogen, total phosphorus, total suspended solid, BOD, and COD values while Sarıçay Creek was third class in terms of these parameters [11,12]. Thus, Atikhisar Reservoir is assumed as unpolluted surface water, but Sarıçay Creek is assumed surface water.

Water pollutants in Sarıçay Creek elevated serum glucose concentrations in chronic stress impact upon European chub. The increased glucose level in the serum of fish in Sarıçay could be a result of hyperglycemia induced by lifelong exposure to environmental stressors such as hypoxia, high salinity, total nitrogen, total phosphorus, total suspended solid, BOD, COD. Indeed, hyperglycemia (high blood glucose) in teleost was believed to be caused by a wide range of environmental stress factors [13-17], nephritis, hepatic disorder [18] and muscle tissue dysfunction [19]. The elevated serum/plasma glucose is an indicative of disrupted carbohydrate metabolism, which may be mainly due to the enhanced breakdown of liver glycogen [18]. In this study, these theories may be supported by the results of serum enzyme analyses showing a statistically significant decreased AST and ALT levels of the fish in Sarıçay Creek. The decreased serum AST and ALT values of the fish might be observed in pollutant exposure [15] as seen in this study. The AST and ALT activities represent protein metabolism, which are the aminotransferases, catalyzing the intermolecular transfer of amino groups between amino acids and α-ketoacidosis [20]. Lower AST activity shows that oxaloacetate and glutamate are not available to Krebs cycle through this route of transamination. The decreased serum AST and ALT activities signal liver cell insufficiency. The enzyme-abundant tissues contribute to the aspect of the circulating enzyme pattern in the serum. If damage occurs in the enzyme-abundant tissue, some enzymes

may leak from injured cells and thus the activities of serum enzymes would change.

Serum's total protein and globulin concentrations of fish in Sarıçay Creek were higher than those of in Atikhisar Reservoir. Exposure to pollutants had a detrimental effect on the immunological response in fish [21]. The significance of the increased globulin levels to meet the immunotoxic challenges lends evidence to the differential response of the constituent plasma protein. Total protein is used to evaluate protein metabolism. Hyperproteinemia, seen with hemoconcentration and shock, contributed to the elevated osmolality [13] and nutritional status [16]. In addition, increased concentrations of total protein can be caused by structural liver alterations reducing aminotransferase activity, (as observed as a reduction of AST and ALT levels in this study) with concurrent reduced deamination capacity [17] and impaired control of fluid balance [22].

Also, bilirubin is the predominant bile pigment found in the circulation in fish derived from disruption of hemoglobin [23]. In this study, the less value of bilirubin in European chub of Sarıçay Creek was a consequence of the increased level of hemoglobin. Bilirubin is a breakdown product of hemoglobin and could be logically expected to decrease adversely with increasing levels of hemoglobin. Total bilirubin comes to the muscle and liver by binding it to the albumin and globulin in the serum through filtering by kidney. Serum bilirubin levels of fish can change with hepatic and nephritic diseases [24].

In this work, low concentration of cholesterol (hypocholesterolemia) in serum of European chub in Sarıçay Creek may have come from the effect of pollution. It is known that cholesterol and lipoproteins values are related with each other, and these are connected with the metabolism of lipids and functions of the liver and kidney. Serum cholesterol level of fish can be affected from nutritional factors [25], nephritic syndrome, glycogen storage disease, seasonal changes, physiological activity and toxic agents [19,25,26].

Increased leucocyte count in blood of European chub establishes leucocytosis, which is considered to be of adaptive value for the tissue under pollution-induced chronic stress. This also helps in the removal of cellular debris of necrosed tissue. In the presence of foreign substances or under pathological conditions leukocytosis in fish can be the consequence of direct stimulation of immunological defense [18].

The MCV, MCH, hemoglobin and hematocrit increased considerably in blood of fish in polluted Sarıçay Creek compared to the fish of Atikhisar Reservoir. These high values indicate an adaptation to polluted condition where more oxygen is needed for metabolic processes. These alterations in European chub of Sarıçay Creek were attributed to direct or feedback responses of structural damage to erythrocyte membranes resulting in hemolysis and impairment in hemoglobin synthesis, stress-related release of erythrocytes from the spleen and hypoxia, induced by exposure to pollution as been reported for several other fish species [16,27]. Hemoglobin levels in the blood of *Cyprinion watsoni* were significantly increased when the fish were exposed to hypoxia [28]. Furthermore, different study showed that an increased ability of hemoglobin to bind with oxygen [29]. The significant reduction of erythrocyte count and elevation of MCV in blood of fish in polluted Sarıçay Creek may be attributed to endocrinopathy, however; to what extent this endocrinopathy occurs in fish with water pollution requires further investigations. The phenomenon of physiological macrocytosis under polluted environmental condition, as evidenced by an increase in the MCV level, was seen in this study as well.

In conclusion, the blood leucocyte, erythrocyte, hematocrit, hemoglobin, MCV and MCH levels and the serum total protein, globulin, total bilirubin, cholesterol, glucose, AST and ALT concentrations in European chub could be useful to evaluate the physiological effects of water pollution, but the application of these findings to prepare laboratory diagnostics will need more elaborated investigations and must be validated *in situ* before establishing them as biomarkers.

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