

Seasonal variations of heavy metals in some tissues of *Chondrostoma regium* (Heckel, 1843) from Batman Dam, Turkey

Emel KAÇAR^{1*}, Hülya KARADEDE AKIN²

ABSTRACT: In this study, it was aimed to investigate seasonal changes in levels of Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Cadmium (Cd) and Lead (Pb) in liver, gill and muscle tissues of *Chondrostoma regium* from Batman Dam. Inductively coupled plasma mass spectrometry (ICP-MS) was used to analyze heavy metal concentration levels in the fish. The metal concentrations in the same tissues of samples taken from Batman Dam were slightly variable. Among the metals that were studied, concentration of Fe in the tissues of the fish was the highest. The metal concentrations in the liver and gill of *Chondrostoma regium* were higher than those in the muscle. The average heavy metal concentration in the muscle of *Chondrostoma regium* was higher in the autumn and winter. The amounts of metal in muscle tissues of the fish were found lower than the acceptable values in fish tissues.

Keywords: Heavy metals, Seasonal variation, *Chondrostoma regium*, Batman Dam.

Batman Barajı'nda (Türkiye) Yaşayan *Chondrostoma regium*'un (Heckel, 1843) Bazı Dokularında Ağır Metallerin Mevsimsel Değişimi

ÖZET: Bu çalışma Batman Barajı'ndan alınan *Chondrostoma regium*'un karaciğer, solungaç ve kaslarında Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd ve Pb metallerinin düzeylerinin mevsimsel değişimlerini analiz etmek için gerçekleştirilmiştir. Balıklarda ağır metal birikim düzeyleri ICP-MS (endüktif eşleşmiş plazma kütle spektrometresi) kullanılarak belirlenmiştir. Batman Barajı'ndan elde edilen örneklerin aynı dokularında ölçülen metal konsantrasyonlarında değişiklik görülmüştür. Fe balık dokularında en yüksek konsantrasyonda bulunmuştur. *Chondrostoma regium*'un karaciğer ve solungaçlarındaki metal konsantrasyonları, kasla mukayese edildiğinde daha yüksek olduğu görülmüştür. Mevsimsel olarak da *Chondrostoma regium*'un kasında ortalama ağır metal konsantrasyonu sonbahar ve kış aylarında yüksek bulunmuştur. Balıkların kas dokularındaki metal düzeyleri balık dokularında kabul edilebilir değerlerden düşük olduğu tespit edilmiştir.

Anahtar Kelimeler: Ağır metaller, Mevsimsel değişim, *Chondrostoma regium*, Batman Barajı

¹ Emel KAÇAR (Orcid ID: 0000-0003-4249-921X), Plant and Animal Production Department, Avanos Vocational School, University of Nevşehir, TR-50300 Nevşehir, Turkey

² Hülya KARADEDE AKIN (Orcid ID: 0000-0001-5634-5831), Department of Biology, Section of Hydrobiology, Faculty of Science, University of Dicle, TR-21280 Diyarbakir, Turkey

*Sorumlu Yazar / Corresponding Author: Emel KAÇAR, e-mail: emelkacar@nevsehir.edu.tr

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INTRODUCTION

Contamination of aquatic ecosystems with heavy metals due to urban, industrial and agricultural wastes has become a serious problem. Due to their bioaccumulative properties and toxicities in the food chain, heavy metals are an important group of pollutants in aquatic environments (Uysal et al., 2008). High metal concentrations in water, sediments and organisms can cause serious ecological consequences. When metal accumulate in the tissues of aquatic organisms at high levels, this can have toxic effects for both fish and people fed with fish (Dural et al., 2007). Hence, the amounts of heavy metals in fish tissues are used as indicators of the extent of pollution in the water (Selvi et al., 2015). Fish are important organisms of aquatic ecosystems and they constitute the nutrients of humans, so it should be unsurprising that numerous investigations have been performed on the determination of metal accumulations in various fish species (Maceda-Veiga et al., 2013; Monroyet et al., 2014; Qu et al., 2014; Firat, 2016).

Chondrostoma regium (Heckel 1843) belongs to *Cyprinidae* family. *C. regium* was preferred in this study because it is an important nutrient for those living in that region and it is abundant in Batman Dam.

Batman Dam are located in the Tigris River basin of the Southeastern Anatolia region of Turkey Batman Dam and hydroelectric power plant are located on Batman stream. They were built in 1999 for irrigation and power generation besides preventing flooding (DSI, 2018). Pollution mainly comes from municipal wastewater discharges and agricultural drainage of the villages. Furthermore, during the rainy season materials are carried by the rivers into the lake, which contribute to pollution. Nevertheless, an industrial pollution source around the reservoirs does not exist (Varol et al., 2012).

The present study aims to assess seasonal change of several heavy metals such as chromium

(Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) concentrations in liver, gill and muscle of *C. regium* obtained from Batman Dam. The results of this work will provide information on food safety for potential consumers. The study will also shed light on for both quality of the water and health of the fish, which are commercially important in Batman Dam.

MATERIALS AND METHODS

Study Area

The study was performed with samples taken seasonally from different stations of Batman Dam between July 2013 and April 2014 (Figure 1). Study Site I, Altinkum Village-Silvan (38° 14' 46.37" N 41° 06' 20.48" E) is the waste waters of Kulp and Lice districts which flows here. Site II Yayık Village-Kulp (38° 14' 46.37" N 41° 06' 20.48" E) is the location where intense agricultural activities occur around the sampling site. Finally, Site III Kumgözü Village (38° 14' 41.56" N 41° 09' 57.48" E) is the region where livestock and agricultural activities. The Batman Stream forms a natural border between Batman and Diyarbakır and it hosts Batman Dam. The coordinates of Batman Dam are 38° 14' 5.72" N 41° 5' 55.37" E, altitude is 659 m and has 49 km² of the lake area. Lake's volume in normal water level is 1175 hm³, the dam's body volume is 7.181 dam³, and its height from the streambed is 85 m. The dam was built from rocks, one of Tigris River's primary tributaries, and it was constructed for irrigation, flood prevention and energy production (Varol et al., 2012). Recently, in addition to the rise in pollution, fish were contaminated with heavy metals due to substantially increase in agricultural activities. Therefore, the quality of this ecosystem has been deteriorating due to human and agriculture activities. Although the people living near Batman Dam mainly eat fish, there has not been any studies conducted evaluating heavy metal concentrations in fish from Batman Dam.



Figure 1. Map of Batman Dam (Tigris River, Turkey)

Sampling and Chemical Analyses

Fish were obtained seasonally from the commercial fish which were maintained from the region surrounding Batman Dam. On the same day, fish specimens were transferred to the laboratory within ice. Each fish species' length and weight was measured. Until analysis, fish samples were stored at -30°C . The sediment samples were collected in triplicates by using a box sediment grab and water samples were also taken from the same localities. Their analysis methods and results were presented in our previous paper (Kaçar et al., 2017). This study was performed according to the Ethical Rules (2013/22) of Institute of Natural and Applied Science of Dicle University.

Gill racers, the whole liver and nearly 5 g of the epaxial muscles on the dorsal surface of fish samples were taken. The samples were exposed to nearly 85°C for 72 h to dry, and then 0.1 g (d.w.) of individual sample was measured and transmitted to a digestion microwave. 7 ml 65% HNO_3 and 1 ml 30% H_2O_2 solutions were put into each sample tube. After digestion, the tubes have been refrigerated to room temperature and diluted to 15 ml by using ultra-pure water. For determination of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb by using an Agilent 7700 Inductively

Coupled Plasma Mass Spectrometer (ICP-MS), the fish samples were analyzed three times. The detection limits of ICP-MS were as follows: Cr; $0.036\ \mu\text{g L}^{-1}$, Mn; $0.037\ \mu\text{g L}^{-1}$, Fe; $0.125\ \mu\text{g L}^{-1}$, Co; $0.002\ \mu\text{g L}^{-1}$, Ni; $0.805\ \mu\text{g L}^{-1}$, Cu; $0.160\ \mu\text{g L}^{-1}$, Zn; $1.483\ \mu\text{g L}^{-1}$, Cd; $0.002\ \mu\text{g L}^{-1}$ and Pb; $0.121\ \mu\text{g L}^{-1}$. The stock solutions ($1\ \text{mg l}^{-1}$ for ICP-MS) were diluted to obtain working standard solutions for system calibration and regulation of analytical certainty. Metal concentrations' accumulation levels were displayed as mg kg^{-1} wet weight (ww) for fish tissues. International certified reference materials were employed for fish muscle (DORM-2, National Research Council Canada) and liver (DOLT-3, National Research Council Canada), which were analyzed in the beginning and at end of each batch of samples to assess certainty and sensibility of the analytical method. Repeated analysis of these reference materials displayed good accuracy and recovery rates for metals were between 97% and 125% for DORM-2, 103% and 119% for DOLT-3.

Statistical Analyses

The varieties between fish tissues were evaluated with variance analysis (one-way ANOVA) and the significance of difference between average values was evaluated by Duncan

Test. Non-parametric Kruskal–Wallis test was employed for determination of the significance of variation between different groups in seasonal comparisons. When statistical variations were detected in Kruskal–Wallis test, Mann–Whitney U was used for multiple comparisons. Differences were regarded as significant at $p < 0.05$.

RESULTS AND DISCUSSION

The seasonal variation of heavy metals in the liver, gill and muscle tissues of *Chondrostoma regium* is outlined in Table 1 and Figure 2. According to Table 1, Fe, Co, Cu, Zn and Cd concentrations in the liver and Cr, Mn, Ni and Pb concentrations in gills of *C. regium* were determined at the highest levels. Among heavy metals, especially, Fe and Zn concentrations were very high in all tissue samples (Figure 2). In addition, Fe and Zn levels in fish tissues were significantly different ($p < 0.05$). In the study, it was found out that Fe concentration was the highest and Co concentration was the lowest in all tissues of *C. regium*. Concentrations of heavy metals detected in the various tissues of fish were found to be different. There were significant differences in the level of a given metal between the tissues throughout the year in the fish samples. Although heavy metal concentrations were higher in the liver and gill, they were lower in the muscle.

Changes in heavy metal concentrations in the tissues of fish that were taken seasonally from Batman Dam are outlined in Figure 2 for *C. regium*. It was determined that the heavy metal concentrations in *C. regium* changed seasonally. Although concentrations of Cr, Co and Zn were the highest in the spring in liver tissues, concentrations of Mn and Ni were the highest in the winter. Heavy metals examined in liver tissues were generally low in autumn and summer. Accumulated Cr, Mn, Fe, Ni, Cu, Cd and Pb concentrations in the liver in terms of seasons were not different, except Co and Zn ($p > 0.05$).

Although concentrations of Fe, Co, Ni and Zn in gills were statistically the highest in the winter, Mn concentration was the lowest in the spring and there was a statistically significant difference among the other seasons ($p < 0.05$). On the other hand, Mn concentration was highest in autumn; while Cd concentration had the highest values in the spring. In contrast, the highest Pb concentration was observed in the summer. However, difference in Cr, Cu, Cd and Pb concentration was not significant ($p > 0.05$).

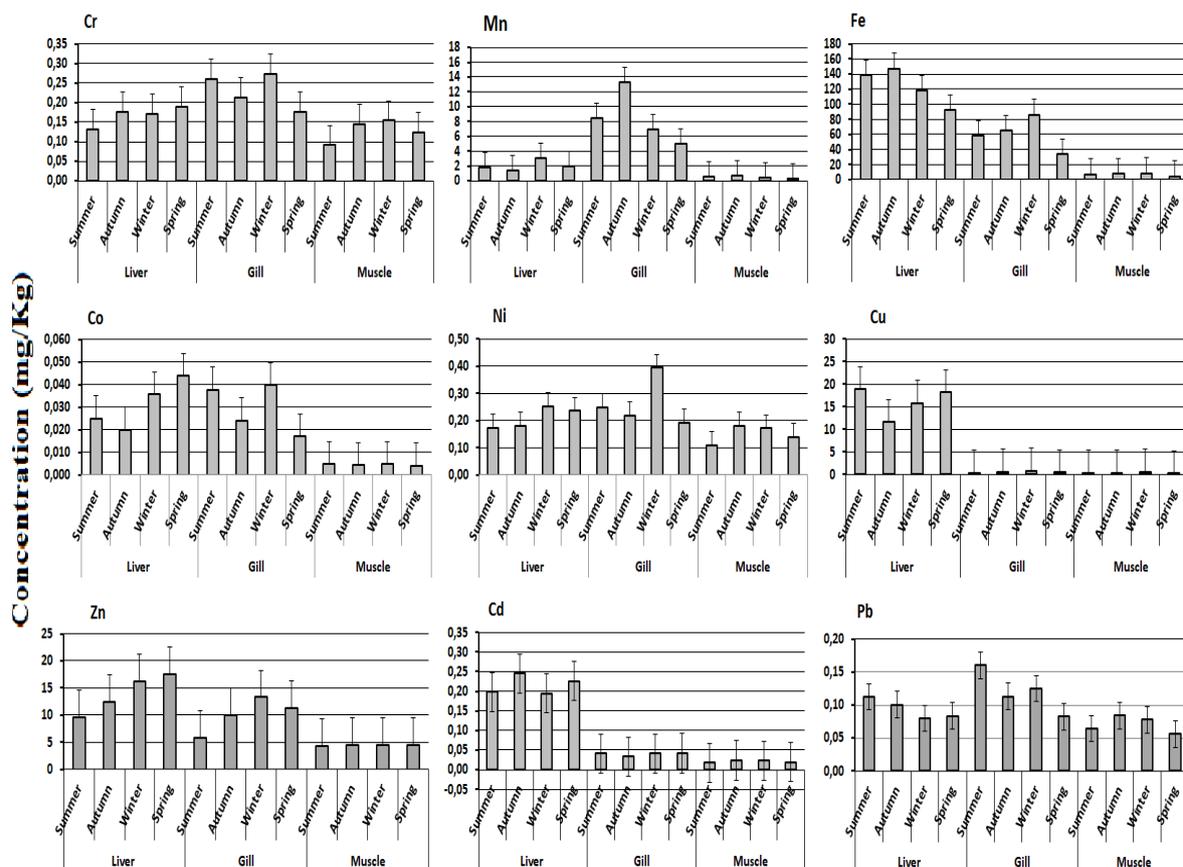
The heavy metal concentrations in the *C. regium* muscle are shown in Table 1. The concentrations of Cr, Fe, Cu and Zn were the highest in winter; Mn, Ni, Cd and Pb were highest in autumn; Co was highest in spring. Furthermore, Mn, Fe, Co, Zn, Cd and Pb had not any statistically significant difference among seasons ($p > 0.05$).

The concentration levels of some heavy metals in various tissues of *C. regium* from Batman Dam were considerably lower than those found in the previous studies in the Tigris River (Gungum et al., 1994; Varol et al., 2010). According to the results, there are significant differences of heavy metal concentration in the investigated tissues. In our work, concentrations of Fe, Co, Cu, Zn and Cd were the highest in the liver, while they were found to be the lowest in the muscle. Particularly, Fe concentration in *C. regium* was the highest in liver. When the tissues of *C. regium* were considered, it was found that the highest metal accumulation was in metabolically active organs such as liver. High metal concentrations in liver tissues is caused by the presence of metallothionein which is a protein in fish liver. This protein has a highly complex structure and is capable of binding a large number of metals. Another reason for the high metal concentrations in the liver is due to the fact that this organ is a metal detoxification site (Hogstrand and Haux, 1991; Kalay and Erdem, 1995; Unlu et al., 1996).

Table 1. The heavy metal concentrations (mg kg⁻¹ wet wt) in tissues of *Chondrostoma regium* in different seasons from the Batman Dam

	N	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	
LIVER	Summer	4	0.13±0.02	1.86±0.86	138.17±6.34	0.02±0.001 ^c	0.17±0.04	18.82±7.04	9.51±1.72 ^{cd}	0.20±0.03	0.11±0.03
	Autumn	4	0.18±0.03	1.41±0.57	147.28±33.26	0.02±0.004 ^c	0.18±0.03	11.56±2.22	12.43±2.66 ^d	0.24±0.11	0.10±0.01
	Winter	4	0.17±0.03	3.00±0.74	118.61±37.43	0.04±0.004 ^{ab}	0.25±0.04	15.74±11.67	16.14±4.72 ^a	0.19±0.07	0.08±0.01
	Spring	4	0.19±0.03	1.90±1.40	92.11±48.24	0.04±0.03	0.23±0.07	18.13±10.21	17.50±3.05 ^{ab}	0.23±0.06	0.08±0.02
	Mean	16	0.17±0.03 ^x	2.04±1.04 ^x	124.04±38.10 ^z	0.03±0.02 ^y	0.21±0.05 ^y	16.06±8.22 ^y	13.89±4.34 ^z	0.22±0.07 ^y	0.09±0.02 ^{xy}
P value		0.06	0.21	0.21	0.03	0.11	0.48	0.04	0.72	0.15	
GILL	Summer	4	0.26±0.10	8.48±1.05 ^{bd}	58.25±4.94 ^{cd}	0.04±0.01 ^d	0.25±0.09 ^c	0.40±0.21	5.76±1.80 ^{bcd}	0.04±0.03	0.16±0.09
	Autumn	4	0.21±0.06	13.28±4.21 ^{acd}	64.84±23.66	0.02±0.01	0.22±0.06 ^c	0.57±0.15	10.02±1.91 ^{ac}	0.03±0.01	0.11±0.01
	Winter	4	0.27±0.02	6.89±1.40 ^b	86.16±10.45 ^{ad}	0.04±0.004 ^d	0.39±0.02 ^{abd}	0.81±0.40	13.27±1.94 ^{ab}	0.04±0.01	0.12±0.01
	Spring	4	0.18±0.03	5.01±1.33 ^{ab}	33.43±10.82 ^{ac}	0.02±0.01 ^{ac}	0.19±0.03 ^c	0.47±0.09	11.29±1.60 ^a	0.04±0.01	0.08±0.01
	Mean	16	0.23±0.07 ^y	8.42±3.81 ^y	60.67±23.22 ^y	0.03±0.01 ^y	0.26±0.10 ^y	0.56±0.27 ^x	10.08±3.28 ^y	0.04±0.01 ^x	0.12±0.05 ^y
P value		0.21	0.007	0.02	0.03	0.02	0.17	0.01	0.51	0.05	
MUSCLE	Summer	4	0.09±0.004 ^{bcd}	0.62±0.54	7.32±1.73	0.01±0.001	0.11±0.01 ^{bc}	0.33±0.16	4.21±0.13	0.02±0.003	0.06±0.01
	Autumn	4	0.14±0.01 ^a	0.74±0.08	8.03±1.44	0.004±0.001	0.18±0.01 ^{ad}	0.38±0.07 ^d	4.39±0.41	0.02±0.003	0.08±0.02
	Winter	4	0.15±0.02 ^a	0.49±0.14	8.73±0.76	0.01±0.001	0.17±0.02 ^a	0.59±0.16 ^d	4.55±0.37	0.02±0.001	0.08±0.01
	Spring	4	0.12±0.02 ^a	0.35±0.05	4.67±1.89	0.004±0.001	0.14±0.03 ^b	0.24±0.03 ^{bc}	4.42±0.73	0.02±0.004	0.06±0.01
	Mean	16	0.13±0.03 ^x	0.55±0.29 ^x	7.19±2.09 ^x	0.004±0.001 ^x	0.15±0.03 ^x	0.38±0.17 ^x	4.39±0.43 ^x	0.02±0.004 ^x	0.07±0.02 ^x
P value		0.01	0.05	0.05	0.21	0.01	0.02	0.54	0.12	0.06	

Mean values and ± standard deviation. Values in parentheses indicate the minimum and maximum levels. Values with different letters are significantly different at p<0.05 level, N number of samples. (a Summer, b Autumn, c Winter, d Spring) Seasonal differences in tissues, .x, y, z Mean differences between the tissues

**Figure 2.** The heavy metal concentrations in tissues of *Chondrostoma regium* in different seasons from Batman Dam

The concentration of Fe and Zn in the liver of *C. regium* was lower than that of the same species living in the Atatürk Dam (Karadede and Ünlü, 2000; Fırat et al., 2018), but Mn level was similar (Karadede and Ünlü, 2000). Also, Cr, Cd and Pb levels were higher than those found by Fırat et al. (2018).

The concentrations of Cr, Cu, Cd and Pb in the gill of *C. regium* samples collected in the Seyhan River (Canlı et al., 1998) and Atatürk Dam Lake (Sıtılce samples) (Fırat et al., 2018) were higher as compared with those found by the present study. On the other hand, Fe, Mn and Pb values are rather higher than the values in the gill of *C. regium*, which live in Sır Dam Lake (Erdoğan and Erbilir, 2007). Also, our Co level was similar to those determined by the study for *C. regium* conducted in the Sır Dam Lake (Erdoğan and Erbilir, 2007). In addition, the metal concentrations in the all tissues of *C. regium* in the present study were found to be higher than those reported for *C. regium* in the Karasu River, Turkey (Aydoğan et al., 2017). High accumulation determined in the gills is due to the fact that this organ carries out numerous metabolic activities such as osmotic and ionic regulation and is in constant contact with the external environment and plays an important role in the entry of water-dissolved metals into the body (Healt, 1995; Au, 2004; Soto et al., 2008).

As reported in Table 2, heavy metal levels at muscle tissues of *C. regium* in the present study were lower than those found in other studies at different localities such as in the Atatürk Dam (Karadede and Ünlü, 2000; Fırat et al., 2018), in the Keban Dam Lake (Çalta and Canpolat, 2006), in the Seyhan River (Canlı et al., 1998). Pb concentration in the muscle of *C. regium* is in agreement with those found for *C. regium* in Sır Dam Lake (Erdoğan and Erbilir, 2007). Cr, Fe, Co, Ni, Cu, Zn, Cd and Pb concentrations in muscle of *C. regium* in the present study were determined to be lower than those found by Kacar et al., (2017) in a study performed on *Cyprinion macrostomum* in the Batman Dam and values of Mn, Fe, Cu were higher than those found for *Barbus grypus* in the Batman

Dam by Kacar et al, (2017). Muscle of a fish is not a metabolic active organ and this may be result of lowness of the metal concentration in this tissue (Bajc et al., 2005). Thus, we can conclude that our findings are in accord with those in the literature.

The present study also aimed to ascertain the seasonal changes in heavy metal concentration in muscle of *C. regium*. The concentrations of heavy metals were found to be maximum in autumn and winter. The heavy metal concentrations were also relatively high in autumn and winter in *Capoeta trutta* in Batman Dam (Kacar et al., 2017). Karadede- Akin, (2009), reported that heavy metal values in the muscle of *Capoeta capoeta umbla* obtained from Hazar Lake had increased in summer and winter. Başyigit and Tekin-Özan (2013) determined the highest metal values in autumn in *Sander lucioperca* collected from Karakaş Dam. It was reported that metal concentrations in fish seasonally changes, which depends on physical and chemical parameters of water, feeding age (Dural et al., 2010; Sauliute and Svecevicus 2017). The seasonal variation may be related to reproductive cycle of fish (Zyadah, 1999; Nussey et al., 2000). The spawning period of cyprinids in the Batman Dam occurs from April through July. Therefore, the concentrations of heavy metal were found lower in muscle tissues of fish in spring and summer when gonads of fish are well developed. On the contrary, in autumn, the highest mean concentrations of heavy metals were recorded in muscle tissues of fish because no gonadal development is present in autumn, fish take more food in this season (Varol and Sünbül, 2018). Another possible source of seasonal variation can cause increases or decreases in the levels of metals in reservoir water. Whereas metal concentrations in reservoir water can decrease, despite increasing agricultural activities, during spring and early summer because of the rise in water level of the reservoir caused by increased rainfall and melting snow, metal concentrations can increase late summer and during autumn because of decrease in water level caused by evaporation and rainfall absence (Varol and Sünbül, 2018).

Table 2. Some heavy metal concentrations (mg kg⁻¹) determined in the muscle tissue of *Chondrostoma regium* and some fish species

	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
The present study									
<i>C. regium</i>	0.139±0.03	0.55±0.29	7.19±2.09	0.004±0.001	0.15±0.03	0.38±0.17	4.39±0.43	0.02±0.004	0.07±0.02
Fırat et al., 2018									
<i>Chondrostoma regium</i>	0.13	-	22.23	-	-	0.75	21.18	0.09	0.24
Kacar et al., 2017									
<i>Barbus grypus</i>	0.132	0.448	3.322	-	-	0.215	5.354	-	-
Kacar et al., 2017									
<i>Cyprinion macrostomum</i>	0.16	0.31	9.30	0.01	0.17	0.43	4.69	0.02	0.07
Erdoğan and Erbilir, 2007									
<i>Chondrostoma regium</i>	-	0.27	0.91	-	0.77	-	-	-	0.06
Çalta and Canpolat 2006									
<i>Chondrostoma regium</i>	-	-	22.51	-	-	3.13	38.66	-	-
Karadede and Ünlü, 2000									
<i>Chondrostoma regium</i>	-	1.44	9.74	-	-	2.29	7.93	-	-
Canlı et al., 1998									
<i>Chondrostoma regium</i>	3.96	-	-	-	-	20.09	-	3.03	20.10

Table 3. Heavy metal concentration (mg kg⁻¹) in the muscle tissue of *Chondrostoma regium* and acceptable values suggested by global standards

	Heavy metals								
	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
FAO, 1983	1.0	-	200.0	1.0	10	10.0	150.0	0.2	1.5
EPA, 1989	4.1	-	410	-	-	54	410	1.4	-
WHO, 1993	-	-	-	-	-	20	50	0.05	-
TFC, 2002	-	-	50	-	-	20	50	0.05	0.2
The current study	0.13	0.55	7.19	0.004	0.15	0.38	4.39	0.02	0.07

CONCLUSION

This study was performed to gain insight into heavy metal concentrations in *Chondrostoma regium* from the Batman Dam. The metal concentrations in the muscles of *C. regium* are lower than the acceptable values for fishes specified by the Food and Agriculture Organization (FAO, 1983), United States Environmental Protection Agency (EPA, 1989),

World Health Organization (WHO, 2004) and Turkish Food Codex (TFC, 2002), as seen in Table 3. It can be expressed that the muscle tissues of *C. regium*, which lives in Batman Dam Lake, is consumed as a nutrient by the local people and has economic importance for the region, are proper for human consumption and do not carry any risk. However, we believe that such studies should be conducted regularly in order

that the aquatic ecosystems we work in do not pose a potential risk for future environmental pollution and are a reference to future studies.

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