

Relationship of Concentrations of Some Heavy Metals with Fish Size in Muscle Tissue of *Carassius gibelio* (Bloch, 1782) from the Tigris River (Turkey)

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Abstract

The levels of some heavy metals in muscle tissues (As, Cr, Mn, Co, Ni, Cu and Cd) of *Carassius gibelio* collected from the Ilısu region of the Tigris River were analyzed using inductively coupled plasma mass spectrometry (ICP-MS/MS). All the metals in fish muscle were determined. The highest metal concentration in the muscle tissue was found to be Cu and the lowest Co. Regarding heavy metal levels and fish size there were remarkable positive and negative relationships. There were no gender differences in terms of heavy metal accumulation in muscle tissues of *Carassius gibelio* ($p > 0.05$). The amounts of metal in muscle tissues of the fish were found lower than the acceptable values in fish tissues.

Keywords: Heavy metals, *Carassius gibelio*, Fish size, Tigris River

Dicle Nehri'nden (Türkiye) *Carassius gibelio*'nun (Bloch, 1782) Kas Dokularındaki Bazı Ağır Metal Konsantrasyonlarının Balık Boyutu İle İlişkisi

Öz

Bu çalışmanın amacı, Eylül 2018 tarihinde Dicle Nehri'nden toplanan *Carassius gibelio*'nun kas dokularındaki (As, Cr, Mn, Co, Ni, Cu ve Cd) bazı ağır metal düzeylerinin endüktif eşleşmiş plazma kütle spektrometresi (ICP-MS/MS) ile belirlenmesidir. Balık kas dokularında analiz edilen tüm metaller belirlenmiştir. Kas dokularındaki en yüksek metal konsantrasyonu Cu ve en düşük Co olarak tespit edilmiştir. Ağır metal seviyeleri ile balık boyutu arasında önemli pozitif ve negatif ilişkiler bulunmuştur. *Carassius gibelio*, kasta ağır metal birikiminde cinsiyet farkı göstermemiştir ($p > 0.05$). Balıkların kas dokularındaki metal düzeyleri balık dokularında kabul edilebilir değerlerden düşük olduğu tespit edilmiştir.

Anahtar Kelimeler: Ağır metaller, *Carassius gibelio*, Balık boyutu, Dicle Nehri.

1. Introduction

Today, one of the most important dangers for all living things in the ecosystem is environmental pollution. Environmental pollution emerged especially with the start of urban life and increased in parallel with the industrial revolution [1, 2]. Especially in the century we live in, the industrial, agricultural and social activities of human beings cause the pollution of the environment and the deterioration of the balance of the ecosystem. As a result, the aquatic ecosystem is affected the most from this pollution in our country as in the whole world. Lakes and streams with potable water started to decrease; Many wetlands have been destroyed by pollution [2, 3].

Metals, which are densely found in the aquatic ecosystem, cause a wide range of pollution due to their presence in many different substances. As a result of their very long stay in nature and easy entry into the living body through the food chain, they can accumulate intensely in the body and cause negative effects [4].

Fish tissues contain many heavy metals. They absorb them through the gills, skin and digestive system [5]. Ingested heavy metals are transported to tissues and organs through the blood pathway, bound to carrier proteins, bound by metal-binding proteins in the tissues and reach high amounts [6]. The amount of heavy metals accumulated in fish varies according to the age of the fish, body size, the stage of development, metabolic activities, interaction process with heavy metal, amount of metal in the environment, tissue and organ where it is accumulated [7].

Carassius gibelio (Bloch, 1782) is belonging to *Cyprinidae* family. *C. gibelio* is an extremely rapidly growing invasive species of freshwater ecosystems, especially in Europe and the Middle East. *C. gibelio*, which joined the inland water fauna of Turkey by way of the Meriç River in the 1980s, has been contaminated almost all of Turkey inland waters, especially with the fisheries studies carried out by the authorized public institutions with *C. carpio*.

Tigris River is among Turkey's most important and biggest streams. However, there are contamination concerns thought to be created by a variety of anthropogenic activities throughout the river. The primary causes of the Tigris River contamination are domestic and industrial polluters, along with local agricultural [8]. There is research on heavy metal accumulation in the Tigris River fish, nonetheless, these studies have been confined to only a few elements and the Tigris River's upstream area [9, 10]. This research investigates the heavy metal accumulation, such as As, Cr, Mn, Co, Ni, Cu and Cd, in the muscle tissues of *C. gibelio* retrieved from downstream Tigris River and compares the rate of metal accumulation in the muscle tissues according to genders and fish length and weight to reveal any possible correlation.

2. Materials and methods

2.1. Study Area

The Tigris River, the study area, is an important river that originates in Turkey, has many branches, passes through Iraqi territory and merges with the Euphrates there and empties into

the Persian Gulf in Shatt al-Arab (Figure 1). The coordinates of the location where the samples were taken from are 37° 31 '15.57 "N 41° 50' 34.25"E. Locals use the water from Tigris River for fishing, watering crops and recreational purposes. Turkey is home to two different bodies of water on Tigris River as dams built to provide potential energy for Kralkızı and Dicle hydroelectric plants [11].

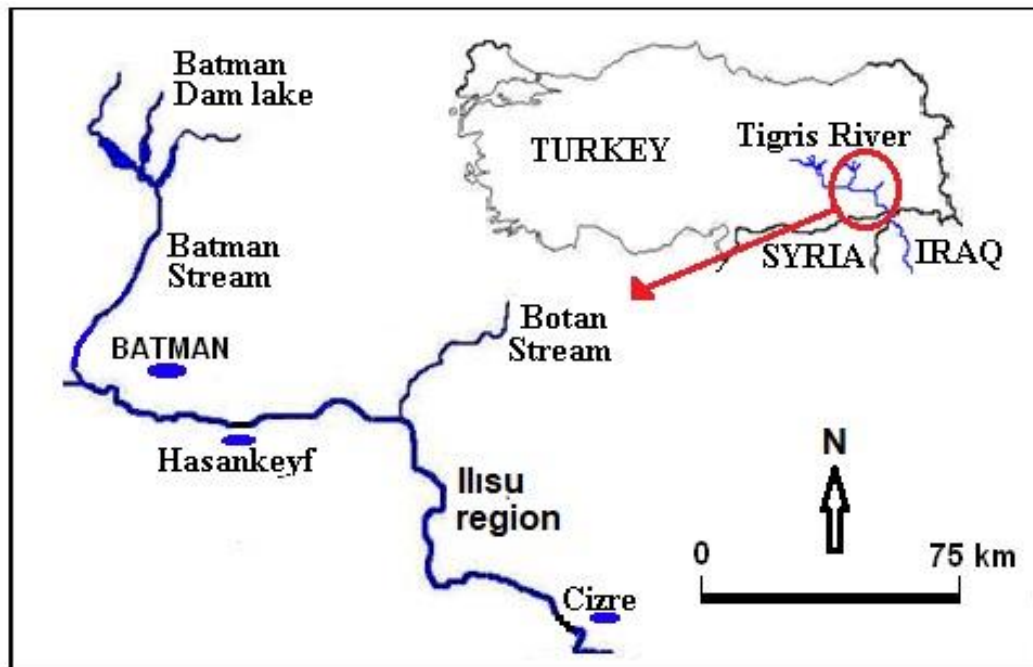


Figure 1. Map of the Ilisu region of the Tigris River, Turkey

2.2. Sample collection and analysis

Research sampling was carried out in the Ilisu region of the Tigris River (September 2015). Fish samples were obtained from the fishermen in the legally determined region. Supplied fish samples were brought to the laboratory on the same day with thermos containing ice molds. Standard forks lengths of the fish samples were measured on a board with a 50 cm long scale, and their weights were measured with 2 g precision market type digital electronic balance. The number, minimum, maximum, and mean values of fork length and body weight of the *C. gibelio* were shown in Table 1. Genders of the samples were determined visually post-dissection. The muscle tissues were kept at -20 °C, separately packed.

Table 1. Minimum, maximum, and mean values of fork length and body weight of the *Carassius gibelio*

Species	N	Female/Male	Fork length (cm)	Body weight (g)
<i>Carassius gibelio</i>	10	6 ♀ / 4 ♂	158.60±4.402 (150-165)	82.30±9.429 (66-94)

For the analysis of heavy metal (As, Cr, Mn, Co, Ni, Cu, and Cd) accumulations in muscle tissues, 0.5 g samples were placed in digestion tubes, then HNO₃ (7 mL) and H₂O₂ (1 mL) were added. Milestone Start D microwave digestion system was used for digestion and then the tubes were let cool down. After cooling down, the tubes were opened, and the contents were transferred to falcon tubes where they were diluted using ultrapure water (15 mL). The levels of heavy metals present in the digested muscle tissues were measured using inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) with an Agilent 8800 (USA). The tool consists of two hyperbolic profile quadrupoles separated by an octopole reaction system (ORS) cell, enabling for MS/MS operation. The first one filters mass and allows only the target analyte's mass go through and denies access to remaining matrix-derived and plasma-based ions. As a result, this arrangement effectively removes polyatomic and isobaric interferences. DORM-2 was used as the standard reference material to ensure the accuracy of the dissolution process and results of analysis in the muscle tissues of the fish. The mean recoveries ranged from 85.9 to 105.3%.

2.3. Statistical analyses

Student's *t*-test was used to determine significant differences in metal concentrations and males and females ($p < 0.05$). Pearson correlation test was conducted to check for significant relationships between metal concentrations and fish size (length and weight). Statistical analyses were carried out using SPSS 11.5 package program.

3. Results and Discussion

In the present study, As, Cr, Mn, Co, Ni, Cu, and Cd heavy metal levels were determined. Mean, standard deviation, minimum and maximum values of heavy metals determined in muscle tissues of *C. gibelio* obtained from Tigris River are given in Table 2. As a result of the analysis of fish samples, it was determined that the accumulation was Cu > Ni > Mn > As > Cd > Cr > Co according to the average values of metal concentrations in the muscle. The highest metal concentration in the muscle tissue was found to be Cu and the lowest Co. Metal values in muscle As 0.351 ± 0.031 mg/kg; Cr, 0.079 ± 0.018 mg/kg; Mn, 0.622 ± 0.073 mg/kg; Co, 0.016 ± 0.004 mg/kg; Ni, 1.117 ± 0.788 mg/kg; Cu, 2.607 ± 0.311 mg/kg and Cd, 0.086 ± 0.008 mg/kg. As you can see, the metal concentrations determined in muscle tissues were found to be quite low. Findings of this study are in line with earlier research that revealed that the concentration of heavy metals in muscle tissues is low [12, 13, 14, 15].

In fish, muscle tissue is the tissue with the lowest metal binding among fish tissues. The low rate of metal accumulation in muscle tissue can be explained by the absence of an active organ in the metabolism of fish [16]. Also, although muscle tissue in fish is not effective in binding heavy metals, it is very important to analyze muscle tissue because it is consumed by humans as food and its consumption poses a health risk [17].

Table 2. The heavy metal concentrations (mg/kg ww) in muscle tissues of *Carassius gibelio*

	As	Cr	Mn	Co	Ni	Cu	Cd
Minimum	0.319	0.055	0.50	0.01	0.351	2.19	0.076
Maximum	0.396	0.113	0.732	0.023	2.762	3.088	0.096
Mean	0.351	0.079	0.622	0.016	1.117	2.607	0.086
Standard	0.031	0.018	0.073	0.004	0.788	0.311	0.008

The relationships fish length and weight the heavy metal amounts detected in the muscle tissues of *C. gibelio* used in our study and the length and weight of the fish were determined using the Pearson correlation test and given in the Table 3. Considering the results obtained, it was determined that the relationships between fish length and weight and the amount of metal in the tissues showed a positive and negative correlation. While a significant positive correlation was determined between the amount of as in muscle tissue and length ($p < 0.05$), no significant correlation was found with weight ($p > 0.05$). In addition, the amount of Ni in the muscle was found to have a significant negative correlation in terms of both length and weight ($p < 0.05$). When Cr, Mn, Co, Cu and Cd concentrations were evaluated in terms of both length and weight, it was found that there was no significant correlation ($p > 0.05$). Likewise, many studies have revealed relationships (positive and negative) between metal levels in fish muscle tissue and their size. For instance, Tekin-Özan and Aktan [18] found that there was both a positive and a negative relationship between heavy metal levels in the tissues and organs of *Cyprinus carpio* from Işıklı Lake and its size. Merciai et al., [19] found that there was a negative and statistically significant relationship between the size of fish from Spain's Llobregat River and heavy metal content. Different research of [20] suggest that the negative size metal concentration correlation might be because old and young fish have different metabolic activities. Younger fish require higher levels of oxygen because provide energy is more active [21]. Furthermore, as per the *t*-test results, there was no significant difference between male and female fish with regards to metals tested ($p > 0.05$). There are several mechanisms that cause negative associations between TE concentrations and fish size. This can be explained by the fact that small fish have a faster metabolism than large fish, their immune systems are not developed, and they are more active in feeding. In addition, the high metal accumulation in the gills of small fish can be explained by the fact that small fish need more oxygen than large fish, and therefore more water passes through their gills for respiration. It has been observed that more metals accumulate in their bodies due to the fact that small fish are more active in metabolic activities than large fish [21].

Table 3. Pearson correlation coefficients between heavy metals and fish size (length and weight)

	Length	Weight	As	Cr	Mn	Co	Ni	Cu	Cd
Length	1								
Weight	0.609	1							
As	0.803 ^a	0.367	1						
Cr	-0.369	-0.389	0.099	1					
Mn	0.140	0.359	0.445	0.215	1				
Co	0.210	0.366	0.419	0.562	0.274	1			
Ni	-0.664 ^b	-0.777 ^b	-0.451	0.661 ^b	-0.412	0.167	1		
Cu	0.255	0.282	0.593	0.503	0.381	0.902 ^a	0.051	1	
Cd	0.070	-0.064	0.524	0.613	0.146	0.725 ^b	0.288	0.856 ^a	1

^a Correlation is significant at the 0.01 level

^b Correlation is significant at the 0.05 level

Table 4 presents the comparison of the levels of heavy metals found in *C. gibelio* tissue in this study and the results of other studies researching the concentration of heavy metal in riverine fish tissues. Töre et al., [22] found that *C. gibelio* exhibited significantly lower levels of As, Mn, Ni, Cu, and Cd but higher Cr and Co in muscle tissues in comparison to the same species in our study. In our study, in the muscle tissues of *C. gibelio* there were significantly lower levels of As, Cr and Mn when compared *Luciobarbus mystaceus* specimens collected from the Tigris River by Varol et al., [12], however, the Co, Ni, Cu, and Cd were higher. Mn, Ni, Cu and Cd levels in the muscle tissue samples of *C. gibelio* in this study were higher than the samples of *Barbus xanthopterus* retrieved from Atatürk Dam Lake by Alhas et al., [23]. Average Cr and Co levels measured in tissue by Alhas et al., [23] were greater than our study. The average As, Mn, Cu and Cd levels in the *C. gibelio* muscle tissues in this research were higher than the ones retrieved for *Carassius auratus* in the Xiang River in China by Jia et al., [14] and for *Abramis brama* in the Rivers in France by Noel et al., [24]. The research by Kaya and Türkoğlu [25] revealed that *C. gibelio* from Keban Dam Lake had significantly lower As, Mn, Ni, and Cd levels in their tissue when compared with this research but had higher levels of Cr and Co. Likewise, the average Mn, Co, Ni, Cu, and Cd (excluding Cr) found in *Chondrostoma regium* tissue from the Batman Dam in Turkey by Kaçar and Karadede-Akın [26] were significantly lower compared to our research. In this research, the average Cu, Mn, and Ni concentrations in the muscle tissue samples of *C. gibelio* were greater than those for *Tor grypus* in Atatürk Dam Lake (Turkey) but Cr and Co levels were lower [27].

Table 4. Comparison of the concentrations (mg/kg ww) of heavy metals in this study with those of fish from other rivers

Location	Species	As	Cr	Mn	Co	Ni	Cu	Cd	Reference
Tigris River, Turkey	<i>Carassius gibelio</i>	0.351	0.079	0.622	0.016	1.117	2.607	0.086	This study
Tigris River, Turkey	<i>Carassius gibelio</i>	0.02	0.78	0.10	0.04	0.16	0.89	0.01	[22]
Tigris River, Turkey	<i>Luciobarbus mystaceus</i>	0.398	0.081	0.856	0.008	0.657	2.311	0.083	[12]
Rivers, France	<i>Abramis brama</i>	0.109						0.004	[24]
Batman Dam, Turkey	<i>Chondrostoma regium</i>		0.139	0.55	0.004	0.15	0.38	0.02	[26]
Keban Dam Lake, Turkey	<i>Carassius gibelio</i>	0.150	0.198	0.165	0.033	0.280		0.006	[25]
Atatürk Dam Lake, Turkey	<i>Tor grypus</i>		0.22	0.45	0.15	0.16	0.56		[27]
Xiang River, China	<i>Carassius auratus</i>	0.042		0.215			0.118	0.021	[14]
Atatürk Dam Lake, Turkey	<i>Barbus xanthopterus</i>		0.12	0.20	0.09	0.08	0.27		[23]

In the present study, according to international criterias and Turkish regulation given, heavy metal concentrations in the muscle of *C. gibelio* were found to be lower than the acceptable limits. The acceptable values for fishes specified by the Food and Agriculture Organization (FAO), United States Environmental Protection Agency (EPA), World Health Organization (WHO) and Turkish Food Codex (TFC) are given in Table 5.

Table 5. Heavy metal concentration (mg/kg) in the muscle tissue of *Carassius gibelio* and acceptable values suggested by global standarts

	Heavy metals						
	As	Cr	Mn	Co	Ni	Cu	Cd
FAO, 1983	-	1.0	-	1.0	10	10	0.2
EPA, 1989	-	4.1	-	-	-	54	1.4
WHO, 1993	-	-	-	-	-	20	0.05
TFC, 2002	-	-	-	-	-	20	0.05
The current study	0.351	0.079	0.622	0.016	1.117	2.607	0.086

4. Conclusion

As a result of this study, it can be said that the muscle tissues of *C. gibelio* obtained from Ilisu region of the Tigris River are suitable for human consumption and do not carry any risk. Heavy metal concentrations were low in muscle tissues. Metal levels in muscle tissues and the size - length and weight- were correlated negatively and positively. Fish gender was not influential in terms of muscle tissues heavy metal accumulation. Our results were below the limits for fish proposed by [28, 29, 30, 31]. It can be stated that the muscle tissues of *C. gibelio* living in the Ilisu region of the Tigris River are consumed as food by the local people, are suitable for human consumption and do not pose any risk.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

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