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The First Study of the Effect of Metal Contamination on Human Health due to Consumption of *Capoeta capoeta* from the Cheshme Kile River in Northern Iran

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Authors
Hassan Malvandi, *Ph.D.*[1,2*]

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[1] Associate Professor, Department of Environmental Sciences and Engineering, Hakim Sabzevari University, 379 post box, 9617916487 Sabzevar, Khorasan Razavi, Iran.
[2] EthnoBiology Core (EBC), Hakim Sabzevari University, Sabzevar, Khorasan Razavi, Iran.

**** Correspondence***
Address: Associate Professor, Department of Environmental Sciences and Engineering, Hakim Sabzevari University, Sabzevar, Khorasan Razavi, Iran.
E-mail: h.malvandi@hsu.ac.ir
Tel: +985144012665

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ABSTRACT

Aims: The primary aims of the current research were to quantify the levels of some elements in the edible tissue of *Capoeta capoeta* and to evaluate the potential health risks to consumers using THQ, TTHQ, and CR indices.
Materials & Methods: For this purpose, samples of the mentioned fish species were taken along the Cheshme Kile River in Mazandaran province. A graphite atomic absorption spectrophotometer was employed to carry out the analysis of elements.
Findings: The mean levels of Cu, Co, Fe, and Ni elements were 0.039, 0.031, 4.451, and 0.987 $\mu g.g^{-1}$ ww, respectively. The mean concentrations of elements measured in the examined species were below the thresholds established by several international organizations, including the EPA, FDA, WHO, NOAA, and EC. The target hazard quotient levels ranged from 2.86×10^{-4} to 267.9×10^{-4}, and estimated weekly intake levels varied from 37.43×10^{-4} to 592.85×10^{-4}. Daily intake levels were between 0.0085 and 2.5313 $mg.kg^{-1}.day^{-1}$, while weekly intake levels ranged from 0.0595 to 17.7194 $mg.kg^{-1}.week^{-1}$.
Conclusion: Evaluating the measured contents against international benchmarks, along with the THQ, TTHQ, and CR_{lim} indices, suggests that consumers are unlikely to face any health risks. It is essential to acknowledge that this research represents the initial study on the health risk evaluation of these metals in this species. Therefore, future studies should evaluate and monitor the risks posed by various pollutants due to the consumption of this species in different aquatic ecosystems.

Keywords: Toxic Metal; Fish Consumption; Sea; Hazard Quotient; Index; Caspian Sea.

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Introduction

The excessive increase in population, urbanization, and agricultural and industrial activities have caused vast amounts of pollutants to enter the river ecosystems through different ways and finally enter the wetlands, lakes, and seas [1]. The health of aquatic species, especially fish and, ultimately, humans, is threatened due to the presence of pollutants in water bodies. Among the various pollutants introduced into marine environments, heavy elements are significant pollutants due to their persistent nature, resistance to degradation, toxic properties, and potential for bioaccumulation. In addition, they become biomagnified along the food chain. They can potentially lead to serious consequences for living organisms, especially the organisms at the top of the food chain, including humans [2,3]. These compounds can adversely affect several bodily functions. They may interfere with heme synthesis, disrupt endocrine function, compromise respiratory performance, and cause issues with blood circulation. Additionally, they can negatively impact bone and kidney health and affect the cells in the central nervous system [4,5]. Consuming aquatic animals, particularly fish, is advised because they are rich in vitamins like B12, D, and Omega 3, which are beneficial for enhancing health and assisting in the control of various diseases. However, eating fish that are contaminated or inhabit polluted waters is discouraged, as consuming such fish is a significant pathway for pollutants to enter the human body. Therefore, there are contradictions to consuming fish because some studies recommend consuming it because of its benefits, and some recommend not consuming it because of the risks involved. Given the situations described and the significance of consumer health, it is essential to evaluate the potential hazards of heavy metals from fish consumption in a thorough, holistic, and scientific way [6–10]. To achieve this goal and mitigate potential adverse health effects from heavy metals, various organizations-such as the Food and Agriculture Organization (FAO), European Community (EC), World Health Organization (WHO), National Oceanic and Atmospheric Administration (NOAA), and Food and Drug Administration (FDA)-recommend permissible limits. Additionally, it is advised to calculate indices like target hazard quotients (THQ), estimated weekly intakes (EWI), total target hazard quotients (TTHQ), estimated daily intakes (EDI), and maximum allowable fish consumption rates (CR)[11–14].

Rivers are considered the vital arteries of the planet. However, due to the entry of toxic pollutants, especially heavy metals, they are polluted, and as a result, the organisms dependent on them are affected. Consequently, researchers have focused on assessing heavy metals levels in river environments and associated aquatic life. For example, we can refer to the research of Malvandi (2017) in the Zarin Gol River [15], Allahabadi and Malvandi (2018) in the Tajan River [16], Majlesi et al. (2018) in the Khersan River, Iran [17], Khan and (2023) in the Swat River, Pakistan [18], Varol et al. (2020) in the Tigris River, Turkey [19], and de Melo Albuquerque et al. (2023) in the Perizes River, Brazil [20].

The Cheshme Kile River is one of northern Iran's most important and permanent rivers. The river plays a crucial role for aquatic organisms, particularly concerning both resident and spawning species. Nevertheless, the health of this vital ecosystem and its related organisms is at risk from various pollutants linked to human actions, including urban, rural, industrial, horticultural, and agricultural wastewaters, fish breeding stations, and mining activities [21,22]. Given the significance of heavy metals in river

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ecosystems and their detrimental impacts on the health of fish and humans, along with the insufficient data regarding heavy metal levels and risk assessment in fish residing in the Cheshme Kile River, this research was essential. In this research, we chose the species *Capoeta capoeta* for several reasons: (1) it is one of the most abundant species in the rivers of northern Iran, including Cheshme Kile; (2) it is frequently caught in significant quantities by local fishermen; (3) it has significant economic potential for aquaculture; and (4) it is easy to sample, requiring minimal effort.

As a result, the following goals were pursued in this research: a) to assess the levels of Co, Ni, Fe, and Cu in the muscle tissue of *C. capoeta* from the Cheshme Kile River, b) to estimate both the daily and weekly intake of the investigated elements, in addition to determining the allowable consumption of *C. capoeta*, c) to analyze the possible health risks associated with the studied elements for consumers by calculating THQ and TTHQ indices and comparing with international standards. It is essential to mention that this research represents the first study with these specific objectives focusing on the species *C. capoeta*.

Materials & Methods

Study Area

The Cheshme Kile River originates from the Takhte-Suleiman and Alamut mountains and finally flows into the Caspian Sea near the urban area of Tonekabon. This research selected five stations along the river from upstream to downstream, considering ecological characteristics, human activities, and the absence of polluting sources. However, *C. capoeta* was present only in two stations, 4 and 5. Station 4 in the middle of the river was considered an upstream station (this station was located upstream of concentrated residential areas and Tonekabon City), and station 5 was considered a downstream station (this station was located next to and downstream of Tonekabon City) (Figure 1).

During the initial month of summer, 36 fish samples were gathered, with 18 samples obtained from each of the two specified stations. These samples' total weight and length were measured at the respective stations. Eq. (1) was used to determine the required sample size for this research.

$$n \cong \frac{2\left(Z_{\alpha} \times Z_{\beta}\right)^{2} \times S^{2}}{d^{2}} \qquad \text{Eq. (1)}$$

where n represents the necessary sample size from both populations, Zβ is the standard normal deviate for the probability of a type II error, d is n represents the size of sample required from both populations -β, and S2 is the variance of measurements, Zα is standard normal deviate for the level of probability [23].

The samples were kept on ice before being stored at -24 ºC in the lab. To determine the concentration of elements, muscle tissues were separated from each sample and then thoroughly dried in an oven at 105 °C for 48 hours. Following that, the samples were ground into a powder, and 1 g of each sample was subjected to digestion using a combination of perchloric acid and nitric acid (1:3). Ultimately, the mixture was diluted to a final volume of 10 ml with deionized water [4]. Following that, the samples were ground into a powder, and 1 g of each sample was subjected to digestion using a combination of perchloric acid and nitric acid (1:3). Ultimately, the mixture was diluted to a final volume of 10 ml with deionized water [4,14]. Elemental analysis was conducted with a Shimadzu AA-6800 graphite atomic absorption spectrophotometer. Sampling and laboratory containers were immersed in 10% nitric acid for 48 hours and subsequently rinsed with deionized

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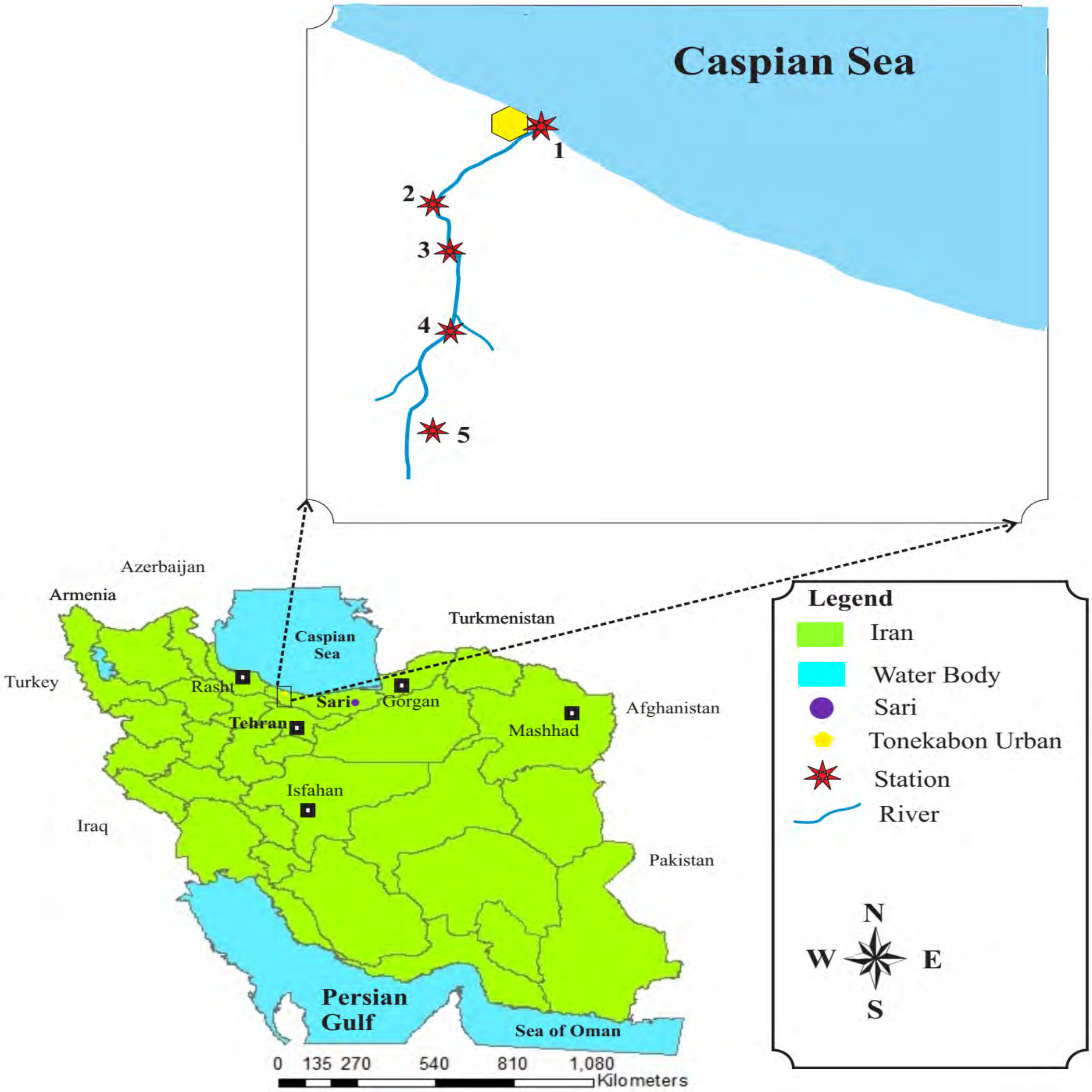

Figure 1) The Cheshme Kile River location and sampling stations, Mazandaran Province, Iran.

water in this study, which helped prevent contamination and ensured result accuracy. The Standard Reference Materials (SRM) 2711 and 1633b, along with blank samples, were employed to evaluate the precision and accuracy of the analytical methods. The findings indicated that recovery rates ranged from 92% to 107%, while the detection limit varied between 0.001 and 0.035 $\mu g.g^{-1}$ of wet weight. It should be noted that the elements Cu, Co, Fe, and Ni were selected based on available facilities, the presence of measurement equipment, laboratory limitations, and the focus of other similar studies on their measurement.

Statistical Analyses

During the initial phase of our study, we conducted a Kolmogorov-Smirnov test to evaluate the normality of the data distribution. In light of the data's non-normal distribution, the Mann-Whitney U test was employed to assess the differences in element concentrations between the upstream and downstream stations. A one-sample Wilcoxon

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Signed-Rank test was employed to analyze the values of the examined elements against the standard limits [7,24].

Risk Assessment of Studied Elements

Calculation of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI)

The values of EDI and EWI indices are obtained according to the concentration of the elements being studied in the species and the rate at which the species is consumed. These indices were calculated based on the Eq. (2) and Eq. (3):

$$EDI=\frac{(C \times IR_d)}{Bw} \quad \text{Eq. (2)}$$

$$TTHQ=THQ_{Co}+THQ_{Cu}+THQ_{Ni}+THQ_{Fe} \quad \text{Eq. (3)}$$

where C represents the concentration of analyzed elements within the tissues of *C. capoeta* ($\mu g.g^{-1}$ wet weight); IR denotes the daily consumption rate of fish ($g.day^{-1}$); Bw refers to the weight of a mean adult human (70 kg), while IRw indicates the weekly consumption rate of the examined species ($g.week^{-1}$) [13].

Target Hazard Index (THQ) and Total Target Hazard Quotient Index (TTHQ)

THQ and TTHQ are among the indices that are often used to evaluate the health risks faced by consumers from exposure to various elements. If the values of THQ and TTHQ are less than 1, it suggests that the affected population is not likely to suffer significant adverse health effects from the studied metals [4,7]. THQ and TTHQ indices were obtained based on the Eq. (4) and Eq. (5):

$$THQ=\left(\frac{FE \times ED \times FIR \times C}{RFD \times Bw \times AT}\right) \quad \text{Eq. (4)}$$

$$TTHQ=THQ_{Co}+THQ_{Cu}+THQ_{Ni}+THQ_{Fe} \quad \text{Eq. (5)}$$

where FE represents the frequency of exposure (365 $days.year^{-1}$), C is explained in previous indices, FIR denotes the amount of food consumption (38 g per $person.day^{-1}$), ED indicates the length of exposure (comparable to the mean lifespan), RFD refers to the oral reference doses (0.003, 0.04, 0.02 and 0.7 $mg.kg^{-1}.day^{-1}$ for Co, Cu, Ni and Fe, respectively), Bw is explained in the previous indices and AT signifies the mean exposure duration for non-carcinogens (ED multiplied by days per year) [13].

Maximum Allowable Fish Consumption

The highest allowable consumption rate of *C. Capoeta* was calculated using the Eq. (6):

$$CR_{lim}=\frac{(RfD \times Bw)}{C} \quad \text{Eq. (6)}$$

Where CR_{lim} represents the highest allowable limit of fish consumption ($kg.day^{-1}$), C, RfD, and other variables are defined in the earlier indices [14]. Also, the allowable number of fish servings per month was obtained from the maximum permissible limit of fish consumption based on the Eq. (7):

$$CR_{mm}=CR_{lim} \times \frac{T}{MS} \quad \text{Eq. (7)}$$

where CR_{mm} represents the highest permissible amount of fish consumption per month, measured in meals per month; the definition of CR_{lim} can be found in the earlier index; T and MS are the numbers of days in each month (averaged at 30.44 days) and the quantity of fish eaten in a single meal (0.227 kg), respectively [25].

In this study, the equation suggested by Malvandi et al. (2014), presented in Eq. (8), was used to convert the wet weight measurements of each sample into their corresponding dry weight values for easier comparison of mercury concentrations.

$$WWC=DWC\left[1-\left(\frac{PM}{100}\right)\right] \quad \text{Eq. (8)}$$

Where WWC refers to the concentration expressed as wet weight, DWC denotes the concentration represented as dry weight, and PM indicates the moisture percentage present in each sample [26].

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Findings

The mean concentration of Co, Cu, Ni, and Fe for *C. capoeta* species from the upstream station of Cheshme Kile River were 0.029, 0.955, 0.044, and 4.663 μg.g^{-1} ww, respectively, and those from the downstream station were 0.033, 0.035, 1.016, and 4.264 μg.g^{-1} ww, respectively (Table 1). The specimens' mean length and weight were 16.2 cm and 32.4 g at the upstream station and 14.5 cm and 27.5 g at the downstream station, respectively.

In Figure 2, the mean levels of Cu, Co, Fe, and Ni were compared in *C. capoeta* collected from the upstream and downstream stations of the river. The estimated daily intake (EDI) levels ranged from 0.0085 to 0.0168 mg.kg^{-1}.day^{-1} for Co, 0.0114 to 0.0239 mg.kg^{-1}.day^{-1} for Cu, 0.2797 to 0.5358 mg.kg^{-1}.day^{-1} for Ni, and 1.3035 to 2.5313 mg.kg^{-1}.day^{-1} for Fe. The estimated weekly intake (EWI) levels ranged from 0.0595 to 0.1178 mg.kg^{-1}.week^{-1} for Co, 0.0800 to 0.1672 mg.kg^{-1}.week^{-1} for Cu, 1.9578 to 3.7506 mg.kg^{-1}.week^{-1} for Ni, and 9.1246 to 17.7194 mg.kg^{-1}.week^{-1} for Fe (Table 2).

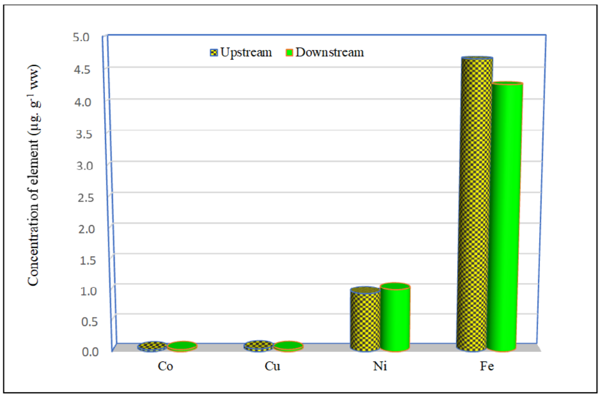

Figure 2) The difference in element concentration (μg.g^{-1} ww) in *C. capoeta* muscle tissue between upstream and downstream stations.

The values of the target hazard quotient (THQ) index were in the range of 28.31 × 10^{-4} and 56.1 × 10^{-4} for Co, 2.86 × 10^{-4} and 5.97 × 10^{-4} for Cu, 139.84 x 10^{-4} and 267.9 × 10^{-4} for Ni and 18.62 × 10^{-4} and 36.16 × 10^{-4} for Fe (Figure 3). The values of the total target hazard quotient (TTHQ) index were also in the range of 37.43 × 10^{-4} and 592.85 x 10^{-4} (Figure4).

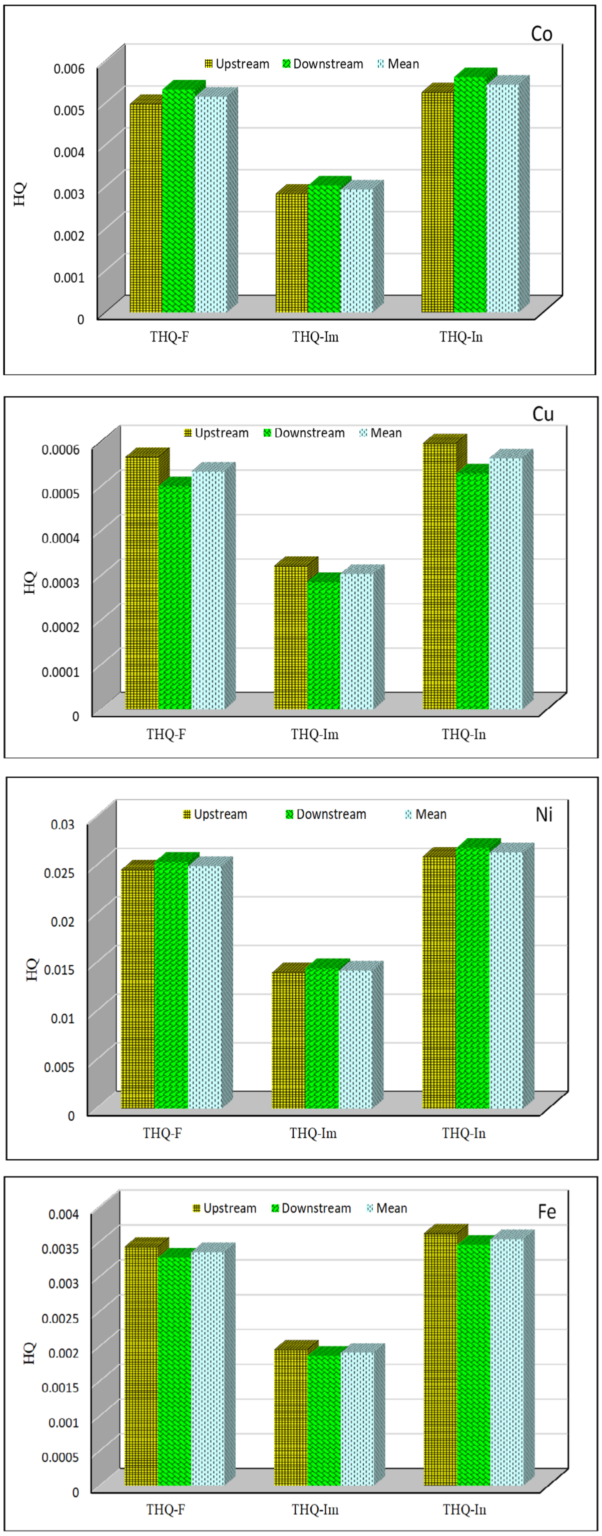

Figure 3) Estimated THQ for the studied elements caused by consuming *C. capoeta*. (THQ-F: Represents the HQ index derived from the FAO's Rate of Food Ingestion. THQ-Im refers to the HQ Index based on the food ingestion rate throughout Iran. THQ-In denotes the HQ Index calculated using the Food Ingestion Rate Specific to the Coastal Provinces in Northern Iran).

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Table 1) The Values of Some Metals (μg.g^{-1} ww) in *C. capoeta* Muscle Tissue from the Cheshme Kile River, Iran.

Station	Parameters	Elements			
		Co	Cu	Ni	Fe
Upstream	Mean	0.029	0.044	0.955	4.663
	S. D	0.005	0.007	0.141	0.886
	Min	0.008	0.015	0.284	1.529
	Max	0.067	0.075	2.007	13.604
Downstream	Mean	0.033	0.035	1.016	4.264
	S. D	0.007	0.015	0.132	0.839
	Min	0.008	0.004	0.186	0.683
	Max	0.139	0.137	2.509	12.123
Total	Mean	0.031	0.040	0.987	4.451
	S. D	0.004	0.009	0.108	0.547
	Min	0.008	0.004	0.186	0.683
	Max	0.139	0.137	2.509	13.604
Standards	FAO	0.5	30	55	180
	WHO	0.5	30	30	109
	FDA	2	-	70	-
	NOAA	2	149	52	250

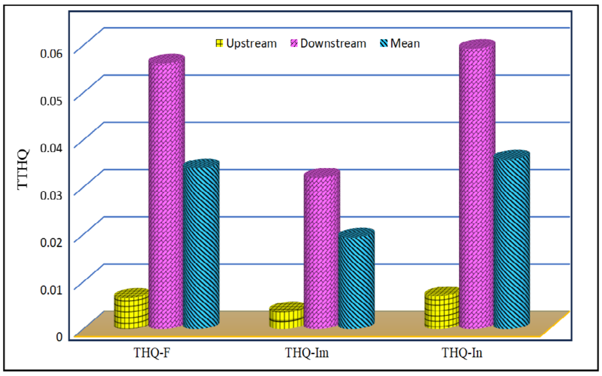

Figure 4) TTHQ Values for the Studied Elements for *C. capoeta*. The Cheshme Kile River, Iran.

Table 3 provides a detailed overview of the maximum recommended limits for fish consumption, broken down by daily, weekly, and monthly allowances. For Co, the daily limits were 7.0 kg for adults and 1.5 kg for children. The weekly limits were 49.0 kg for adults and 10.2 kg for children, with maximum monthly meal servings of 938 for adults and 194 for children. For Cu, the daily limits were 67.5 kg for adults and 14.0 kg for children, the weekly limits were 472.3 kg for adults and 97.8 kg for children, and the maximum monthly meal servings were 9,047 for adults and 1,874 for children. For Ni, the daily limits were 1.4 kg for adults and 0.3 kg for children. The weekly limits were 10.1 kg for adults and 2.1 kg for children, with maximum monthly meal servings of 193 for adults and 40 for children. For Fe, the daily limits were 10.8 kg for adults and 2.2 kg for children, the weekly limits were 75.3 kg for adults and 15.6 kg for children, and the maximum monthly meal servings were

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Table 2) The Values for the Indices of Estimated Daily Intake (EDI) in $mg.kg^{-1}.day^{-1}$ as well as Estimated Weekly Intake (EWI) in $mg.kg^{-1}.week^{-1}$ Concerning Heavy Metals in *C. capoeta*.

Element	Location	Index			Index		
		EDI_{F} [a]	EDI_{Im} [b]	EDI_{In} [c]	EWI_{F} [a]	EWI_{Im} [b]	EWI_{In} [c]
Co	Upstream	0.0149	0.0085	0.0157	0.1044	0.0595	0.1102
	Downstream	0.0159	0.0091	0.0168	0.1116	0.0636	0.1178
	Total	0.0154	0.0088	0.0163	0.108	0.0615	0.114
Cu	Upstream	0.0226	0.0129	0.0239	0.1584	0.0902	0.1672
	Downstream	0.0201	0.0114	0.0212	0.1404	0.08	0.1482
	Total	0.0213	0.0122	0.0225	0.1494	0.0851	0.1577
Ni	Upstream	0.4911	0.2797	0.5184	2.3981	1.3656	2.5313
	Downstream	0.5076	0.2891	0.5358	2.2891	1.3035	2.4163
	Total	0.4994	0.2844	0.5271	2.3436	1.3346	2.4738
Fe	Upstream	2.3981	1.3656	2.5313	16.7868	9.5592	17.7194
	Downstream	2.2891	1.3035	2.4163	16.0236	9.1246	16.9138
	Total	2.3436	1.3346	2.4738	16.4052	9.3419	17.3166

[a] According to the FAO, the food consumption rate is 36 g per $person.day^{-1}$.
[b] The mean food consumption rate throughout Iran is 20.5 g per $person.day^{-1}$.
[c] In the coastal regions of northern Iran, the food consumption rate is 38 g per $person.day^{-1}$.

Table 3) The Permissible Daily, Weekly, and Monthly Consumption Thresholds of *C. capoeta* for both Child and Adult Populations.

Elements	Sampling Station	Index					
		CR lim ($kg.day^{-1}$)		CR lim ($kg.week^{-1}$)		CR mm ($meals.month^{-1}$)	
		Adult	Children	Adult	Children	Adult	Children
Co	Upstream	7.2	1.5	50.7	10.5	971.1	201.2
	Downstream	6.8	1.4	47.4	9.8	908.4	188.2
	Mean	7.0	1.5	49.0	10.2	938.7	194.4
Cu	Upstream	63.6	13.2	445.5	92.3	8533.4	1767.6
	Downstream	71.8	14.9	502.6	104.1	9627.5	1994.3
	Mean	67.5	14.0	472.3	97.8	9047.5	1874.1
Ni	Upstream	1.5	0.3	10.3	2.1	196.6	40.7
	Downstream	1.4	0.3	9.9	2.1	190.2	39.4
	Mean	1.4	0.3	10.1	2.1	193.3	40.1
Fe	Upstream	10.5	2.2	73.6	15.2	1409.1	291.9
	Downstream	11.0	2.3	77.1	16.0	1476.2	305.8
	Mean	10.8	2.2	75.3	15.6	1441.9	298.7

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1442 for adults and 298 for children.

Discussion

The mean Co, Cu, Ni, and Fe concentrations for *C. capoeta* from the studied river were 0.031, 0.039, 0.987, and 4.451μg.g^{-1} ww, respectively. The concentration differences of the studied elements were evaluated among the fish samples caught from the upstream and downstream stations of the river (Figure 2). The findings showed no significant differences between the concentrations of elements in the studied species among the stations. Therefore, it can be said that there are probably no significant sources of pollution in the downstream stations, especially in the city of Tonkabon, for the studied elements. It can be said that the pollution sources at the two stations are similar, and they are most likely derived from natural and geological sources.

Similar results were reported in a study on *Lepomis gibbosus* and *Leuciscus cephalus* from the Saricay River, Turkey. In that study, no significant difference was found in metal concentrations between the upstream and downstream stations [27]. In contrast, in research conducted in the Liujiang River, China, metal levels in fish species, including *Cyprinus carpio*, *Siniperca chuatsi*, *Mystus guttatus*, *Acrossocheilus fasciatus*, and *Pseudohemiculter dispar*, were higher upstream than downstream [28]. Also, in studies conducted on the species *Phoxinus phoxinus* and *Leuciscus cephalus* from the Crișul Negru River, Romania [29], and *Oligosarcus hepsetus*, *Geophagus brasiliensis* and *Hypostomus luetkeni* from the Tropical Brazilian River, Brazil [30], the middle stations in the mentioned rivers had higher concentrations of metals than the upstream and downstream stations. The high concentration in the middle stations was due to untreated organic and industrial wastewater being discharged into the rivers. To assess the safety of fish consumption examined in this study, the concentrations of elements present in the muscle tissue of *C. capoeta* were compared with international standards. The findings indicated that the concentrations of elements in every sample fell below the acceptable limits established by the National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Agency (EPA), the World Health Organization (WHO), the European Community (EC), and the Food and Drug Administration (FDA) [31,32] (Table 1). Therefore, the results indicate that the elemental concentrations in the examined fish do not present a health risk to consumers. Similar results have been reported in other studies, for example, heavy metal levels in *Liza abu* and *Chondrostoma regium* from the Tigris River, Turkey [33], in *Euryglossa orientalis*, *Liza abu*, *Psettodes erumei* and, *Otolithes ruber*, from the Persian Gulf, Iran [32], and in *Lateolabrax japonicas*, and *Liza haematocheila* from the Yellow River, China [34] were lower than international standards. In contrast, some studies have reported different results. For example, Fe content in Liza abu from Karkheh River, Iran [35] and Ni content in *Euryglossa orientalis* from the Persian Gulf, Iran [32] were reported to be higher than the mentioned standards.

Since the values of the studied elements in *C. capoeta* have not been examined in any research so far, the values of the elements obtained in this species were compared with the concentrations of elements obtained from other species. Among the species mentioned in Table 4, the lowest concentration of Co was found in *Lateolabrax japonicus* from the Yellow River, China [34]. The mean concentration of Co in the present study was similar to the values obtained in *Liza haematocheila* from the Yellow River, China [34] and *Capoeta trutta* from the Tigris River, Turkey [33]. The mean concentration of Co in this study was lower than other species

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Table 4) Analysis of the Mean Levels of the Examined Elements in *C. capoeta* Compared to Their Levels in Various Other Fish Species.

Scientific Name	Elements				Sampling Location	Country	Reference
	Co	Cu	Ni	Fe			
Capoeta capoeta	0.031 [a]	0.039 [a]	0.987 [a]	4.451 [a]	CheshmeKile River	Iran	Present study
Liza abu	-	0.240 [b]	-	12.060 [b]	Karkheh River	Iran	(35)
Esox lucius	-	7.351 [c]	0.262 [c]	22.907 [c]	Siah Darvishan River	Iran	(38)
Euryglossa orientalis	0.62 [c]	5.29 [c]	42.05 [c]	73.82 [c]	Khuzestan shore, Persian Gulf	Iran	(32)
Otolithes ruber	0.63 [c]	4.09 [c]	42.01 [c]	87.02 [c]	Khuzestan shore, Persian Gulf	Iran	(32)
Rutilus frisii kutum	-	1.680 [a]	2.650 [a]	5.600 [a]	Tajan River	Iran	(39)
Liza abu	0.04 [a]	2.81 [a]	1.11 [a]	97.31 [a]	Tigris River	Turkey	(33)
Chondrostoma regium	0.04 [a]	0.75 [a]	0.22 [a]	16.55 [a]	Tigris River	Turkey	(33)
Cyprinion macrostomus	0.05 [a]	0.77 [a]	2.76 [a]	175.88 [a]	Tigris River	Turkey	(33)
Barbus rajanorum mystaceus	0.04 [a]	0.29 [a]	0.38 [a]	50.33 [a]	Tigris River	Turkey	(33)
Capoeta trutta	0.03 [a]	0.05 [a]	0.18 [a]	84.18 [a]	Tigris River	Turkey	(33)
Carassius gibelio	0.04 [a]	0.89 [a]	0.16 [a]	57.38 [a]	Tigris River	Turkey	(33)
Oreochromis niloticus	-	2.85 [c]	-	13.21 [c]	Nile River	Egypt	(40)
Lateolabrax japonicus	0.02 [a]	0.85 [a]	0.33 [a]	11.39 [a]	Yellow River	China	(34)
Liza haematocheila	0.03 [a]	0.96 [a]	0.40 [a]	18.99 [a]	Yellow River	China	(34)
Mormyrus rume	-	87.00 [b]	41.50 [b]	301 [b]	Igbokoda River	Nigeria	(37)
Heterobranchus longifilis		65.00 [b]	21.00 [b]	301 [b]	Igbokoda River	Nigeria	(37)
Anguilla labiate		10.560 [b]	0.322 [b]	1.927 [b]	Niger River	Nigeria	(36)
Heterotis niloticus		2.197 [b]	0.514 [b]	1.234 [b]	Niger River	Nigeria	(36)
Clarias garipinus		2.161 [b]	0.419 [b]	1.755 [b]	Niger River	Nigeria	(36)

[a] $\mu g.g^{-1}$ ww; [b] $\mu g.g^{-1}$; [c] $\mu g.g^{-1}$ dw

mentioned in Table 4. The lowest levels of Fe, Ni, and Cu were observed in *C. capoeta* from the Cheshme Kile River (current study), in *Carassius gibelio* from the Tigris River, Turkey [33], and *Heterotis niloticus* from the Niger River, Nigeria, respectively [36]. The highest levels of Fe and Cu were observed in *Mormyrus rume* and *Heterobranchus longifilis* from Igbokoda River Nigeria [37], and Co and Ni were found in *Euryglossa orientalis* and *Otolithes ruber* Khuzestan shore, Persian Gulf, Iran [32]. Several factors may contribute to the high values of Ni and Co in the last two mentioned species. We can mention many sources of pollution, including the presence of oil and gas wells, petroleum extraction activities, and marine transportation, especially the heavy traffic of oil tankers. Indeed, it is crucial to approach the comparison of element values across various species with care for these reasons: (1) variations in the species' positions within the food chain; (2) habitat difference; 3) diet difference; 4) age and size difference.

The values for estimated weekly intakes (EWI) and estimated daily intakes (EDI) were derived from the per capita fish consumption stated by the FAO, along with the mean per capita fish consumption figures for Iran and its northern coastal regions (Table 2). The lowest and highest values of EDI and WDI were related to Cu and Fe, respectively .Daily and weekly intake values were compared with the provisional tolerance daily intake (PTDI) and provisional tolerance weekly intake (PTWI) to assess consumer health risks better. PTDI and PTWI define the maximum levels of pollutant exposure that are deemed safe for individuals over their lifetime, ensuring they avoid significant health risks. Understanding these benchmarks is crucial for protecting our health and well-being. The recommended value of PTDI and PTWI is shown in Table 5. This study's findings showed that EDI and EWI values for an adult were lower than those of PTDI and PTWI. As a result, consuming *C. capoeta* does not present health risks to consumers.

Comparable findings have been reported in additional research. For example, EDI and EWI values in *Oncorhynchus mykiss* from Chaharmahal and Bakhtiari Province, Iran [31], in *Rutilus rutilus* from the Miankaleh wetland, Iran [41], in Oreochromis niloticus from Lake Kariba, Zambia [42], in *Mullus barbatus*, *Sardina pilchardus*, and *Solea solea*, from Mersin, Turkey [43], and *Sander lucioperca* and *Perca fluviatili* from the Caspian Sea [14] were lower than PTDI and PTWI values. In contrast, EDI and EWI values in *Tilapia zillii*, *Sarotherodon galilaeus*, *Oreochromis niloticus*, *Oreochromis aureus*, and *Larias gariepinus*, from Manzalah Lake, Egypt [44] were significantly greater than the PTWI values.

Table 5) The Provisional Tolerable Daily Intake (PTDI) ($mg.kg^{-1}.day^{-1}$) and Provisional Tolerable Weekly Intake (PTWI) ($mg^{-1}.week^{-1}.kg^{-1}$ body weight) Levels for Heavy Metals are Established According to FAO/WHO Guidelines.

Elements	PTDI[a]	PTDI[b]	PTWI[a]	PTWI[a]
Co	-	-	-	-
Cu	0.500	35.000	3.500	245.000
Ni	0.035	2.450	0.245	17.150
Fe	5.600	392.000	39.200	2744.000

[a] PTDI: Provisional Tolerable daily intake
[b] PTDI: For a 70 kg adult
[c] PTWI: Provisional Tolerable Weekly Intake
[d] PTWI: For a 70 kg adult

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Figures 3 and 4 show the values of THQ and TTHQ for the studied species individually. The THQ represents the risk of adverse effects from a specific contaminant per exposure unit, while the TTHQ aggregates these risks across multiple contaminants. Understanding these metrics is essential for assessing the environmental health and safety of the species in question. It is crucial to highlight that if the THQ and TTHQ values exceed one, it suggests that the consumption of the fish being studied may be hazardous to health.

As a result, based on the values of these indices in this research, it can be said that the consumption of *C. ca*poeta is safe and without risk to consumer health. Comparable findings have been observed in various studies across various fish species. For instance, the THQ values recorded for the species *Macrobrachium rosenbergii*, *Liza parse*, *Otolithoides pama*, *Pseudapocryptes elongatus*, *Notropis atherinoides*, *Rhinomugil corsula*, and *Apocryptes bato* from the Halda River, Bangladesh [45], *Otolithoides pama*, *Awaous grammepomus*, *Setipinna phasa*, *Polynemus paradiseus*, *Cirrhinus cirrhosus*, *Apocryptes bato*, *Macrobrachium rosenbergii*, *Metapenaeus dobsoni*, and *Neritina smithi* from Karnaphuli River, Bangladesh [46], *Alosa spp*. [4], *Chelon saliens*, *Chelon auratus*, *Persian sturgeon*, and *Stellate sturgeon* [7] from the Caspian Sea, Iran, along with sixteen different fish species from the Mediterranean Sea [47] were all found to be below one.

The daily, weekly, and monthly limits for fish consumption for adults and children were established and are shown in Table 3. The permissible levels of fish intake for adults varied from 1.4 to 71.8 kg.day^{-1} and from 9.9 to 502.6 kg.week^{-1}. For children, the allowable limits varied from 0.3 to 14.9 kg.day^{-1} and from 2.0 to 104.1 kg.week^{-1}. Additionally, the maximum allowable monthly consumption of *C. capoeta* ranged from 39 to 194 and 190 to 9627 meals.month^{-1} for children and adults, respectively. According to the United States Environmental Protection Agency (USEPA, 2000), consuming more than 16 monthly meals is considered safe fish consumption [48]. Therefore, adults and children can safely consume at least 39 meals of *C. capoeta* fish from the studied river per month.

Various studies have documented the permissible limits for monthly fish consumption for different species. For instance, the reported values indicate that children can safely consume *Rutilus frisii kutum* up to 9 meals, while adults may have up to 42 meals. Similarly, for *Chelon saliens*, the limits are 29 meals for children and 138 meals for adults, whereas, for *Chelon auratus*, the figures are 27 meals for children and 117 meals for adults. In the case of *Acipenser persicus*, the recommended consumption is five meals for children and 26 meals for adults. At the same time, *Acipenser stellate* limits 11 meals for children and 51 meals for adults [7]. Additionally, for *Alosa spp*., the suggested consumption is 25 meals for children and 5 for adults [4]. These variations underscore the importance of considering age and species when making dietary choices regarding fish consumption.

Conclusion

Overall, based on the results obtained, the levels of cobalt (Co), iron (Fe), nickel (Ni), and copper (Cu) in the muscle tissue of *C. capoeta* were within acceptable limits as defined by international standards. Therefore, consuming this species does not pose a significant health risk to consumers. The values of THQ, TTHQ, and CRlim indices also indicated the safety of consuming this fish and the lack of health risks for consumers due to its consumption. Since the concentrations of various contaminants, including heavy metals, pesticides, and persistent organic pollutants in river ecosystems, are increasing, assessing and monitoring the potential risks of various pollutants due to the consumption of *C. capoeta* is advisable. Finally, it should be noted that we believe that this study is the first to evaluate the health risks of these metals in the fish species being studied.

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Ethical Permission: All the experimental procedures and sample collection complied with the ethical considerations of the legislation on the protection of animals used for scientific purposes.

Consent to Participate: “Not applicable”

Availability of Data and Material

“The datasets generated during and/ or analyzed during the current study are available from the corresponding author on reasonable request.”

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