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Bioaccumulation of heavy metals in *Capoeta tinca* fish and health risk assessment

Bioacumulación de metales pesados en peces *Capoeta tinca* y evaluación de riesgos para la salud

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ABSTRACT

The This study evaluates the potential human health risks associated with five heavy metals (Zn, Pb, Cu, Cd, and Cr) in *Capoeta tinca* fish. It assesses the heavy metal burden in the muscle, gill, and liver tissues of C. tinca, and estimates the potential health risks for consumers by employing estimated daily intake (EDI) and standard hazard ratios (THQ) related to heavy metal consumption. Fish and water samples were taken from three different Regions as Sincan Brook (Sivas–Hafik), Habeş Brook (Sivas–Zara), and Tozanlı Brook (Sivas–Hafik), Turkey. The heavy metal concentrations in the brook water were found to be higher than the established safe for safety threshold in all the sampling points. Besides that, the values were observed to be lower than the allowed limits. Considering the fish tissues, the Pb, Cd, and Cr concentrations were found to be higher than the safe limits predicted by WHO. The findings indicate that the liver of *C. tinca* fish exhibited the highest accumulation of heavy metals across all sampling areas. The highest heavy metal concentrations found in fish muscles were found to be (Cu) 2.51 ± 0.91 $\mu g \cdot g^{-1}$, (Cr) 0.45 ± 0.03 $\mu g \cdot g^{-1}$, (Cd) 0.88 ± 0.04 $\mu g \cdot g^{-1}$, (Pb) 2.04 ± 0.03 $\mu g \cdot g^{-1}$, and (Zn) 13.12 ± 1.08 $\mu g \cdot g^{-1}$. The descending order of heavy metal accumulation in gills was found to be Zn > Cu >Pb > Cd > Cr. Moreover, for each heavy metal, the Bio–concentration factor (BCF) index, Acceptable Daily Intake, EDI, and THQ (<1) values were found to be lower than the limits set in the international standards, indicating that no elements posing a threat to public health were encountered, thus not posing a short–term risk.

Key words: Water quality; fish quality; heavy metal; human health; risk assessment

ABSTRACT

Este estudio evalúa los posibles riesgos para la salud humana asociados con cinco metales pesados (Zn, Pb, Cu, Cd y Cr) en el pez *Capoeta tinca*. Evalúa la carga de metales pesados en los tejidos musculares, branquias e hígado de *C. tinca*, y estima los riesgos potenciales para la salud de los consumidores mediante el uso de la ingesta diaria estimada (EDI) y las razones de riesgo estándar (THQ) relacionadas con el consumo de metales pesados. Se tomaron muestras de peces y agua de tres regiones diferentes: Arroyo Sincan (Sivas–Hafik), Arroyo Habeş (Sivas–Zara) y Arroyo Tozanlı (Sivas–Hafik), Turquía. Las concentraciones de metales pesados en el agua del arroyo resultaron ser más altas que el umbral de seguridad establecido en todos los puntos de muestreo. Además, se observó que los valores eran más bajos que los límites permitidos. Considerando los tejidos de los peces, las concentraciones de Pb, Cd y Cr resultaron ser más altas que los límites seguros predichos por la OMS. Los resultados indican que el hígado del pez C. tinca mostró la mayor acumulación de metales pesados en todas las áreas de muestreo. Las concentraciones más altas de metales pesados encontradas en los músculos de los peces fueron (Cu) 2.51 ± 0.91 $\mu g \cdot g^{-1}$, (Cr) 0.45 ± 0.03 $\mu g \cdot g^{-1}$, (Cd) 0.88 ± 0.04 $\mu g \cdot g^{-1}$, (Pb) 2.04 ± 0.03 $\mu g \cdot g^{-1}$ y (Zn) 13.12 ± 1.08 $\mu g \cdot g^{-1}$. El orden descendente de acumulación de metales pesados en las branquias resultó ser Zn > Cu > Pb > Cd > Cr. Además, para cada metal pesado, se encontró que los valores del índice de bioconcentración (BCF), la ingesta diaria aceptable, la EDI y THQ (<1) eran más bajos que los límites establecidos en las normas internacionales, lo que indica que no se encontraron elementos que representaran una amenaza para la salud pública, por lo tanto, no representan un riesgo a corto plazo.

Palabras clave: Calidad del agua; calidad del pescado; metal pesado; salud humana; evaluación de riesgos

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INTRODUCTION

Water bodies are subjected to the influence of numerous pollutants, among which heavy metals stand out as particularly hazardous substances due to their elevated toxicity levels and carcinogenic properties, posing significant threats to both human health and the environment [1, 2]. Since they are not biologically degraded, as well as their bioaccumulation, persistence, and potential danger for aquatic life, and humans, the heavy metal pollution in water is of very significant importance [3]. Heavy metals causing degradation of water quality may be human-origin or nature-origin. The natural-origin heavy metals in waters originate from bedrock/soil transfer, erosion, volcanic eruption, and atmospheric precipitation. Human-origin heavy metal content in waters originates mainly from mining, industrial and agricultural activities, and domestic wastewaters [4]. The toxicity of any pollutant and its negative effects on the environment and humans depend on the concentration and exposure ways of pollutants. The heavy metal accumulation in tissues arises from the absorption of heavy metals in the aquatic environment by the organisms and transfer to humans through the food chain [5]. Thus, they may cause various diseases including life-threatening cancers. It is an important threat for humans and a fundamental source of concern. For this reason, water resources should be continuously monitored for sustainable management of water quality [6].

The remarkable change in agricultural and industrial activities in the last 30 years is one of the human-origin factors influencing the water and soil resources in Sivas (Turkey). Opening the lands for agriculture, salt accumulation in soil, intense use of fertilizers, erosion, and decrease in organic matter and plant diversity threaten the water resources as the most important environmental problems [2]. For this reason, the characteristics of waters from wetlands protected within the scope of RAMSAR convention and planned for aquaculture should be known and the ecological balance in waters should be protected. In order to take the required measures, the physical and chemical factors in the aquatic medium should be periodically investigated. Determining the water quality and water pollution is important especially for the media hosting intense aquatic life and being protected.

Since it contains high-quality protein, a low level of saturated fat, and vitamins and minerals, fish is an important food for a healthy life [7]. Moreover, since it includes a high level of omega-3 polyunsaturated fatty acids (PUFAs), fish plays an important role in the human diet [8]. On the other hand, in parallel with the advancement of technology and industry and growth of population, increasing domestic and industrial wastes mix into waters through various pathways, cause the pollution of water, and have many sea organisms be exposed to many toxic matters [9].

In previous studies, it was emphasized that, due to the chemical pollutants in sea products, the consumption of fish especially by children and pregnant women may pose significant health problems [10]. Moreover, the accumulation of heavy metals in the tissues and organs of fishes varies depending on the parameters such as species, metal, metal's environmental concentration, activity time, age, temperature, salinity rate, and pH [2, 11].

For this reason, the studies on determining the concentration of heavy metals in sea creatures from various media drew significant interest Worldwide and it was always emphasized that risk analysis should be periodically performed for this purpose. It was reported in the literature that, when they exceed the daily tolerable intake limit since they cannot be eliminated from the organisms through natural physiological pathways, heavy metals such as cadmium, lead, mercury, nickel, arsenic, and chromium causes toxic effects [12].

Capoeta tinca (Heckel, 1843; Anatolian Khramulya) is a species from the *Cyprinidae* family and it has a wide distribution in western Asia. In Turkey, it shows a wide dispersion in Northern and NorthWestern Anatolia, and, from hydrological aspect, they live in the systems that are connected to the Black Sea. *C. tinca* can easily adapt to the changes in water regime. Since it lives in both lotic and lentic habitats, it is a fish that is of economic value and found in natural and man-made lakes. It is widely preferred by consumers for its delicious meat [13, 14]. In a previous study, heavy metal concentrations (Ag, Cd, Co, Cu, Ni, Pb, and Zn) in *C. tinca* (muscle, skin, and liver) collected from Çamlıgöze Dam Lake were analyzed and it was reported that the metal concentrations of the fishes widely consumed by the community should be periodically monitored [13].

In the present literature review, no study examining the heavy metal concentrations of *C. tinca* collected from Sincan Brook (Sivas-Hafik), Habeş Brook (Sivas-Zara), and Tozanlı Brook (Sivas-Hafik) together could be found. Besides the health risk assessment, relating the heavy metal concentrations of *C. tinca*, which have been collected in these three Regions, to the water quality parameters is the novelty of the present study.

Within the purpose of this study, fish and water specimens were collected from three regions namely Sincan Brook (Sivas-Hafik), Habeş Brook (Sivas-Zara), and Tozanlı Brook (Sivas-Hafik). In this parallel, the aim of this study is to:

1. Assess the heavy metal burden (those posing a threat to public health and the most risky ones) in the muscle, gill, and liver tissues of *C. tinca*.
2. Estimate the potential health risk for consumers regarding heavy metal intake by using estimated daily intake (EDI) and standard hazard ratios (THQ).

MATERIALS AND METHODS

Study area

For this research, approval certifcate was obtained from Kastamonu University Animal Experiments Local Ethics Committee (Decision No: 19.12.2014/2014.10).

The study area consists of three stations. The first station is located in Habeş (Arap) Brook, the second one in Sincan Brook, and the third one in Tozanlı Brook. Habeş Brook the first station (40°16´53´´N | 37°19´14´´E) originates from the piedmont of Mount Kösedağ, whereas Sincan Brook – the second station (39°52´59´´N | 37°35´47´´E) from Mount Gülek and Tozanlı Brook the third station – (39°27´53´´N | 37°52´41´´E) from the western shoulder of Mount Kösedağ.

Collection of samples

Water samples

Water samples were collected on a monthly basis in years 2017 and 2018. A total of 72 water samples were examined. The samples were collected using 1 L polyethylene bottles (bottles were rinsed twice using deionized water) and transferred to the laboratory by using a portable icebox (Icepeak 300083 Icebox, 26L, Asorti, Turkey). The filtered water samples bottled in 100 mL were digested at 100°C with concentrated HNO_3 (20 mL). The digested water samples were cooled to room temperature, diluted, and then filtered using Whatmann–42 filter paper.

Fish samples

Throughout the study, we collected *C. tinca* species, which are extensively captured in the Region, on a monthly basis. It was formed three distinct groups, with each group consisting of 17 samples. The collected samples were carefully placed in polyethylene bags and transported to the laboratory under ice storage conditions. In the laboratory, the samples were subjected to various procedures including biometrics, dissection, and collection of fish tissue for heavy metal analysis. To ensure surface cleanliness, the samples were washed using tap water. Following the cleaning process, the fish tissue was isolated and finely diced using a stainless steel knife (North Knife). Afterward, the tissues underwent an additional cleaning process using deionized water and were left to air dry, allowing for the removal of excess water and debris. Subsequently, the dried tissues were homogenized in a food processor (Karaca Pro–Multimax 2000 W, Turkey), and a specific amount of 200 g of tissue was carefully stored (Nüve Fr 290,Turkey) at a temperature of -20°C for preservation. 5 g identified tissue (dry) was digested in analytical grade HNO_3:$HClO_4$: HCl (3:2:9) for 4–6 hours on a hot plate. Following digestion, the samples were cooled down and passed through filter paper for filtration. To prepare the samples for analysis, they were then diluted with distilled water up to a volume of 50 mL [6].

Experimental analysis

Measurements were carried out on atomic absorption spectrometer (AAS, AA 800 Series, Germany) equipped with a graphite furnace autosampler. AAS was used to measure Zn, Pb, Cu, Cd, and Cr in the samples [(water and fish] collected. The purity of standard and acetylene gases was 99.99 to 99.99%, respectively. Atomic signals for Zn, Pb, Cu, Cd, and Cr were measured in peak area mood. The concentration of heavy metals in water sample was calculated using the following formula.

$$Heavy\ metal\ concentration\left(\mu\frac{g}{mL}\right)=\frac{AAS\ reading\times V}{Volume\ of\ the\ sample\ (mL)}$$

where, V = volume of dilution solution

The concentration of heavy metals in fish tissue was calculated using the following formula;

$$Heavy\ metal\ concentration\left(\mu\frac{g}{mL}\right)=\frac{AAS\ reading\times V}{Weight\ of\ the\ sample\ (g)}$$

where, V = volume of dilution solution

Quality assurance and quality control

Heavy metal analysis followed the World Health Organization (WHO) standards. The calibration curve was guaranteed with the correlation coefficient (R^2), where, $Pb^{0.9992}$,$Cr^{0.9999}$,$Cu^{0.9996}$,$Cd^{0.9988}$.

Bioaccumulation factor

The bioaccumulation factors (BAF) are the ratio of heavy metals concentration in fish organ to that in water. BAF was determined using the formula suggested by Maurya *et al*. [6].

$$BAF=\frac{Concentration\ of\ heavy\ metals\ in\ fish}{Concentration\ of\ heavy\ metals\ in\ water}$$

Quantitative health risk assessment

The fish muscles are mainly consumed by the human population as food. Therefore, In this study used fish muscles for evaluating the human health risk through an estimated daily intake (EDI) of metals and target hazard quotients (THQ) [15].

Estimated daily intake of metals

The estimated daily intake of heavy metals was calculated using the following equation.

$$EDI=\frac{C\times V}{BW}$$

where, C is the mean heavy metals concentration in fish muscle ($\mu g\cdot g^{-1}$) of dry weight basis. For conversion from dry weight to wet weight, 4.8 conversion factor is taken [16]. FIR (Food Ingestion Rate) is the daily consumption of freshwater fish (gram per day: $g\cdot day^{-1}$) per capita. The average FIR was 0.019 g $person^{-1}$ day^{-1} (FAO, 2016). BW is the average body weight, 70 kg for adults [17, 18].

Target hazard quotient (THQ)

The THQ is the estimate of non-carcinogenic risk level due to heavy metals exposure. It was calculated using the following equation [17].

$$THQ=\frac{Efr\times ED\times FIR\times C\times 0.001}{RfD\times BW\times ATn}$$

where Efr (Exposure frequency) is 365 day^{-1}, and ED (Exposure Duration) is 70 years (as set for this study). RfD (Reference Dose) assesses the health risk of consuming fish, and ATn is the time of average exposure for non-carcinogenic (365 day × no. of exposure year) [2, 19].

Statistical analysis

The data were statistically analyzed using the statistical package SPSS (version 16.0). The mean ± standard deviations of the metal concentration in fish species were calculated. Regarding the correlation coefficient level, if $P<0.05$, it was evaluated as there was a statistically significant difference between the groups.

RESULTS AND DISCUSSION

Analysis of water quality and physicochemical parameters

The results of the physicochemical qualities of Brook water samples gathered from Sivas (Hafik, Divrigi, Zara) in three different sites (Tozanli Brook, Sincan Brook, Habes Brook) are shown in FIG. 1. In the aquatic environment, temperature stands out as a crucial parameter due to its significant impact on various physico–chemical factors. Temperature plays a vital role as it directly influences the metabolism and growth of fishes, which are ectothermic animals.

Being cold–blooded creatures, fish adapt their body temperature in response to the surrounding environment, thereby affecting their physiological processes [20]. The temperature of the brook water was observed in the range between 22.70–5.80°C with an average temperature of 14.16°C. Temperature changes were parallel to seasonal transitions. Temperature data are compatible with other rivers of the Region [21]. Various research reports have documented that the construction of dams and barrages can lead to water blockage, consequently causing alterations in water temperature. The presence of dams/barrages has been associated with significant changes in the thermal characteristics of the water bodies, as reported in scientific studies [22].

FIGURE 1. Seasonal water quality parameters of water samples taken from three stations (Sincan, Tozanli, Habes). DO: Dissolved oxygen (mg·L^{-1}), Saltiness (%), pH, EC: Electrical conductivity, SSM: Suspended solid matter (mg·L^{-1}), COD: Chemical oxygen demand (mg·L^{-1}), NO_2:Nitrite (mg·L^{-1}), Cl: Chloride (mg·L^{-1}), PO_4: Phosphate (mg·L^{-1}), SO_4: Sulfate (mg·L^{-1}), Na: Sodium (mg·L^{-1}), K: Potassium (mg·L^{-1}), $CaCO_3$: Total hardness (mg·L^{-1}), TA: Total alkalinity (mg·L^{-1}), Mg: Magnesium (mg·L^{-1}), Ca: Calcium (mg·L^{-1}), Fe: Iron (mg·L^{-1}), AN: Ammonium nitrogen (mg·L^{-1}), NO_3: Nitrate (mg·L^{-1}), SO_3: Sulfide (mg·L^{-1}),T°C: Temperature

The pH values of the samples ranged from 8.32 to 8.97 with a mean value of 8.68. In same sites another study, Serefiye Dam pH was observed ranging from 7.83 to 8.27 and with an average of 8. The pH values of this study are in line with the standards. Studies have interpreted changes in pH values differently.

In the river, it was consistently observed that the pH values of water near sewage discharge points were generally lower compared to those measured in other sections of the river. This finding indicates a notable disparity in pH levels between the water samples collected at sewage discharge points and elsewhere along the river [6]. When assessing the quality of Brook water, the pH value emerges as the second most crucial parameter. Additionally, the survival and growth of aquatic organisms predominantly transpire within a limited range of water pH, underscoring its significance. Fish have a tolerance threshold for pH, with their survival typically within the range of 6.7 to 9.5. However, for optimal growth, fish thrive in an ideal pH range that spans from 7.5 to 8.5. The pH of water is extremely sensitive to changes in CO_2 ratio and nature of sediments. The pH of water is influenced by two main factors: the concentration of carbon dioxide (CO_2) dissolved in the water and the nature of sediments in aquatic environments. When CO_2 dissolves in water, it forms carbonic acid (H_2CO_3), lowering the pH and making it more acidic. Conversely, when CO_2 is released from water, the pH tends to rise, making it more alkaline. Sediments can affect pH by releasing ions from weathered bedrock, such as calcium, magnesium, and bicarbonate, which can buffer pH and prevent large fluctuations. However, the composition of sediments varies, and some may increase acidity while others contribute to alkalinity. Additionally, organic matter in sediments can influence pH through processes like decomposition, releasing acidic compounds. Fluctuations in these factors can impact the pH of water bodies, which in turn affects the survival and growth of aquatic organisms.Studies indicate that the alkalinity of water experiences fluctuations during both winter and summer seasons due to significant biological activity and the presence of ions from weathered bedrock on the water's surface, particularly in areas with slow flow rates [6]. The total alkalinity of the Brook water was observed in the range between 215–112 $mg \cdot L^{-1}$ with an average value of 160.250 $mg \cdot L^{-1}$ (FIG. 1).

The measurement of dissolved oxygen serves as a vital indicator of water purity. The quantity of dissolved oxygen present in water serves as a gauge for assessing the biological activity within aquatic environments, making it an essential parameter in water quality research and the regular functioning of water treatment facilities [2, 6]. The dissolved oxygen of the Brook water was observed in the range between 13.920–8.520 $mg \cdot L^{-1}$ with an average value of 11.198 $mg \cdot L^{-1}$ (FIG. 1). When FIG. 1 is examined in detail, the total hardness is high in the summer season (192.340 ± 20.286). It is thought to be caused by metal concentration in the water. Metal concentration can typically increase water hardness because hardness measures the total dissolved mineral ions in water. Metal ions, especially calcium and magnesium, can elevate water hardness by increasing the levels of total hardness. Therefore, the association of high total hardness values with water is directly linked to an increase in metal concentration in the water. On the other hand, ammonium nitrogen, nitrate, sulfide, nitrite, phosphate and sulfate ratios are affected by temperature and pH changes.

FIG. 2 illustrates the metal concentrations found in the water samples collected from three specific stations at the selected sites. The mean heavy metals load in the brook water of stations were in the following order Tozanli> Habes> Sincan. The mean heavy metals load in the all brooks were in the following order: Zn > Cu > Pb > Cd >Cr. Within the scope of this study, it was determined that all of the selected heavy metals, with the exception of zinc (Zn), were found to fall within the permissible limits set by the World Health Organization (WHO).

On the other hand, the lowest Cu concentration was observed at Sincan. The highest Pb concentration was found at Tozanli station followed by Habes, while the lowest Pb concentration was observed at the Sincan. The highest and lowest levels Cd concentrations were recorded at the Tozanli and Sincan sampling sites, respectively. Highest and lowest Cr concentrations were observed at the Habes and Sincan stations, respectively.

Literature studies have revealed that the environmental transport of heavy metals is predominantly governed by their interactions with water, sediments, and aquatic organisms, as well as their interplay with other metals and various environmental conditions. These intricate reactions play a pivotal role in shaping the dynamics of heavy metal transport within ecosystems [23, 24].

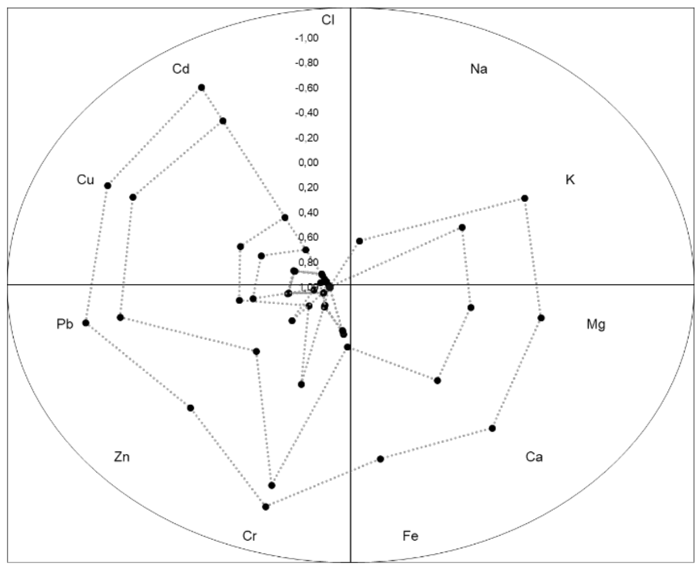

FIGURE 2. Inter–elemental correlation matrix of metals in the fish of the three stations (Sincan, Tozanli, Habes). Cl: Chloride (Cl^-), Cd: Cadmium (Cd^{2+}), Cu: Copper (Cu^{2+}), Pb: Lead (Pb^{2+}), Zn: Zinc (Zn^{2+}), Cr: Chromium (Cr^{3+}), Fe: Iron (Fe^{2+}, Fe^{3+}), Ca: Calcium (Ca^{2+}), Mg: Magnesium (Mg^{2+}), K: Potassium (K^+), Na: Sodium (Na^+)

Based on the data presented in FIG. 2, it is evident that the cadmium (Cd) levels in all the analyzed samples were found to be below the maximum permissible limits set by both the Turkish Standards (0.1 $mg \cdot kg^{-1}$) and the EU commission (0.05 $mg \cdot kg^{-1}$) for Cd concentration [24, 25, 26]. The water metal concentrations observed in this study were attributed to anthropogenic waste, industrial residue discharge, and the use of agricultural chemicals. These factors contribute to seasonal water pollution with heavy metals, posing a significant risk to the fish population due to the accumulation of these persistent pollutants.

Analyses of heavy metal concentrations in fish tissue

The concentrations of heavy metals in the *C. tinca* fish specy was in the magnitude order of liver >gill > muscle (F–1; Sincan, F–2; Habes, F–3; Tozanli). Fish muscles are widely consumed as a primary source of food Worldwide. The consumption of fish muscle is prevalent across the globe due to its nutritional value and culinary versatility. Fish muscle is preferred in canned food in the food industry. *C. tinca* fish consumed thought Region people. Coastal ecosystems in Regions characterized by intensive industrial and agricultural activities often exhibit elevated metal concentrations, as highlighted by Naser [27]. Within these ecosystems, aquatic organisms have a tendency to accumulate these metals within their bodies, further emphasizing the potential impacts of metal pollution on the marine food web.

Consequently, we specifically selected *C. tinca* fish species for this study and conducted analyses to assess their exposure to various heavy metals. Among the studied water stations, the highest concentration of zinc (Zn) was consistently observed, with Tozanli, Sincan, and Habes stations displaying progressively lower Zn levels. The hevy metal concentration trend was Zn>Cu>Pb>Cd>Cr in almost all fish groups.

The findings of our study align with the results reported in the literature, specifically corroborating the findings presented by Maurya *et al.* [6]. The presents study's outcomes provide additional support to the existing body of research, further strengthening the validity and reliability of the reported results in relation to the topic under investigation [6, 19]. Furthermore, significant disparities in heavy metal levels were observed across various water stations, as depicted in FIG. 2. Previous studies have identified that the variations in heavy metal concentrations can be attributed to factors such as fish species, fish age, seasonal fluctuations, and the overall quality parameters of the aquatic environment. These findings underscore the complex interplay of multiple factors influencing heavy metal accumulation in aquatic ecosystems [28].

Moreover, it is crucial to consider metal speciation, pH levels, and temperature as key factors when examining metal accumulations within aquatic systems. The interplay between metal speciation, pH, and temperature plays a pivotal role in determining the extent of metal accumulation and its potential impact on aquatic ecosystems. Therefore, these factors warrant significant attention and consideration in studies pertaining to metal accumulation dynamics. In this study chromium (Cr) levels among the selected area of fish tissue ranged from 0.27–0.45 $\mu g \cdot g^{-1}$. Levels of the Cr concentrations in muscle were recorded as 0.31 ± 0.02 $\mu g \cdot g^{-1}$ in *C. tinca* (Sincan), 0.33 ± 0.08 $\mu g \cdot g^{-1}$ (Tozanli) and 0.45 ± 0.03 $\mu g \cdot g^{-1}$ (Habes), in *C. tinca*, respectively. European Union Commission suggested the daily tolerable Cr concentration to be 1 $mg \cdot kg^{-1}$ [25], WHO and Federal Environmental Protection Agency (FEPA) commissions were suggested 0.15 $mg \cdot kg^{-1}$ [19, 29]. The Turkish Standards do not provide specific information regarding the maximum permissible intake of chromium (Cr) in fish. The regulatory guidelines for fish consumption in relation to chromium levels are not explicitly outlined in the Turkish Standards [30]. Additionally, in all samples Cr concentrations in muscle, gills and liver's are below the legal limit of EU commission [31]. In the literature, Jayaprakash *et al.* reported that the obtained Cr concentrations were 1.09 $mg \cdot kg^{-1}$ *Sillago sihama*, which were caught from the coast of India [32].

Copper (Cu) plays a vital role in the synthesis of hemoglobin and certain enzymes in the human body, highlighting its essentiality. However, excessive intake of copper can lead to adverse effects on the liver and kidneys, potentially causing damage to these vital organs. Copper (Cu) is vital for various physiological processes, including hemoglobin synthesis and enzyme function. While necessary in small amounts, excessive intake can lead to toxicity, primarily affecting the liver, kidneys, and nervous system. Hepatic effects may include hepatitis or cirrhosis, while renal damage can lead to tubular necrosis. Neurological symptoms like tremors and cognitive impairment may also occur. Excessive copper levels have been linked to oxidative stress and chronic diseases. Maintaining a balanced diet is crucial to avoid toxicity, especially for individuals with conditions like Wilson's disease. It is important to maintain a balanced and appropriate intake of copper to ensure its beneficial effects while avoiding potential harm [33]. The lowest Cu concentration was observed in the (Habes Brook) *C. tinca* with 2.14 ± 0.82 $\mu g \cdot g^{-1}$ in it is muscle, while the highest levels was found in *C. tinca* (Sincan Brook) 5.32 ± 1.02 $\mu g \cdot g^{-1}$ in it is gills. These findings indicate that the levels of copper (Cu) did not surpass the permissible limit recommended by international agencies, such as the Food and Agriculture Organization (FAO). The Cu concentrations observed in the study were within the acceptable range defined by the FAO, demonstrating compliance with the established guidelines set for safe consumption [34]. According to the World Health Organization (WHO) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the persistent elevation of copper (Cu) levels in the Brook ecosystem presents a significant and alarming health risk to human consumers through the consumption of fish. The continuous rise in Cu concentrations poses a serious threat to human well–being, underscoring the urgent need for mitigation measures to safeguard Public Health [35].

In the study, the Cadminium (Cd) concentrations in the muscle of *C. tinca* was determined to be between 0.61–0.88 $\mu g \cdot g^{-1}$; in the gills Cd levels were determined to be 0.81–1.07 $\mu g \cdot g^{-1}$; in the liver Cd concentrations were determined to be 0.86–1.19 $\mu g \cdot g^{-1}$. TABLE I shows, that the Cd concentrations of all examined samples were below the maximum allowed Cd levels by Turkish Standards which is 0.1 $mg \cdot kg^{-1}$ [24] and EU commission's allowed Cd concentration, which is 0.05 $mg \cdot kg^{-1}$ [26]. Cadmium (Cd) is a highly toxic and concerning contaminant that can be found in various sources and is transported through both water and air pathways. It poses a significant threat due to its detrimental effects on environmental and human health. Cd is known to be a serious pollutant with harmful implications, emphasizing the importance of monitoring and addressing its presence in the environment [18].

The lead (Pb) concentration ranged from 1.67±0.02 $\mu g \cdot g^{-1}$ to 2.18±0.23 $\mu g \cdot g^{-1}$ (same value Sincan Brook and Tozanli Brook) among the *C. tinca* from the study areas. The highest Pb concentrations were detected in liver for Sincan Brook and Tozanli Brook. The lowest Pb levels of gills tissue were detected in Tozanli Brook (2.04 ± 0.05 $\mu g \cdot g^{-1}$) (TABLE I). According to the Turkish Food Codex [36], TABLE I presents the recommended maximum tolerable concentrations of lead, set at 0.3 $mg \cdot kg^{-1}$. These guidelines serve as a reference for assessing lead levels and ensuring compliance with regulatory standards in relation to food safety and public health. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recommended a limit of 0.5 $\mu g \cdot g^{-1}$ for lead (Pb) in food, whereas the Federal Environmental Protection Agency (FEPA) has set a value of 2.0 $\mu g \cdot g^{-1}$. In several literature studies focusing on Iskenderun Bay, metal analyses were performed on a variety of fish species, revealing lead levels in the muscle and skin of *Solea lascaris* ranging from 0.39 to 2.09 $mg \cdot g^{-1}$.

These findings highlight the importance of assessing lead contamination in fish and considering the variations observed across different species [37]. The obtained findigs are appropriated with literature findings.

TABLE I
Concentrations of heavy metals (µg·g⁻¹ wet weight) in some organs of fish species collected from the three stations (Sincan, Habes, Tozanli)

	Cu			Cr			Cd			Pb			Zn		
	Muscle	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**
F–1	2.51 ± 0.91	5.32 ± 1.02	4.63 ± 1.23	0.31 ± 0.02	0.39 ± 0.01	0.28 ± 0.01	0.78 ± 0.03	1.02 ± 0.08	1.08 ± 0.02	2.04 ± 0.03	2.16 ± 0.02	2.18 ± 0.02	10.12 ± 0.88	16.13 ± 1.16	19.22 ± 1.05
F–2	2.14 ± 0.82	3.12 ± 1.15	3.12 ± 1.08	0.45 ± 0.03	0.38 ± 0.03	0.41 ± 0.02	0.61 ± 0.09	0.81 ± 0.02	0.86 ± 0.05	1.86 ± 0.08	2.06 ± 0.04	2.14 ± 0.03	11.17 ± 1.12	18.28 ± 1.12	20.12 ± 0.98
F–3	2.28 ± 0.86	3.08 ± 1.21	3.88 ± 1.24	0.33 ± 0.08	0.27 ± 0.02	0.38 ± 0.03	0.88 ± 0.04	1.07 ± 0.03	1.19 ± 0.06	1.67 ± 0.02	2.04 ± 0.05	2.18 ± 0.07	13.12 ± 1.08	17.63 ± 0.86	19.21 ± 1.13
FAO		30			1			0.5			0.5			30	
WHO		30			0.15			*			0.5			40	
FEPA		*			0.15			*			2			*	
CCFAC		*			*			0.5			0.2			*	
TFC		20			*			0.05			0.3			50	

(Mean ± SD), **FAO**: Food and Agriculture Organisation, **WHO**: World Health Organization, **FEPA**: Federal Environmental Protection Agency, **CCFAC**: Codex Committee on Food Additives and Contaminants, **TFC:** Turkish Food Codex, **F–1**: Sincan, **F–2**: Habes, **F–3**: Tozanlı

Zinc (Zn) plays a vital role in the functioning of crucial enzymes (carbonic anhydrase, transferrin, ferritin) in all living organisms. Among all the heavy metals analyzed, zinc consistently exhibited the highest levels across all stations. This finding underscores the prominence of zinc as an essential element and highlights its prevalence within the studied aquatic environment.

The highest (19.22 ± 1.05 µg·g⁻¹) and lowest (10.12 ± 0.88 µg·g⁻¹) concentration of Zn was observed in the liver and muscle of *C. tinca* (Sincan Brook), respectively (TABLE I). The highest Zn in the muscle (13.12 ± 1.08 µg·g⁻¹) and gills (18.28 ± 1.12 µg·g⁻¹) were observed in *C. tinca* (Tozanli Brook and Habes Brook), respectively. The FAO proposed a limit of 30 µg·g⁻¹ for Zn in food.

In a separate study, the concentration of zinc (Zn) revealed a heterogeneous pattern of heavy metal accumulation within various fish tissues, potentially attributed to the feeding behavior exhibited by different fish species [6]. Notably, some literature findings have reported exceptionally high levels of zinc concentration. However, our findings align with established standards limits, demonstrating compliance with regulatory guidelines and providing reassurance regarding the safety of the observed Zn concentrations.

Determination of bioconcentration factor and correlation analysis of heavy metal in *C. tinca* tissues

Bioconcentration factors (BCFs) in various fish tissues represent the relationship between the concentrations of heavy metals found in the tissues and the corresponding indicators of the surrounding water. These BCF values serve as ratios that provide insights into the extent of heavy metal accumulation within different fish tissues, shedding light on the potential bioaccumulation dynamics and the interaction between the fish and their aquatic environment [20] (TABLE II). In this study, BCFs of heavy metals in the *C. tinca* species and different station (Tozanli, Sincan, Habes Brooks) and different fish tissues (muscle, liver, gill). The analysis of *C. tinca* fish organ tissues revealed notable variations in the bioaccumulation of different heavy metals. Among the selected brooks (Tozanli, Sincan, Habes), the gills exhibited higher bioconcentration factors (BCFs), while the liver and muscle displayed lower BCF values. These findings suggest that the transfer of heavy metal concentrations from water to fish tissues occurs across all the studied areas. Overall, the BCF values, as shown in TABLE II, indicate that the levels of heavy metals in fish tissues follow the order of gill>liver>muscle. Consistent with the existing literature [38], metabolically active tissues such as gills, liver, and kidneys tend to exhibit higher accumulations of heavy metals compared to other tissues like skin and muscle.

TABLE II
Bio-concentration factor (BCF) index of the selected *Capoeta tinca* in different heavy metals

	Tissue	F–1	F–2	F–3
Cu	Muscle	0.533	0.454	0.484
	Gills	1.130	0.662	0.654
	Liver	0.983	0.662	0.824
Cr	Muscle	0.764	1.108	0.813
	Gills	0.961	0.936	0.665
	Liver	0.690	1.010	0.936
Cd	Muscle	0.848	0.663	0.957
	Gills	1.109	0.880	1.163
	Liver	1.174	0.935	1.293
Pb	Muscle	1.207	1.101	0.988
	Gills	1.278	1.219	1.207
	Liver	1.290	1.266	1.290
Zn	Muscle	0.638	0.704	0.827
	Gills	1.017	1.153	1.112
	Liver	1.212	1.269	1.211

F–1: Sincan, F–2: Habes, F–3: Tozanli

The correlation analyses, as illustrated in the Pearson's correlation matrix (FIG. 2), unveiled a statistically significant relationship between lead (Pb) and copper (Cu) concentrations ($r = 0.78$; $P<0.05$). This robust correlation indicates a potential interplay between the accumulation patterns of these two heavy metals within the samples of *C. tinca* collected from Sincan Brook and Tozanli Brook. The observed co–accumulation of Pb and Cu across various tissues suggests a complex interaction, possibly influenced by anthropogenic activities such as chemical–intensive industries and their associated waste discharge.

FIG. 3 shows that Scatterplot matrix of metals in the fish of the three stations (Sincan, Tozanlı, Habes).

Health risk assessment

TABLE III demonstrates that the average concentrations of chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) in the muscle, gills, and liver of *C. tinca* (Tozanli, Sincan, Habes Brooks) were

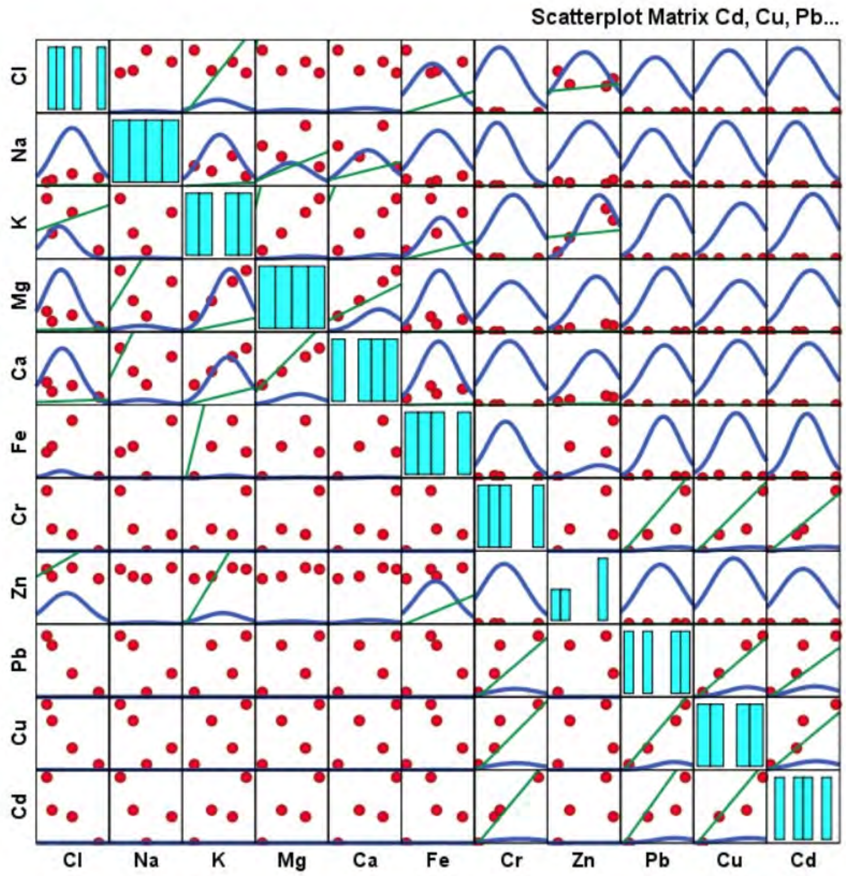

FIGURE 3. Scatterplot matrix of metals in the fish of the three stations

TABLE III
Target hazard quotient (THQ) estimated for individual heavy metals through consumption of from different area to *Capoeta tinca*

Heavy metals	Fish species	Average concentration	Recommended daily allowance mg·day^{-1}·70 kg^{-1} body weight	EDI 70 kg^{-1} body weight	RfD µg·kg^{-1}·day^{-1}	Target hazard quotient (THQ)
Cr	F–1	0.35	0.230	0.085	0.003	0.0469
	F–2	0.48		0.116	0.003	
	F–3	0.39		0.094	0.003	
Cu	F–1	4.15	35	0.251	0.040	0.1813
	F–2	5.10		0.314	0.040	
	F–3	4.88		0.301	0.040	
Cd	F–1	0.893	0.067	0.403	0.001	0.1723
	F–2	0.911		0.395	0.001	
	F–3	0.982		0.434	0.001	
Pb	F–1	2.036	0.248	0.287	0.0035	0.0392
	F–2	2.073		0.237	0.0035	
	F–3	2.101		0.240	0.0035	
Zn	F–1	16.168	70	2.311	0.300	0.1519
	F–2	15.215		2.173	0.300	
	F–3	16.205		2.340	0.300	

F–1: Sincan, F–2: Habes, F–3: Tozanli

significantly lower than the maximum allowable levels set by prominent regulatory bodies such as FAO, WHO, FEPA, EU, and Turkish Standards.

These findings indicate that the studied fish specimens are within the permissible limits for heavy metal concentrations, providing reassurance regarding their safety and compliance with established guidelines. The accumulation of heavy metals in fish poses an immediate concern, necessitating a thorough health risk assessment, particularly for fish sourced from contaminated environments. Given the toxicity of heavy metals and their potential impact on human health, various methods have been developed to evaluate the potential health risks associated with their consumption. These methods aim to assess and mitigate the risks posed by heavy metal exposure to individuals who consume these contaminated fish, ensuring the safety and well-being of the population [39].

TABLE III presents the calculated Target Hazard Quotient (THQ) values for each heavy metal, reflecting the potential health risks associated with the consumption of *C. tinca* from different areas. The THQ values serve as indicators of the extent to which the intake of these heavy metals may pose hazards to human health. The acceptable guidline value for THQ is 1 [17]. The estimated daily intake of Cr, Cu, Cd, Pb and Zn were below the guideline references doses of 0.003, 0.040, 0.001, 0.0035 and 0.3, respectively.

CONCLUSION

Based on the findings of this study, it has been identified that the study area encompassing Sincan, Tozanli, and Habes Brooks is subject to significant pollution pressure. In accordance with the guidelines outlined in the RAMSAR convention, it is imperative that strict implementation of protective laws is carried out to mitigate ecological degradation and restore the ecological balance of these regions. Despite the overall good water quality observed in all three sampling regions, the biological accumulation of heavy metals in *C. tinca* specimens surpassed the maximum limits established by prominent regulatory bodies including FAO, WHO, FEPA, CCFAC, TFC, and EC, particularly for Cd and Pb. To prevent further pollution of the analyzed water resources and maintain the natural ecological balance comprising native fish populations and other aquatic organisms, periodic monitoring is essential. Implementing a comprehensive monitoring program that incorporates thorough data analysis would offer valuable insights for effective water quality management in the lake.

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Data availability statement

The data are available by the corresponding author upon.

Conflicts of interest

The authors declare no conflict of interest.

Sample availability

Samples of the compounds are available from the authors.

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