

Comparison of heavy metal levels of farmed and escaped farmed rainbow trout and health risk assessment associated with their consumption

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Abstract In this study, levels of ten metals (arsenic, cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead, and zinc) in muscles of farmed and escaped farmed rainbow trout (*Oncorhynchus mykiss*) in the Keban Dam Reservoir (Turkey) were determined. Also, human health risks associated with their consumption were assessed. Of ten metals, only Co and Fe levels in escaped rainbow trout were significantly higher than those in farmed rainbow trout. The metal levels in farmed and escaped rainbow trout were below the maximum permissible limits. The estimated daily intake (EDI) of each metal in both farmed and escaped farmed rainbow trout was much lower than the respective tolerable daily intake (TDI). The target hazard quotient (THQ) values for individual metal and the total THQ values for combined metals were lower than 1 in both farmed and escaped rainbow trout, indicating no health risk for humans. The cancer risk (CR) values estimated for inorganic As in both farmed and escaped rainbow trout indicated low carcinogenic risk to the consumers. According to the maximum allowable monthly consumption limits (CR_{mm}), adults may safely consume 24 meals of farmed rainbow trout per month or 39 meals of escaped rainbow trout per month, with minimal adverse carcinogenic and non-carcinogenic health effects. This study revealed that the risk

from consuming farmed and escaped farmed rainbow trout in the Keban Dam Reservoir due to these trace elements is minimal.

Keywords Rainbow trout · Metals · Health risk assessment · Carcinogenic and non-carcinogenic effects · Fish consumption advisories

Introduction

Fish consumption has positive human health benefits since fish contain high levels of omega-3 polyunsaturated fatty acids that are not commonly found in other foods. Omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) in fish may prevent cardiovascular diseases and inflammation, maintain brain and eye health, and decrease the risk of depression, Alzheimer's disease and diabetes (Neff et al. 2014; Assisi et al. 2006). Consequently, global demand for fish has been increasing rapidly in recent years. However, fish can also contain contaminants, such as heavy metals, which may have harmful effects on human health. Some heavy metals, such as Cu and Zn, have an important role in all living organisms, but they can produce toxic effects when their levels are above optimal levels, whereas As, Cd, and Pb have not beneficial effects on human health and are toxic even at low concentrations (Copat et al. 2013; Rahman et al. 2012; Kalantzi et al. 2016; Makedonski et al. 2017).

Because fish are at the top of the food chain in aquatic ecosystems, they accumulate metals in their tissues from the surrounding water and their diet. Therefore, fish are important bioindicators of heavy metal contamination in aquatic ecosystems. Heavy metals in fish can cause a significant risk to human health when consumed in amounts exceeding safe consumption levels. From the human health perspective, it is

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important to determine the concentrations of heavy metals in widely consumed fish species (Kelly et al. 2008; Kalantzi et al. 2016; Qin et al. 2015).

Heavy metals can be classified as carcinogen and non-carcinogen, which can cause carcinogenic and non-carcinogenic effects on humans. Some methods have been developed by USEPA for the assessment of human health risks for carcinogenic and non-carcinogenic metals associated with fish consumption. The target hazard quotient (THQ), and total THQ are used to evaluate the potential non-carcinogenic health risks. The USEPA also has reported cancer slope factors for carcinogenic metals to determine the lifetime cancer risk (CR) (Griboff et al. 2017; Yu et al. 2014; Saha et al. 2016). In addition, the USEPA (2000) has recommended that the maximum allowable fish consumption rates should be calculated to minimize carcinogenic and non-carcinogenic health effects.

Aquaculture is one of the fastest growing food sectors in the world due to the increasing global demand for fish, the rising human population, and the global decline in wild fish stocks. The aquaculture sector in Turkey started with the farming of rainbow trout (*Oncorhynchus mykiss*) in the early 1970s (FAO 2005). Today, Turkey is one of the most important producers of rainbow trout in Europe with the total annual production of 108,038 tons, which is about 45% of the country's total aquaculture production. Rainbow trout is the main freshwater fish species farmed in Turkey and is cultured mostly in net cages on dam reservoirs of Turkey (GDFA 2016).

Keban Dam Reservoir (KDR) is one of the most important dam reservoirs on the Euphrates (Firat) River in Turkey. The KDR is widely used for fishing, recreation, irrigation, and as sources of hydroelectric power. Also, it has the biggest rainbow trout production capacity in Turkey. Rainbow trout have been cultured in net cages on the dam reservoir since 2000. While the total annual production of rainbow trout in the KDR was 523 tons in 2004, it reached 19,709 tons in 2013 (Güner 2015). However, escapes of rainbow trout from the facilities into the reservoir occur each year due to holes in the nets or transportation of fish among cages. Therefore, both farmed and escaped farmed rainbow trout are extensively consumed by the local population.

Despite the increasing awareness of the differences in metal levels between farmed and wild fish, there is limited data available on this issue and the investigations comparing their metal contents are scarce (Urena et al. 2007; Alam et al. 2002; Minganti et al. 2010; Kelly et al. 2008; Foran et al. 2004; Lundebye et al. 2017; Percin et al. 2011; Yildiz 2008). These investigations indicated that the variability in their environmental conditions and diet is regarded as the main reason for the observed distinctions. In addition, there are few studies on the differences between farmed and escaped farmed rainbow trout in terms of metal levels (Fallah et al. 2011; Karataş 2014).

The main objectives of this study were to determine and compare the levels of heavy metals in farmed and escaped

farmed rainbow trout in the Keban Dam Reservoir, to assess human health risks for carcinogenic and non-carcinogenic metals ingested via consumption of farmed and escaped rainbow trout, and to estimate bioconcentration factors for each metal. For these purposes, in this study, the concentrations of ten heavy metals (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were determined in the muscles of farmed rainbow trout (*O. mykiss*) from four fish cage facilities and escaped rainbow trout from seven sampling sites in the Keban Dam Reservoir. In addition, the concentrations of these elements were measured in water samples from cage sites and sampling sites.

Materials and methods

Study area

The KDR was formed on the Euphrates River in eastern Anatolia. It is the second largest reservoir in Turkey. Located between latitudes 35°20' and 38°37'N and longitudes 38°15' and 39°52'E, the KDR has a surface area of 675 km² and a volume of 30.6 km³ at 845 m above sea level. The Keban Dam was erected for hydroelectric power generation in 1974. The KDR has a significant potential in terms of fisheries and aquaculture production (Güner 2015).

Sample collection and preparation

Between autumn 2014 and spring 2015, farmed rainbow trout (*O. mykiss*) were collected from four cage farming facilities located in the different regions of the Keban Dam Reservoir, while escaped farmed rainbow trout (*O. mykiss*) were collected from seven sampling sites on the KDR (Fig. 1). A total of 3–5 fish for each season were taken from each cage facility and sampling site. Immediately after collection, fish were transferred to the laboratory on ice boxes. Total lengths and body weights of the fish samples were measured (Table S1). Fish samples were dissected using a precleaned stainless steel knife and approximately 75 g of muscle tissue was taken, packed in labeled zip-lock bags, and then stored at –20 °C until analysis. In addition, water samples from four cage facilities and seven sampling sites were taken. Water samples were filtered and acidified to pH < 2 using suprapure nitric acid on the sampling day, and then they were stored refrigerated until analysis.

Analysis of fish and water samples

Approximately 1.0 g fish muscle sample was digested in teflon vessels with 8 mL HNO₃ (65%) and 2 mL H₂O₂ (30%) in a microwave digestion unit (MARSXpress, CEM). The microwave digestion program used is presented in Table S2. After cooling to room temperature, the digested samples were

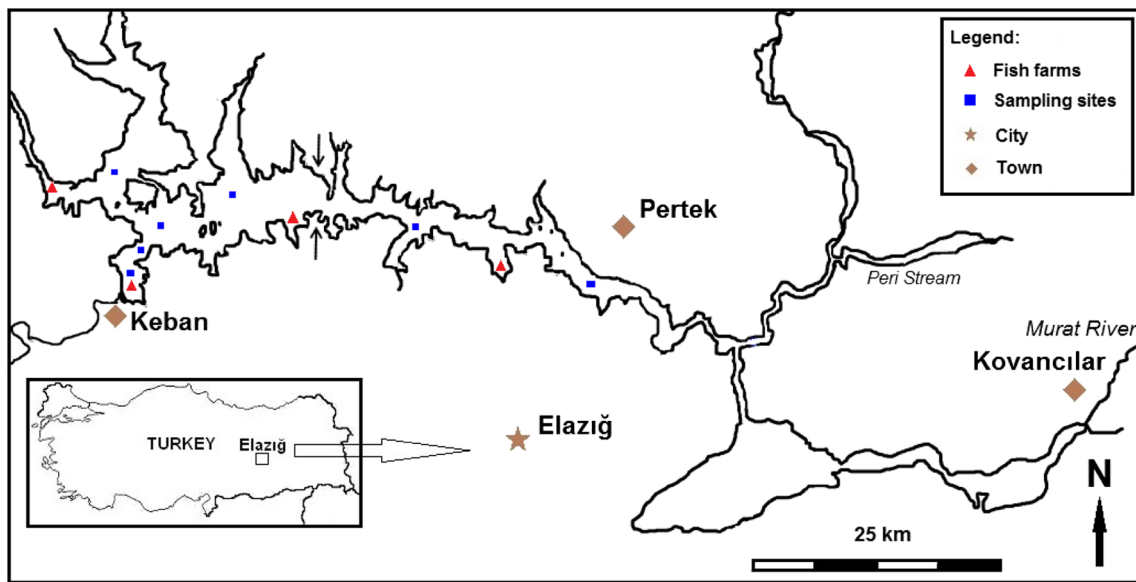


Fig. 1 Map showing study area, rainbow trout farming facilities and sampling sites for escaped rainbow trout

diluted to a total volume of 50 mL with ultrapure water. The fish extracts were analyzed for Co, Cr, Cu, Fe, Mn, Ni, and Zn by a flame atomic absorption spectrometry (Thermo Scientific iCE 3000). As, Cd, and Pb in extracts were measured by using a graphite furnace atomic absorption spectrometry (GFAAS) (Thermo Scientific iCE 3000). In water samples, GFAAS was used to determine concentrations of ten metals. Method accuracy was verified by analysis of certified reference material (CRM, TORT-3, lobster hepatopancreas, National Research Council of Canada). The percentage recoveries of ten elements ranged from 94.4 to 105.7% (Table S3).

Human health risk assessment

In this study, to assess the human health risks for carcinogenic and non-carcinogenic metals associated with fish consumption, multiple approaches were used: (1) estimated daily intake (EDI, $\mu\text{g}/\text{kg bw}/\text{day}$) (Copat et al. 2013; Griboff et al. 2017), (2) target hazard quotient (THQ) and total target hazard quotient (TTHQ) (Copat et al. 2013; Taweel et al. 2013; Alamdar et al. 2017), (3) lifetime cancer risk (CR) (Griboff et al. 2017; Saha et al. 2016; Monferran et al. 2016), (4) maximum allowable fish consumption limits (CR_{lim} , kg/day ; CR_{mm} , meals/month) (Yu et al. 2014; Copat et al. 2013), (5) comparison of mean concentrations of heavy metals with the maximum permissible levels for fish. Detailed information describing human health risk assessment methods and the equations for calculating EDI, THQ, TTHQ, CR, CR_{lim} , and CR_{mm} can be found in the text of [Supplementary Material](#).

In the present study, the mean concentrations of ten metals in the muscle of farmed and escaped rainbow trout were used for the calculations of EDI, THQ, CR, and CR_{lim} . An average body weight of 70 kg, an average daily fish ingestion rate of

20 g/day (GDFA 2016), and a meal size of 227 g (USEPA 2000) for adults were used for the estimation of EDI, THQ, CR, CR_{lim} , and CR_{mm} . THQ and CR_{lim} for non-carcinogenic effects of ten metals were calculated from the oral reference dose (RfD, $\text{mg}/\text{kg}/\text{day}$) of each metal (USEPA 2016); CR and CR_{lim} for carcinogenic effect of arsenic were calculated from the cancer slope factor (CSF, $\text{mg}/\text{kg}/\text{day}$) of arsenic (USEPA 2016). The risk factors (EDI, THQ, CR) and consumption limits (CR_{lim}) of arsenic were estimated only for inorganic arsenic which is the toxic form of As. The proportion of inorganic arsenic in fish is 1–10% of the total arsenic (Kalantzi et al. 2016). In the present study, we assumed that inorganic arsenic was 3% of the total As (Copat et al. 2013; Li et al. 2015). The CSF is 1.50 ($\text{mg}/\text{kg}/\text{day}$) for inorganic As (USEPA 2016).

Bioconcentration factor

The bioconcentration factor (BCF) expresses the accumulation in organism tissue of contaminants from water. It was calculated as (Tao et al. 2012):

$$\text{BCF} = C_{\text{fish}} / C_{\text{water}} \quad (1)$$

where C_{fish} is the metal concentration in the muscle of fish ($\mu\text{g}/\text{kg}$ wet weight) and C_{water} is the metal concentration in water ($\mu\text{g}/\text{L}$).

Statistical analysis

Analysis of variance (ANOVA) was performed to analyze the significant spatial differences in metal concentrations among seven sampling sites and among four fish cage facilities

($p < 0.05$). Also, a t test was used to evaluate differences in metal levels between farmed and escaped rainbow trout ($p < 0.05$). ANOVA and t test were performed by using SPSS 11.5 for Windows.

Results and discussion

The descriptive statistics of ten metals in farmed and escaped farmed rainbow trout are presented in Table 1. During the study period, the mean concentrations of ten metals in both farmed and escaped rainbow trout did not show significant spatial variations (ANOVA, $p < 0.05$). In this study, the total mean concentrations of ten metals varied from 11.55 to 13.62 mg/kg ww and escaped rainbow trout contained significantly higher total metal concentration than farmed rainbow trout (t test, $p < 0.05$) (Table 1).

Metals such as Cu, Zn, Fe, and Co are essential metals. These metals were the most abundant in the muscles of both farmed and escaped rainbow trout. Of the essential metals, Fe was the predominant metal in escaped rainbow trout, while Zn was the predominant metal in farmed rainbow trout. The contribution of Fe accounted for 35.1% of the total metal concentration in escaped rainbow trout. However, Zn accounted for 38.3% of the total in farmed rainbow trout. Of the essential metals, Cu had the lowest mean concentration in both farmed and escaped rainbow trout. Cu accounted for 3.6 and 4.5% of the total metal concentration in farmed and escaped rainbow trout, respectively. Metals such as As, Cd, and Pb are toxic metals. The concentrations of these metals were much lower compared with the essential metals. Of the toxic metals, As was the predominant metal, followed by Pb in both farmed and escaped rainbow trout. Cd had the lowest mean concentration, and it accounted for only 0.003% of the total metal concentrations in both farmed and escaped rainbow trout.

Heavy metal concentrations in the muscle of escaped rainbow trout followed the order of Fe > Zn > Ni > Mn > Cr > Co > Cu > As > Pb > Cd, while they followed the order of Zn > Fe > Ni > Mn > Cr > Co > Cu > As > Pb > Cd in farmed rainbow trout.

Metal levels in farmed and escaped rainbow trout

The mean concentrations of As ranged from 52.39 (escaped rainbow trout) to 85.52 (farmed rainbow trout) µg/kg ww. However, there was no statistically significant difference between levels of As in muscles of farmed and escaped rainbow trout (t test, $p > 0.05$) (Table 1). In a previous study, no significant difference was also found in levels of As between farmed and escaped rainbow trout (Fallah et al. 2011). According to Food Standards Australia and New Zealand (FSANZ 2013) and Chinese Health Ministry (MHPRC 2013), the maximum permissible limits for inorganic As were 2.0 and 0.10 mg/kg wet weight, respectively. In this study, inorganic arsenic concentrations (assuming inorganic As is 3% of total As) in both farmed and escaped rainbow trout did not exceed the maximum permissible limits established by FSANZ (2013) and MHPRC (2013) (Table 2). In addition, the mean As concentration in farmed rainbow trout was lower than that reported for farmed rainbow trout from Iran (Fallah et al. 2011), China (Jiang et al. 2016), and Czech Republic (Svobodova et al. 2002), whereas it was higher than that reported from the southern USA (Santerre et al. 2001). The mean As concentration in escaped rainbow trout was lower than that reported for escaped rainbow trout from Iran (Fallah et al. 2011), while it was comparable to that reported from Turkey (Celik et al. 2008) (Table 2).

The mean concentrations of Cd were low and comparable in farmed (0.38 µg/kg ww) and escaped rainbow trout (0.37 µg/kg ww) in the study (t test, $p > 0.05$) (Table 1) and

Table 1 Descriptive statistics of metal concentrations in the muscles of farmed and escaped rainbow trout (units µg/kg ww for As, Cd, and Pb, mg/kg ww for other metals and total metal)

	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Total
<i>Farmed rainbow trout (N = 12)</i>											
Min	4.26	0.11	0.38	0.58	0.06	1.72	0.32	0.36	10.49	3.58	8.73
Max	193.23	1.43	0.92	1.04	0.81	4.86	1.47	1.38	92.08	5.31	12.81
Mean	85.52 ^a	0.38 ^a	0.52 ^a	0.81 ^a	0.42 ^a	3.52 ^a	0.82 ^a	0.89 ^a	54.21 ^a	4.42 ^a	11.55 ^a
SD	53.13	0.42	0.18	0.13	0.21	0.89	0.37	0.32	19.30	0.53	1.15
<i>Escaped rainbow trout (N = 21)</i>											
Min	1.42	0.06	0.36	0.49	0.15	2.78	0.41	0.23	5.26	2.69	10.25
Max	142.14	1.80	1.38	1.16	1.18	8.73	1.43	1.77	83.56	7.01	17.90
Mean	52.39 ^a	0.37 ^a	0.72 ^b	0.85 ^a	0.61 ^a	4.78 ^b	0.95 ^a	1.20 ^a	51.5 ^a	4.41 ^a	13.62 ^b
SD	47.84	0.46	0.24	0.21	0.32	1.58	0.41	0.52	25.67	0.99	2.09

The different letters indicate statistical difference between farmed and escaped rainbow trout at $p < 0.05$; t test

Table 2 The mean concentrations of heavy metals in farmed and escaped rainbow trout measured in different studies and maximum permissible limits for fish (units mg/kg ww)

	iAs	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	
<i>Farmed rainbow trout</i>												
Turkey (Keban Dam Reservoir)	0.0026	0.086	0.00038	0.52	0.81	0.42	3.52	0.82	0.89	0.054	4.42	This study
Czech Republic		0.498–1.133										Svobodova et al. (2002)
Southern USA		0.053	nd	0.313	0.360	0.724				0.724		Santerre et al. (2001) ^c
Iran		0.234	0.024	0.047	0.141	5.453	3.867	1.566	0.095	0.277	5.243	Fallah et al. (2011) ^a
Portugal			0.013	0.23	0.23	0.42	4.5	0.18	0.02	0.05	6.3	Lourenço et al. (2012)
Turkey (Kuzgun Dam Lake)				0.116	0.116	0.828	0.913	0.232	0.085		4.365	Karataş (2014) ^a
China		0.605	nd	0.02	0.059	0.348	6.964	0.358	0.014	0.009	4.185	Jiang et al. (2016)
<i>Escaped rainbow trout</i>												
Turkey (Keban Dam Reservoir)	0.0016	0.052	0.00037	0.72	0.85	0.61	4.78	0.95	1.20	0.052	4.41	This study
Iran		0.280	0.033	0.054	0.158	2.100	8.115	3.483	0.102	0.300	11.686	Fallah et al. (2011) ^a
Turkey (Atatürk Dam Lake)		0.05	0.01	20.82		8.19	4.15	1.91	0.12	0.65	5.45	Celik et al. (2008)
Turkey (River Yeşilirmak)			0.11			0.475	8.425	0.70	0.575	0.053	11.55	Mendil et al. (2010) ^{a, b}
Turkey (Kuzgun Dam Lake)					0.149	2.031	2.459	1.712	0.110		8.688	Karataş (2014) ^a
<i>Maximum permissible limits</i>												
Food Standards Australia New Zealand	2.0									0.5		FSANZ (2013)
Chinese Health Ministry	0.1		0.1	2.0						0.5	30	MHPRC (2013)
Food and Agriculture Organization												FAO (1983)
European Commission			0.05							0.3		EC (2006)
Codex Alimentarius Commission										0.3		WHO/FAO (2015)

iAs: inorganic As; nd: not detected

^aOriginal values expressed in dry mass and here calculated in wet mass (wet mass = dry mass divided by 4)^bData are maximum^cData are mean for the positive samples (9 out of total 33 samples are positive for As, 12 samples for Cr, 29 samples for Cu, 5 samples for Pb)

well below the maximum permissible levels for Cd in fish established by the MHPRC (2013) and European Commission (EC 2006) (Table 2). Similarly, Fallah et al. (2011) reported that no statistically significant difference was observed in Cd levels between farmed and escaped rainbow trout. The mean concentrations of Cd determined in both farmed and escaped rainbow trout were much lower than those found in other studies of both farmed (Fallah et al. 2011; Lourenço et al. 2012) and escaped rainbow trout (Fallah et al. 2011; Mendil et al. 2010; Celik et al. 2008) (Table 2).

The mean concentrations of Co were between 0.52 and 0.72 mg/kg ww. The content of Co was significantly higher in escaped rainbow trout (t test, $p < 0.05$) (Table 1), in contrast to no significant difference in Co concentrations found by Fallah et al. (2011) in farmed rainbow trout compared to escaped rainbow trout. The mean Co concentration in farmed rainbow trout was found higher than that reported for farmed rainbow trout from Iran (Fallah et al. 2011) and China (Jiang et al. 2016). Also, the mean Co concentration in escaped rainbow trout was higher than that reported for escaped rainbow trout from Iran (Fallah et al. 2011), while it was found lower than that reported from Turkey (Celik et al. 2008) (Table 2).

The mean concentrations of Cr were comparable in farmed (0.81 mg/kg ww) and escaped rainbow trout (0.85 mg/kg ww) in the study (t test, $p > 0.05$) (Table 1). Similarly, Fallah et al. (2011) and Karataş (2014) reported that Cr concentration in escaped rainbow trout was not significantly different compared to farmed rainbow trout. The mean concentrations of Cr determined in both farmed and escaped rainbow trout were much lower than those found in other studies of both farmed (Fallah et al. 2011; Lourenço et al. 2012; Santerre et al. 2001; Jiang et al. 2016; Karataş 2014) and escaped rainbow trout (Fallah et al. 2011; Karataş 2014) (Table 2). The maximum permissible limit for Cr in fish set by the MHPRC (2013) is 2 mg/kg. Cr concentrations in both farmed and escaped rainbow trout were below this limit (Table 2).

The mean concentrations of Cu ranged from 0.42 (farmed rainbow trout) to 0.61 (escaped rainbow trout) mg/kg ww. In the study, there was no statistically significant difference between levels of Cu in muscles of farmed and escaped rainbow trout (t test, $p > 0.05$) (Table 1). Fallah et al. (2011) found Cu concentration in farmed rainbow trout to be significantly higher than in escaped rainbow trout, whereas Karataş (2014) found statistically higher Cu concentration in escaped rainbow trout compared to farmed rainbow trout. The maximum permissible level in fish for Cu was 30 mg/kg established by Food and Agriculture Organization (FAO 1983). In this study, Cu concentrations in farmed and escaped rainbow trout were significantly below this value (Table 2). The mean concentrations of Cu determined in both farmed and escaped rainbow trout were comparable to those found in other studies of both farmed (Santerre et al. 2001; Lourenço

et al. 2012; Jiang et al. 2016; Karataş 2014) and escaped rainbow trout (Mendil et al. 2010), whereas they were lower than those found in other studies of both farmed (Fallah et al. 2011) and escaped rainbow trout (Fallah et al. 2011; Karataş 2014; Celik et al. 2008) (Table 2).

Of ten metals, Fe was the most abundant metal in escaped rainbow trout, while it was the second most abundant metal in farmed rainbow trout. The mean concentrations of Fe ranged from 3.52 to 4.78 mg/kg ww. Fe concentration in escaped rainbow trout was significantly higher compared to farmed rainbow trout (t test, $p < 0.05$) (Table 1). In previous studies, higher levels of Fe were also observed in escaped rainbow trout (Fallah et al. 2011; Karataş 2014). The mean Fe concentration in farmed rainbow trout was comparable to that reported for farmed rainbow trout from Iran (Fallah et al. 2011), Portugal (Lourenço et al. 2012), and China (Jiang et al. 2016), whereas it was lower than that reported from Turkey (Karataş 2014). The mean concentration of Fe in escaped rainbow trout was comparable to that found in other studies of escaped rainbow trout (Fallah et al. 2011; Karataş 2014; Mendil et al. 2010; Celik et al. 2008) (Table 2).

The mean concentrations of Mn were comparable in farmed (0.82 mg/kg ww) and escaped rainbow trout (0.95 mg/kg ww) in the study (t test, $p > 0.05$) (Table 1). However, Fallah et al. (2011) found higher level of Mn in escaped rainbow trout, while Karataş (2014) found that farmed rainbow trout contained significantly higher concentration of Mn. The mean Mn concentration in farmed rainbow trout was higher than that reported for farmed rainbow trout from Turkey (Karataş 2014), Portugal (Lourenço et al. 2012), and China (Jiang et al. 2016), whereas it was comparable to that reported from Iran (Fallah et al. 2011). The mean concentration of Fe in escaped rainbow trout was comparable to that reported for escaped rainbow trout from Turkey (Karataş 2014; Mendil et al. 2010; Celik et al. 2008), while it was lower than that reported from Iran (Fallah et al. 2011) (Table 2).

The mean concentrations of Ni ranged from 0.89 (farmed rainbow trout) to 1.2 (escaped rainbow trout) mg/kg ww. In the study, there was no statistically significant difference between levels of Ni in farmed and escaped rainbow trout (t test, $p > 0.05$) (Table 1). Similarly, Karataş (2014) and Fallah et al. (2011) reported that Ni concentration in escaped rainbow trout was not significantly different compared to farmed rainbow trout. The mean concentrations of Ni determined in both farmed and escaped rainbow trout were higher than those found in other studies of both farmed (Fallah et al. 2011; Lourenço et al. 2012; Jiang et al. 2016; Karataş 2014) and escaped rainbow trout (Karataş 2014; Mendil et al. 2010; Celik et al. 2008; Fallah et al. 2011) (Table 2).

The mean concentrations of Pb were comparable in farmed (54.21 µg/kg ww) and escaped rainbow trout (51.50 µg/kg ww) in the study (t test, $p > 0.05$) (Table 1). Similarly, Fallah et al. (2011) reported that no significant difference was found

in Pb levels between farmed and escaped rainbow trout. In this study, Pb concentrations in both farmed and escaped rainbow trout were below the maximum permissible levels for Pb in fish set by MHPRC (2013), FSANZ (2013), EC (2006), and the Codex Alimentarius Commission (WHO/FAO 2015) (Table 2). The mean Pb concentration determined in farmed rainbow trout was much lower than that reported for farmed rainbow trout from Iran (Fallah et al. 2011) and the southern USA (Santerre et al. 2001), whereas it was comparable to that reported from Portugal (Lourenço et al. 2012) (Table 2) and it was higher than that reported from China (Jiang et al. 2016). The mean Pb concentration in escaped rainbow trout was much lower than that reported for escaped rainbow trout from Iran (Fallah et al. 2011) and Turkey (Celik et al. 2008), whereas it was comparable to that reported from Turkey (Mendil et al. 2010) (Table 2).

Of ten metals, Zn was the most abundant metal in farmed rainbow trout, while it was the second most abundant metal in escaped rainbow trout. However, the mean concentrations of Zn were comparable in farmed (4.42 mg/kg ww) and escaped rainbow trout (4.41 mg/kg ww) in the study (t test, $p > 0.05$) (Table 1). In previous studies, Fallah et al. (2011) and Karataş (2014) found statistically higher Zn concentrations in escaped rainbow trout compared to farmed rainbow trout. The maximum permissible level in fish for Zn was 30 mg/kg established by FAO (1983). Zn concentrations in both farmed and escaped rainbow trout were below this limit (Table 2). The mean concentration of Zn in farmed rainbow trout was comparable to those found in other studies of farmed rainbow trout (Fallah et al. 2011; Lourenço et al. 2012; Jiang et al. 2016; Karataş 2014). The mean Zn concentration in escaped rainbow trout was comparable to that reported for escaped rainbow trout from Turkey (Celik et al. 2008; Karataş 2014), whereas it was lower than that reported from Iran and Turkey (Fallah et al. 2011; Mendil et al. 2010) (Table 2).

Fallah et al. (2011) reported that the differences in metal concentrations between farmed and escaped rainbow trout were probably related to the differences in both their environmental conditions and dietary metal concentrations. Yildiz (2008) and Lundebye et al. (2017) reported that the differences in trace element contents of wild and cultured fish fillets could be directly related to the differences between the levels in natural prey and commercial fish feed. However, Alam et al. (2002) reported that despite their habitat and dietary differences, the differences in metal concentrations between farmed and wild carp are negligible because they were accumulating and distributing metals in the same manner. In this study, of the ten metals, only Co and Fe levels in muscle of escaped rainbow trout were significantly higher than those in farmed rainbow trout, whereas for other metals there were not found significant differences between farmed and escaped rainbow trout. In addition, the total concentration of essential metals (Co, Cr, Cu, Fe, Mn, and Zn) in escaped

rainbow trout (12.32 mg/kg ww) was significantly higher (t test, $p < 0.05$) compared to farmed rainbow trout (10.51 mg/kg ww), while there was no statistically significant difference (t test, $p > 0.05$) for the total concentration of toxic metals (As, Cd, and Cu) between farmed (0.140 mg/kg ww) and escaped rainbow trout (0.104 mg/kg ww).

Human health risk assessment

The EDI values of ten metals from farmed and escaped rainbow trout consumption by adult people are presented in Table 3. The EDI values for the examined both farmed and escaped rainbow trout were well below the tolerable daily intake (TDI) limits, indicating that there was no health risk associated with the intake studied heavy metals through the consumption of examined fish samples.

The THQ values of ten metals due to consumption of farmed and escaped rainbow trout are presented in Table 4. The THQ value (< 0.02) of each metal for both farmed and escaped rainbow trout did not exceed the hazard quotient threshold of 1. In this study, TTHQ values calculated for farmed and escaped rainbow trout were also smaller than 1 (Table 4). These THQ and TTHQ values suggested that there was no health risk for the adults in Turkey due to the intake of either individual metal or ten metals contained in farmed and escaped rainbow trout.

The carcinogenic risk (CR) values for inorganic As (iAs) due to exposure from consumption of farmed and escaped rainbow trout were listed in Table 4. CR values of iAs obtained in the study ranged from 1.09×10^{-6} in farmed rainbow trout to 6.73×10^{-7} in escaped rainbow trout. In general, a lifetime cancer risk above 10^{-4} is considered unacceptable, cancer risk lower than 10^{-6} is considered to be negligible

Table 3 Estimated daily intakes (EDI) from consumption of farmed and escaped rainbow trout by the adults

	Mean ($\mu\text{g}/\text{kg}$ ww)		EDI ($\mu\text{g}/\text{kg}$ bw/day)		TDI ($\mu\text{g}/\text{kg}$ bw/day)
	Farmed	Escaped	Farmed	Escaped	
As ^a	2.57	1.57	0.0007	0.0004	2.14 (JECFA 1989)
Cd	0.38	0.37	0.0001	0.0001	0.8 (JECFA 2011)
Co	520	720	0.1490	0.2053	30 (Finley et al. 2012)
Cr	810	850	0.2321	0.2437	300 (EFSA 2014)
Cu	420	610	0.1186	0.1754	500 (JECFA 1982)
Fe	3520	4780	1.0064	1.3660	800 (JECFA 1983)
Mn	820	950	0.2348	0.2709	140 (USEPA 2016)
Ni	890	1200	0.2552	0.3420	12 (WHO 2011)
Pb	54.21	51.53	0.0155	0.0147	1.50 (EFSA 2010)
Zn	4420	4410	1.2629	1.2593	300 (JECFA 1982)

TDI tolerable daily intake

^a It was assumed that inorganic As was 3% of total As

Table 4 Target hazard quotient (THQ), total THQ (TTHQ), and carcinogenic risk (CR) values due to consumption of farmed and escaped rainbow trout in the Keban Dam Reservoir

	THQ		Rfd (µg/kg/ bw/day)	CR	
	Farmed	Escaped		Farmed	Escaped
As ^a	0.00244	0.00150	0.3 (USEPA 2016)	1.09×10^{-6}	6.73×10^{-7}
Cd	0.00011	0.00010	1 (USEPA 2016)		
Co	0.00497	0.00684	30 (Finley et al. 2012)		
Cr	0.00015	0.00016	1500 (USEPA 2016)		
Cu	0.00296	0.00438	40 (Qin et al. 2015; Kalantzi et al. 2016)		
Fe	0.00144	0.00195	700 (Qin et al. 2015; Kalantzi et al. 2016)		
Mn	0.00168	0.00193	140 (USEPA 2016)		
Ni	0.01276	0.01710	20 (Qin et al. 2015; Kalantzi et al. 2016)		
Pb	0.01033	0.00982	1.50 (EFSA 2010)		
Zn	0.00421	0.00420	300 (USEPA 2016)		
TTHQ	0.04105	0.04799			

^a It was assumed that inorganic As was 3% of total As
RfD oral reference dose

and cancer risk between 10^{-6} and 10^{-4} is generally considered an acceptable range (Ahmed et al. 2016). In this study, the CR value of iAs calculated for farmed rainbow trout was within the acceptable range of 10^{-4} and 10^{-6} , while the CR value of iAs calculated for escaped rainbow trout was below 10^{-6} . According to these results, there was no carcinogenic risk from consumption of both farmed and escaped rainbow trout.

For non-carcinogen effects, the maximum allowable daily consumption limits (CR_{lim}) of both farmed and escaped rainbow trout for all metals were found to be high enough to ensure the human health (Table 5). The calculated CR_{lim} of each metal represents the maximum lifetime daily fish consumption rate (kg) that would not be expected to cause adverse non-carcinogenic health effects (USEPA 2000). Of ten metals, Cd and Cr had the highest CR_{lim} values in both farmed

and escaped rainbow trout, whereas Pb and Ni had the lowest CR_{lim} values (Table 5). In addition, the maximum allowable monthly consumption limits (CR_{mm}) were calculated to determine how many meals of farmed and escaped rainbow trout can safely be eaten per month with no adverse non-carcinogenic health effects. CR_{mm} values of both farmed and escaped rainbow trout for all analyzed metals were > 150 meals/month (Table 5). According to USEPA (2000), CR_{mm} values found in the study are categorized as safe fish consumption (represented by > 16 meals/month). Thus, an adult can safely consume > 150 meals of farmed or escaped rainbow trout per month.

With regard to carcinogenic effects of inorganic As, the maximum allowable daily consumption limits (CR_{lim}) for inorganic As contents of farmed and escaped rainbow trout were

Table 5 The maximum allowable fish consumption rates for non-carcinogenic and carcinogenic health effects (units kg/day for CR_{lim}; meals/month for CR_{mm})

	Consumption limits for non-carcinogenic health effects				Consumption limits for carcinogenic health effects			
	CR _{lim} (kg/day)		CR _{mm} (meals/month)		CR _{lim} (kg/day)		CR _{mm} (meals/month)	
	Farmed	Escaped	Farmed	Escaped	Farmed	Escaped	Farmed	Escaped
As ^a	8.19	13.36	1097	1791	0.182	0.297	24	39
Cd	182.61	191.38	24,487	25,663				
Co	4.03	2.92	539	391				
Cr	129.23	123.12	17,329	16,509				
Cu	6.75	4.56	904	611				
Fe	13.91	10.25	1865	1374				
Mn	11.93	10.34	1599	1386				
Ni	1.57	1.17	210	156				
Pb	1.94	2.04	259	273				
Zn	4.75	4.76	637	638				

^a It was assumed that inorganic As was 3% of total As

Table 6 BCF values of metals for farmed and escaped rainbow trout

	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Farmed rainbow trout	30	10	549	272	141	226	311	285	343	755
Escaped rainbow trout	19	5	511	121	191	307	302	318	330	507

182 and 297 g/day, respectively (Table 5). Because CR_{lim} values for inorganic As contents of farmed and escaped rainbow trout are higher than 20 g/day, which is the average per adult consumption of fish in Turkey (GDFA 2016), no carcinogenic health effects to adult people in Turkey is expected. The maximum allowable monthly consumption limit (CR_{mm}) for inorganic As also was calculated to determine how many meals of fish can safely be eaten per month with no adverse carcinogenic health effects. According to CR_{mm} values of inorganic As, adults may safely consume 24 meals of farmed rainbow trout per month (Table 5), while they may safely consume 39 meals of escaped rainbow trout per month. Thus, CR_{mm} values calculated for both farmed and escaped rainbow trout are categorized as safe fish consumption because they are > 16 meals/month (USEPA 2000).

To protect human health, USEPA (2000) suggests that the lower one of the CR_{mm} values for either carcinogenic or non-carcinogenic effects should be used (Yu et al. 2014). In the present study, the CR_{mm} values (24 and 39 meals/month, respectively) of farmed and escaped rainbow trout for carcinogenic effects was lower than the CR_{mm} values (> 150 meals/month) of both farmed and escaped rainbow trout for non-carcinogenic effects (Table 5). Therefore, 24 meals of farmed rainbow trout per month or 39 meals of escaped rainbow trout per month for adults may be safely eaten for each metal for both non-cancer and cancer health effects endpoints.

Bioconcentration factor

If a metal has a bioconcentration factor (BCF) between 1000 and 5000, it is considered bioaccumulative. If a metal has a BCF less than 1000, it is considered to be not bioaccumulative (Costanza et al. 2012). The calculated values of the BCF of the ten metals for both farmed and escaped rainbow trout are listed in Table 6. BCF values of all metals that were lower than 1000 indicate that both farmed and escaped rainbow trout have not potential to accumulate these metals. In this study, mean BCF values of ten metals for farmed rainbow trout followed the order of $Zn > Co > Pb > Mn > Ni > Cr > Fe > Cu > As > Cd$, while they followed the order of $Co > Zn > Pb > Ni > Fe > Mn > Cu > Cr > As > Cd$ in escaped rainbow trout (Table 6).

Conclusions

The present study indicated that the levels of essential metals Co and Fe in muscle of escaped farmed rainbow trout were

significantly higher than those in muscle of farmed rainbow trout. However, there were no significant differences in the levels of other metals between farmed and escaped rainbow trout. The levels of heavy metals determined in farmed and escaped rainbow trout were below the maximum permissible levels set by international food standards. From the human health perspective, the EDI of each metal in both farmed and escaped rainbow trout was much lower than the respective TDI. The THQ values for individual metal and the TTHQ values for combined metals were lower than 1, indicating no health risk for humans due to the intake of either individual metal or combined metals. The cancer risk (CR) values for inorganic As were 1.09×10^{-6} in farmed rainbow trout and 6.73×10^{-7} in escaped rainbow trout, which do not pose any potential carcinogenic health risk to the consumers. According to CR_{mm} , adults may safely consume 24 meals of farmed rainbow trout per month or 39 meals of escaped rainbow trout per month, with no adverse carcinogenic and non-carcinogenic health effects. BCF values of all metals that were lower than 1000 indicate that both farmed and escaped rainbow trout have not potential to accumulate these metals. In conclusion, this study revealed that the consumption of farmed and escaped rainbow trout in the Keban Dam Reservoir is completely safe for consumers.

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