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Trace Metal Levels in Rainbow Trout (*Oncorhynchus mykiss*) Cultured in Net Cages in a Reservoir and Evaluation of Human Health Risks from Consumption

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Abstract Although fish consumption has positive health effects, metals accumulated in fish can cause human health risks. In this study, the levels of ten metals in rainbow trout (*Oncorhynchus mykiss*) farmed in the Keban Dam Reservoir, which has the biggest rainbow trout production capacity in Turkey, were determined and compared with the maximum permissible levels (MPLs). Also, human health risks associated with rainbow trout consumption were assessed. The metal concentrations in rainbow trout were found below the MPLs. The estimated daily intake of each metal was much lower than the respective tolerable daily intake. The target hazard quotient (THQ) for individual metal and total THQ for combined metals did not exceed 1, indicating no health risk for consumers. The cancer risk (CR) value for inorganic arsenic was within the acceptable lifetime risk range of 10^{-6} and 10^{-4}. For carcinogenic and non-carcinogenic effects, the maximum allowable fish consumption rates were high enough to ensure the human health. According to these results, the consumption of rainbow trout farmed in the Keban Dam Reservoir does not pose a risk on human health.

Keywords Metals · Farmed rainbow trout · Risk assessment · Fish consumption advisories · Bioconcentration factor · Biomagnification factor

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Introduction

Fish consumption is a very important part of a healthy human diet. It is rich in omega-3 polyunsaturated fatty acids, liposoluble vitamins, and essential minerals and is a major source of high-quality proteins [1, 2]. Nevertheless, fish can also accumulate elevated levels of environmental contaminants, such as metals [2–5]. Anthropogenic activities are the main source of metal pollution. In the aquatic ecosystems, metals can be bioaccumulated via food web to hazardous levels, and thus, they can pose potential health risk to humans who consume fish. Therefore, metal contamination in fish has become an important worldwide concern in recent years. Some metals, such as copper, zinc, iron, chromium, and cobalt are considered as essential since they play an important role in the metabolism of living organisms [5, 6]. Whereas, arsenic, cadmium, mercury, and lead are non-essential elements, because they do not have beneficial properties and they are toxic to humans and animals. Moreover, the essential metals can also cause toxic effects at high concentrations [2, 3, 6].

Metals can be classified as either carcinogen or non-carcinogen, which can cause carcinogenic and non-carcinogenic effects on humans. The USEPA [7, 8] has proposed some health risk assessment methods for carcinogenic and non-carcinogenic metals via fish consumption [2, 5, 9–11]. The target hazard quotient (THQ) developed by USEPA [8, 11] is used to determine the potential non-carcinogenic health risks [2]. The USEPA [8] also provided cancer slope factors for carcinogenic metals to determine lifetime cancer risk (CR) [2, 10]. Finally, the USEPA [7] has suggested that the maximum allowable fish consumption rates should be calculated to protect consumers from both the carcinogenic and non-carcinogenic effects of metals [2, 9, 11].

Aquaculture is the fastest growing economic area in the food industry. Aquaculture in Turkey began with the farming of rainbow trout (*Oncorhynchus mykiss*) in the late 1960s [12]. Today, Turkey is one of the countries with the highest trout production in Europe. The annual rainbow trout production in Turkey is 108,038 tons, representing 45% of the country's total aquaculture production in 2015 [13]. Rainbow trout is the main freshwater fish species farmed in Turkey, accounting for about 99% of total freshwater aquaculture production in Turkey [13]. Trout cage culture has grown significantly over the last decade in dam reservoirs of Turkey, especially in Keban Dam Reservoir (KDR), which has the biggest rainbow trout production capacity. Rainbow trout have been cultured in net cages on the dam reservoir since 2000. While the annual production of rainbow trout in the KDR was 523 tons in 2004, it reached 19,709 tons in 2013 [14]. Although some reports on trace metal levels in wild fish from the KDR have been published, there has not been any study on trace metal concentrations in farmed rainbow trout.

The main objectives of this study were to (1) estimate biomagnification factors from feed to fish muscle and bioconcentration factors from water to fish muscle for trace metals and (2) perform human health risk assessment for carcinogenic and non-carcinogenic metals ingested via consumption of farmed rainbow trout in KDR. To achieve this, concentrations of ten elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were determined in the muscles of rainbow trout (*Oncorhynchus mykiss*) from three fish cage facilities in the KDR. In addition, the concentrations of these elements were determined in water samples from cage sites and feeds used for trout production.

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 km^2 and a volume of 30.6 km^3 at 845 m above sea level [14].

Sample Collection and Preparation

Between November 2014 and April 2015, rainbow trout (*Oncorhynchus mykiss*) samples were collected from cage farming facilities located in three different regions of the KDR. Site-1 (the first facility) was located in Ağın region, site-2 (the second facility) was located in Keban region, and site-3 (the third facility) was located in Aydıncık region of the dam reservoir (Fig. 1). A total of five fish for each month were taken from each cage facility. Immediately after collection, fish were transferred to the laboratory on ice boxes. Total lengths and body weights of fish were measured. Trout samples were dissected using a precleaned stainless steel knife, and approximately 25 g of muscle tissue was taken, packed in labeled zip-lock bags, and stored at − 20 °C until analysis. In addition, trout feed and water samples were collected from the cage facilities. In KDR, extruded feeds are commonly used for trout cage culture.

Water samples were filtered and acidified to pH < 2 and then stored refrigerated until analysis. Feed samples were also stored refrigerated until analysis. In this study, the sex difference was not considered. The total lengths and weights of fish samples were 25–33.5 cm and 205–457 g at site-1, 24–27 cm and 149–330 g at site-2, and 24–30.5 cm and 168–388 g at site-3, respectively.

Chemical Analysis

Metal Analysis of Fish, Feed, and Water Samples

A composite sample for muscle tissue of five rainbow trout individuals from each cage facility for each month was prepared. For metal content determinations, 1.0 g fish muscle and 0.5 g feed samples were digested in Teflon vessels with 10 ml HNO_3 (65%):H_2O_2 (30%) (4:1) mixture in a microwave digestion unit (MARSXpress, CEM) using a four-stage program (Table 1). After cooling to room temperature, the digested sample solutions were filtered and adjusted to 50 mL with ultrapure water. The levels of Co, Cr, Cu, Fe, Mn, Ni, and Zn in the fish and feed extracts were measured by a flame atomic absorption spectrometry (Thermo Scientific iCE 3000), while As, Cd, and Pb levels were measured by using a graphite furnace atomic absorption spectrometry (GFAAS) (Thermo Scientific iCE 3000) [15, 16]. 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). The percentage recoveries ranged from 92% (Mn) to 110% (Co) (Table 2).

Bioconcentration Factor

The bioconcentration factor (BCF) expresses the accumulation in fish of metals from water. It was calculated as [17]:

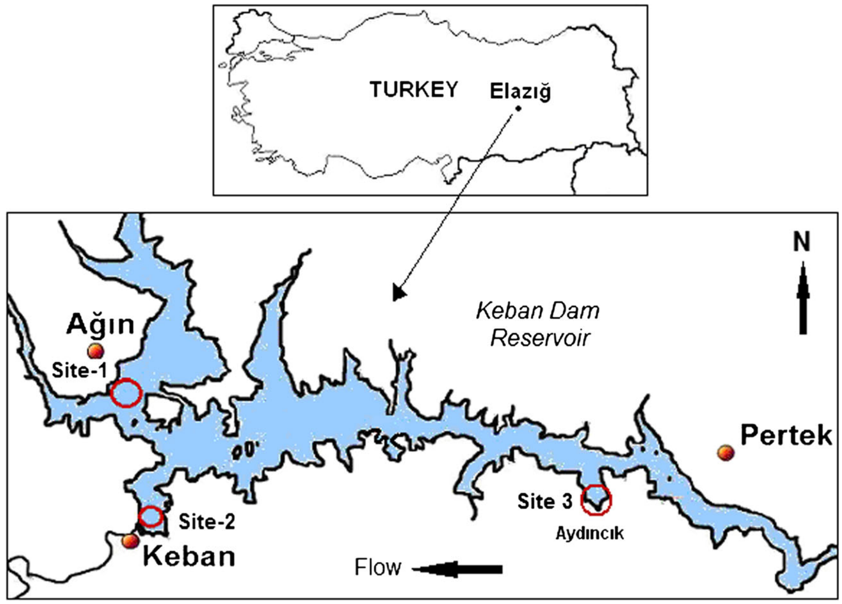
$$\mathrm{BCF} = C_{fish} \Big/ C_{water} \quad (1)$$

where C_{fish} is the metal concentration in the trout muscle and C_{water} is the metal concentration in water.

Biomagnification Factor

Biomagnification factor (BMF) is the ratio of the concentration of an element in fish relative to the concentration in its

Fig. 1 Map showing study area and sampling sites



diet. BMF was calculated by using the following equation [18]:

$$BMF = C_{fish} \Big/ C_{feed} \quad (2)$$

where C_{feed} is the metal concentration in trout feed.

Human Health Risk Assessment

Comparison to Maximum Levels in Food Standards

Metal concentrations detected in rainbow trout samples were compared with the maximum permissible levels (MPLs) recommended by Food Standards Australia and New Zealand (FSANZ) [19], FAO [20], European Commission (EC) [21], the Codex Alimentarius Commission (WHO/FAO) [22], and Chinese Health Ministry (MHPRC) [23].

Estimated Daily Intake of Trace Metals

The estimated daily intake (EDI) of trace metals depends on the metal concentration in fish muscle and the amount of daily fish consumption. EDI values of metals were compared with tolerable daily intake (TDI) values proposed by the Joint FAO/WHO Expert Committee on Food Additives [24–27], European Food Safety Authority (EFSA) [28, 29], and World Health Organization (WHO) [30] or reference dose (RfD) values suggested by USEPA [31] and Finley et al. [32]. The daily intake of metals for adults was calculated using the following equation [2]:

$$EDI = (MC \times IRd) \Big/ BW \quad (3)$$

where MC is the metal concentration in fish muscle (mg/kg wet weight), IRd is the daily average fish ingestion rate (g/day), and BW is the average body weight (kg) for an adult. EDI was expressed as µg/kg bw/day.

Table 1 Microwave digestion program

Stage	1	2	3	4
Temperature (°C)	100	140	160	180
Ramp time (min)	3	3	3	3
Hold time (min)	3	3	1	20
Power (%)	100	100	100	100
Maximum power (watt)	800	1600	1600	1600

Table 2 Concentrations of metals found in certified reference material (TORT-3, lobster hepatopancreas, National Research Council of Canada)

Metal	Certified value (mg/kg)	Found value (mg/kg)	Recovery (%)
As	59.5	57.8	97
Cd	42.3	43.6	103
Cr	1.95	2.12	109
Co	1.06	1.17	110
Cu	497	492	99
Fe	179	167	93
Mn	15.6	14.3	92
Ni	5.5	5.8	105
Pb	0.225	0.239	106
Zn	136	139	102

Non-carcinogenic and Carcinogenic Health Risk

The non-carcinogenic health risks via the consumption of fish species by the consumers were calculated based on the THQ, which is the ratio between the estimated dose of a contaminant and the reference dose (RfD). The method of estimating risk using THQ was developed by the USEPA [8], and it is calculated using the following equation:

$$THQ = \left[(EF \times ED \times IRd \times MC) \big/ (RfD \times BW \times AT)\right] \times 10^{-3} \quad (4)$$

where THQ is target hazard quotient, EF is exposure frequency (365 days/year), ED is the exposure duration (70 years), RfD is the oral reference dose (mg/kg/day), and AT is averaging time for non-carcinogens (365 days/year × number of exposure years, assuming 70 years in this study). When the THQ is below 1, it means that there is no potential health risk [5].

In this study, the hazard index (HI) (also called total THQ) was also performed. The HI was calculated by the sum of the individual metal THQ values [17].

$$HI = THQ(As) + THQ(Cd) + THQ(Co) + \ldots + THQ(Ni) + THQ(Pb) + THQ(Zn) \quad (5)$$

Cancer risk (CR) for inorganic arsenic was calculated using the cancer slope factor, provided by USEPA [31] only for this metal. The lifetime cancer risk is calculated using the following equation [8]:

$$CR = \left[(EF \times ED \times IRd \times MC \times CSF) \big/ (BW \times AT)\right] \times 10^{-3} \quad (6)$$

where CSF is the oral carcinogenic slope factor from the USEPA's Integrated Risk Information System (IRIS) online database [31]. CSF of inorganic As is 1.50 (mg/kg/d).

Maximum Allowable Consumption Rate

To calculate the maximum allowable daily fish consumption rate for non-carcinogenic effects, the following equation was used [7]:

$$CR_{lim} = (RfD \times BW) \big/ MC \quad (7)$$

where CR_{lim} is the maximum allowable fish consumption rate (kg/day).

CR_{lim} represents the maximum lifetime daily fish consumption rate (kg) that would not be expected to cause adverse non-carcinogenic health effects [7].

For carcinogenic health effects, the maximum allowable daily fish consumption limit was also determined by the following equation [7].

$$CR_{lim} = (ARL \times BW) \big/ (MC \times CSF) \quad (8)$$

where ARL is the maximum acceptable risk level (10^{-5} unitless).

CR_{lim} represents the amount of fish (kg) expected to generate a risk no greater than the maximum ARL used, based on a lifetime of daily consumption at that consumption limit [7].

The maximum allowable daily consumption limits calculated in this study were converted to the number of allowable meals per month [7]. It was calculated as:

$$CR_{mm} = \left(CR_{lim} \times T_{ap}\right) \big/ MS \quad (9)$$

where CR_{mm} is the maximum allowable fish consumption rate (meals/month), T_{ap} is time averaging period (365.25 days/12 months = 30.44 days per month), and MS is meal size (0.227 kg fish/meal for adults).

In the present study, the average concentrations of ten metals in the muscles of rainbow trout samples were used for the calculations of EDI, THQ, CR, and CR_{lim}. Calculations were performed assuming body weight (BW) of 70 kg for adults and a 20 g average daily consumption [13]. Furthermore, the risk factors and consumption limits of As were estimated only for inorganic arsenic. In the present study, we assumed that the inorganic arsenic was 10% of the total arsenic.

Statistical Analysis

Analysis of variance (ANOVA) was performed to determine significant differences in metal concentrations between sampling sites (cage farming facilities) and between sampling months ($p < 0.05$).

Results and Discussion

The descriptive statistics (mean, range, and standard deviation) of ten metals measured during the sampling period at three sampling sites are shown in Table 3. Among studied metals, Fe, Zn, and Mn were the most abundant elements in the trout samples taken from the three sites. Metal concentrations in the samples followed the order Fe > Zn > Mn > Ni > Cr > Co > Cu > As > Pb > Cd. Trace metals studied except As, Cr, and Pb did not show significant variations between sampling sites ($p > 0.05$) during the study period. However, Co, Cu, Mn, Ni, and Zn showed significant differences between months ($p < 0.05$).

According to spatial distribution of total mean concentrations of ten metals, the highest total mean concentration was measured at site-3 (16.15 mg/kg). Differences in total mean

Table 3 Descriptive statistics of metal concentrations in the muscles of the examined rainbow trout in this study (units μg/kg ww for As, Cd, and Pb, mg/kg ww for other metals)

	Site-1				Site-2				Site-3			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
As	54.1	110.7	83.9	19.1	97.6	195.3	132.2	45.6	70.7	110.6	89.7	16.9
Cd	0.500	1.847	1.028	0.522	0.084	1.346	0.708	0.425	0.185	1.244	0.726	0.367
Co	0.121	0.692	0.424	0.217	0.313	0.697	0.463	0.171	0.265	0.677	0.409	0.140
Cr	0.663	0.961	0.835	0.107	0.701	0.942	0.877	0.088	0.458	0.910	0.713	0.182
Cu	0.214	0.575	0.378	0.116	0.259	0.480	0.357	0.079	0.305	0.519	0.360	0.082
Fe	3.521	7.740	5.246	1.456	4.599	7.227	6.121	1.054	4.929	10.516	7.113	2.448
Mn	0.674	1.497	1.035	0.353	0.687	1.419	1.032	0.335	0.684	1.576	1.335	0.332
Ni	0.872	1.397	1.089	0.207	0.924	1.331	1.034	0.152	0.955	1.272	1.179	0.116
Pb	44.7	61.3	53.7	5.3	53.1	95.2	67.9	15.8	52.4	65.0	57.9	4.8
Zn	1.625	5.946	3.923	1.543	1.729	6.648	4.244	2.050	2.314	5.890	4.893	1.289

metal concentrations among sites may be attributed to the metal content of fish feed, fish physiology, quantity of feed consumed, fish age, and environmental conditions at each site, because the main sources of metals in farmed fish are feed and water. Kalantzi et al. [4] reported that local environmental conditions and fish feed have important roles in metal bioavailability and accumulation in fish tissues. Temporal distribution of total mean metal concentrations showed an increasing trend from November to April, and the highest total mean metal concentration (16.18 mg/kg) was measured in April, which is the last month of on-growing period. In the Keban Dam Reservoir, rainbow trout are stocked into the cages in November, fed with commercial extruded feeds, and harvested from cages in late April.

Metal Levels in Farmed Rainbow Trout

Excessive arsenic exposure may cause lung cancer, wart formation, decreased nerve conduction velocity, dermatitis, vasospasticity, mild pigmentation keratosis of the skin, and gross pigmentation with hyperkeratinization of exposed areas [33]. In the present study, the average As concentrations ranged from 83.9 to 132.2 μg/kg (Table 3). The highest concentration of As (195.3 μg/kg) was determined at site-2 in November, while the lowest concentration (54.1 μg/kg) was recorded at site-1 in March (Fig. 2). These results were much lower than those values reported by Svobodova et al. [34], who found between 0.498 and 1.133 mg/kg (ww) from rainbow trout farmed in the Czech Republic. In addition, the average As concentration found in this study was lower than that reported for farmed rainbow trout from Iran (0.934 mg/kg dw or 0.243 mg/kg ww; assuming 74% moisture content) [1] and China (0.605 mg/kg ww) [35], whereas it was higher than that reported for farmed rainbow trout from the southern USA (53 μg/kg ww for positive samples) [36]. According to FSANZ [19] and MHPRC [23], the maximum permissible levels (MPLs) for inorganic As were 2.0 and 0.10 mg/kg wet weight, respectively. The results of this study showed that inorganic arsenic concentrations (assuming inorganic As is 10% of total As) did not exceed MPLs established by FSANZ and MHPRC.

Excessive Cd exposure may lead to cancer and hepatic, renal, skeletal, pulmonary, and reproductive effects [37]. The mean concentrations of Cd in muscles of rainbow trout in this study ranged between 0.708 and 1.028 μg/kg (Table 3). The highest and lowest Cd levels were found as 1.847 μg/kg at site-1 in February and 0.084 μg/kg at site-2 in January, respectively (Fig. 2). Cd levels in trout samples were below MPLs recommended by MHPRC (0.1 mg/kg) [23] and by EC (0.05 mg/kg) [21]. The average Cd concentration found in this study was lower than that reported for farmed rainbow trout from Iran (0.025 mg/kg ww) [1] and Portugal (0.013 mg/kg ww) [38].

Cobalt is a component of vitamin B12; therefore, it is an essential element. However, high oral doses of Co have resulted in adverse effects, such as cardiovascular, hematological, immunological, neurological, reproductive, and endocrine responses [32]. In the present study, the highest concentration of Co (0.697 mg/kg) was determined at site-2 in November, while the lowest concentration (0.121 mg/kg) was recorded at site-1 in April (Fig. 2). The mean concentrations of Co ranged between 0.409 and 0.463 mg/kg (Table 3). The average Co concentration found in this study was higher than that reported for farmed rainbow trout from Iran (0.048 mg/kg ww) [1] and China (0.02 mg/kg ww) [35].

Chromium (III) is an essential element, while chromium (VI) is carcinogenic for humans. In contrast, there is little evidence that chromium (III) is toxic to humans [29]. In the present study, the average Cr concentrations ranged from 0.713 to 0.877 mg/kg (Table 3). The lowest and highest Cr

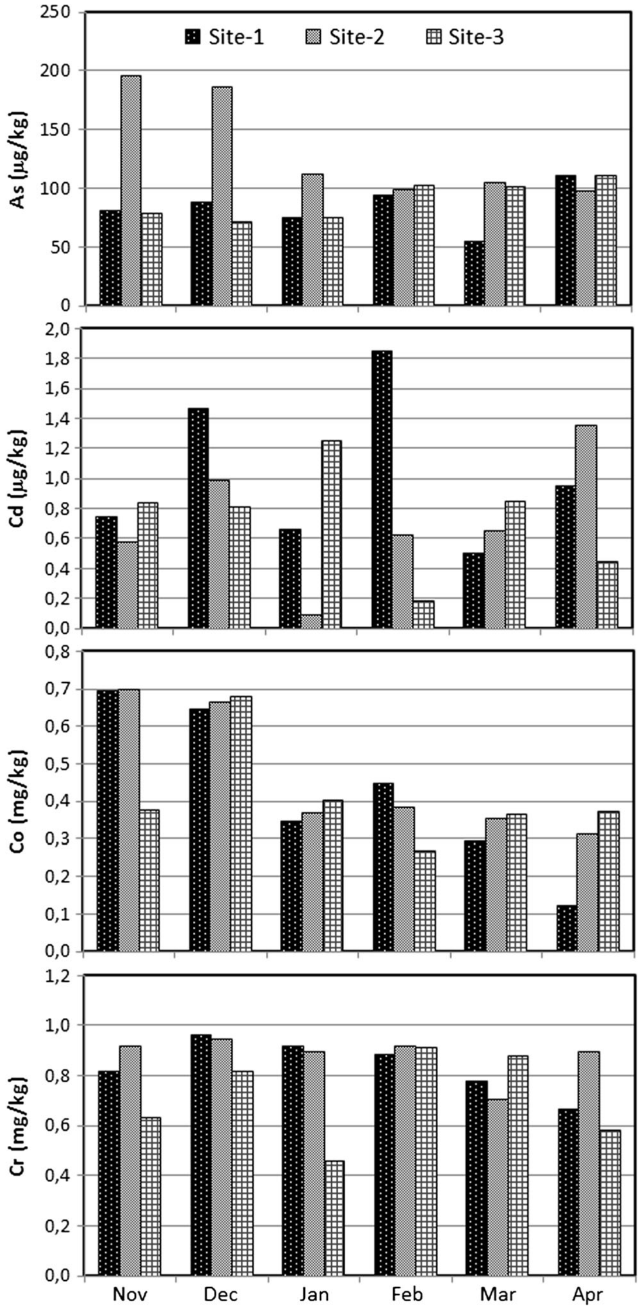


Fig. 2 Spatial and seasonal variations of As, Cd, Co, and Cr elements in rainbow trout

levels were found as 0.458 mg/kg at site-3 in January and 0.961 mg/kg at site-1 in December, respectively (Fig. 2). The maximum permissible limit for Cr in fish set by MHPRC [23] is 2 mg/kg. Trout samples had Cr concentrations below this limit. The average Cr concentration was higher than that reported for farmed rainbow trout from Iran (0.147 mg/kg ww) [1], China (0.059 mg/kg ww) [35], Portugal (0.23 mg/kg ww) [38], and the southern USA (0.313 mg/kg ww for positive samples) [36].

Copper is also an essential element. However, excessive Cu exposure can cause anemia, liver and kidney damage, developmental toxicity, and immunotoxicity [39]. The highest concentration of Cu (0.575 mg/kg) was determined at site-1 in December 2014, while the lowest concentration (0.214 mg/kg) was recorded at site-1 in March (Fig. 3). The average Cu concentrations ranged from 0.357 to 0.378 mg/kg (Table 3). The average Cu concentration found in this study was lower than that reported for farmed rainbow trout from Iran (5.671 mg/kg ww) [1], while it was comparable to that

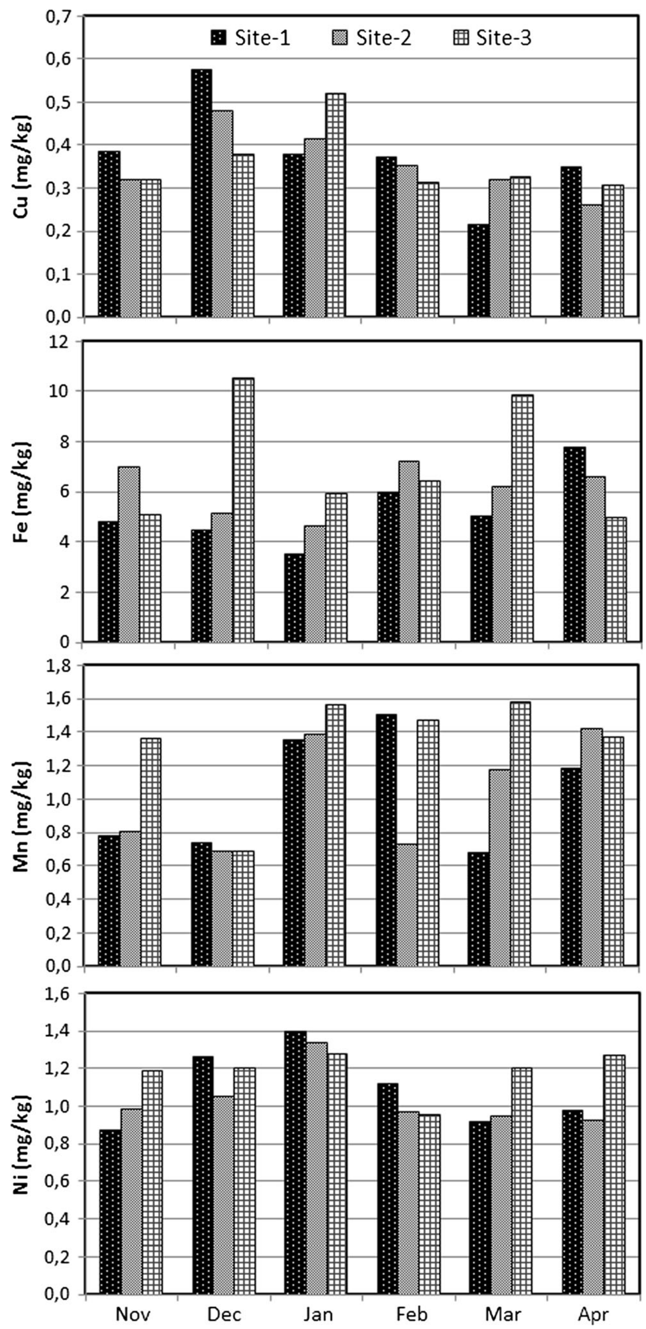


Fig. 3 Spatial and seasonal variations of Cu, Fe, Mn ve Ni elements in rainbow trout

reported for farmed rainbow trout from China (0.348 mg/kg ww) [35], the southern USA (0.360 mg/kg ww for positive samples) [36], Portugal (0.42 mg/kg ww) [38], and Turkey (0.33 mg/kg ww) [40]. The maximum permissible level in fish for Cu was 30 mg/kg, established by FAO [20]. Cu concentrations found in trout samples were significantly below this value.

Iron is an abundant element on earth and a biologically essential component of every organism. However, high iron intake may lead to increased risk of chronic diseases [41]. The lowest and highest Fe levels were found as 3.521 mg/kg at site-1 in January and 10.516 mg/kg at site-3 in December, respectively (Fig. 3). The average Fe concentrations ranged from 5.246 to 7.113 mg/kg (Table 3). The average Fe concentration was higher than that reported for farmed rainbow trout from Iran (4.022 mg/kg ww) [1], Portugal (4.5 mg/kg ww) [38], and Turkey (2.1 mg/kg ww) [40], while it was comparable to that reported for farmed rainbow trout from China (6.964 mg/kg ww) [35].

High levels of manganese can be toxic to humans and can cause adverse neurological effects [42]. In the present study, the average Mn concentrations ranged from 1.032 to 1.335 mg/kg (Table 3). The lowest and highest Mn levels were found as 0.674 mg/kg at site-1 in March and 1.576 mg/kg at site-3 in March, respectively (Fig. 3). The average Mn concentration was lower than that reported for farmed rainbow trout from Iran (1.628 mg/kg ww) [1], while it was higher than that reported for farmed rainbow trout from China (0.358 mg/kg ww) [35], Portugal (0.18 mg/kg ww) [38], and Turkey (0.78 mg/kg ww) [40].

Nickel, which is found in abundance on earth, is an essential trace element for animals. However, its essentiality in humans has not been established. Exposure to excess levels of nickel can lead to reproductive toxicity, hepatotoxicity, gene toxicity, neurotoxicity, nephrotoxicity, and increased risk of cancer [43]. In the present study, the average Ni concentrations ranged from 1.034 to 1.179 mg/kg (Table 3). The highest concentration of Ni (1.397 mg/kg) was determined at site-1 in January 2015, while the lowest concentration (0.872 mg/kg) was recorded at site-1 in November (Fig. 3). The average Ni concentration was higher than those values reported from China (0.014 mg/kg ww) [35], Portugal (0.02 mg/kg ww) [38], and Iran (0.0985 mg/kg ww) [1].

Exposure to excess levels of lead may result in nephrotoxicity, neurotoxicity, and many other negative health effects [37]. The highest concentration of Pb (95.2 μg/kg) was determined at site-2 in March 2015, while the lowest concentration (54.1 μg/kg) was recorded at site-1 in November (Fig. 4). The mean concentrations of Pb ranged between 53.7 and 67.9 μg/kg (Table 3). The average Pb concentration was much lower than those values reported from Iran (0.288 mg/kg ww) [1] and the southern USA (0.724 mg/kg ww for positive samples) [36], while it was higher than that reported from China

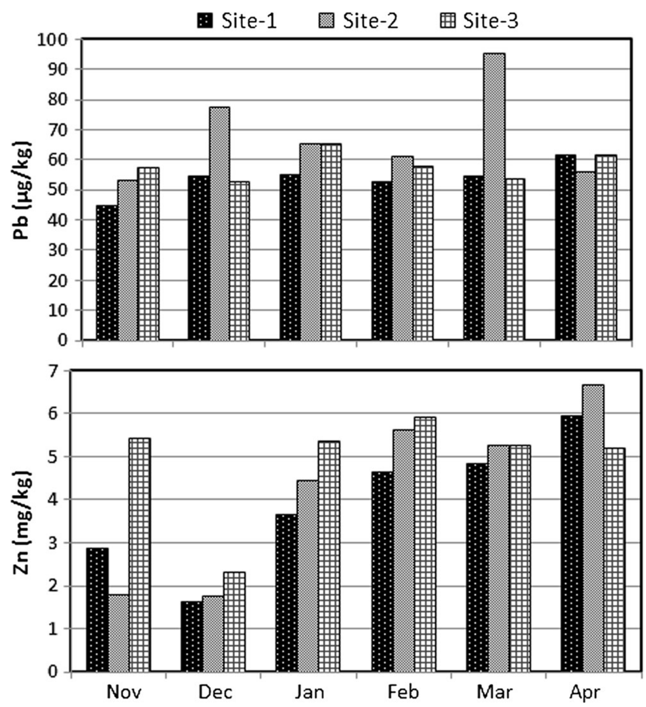


Fig. 4 Spatial and seasonal variations of Pb and Zn elements in rainbow trout

(0.009 mg/kg ww) [35], and it was comparable to that reported from Portugal (0.05 mg/kg ww) [38]. The maximum permissible limits for Pb in fish set by MHPRC [23] and FSANZ [19] are 0.5 mg/kg ww and 0.3 mg/kg ww for EC [21] and WHO/FAO [22]. Pb levels in trout samples were below both MPLs.

Zinc is an essential element. However, exposure to excess levels of Zn may lead to severe neurological diseases attributable to copper deficiency [44]. The mean concentrations of Zn ranged between 3.923 and 4.893 mg/kg (Table 3). The lowest and highest Mn levels were found as 1.625 mg/kg at site-1 in December and 6.648 mg/kg at site-2 in April, respectively (Fig. 4). The average Zn concentration was lower than those values reported from Iran (5.453 mg/kg ww) [1], Portugal (6.3 mg/kg ww) [38], and Turkey (9.68 mg/kg ww) [40], whereas it was comparable to that reported from China (4.185 mg/kg ww) [35]. The maximum permissible level in fish for Zn was 30 mg/kg established by FAO [20]. Trout samples had Zn concentrations below this limit.

Bioconcentration Factor

In this study, bioconcentration factor (BCF) values ranged between 18 and 672. If a metal has a 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 [45]. The calculated values of the BCF of the ten metals for trout are listed in Table 4. Generally, BCF values of all metals were lower than 1000, indicating that farmed rainbow trout in the KDR has low potential to accumulate these metals. Among metals, Zn had the

Table 4 Bioconcentration factors (BCF) and biomagnification factors (BMF) of ten metals

	BCF	BMF
As	30	0.711
Cd	18	0.012
Co	386	0.225
Cr	306	0.959
Cu	85	0.02
Fe	213	0.026
Mn	540	0.022
Ni	414	0.933
Pb	273	0.552
Zn	672	0.024

highest BCF value. Hogstrand [46] reported that because Zn is an essential element, fish will take up as much of Zn as needed from their environment. Therefore, BCF value of Zn can be higher than that of other metals. In the present study, BCF values of ten metals followed the order Zn > Mn > Ni > Co > Cr > Pb > Fe > Cu > As > Cd (Table 4).

Biomagnification Factor (BMF)

Biomagnification factor (BMF) term is used to express the ability of a contaminant to biomagnify. BMF value of a metal is less than 1, suggesting that this metal is not biomagnifying [18]. In this study, BMF values of all ten metals were < 1 (Table 4), indicating that these metals were not biomagnified by rainbow trout from the diet. The fact that concentrations of all metals in trout feeds were higher than concentrations in cultured farmed rainbow. BMF values of ten metals followed the order Cr > Ni > As > Pb > Co > Fe > Zn > Mn > Cu > Cd (Table 4).

Human Health Risk Assessment

The estimated daily intake (EDI) values of ten metals from fish consumption by adult people in Turkey are shown in Table 5. The EDI values for the examined rainbow trout samples were far below the tolerable daily intake (TDI) limits. This indicates that there is no health risk associated with the intake of studied metals from trout consumption. Among metals, Cu and Cd had the highest TDI/EDI ratio (> 4000), whereas Ni and Pb had the lowest TDI/EDI ratio (< 90).

The target hazard quotient (THQ) values of individual metals due to fish consumption are presented in Table 5. The THQ value (< 0.02) of each metal did not exceed 1 (Table 5). In this study, the value of HI or TTHQ (0.053) was also smaller than 1 (Table 5). These THQ and TTHQ values indicated that there was no health risk for the adults in Turkey due to the intake of either individual metal or ten metals contained in rainbow trout. Due to lower RfD values compared to their concentrations, Ni, Pb, and As were the three major contributors to TTHQ, accounted for 29.73, 21.53, and 18.33%, respectively. Whereas, Cr (0.28%) and Cd (0.43%) contributed the least to TTHQ.

The carcinogenic risk (CR) value for inorganic As due to exposure from trout consumption was 4.37×10^{-6}. According to USEPA [8], cancer risks between 10^{-6} and 10^{-4} are generally considered an acceptable range. In this study, the CR value obtained for inorganic As was within the acceptable range of 10^{-4} and 10^{-6}.

For non-carcinogen effects, the maximum allowable daily consumption limits (CR_{lim}) of trout for all metals were calculated and found to be quite high enough to ensure the human health (Table 6). Ni, Pb, and As had the lowest CR_{lim} values, whereas Cr and Cd had the highest CR_{lim} values. In addition, monthly consumption limits (CR_{mm}) were calculated to determine how many meals of rainbow trout from Keban Dam Reservoir can safely be eaten per month with no adverse

Table 5 Estimated daily intakes (EDI), target hazard quotient (THQ) for individual metals, and total THQ (TTHQ) due to fish consumption

	Mean (μg/kg ww)	EDI (μg/kg bw/day)	TDI (μg/kg/ bw/day)	Rfd (μg/kg/ bw/day)	THQ
As	10.19[a]	0.0029	2.14 [26]	0.3 [31]	0.00970
Cd	0.82	0.0002	0.8 [27]	1 [31]	0.00023
Co	432	0.1234	30 [32]	30 [32]	0.00411
Cr	808	0.2309	300 [29]	1500 [31]	0.00015
Cu	365	0.1043	500 [24]	40 [4, 6]	0.00261
Fe	6160	1.7600	800 [25]	700 [4, 6]	0.00251
Mn	1134	0.3240	140 [31]	140 [31]	0.00231
Ni	1101	0.3146	12 [30]	20 [4, 6]	0.01573
Pb	59.83	0.0171	1.50 [28]	1.50 [28]	0.01139
Zn	4353	1.2437	300 [24]	300 [31]	0.00415
TTHQ					0.05291

[a] The mean concentration of inorganic As was calculated as 10% of total As concentration

Table 6 The maximum allowable fish consumption rates for non-carcinogenic and carcinogenic health effects

	Consumption limits for non-carcinogenic health effects		Consumption limits for carcinogenic health effects			
	CR_{lim} (kg/day)	CR_{mm} (meals/month)	CR_{lim} (g/day)	CR_{mm} (meals/month)	CR_{lim} (g/day)	CR_{mm} (meals/month)
As	2.06	> 170	46[a]	6[a]	153[b]	20[b]
Cd	85.37	> 170				
Co	4.86	> 170				
Cr	129.95	> 170				
Cu	7.67	> 170				
Fe	7.95	> 170				
Mn	8.64	> 170				
Ni	1.27	> 170				
Pb	1.76	> 170				
Zn	4.82	> 170				

[a] It was assumed that inorganic As was 10% of total As

[b] It was assumed that inorganic As was 3% of total As

non-carcinogenic health effects. CR_{mm} was > 170 meals/month for all analyzed metals (Table 6). According to USEPA [7], this is categorized as safe fish consumption (represented by > 16 meals/month). Thus, an adult can safely consume 170 meals of rainbow trout from the KDR per month.

With regard to carcinogenic effects of inorganic As, the maximum allowable daily consumption limit (CR_{lim}) for inorganic As content of trout was 46 g/day (Table 6). Because the average per adult consumption of fish in Turkey is 20 g/day [13], no carcinogenic health effects to adult people in Turkey are expected. Monthly consumption limit (CR_{mm}) for inorganic As was also calculated to determine how many meals of rainbow trout can safely be eaten per month with no adverse carcinogenic health effects. CR_{mm} value of inorganic As for adults was six meals per month (Table 6). In the present study, we tested the worst-case scenario by assuming that toxic inorganic arsenic is 10% of total arsenic. Other studies reported that inorganic arsenic accounted for only 3% of total arsenic in fish [2]. By assuming inorganic As to constitute 3% of the total As, the calculated CR_{lim} and CR_{mm} values of As would be 153 g/day and 20 meals/month, respectively (Table 6). Thus, this CR_{mm} value (20 meals/month) is categorized as safe fish consumption because it is > 16 meals/month.

Conclusions

In this study, trace metal concentrations in rainbow trout farmed in cages in the Keban Dam Reservoir were determined. BCF results of ten metals indicated that no metals were bioaccumulative. According to BMF values, none of the metals were biomagnified by rainbow trout. The levels of metals in fish muscle were below the maximum permissible levels for human consumption set by various international food standards. From the human health perspective, the EDI of each metal was much lower than the respective TDI. THQ values of individual metals and TTHQ value of combined metals showed that there was no human health risk for consumption. The cancer risk (CR) value for inorganic As was within the acceptable lifetime risk range of 10^{-6} and 10^{-4}. For non-carcinogenic effects, the maximum allowable fish consumption rates were high enough to ensure the human health. The results of this study revealed that the consumption of rainbow trout farmed in the Keban Dam Reservoir is safe for consumers.

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References

1. Fallah AA, Saei-Dehkordi SS, Nematollahi A, Jafari T (2011) Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (*Oncorhynchus mykiss*) using ICP-OES technique. Microchem J 98:275–279
2. Copat C, Arena G, Fiore M, Ledda C, Fallico R, Sciacca S, Ferrante M (2013) Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. Food Chem Toxicol 53:33–37
3. Rahman MS, Molla AH, Saha N, Rahman A (2012) Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. Food Chem 134:1847–1854
4. Kalantzi I, Pergantis SA, Black KD, Shimmield TM, Papageorgiou N, Tsapakis M, Karakassis I (2016) Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers. Food Chem 194:659–670

5. Ahmed MK, Baki MA, Kundu GK, Islam MS, Islam MM, Hossain MM (2016) Human health risks from heavy metals in fish of Buriganga river, Bangladesh. SpringerPlus 5:1697
6. Qin D, Jiang H, Bai S, Tang S, Mou Z (2015) Determination of 28 trace elements in three farmed cyprinid fish species from Northeast China. Food Control 50:1–8
7. USEPA (US Environmental Protection Agency) (2000) Guidance for assessing chemical contaminant data for use in fish advisories, Volume II risk assessment and fish consumption limits. EPA 823-B-00-008. United States Environmental Protection Agency, Washington, DC
8. USEPA (U S Environmental Protection Agency) (1989) Risk assessment guidance for superfund, volume I. Human health evaluation manual part a, interim final. EPA/540/1–89/002. United States Environmental Protection Agency, Washington, DC
9. Yu Y, Wang X, Yang D, Lei B, Zhang X, Zhang X (2014) Evaluation of human health risks posed by carcinogenic and non-carcinogenic multiple contaminants associated with consumption of fish from Taihu Lake, China. Food Chem Toxicol 69:86–93
10. Saha N, Mollah MZI, Alam MF, Rahman MS (2016) Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control 70:110–118
11. Griboff J, Wunderlin DA, Monferran MV (2017) Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health? Microchem J 130:236–244
12. FAO (Food and Agriculture Organization) (2005) National Aquaculture Sector Overwiew: Turkey http://www.fao.org/fishery/countrysector/naso_turkey/en/
13. GDFA (General Directorate of Fisheries and Aquaculture) (2016) Fisheries statistics. Republic of Turkey Ministry of Food Agriculture and Livestock. http://www.tarim.gov.tr/sgb/Belgeler/SagMenuVeriler/BSGM.pdf
14. Güner B (2015) Aquafishing in Keban Dam Lake. Fırat Univ J Soc Sci 25:1–8
15. Makedonski L, Peycheva K, Stancheva M (2017) Determination of some heavy metal of selected black sea fish species. Food Control 72:313–318
16. Mendil D, Ünal ÖF, Tüzen M, Soylak M (2010) Determination of trace metals in different fish species and sediments from the River Yeşilırmak in Tokat, Turkey. Food Chem Toxicol 48:1383–1392
17. Tao Y, Yuan Z, Xiaona H, Wei M (2012) Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihu Lake, China. Ecotoxicol Environ Saf 81:55–64
18. Kelly BC, Ikonomou MG, Higgs DA, Oakes J, Dubetz C (2008) Mercury and other trace elements in farmed and wild salmon from British Columbia, Canada. Environ Toxicol Chem 27:1361–1370
19. FSANZ (Food Standards Australia and New Zealand) (2013) Australia New Zealand Food Standards Code. Standard 141. Contaminants and natural toxicants http://www.legislation.gov.au/Details/F(2013)C00140/
20. FAO (Food and Agriculture Organization) (1983) Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No 464. Food and Agriculture Organization of the United Nations, Rome
21. EC (Commission of the European Communities) (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006: setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union Legis 364. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881from=EN/
22. WHO/FAO (World Health Organization/Food and Agriculture Organization) (2015) Codex alimentarius commission. General standard for contaminants and toxins in food and feed. CODEX STAN 193–1995
23. MHPRC (Ministry of Health of the People's Republic of China) (2013) National food safety standard, maximum levels of contaminants in foods (GB2762–2012) http://www.seafish.org/media/publications/China_Max_levels_of_contaminants_in_food.pdf
24. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1982) Evaluation of certain food additives and contaminants. Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, no 683. World Health Organization, Geneva
25. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1983) Evaluation of certain food additives and contaminants. Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, no 696. World Health Organization, Geneva
26. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1989) Evaluation of certain food additives and contaminants. Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, no 776. World Health Organization, Geneva
27. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2011) Evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, no 960. World Health Organization, Geneva
28. EFSA (European Food Safety Authority) (2010) Scientific opinion on lead in food. EFSA J 8(4):1570
29. EFSA (European Food Safety Authority) (2014) Scientific opinion on dietary reference values for chromium. EFSA J 12(10):3845
30. WHO (World Health Organization) (2011) Guidelines for drinking water quality, 4th edn. World Health Organization, Geneva
31. USEPA (US Environmental Protection Agency) (2016) Integrated risk information system https://www.epa.gov/iris/
32. Finley BL, Monnot AD, Paustenbach DJ, Gaffney SH (2012) Derivation of a chronic oral reference dose for cobalt. Regul Toxicol Pharmacol 64:491–503
33. Raknuzzaman M, Ahmed MK, Islam MS, Habibullah-Al-Mamun M, Tokumura M, Sekine M, Masunaga S (2016) Trace metal contamination in commercial fish and crustaceans collected from coastal area of Bangladesh and health risk assessment. Environ Sci Pollut Res 23:17298–17310
34. Svobodova Z, Celechovska O, Machova J, Randak T (2002) Content of arsenic in market–ready rainbow trout (*Oncorhynchus mykiss*). Acta Vet Brno 71:361–367
35. Jiang H, Qin D, Mou Z, Zhao J, Tang S, Wu S, Gao L (2016) Trace elements in farmed fish (*Cyprinus carpio*, *Ctenopharyngodon idella* and *Oncorhynchus mykiss*) from Beijing: implication from feed. Food Addit Contam B 9:132–141
36. Santerre CR, Bush PB, Xu DH, Lewis GW, Davis JT, Grodner RM, Ingram R, Wei CI, Hinshaw JM (2001) Metal residues in farm-raised channel catfish, rainbow trout, and red swamp crayfish from the Southern US. J Food Sci 66:270–273
37. Shaheen N, Ahmed MK, Islam MS, Habibullah-Al-Mamun M, Tukun AB, Islam S, Rahim ATMA (2016) Health risk assessment of trace elements via dietary intake of 'non-piscine protein source' foodstuffs (meat, milk and egg) in Bangladesh. Environ Sci Pollut Res 23:7794–7806
38. Lourenço HM, Afonso C, Anacleto P, Martins MF, Nunes ML, Lino AR (2012) Elemental composition of four farmed fish produced in Portugal. Int J Food Sci Nutr 63(7):853–859
39. ATSDR (Agency for Toxic Substances and Disease Registry) (2004) Toxicological profile for Copper. US Department of Health and Human Services, Public Health Service, Atlanta

40. Gokoglu N, Yerlikaya P, Cengiz E (2004) Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). Food Chem 84:19–22
41. EFSA (European Food Safety Authority) (2004) Opinion of the scientific panel on dietetic products, nutrition and allergies [NDA] related to the tolerable upper intake level of iron. EFSA J 125:1–34
42. ATSDR (Agency for Toxic Substances and Disease Registry) (2012) Toxicological profile for Manganese. US Department of Health and Human Services, Public Health Service, Atlanta
43. Guo H, Chen L, Cui H, Peng X, Fang J, Zuo Z, Deng J, Wang X, Wu B (2016) Research advances on pathways of nickel-induced apoptosis. Int J Mol Sci 17:10
44. Hedera P, Peltier A, Fink JK, Wilcock S, London Z, Brewer GJ (2009) Myelopolyneuropathy and pancytopenia due to copper deficiency and high zinc levels of unknown origin II. The denture cream is a primary source of excessive zinc. NeuroToxicology 30: 996–999
45. Costanza J, Lynch DG, Boethling RS, Arnot JA (2012) Use of the bioaccumulation factor to screen chemicals for bioaccumulation potential. Environ Toxicol Chem 31:2261–2268
46. Hogstrand C (2012) Zinc. In: Wood CM, Farrell AP, Brauner CJ (eds) Homeostasis and toxicology of essential metals. Academic press, pp 135–200