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Heavy metal levels in the liver and muscle tissues of the four commercial fishes from Lake Balik, Kızılırmak Delta (Samsun, Turkey)

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ABSTRACT

Objective: To determine the levels of some heavy metals in the liver and edible tissues of four fish species [*Mugil cephalus* L., 1758 (*M. cephalus*), *Cyprinus carpio* L., 1758 (*C. carpio*), *Perca fluviatilis* L., 1758 (*P. fluviatilis*) and *Stizostedion lucioperca* L., 1758 (*S. lucioperca*)] collected from Lake Balik, Kizilirmak Delta (Samsun) for analysis of Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe. These metals were chosen because at higher concentrations there might be toxic to the fish and by extension humans that depends on such fish as food.
Methods: The concentrations of the metals were carried out by the flame atomic absorption spectrophotometer (UNICAM 929).
Results: Large differences in heavy metal levels were observed between liver and muscle tissues within each fish. The results showed that the Fe concentrations were the highest and Cd concentrations were the lowest in livers and edible tissues of the four species. The muscles of *C. carpio* accumulated significant higher levels of Co, Zn and Ni than other species. Cu was higher in the muscles of *S. lucioperca* than those in other species, while *M. cephalus* showed more of Mn and Fe levels. The muscles of *P. fluviatilis* accumulated significant higher levels of Pb and Cd than other species. However, the liver of *M. cephalus* accumulated significant higher levels of Co, Mn and Fe than other species. The level of Cd was the highest in the liver of *P. fluviatilis*, while *S. lucioperca* showed more of Cu and Pb, and the higher levels of Zn and Ni was measured in the liver of *C. carpio*.
Conclusions: The metal levels obtained were compared with the maximum permitted levels in food of the Turkish Food Codex and Commission Regulation (EC). The results of this study showed that estimated daily and weekly intakes of selected metals via consumption of fish were below the permissible tolerable daily intake and provisional tolerable weekly intake values established by Food and Agriculture Organization/World Health Organization.

1. Introduction

Fish is very important food for people because it has high protein supplies, essential amino acids, vitamin and mineral contents[1-3]. They are exposed to pollutants such as heavy metals in polluted waters. Heavy metals from the anthropogenic activities and sources are continually released into the aquatic environment, which creates serious health risks due to their toxicity, long persistence, bioaccumulation and bio-magnifications in the food chain[4-7]. It is vital to determine heavy metal levels in seafood, because heavy metal ions can accumulate more easily in fish as compared with other foodstuffs[8-10]. Non-essential heavy metals are toxic to fish even at low concentrations. For example, Cd and Pb may cause mental and central nervous system damage. On the other hand, essential elements such as of Cu, Co, Zn, Mn, Ni and Fe become toxic at certain concentrations[11,12]. Aquatic systems especially lakes are more sensitive to heavy metal pollutants and are contaminated by chemical substances, industrial, domestic and agriculture wastes in the form of particles, metal ions, organic and inorganic compounds[13].

Kızılırmak is the most important river as wetland and deltaic ecosystem in the Turkish part of the Black Sea[14]. The Kızılırmak Delta occupies 56 000 ha and includes 12 000 ha of freshwater marshes and swamps, coastal lakes, and lagoons on both sides of the Kızılırmak River. The Kızılırmak Delta is the largest and the most significant delta of Turkey, which reflects the natural heritage of the Black Sea coasts. The ecosystem of the delta wetland area is very rich in biological diversity. The delta, especially with its bird heritage and the dune vegetation, is very attractive for fauna and

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flora[15]. Kızılırmak Delta has several habitat types such as marine, river, lake, dunes, forest and others that need to be protected[16].

Kızılırmak Delta is located in the north of the Samsun highway within the border of Bafra. Bafra Basin is the main agricultural basin of Turkey in the region and the Kızılırmak receives many pollutants including heavy metals from the basin as well as from the cities which pass through[17-20]. Moreover, due to the lack of proper sewage and wastewater treatment systems in most cities in the region, wastewater from these cities has been discharged directly to the river or directly to the Black Sea[17-19]. Runoff from agricultural activities contains pollutants including heavy metals, which may contaminate the water, and depending on loads may result in various hazards to the aquatic life[20].

The Kızılırmak Delta which is located in the central Black Sea region of Turkey (41°30' to 41°45' N, 35°43' to 36°08' E), between the Samsun and Sinop highway within the borders of Bafra, Alaçam and Ondokuzmayıs townships of Samsun, is one of the Turkey's most important wetland complexes with its rich biodiversity and critical habitats for globally endangered bird species[21].

This point is located near major settlement in urban and agricultural part of the region where it is very important to determine the contents of heavy metals which can be a risk to human health. It is necessary to follow concentrations of toxic substances such as heavy metals in order to reduce pollutions and to provide a good ecological status.

Therefore, the purposes of this study were to determine if Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe were present in *Mugil cephalus* L., 1758 (*M. cephalus*), *Cyprinus carpio* L., 1758 (*C. carpio*), *Perca fluviatilis* L., 1758 (*P. fluviatilis*) and *Stizostedion lucioperca* L., 1758 (*S. lucioperca*) from Balik Lake, Kızılırmak of Samsun Province, describe if the concentrations of these heavy metals were significantly different among the edible tissues and liver, determine if the concentrations of Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe were significantly different between fish species, and compare the concentrations of any Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe presented with the guidelines set down the Turkish Food Codex and Commission Regulation (EC) for the safe consumption limits of fish.

2. Materials and methods

2.1. Study area

The study area, Balik Lake, situated in the east part of Kızılırmak Delta within the latitude and longitude of 41°36' N, 36°04' E is a lagoon. Its surface area is nearly 1 380 ha. It connects to the sea and its curved sand dune spits which separates from the sea. Water depth shows seasonal variations and it has an average depth of 1.5 m and a maximum depth of 3 m. Bafra Fish Lakes are the coastal wetlands on the east of Kızılırmak Delta in Samsun, comprises a variety of six saline lakes named Balik, Cernek, Uzun, Liman, Gici and Tatli (Figure 1).

Balik Lake was selected as a sampling location because it is in the way of various industrial and domestic effluent discharge sites. Hence, one would expect it to be more polluted segment of Kızılırmak Delta, Samsun.

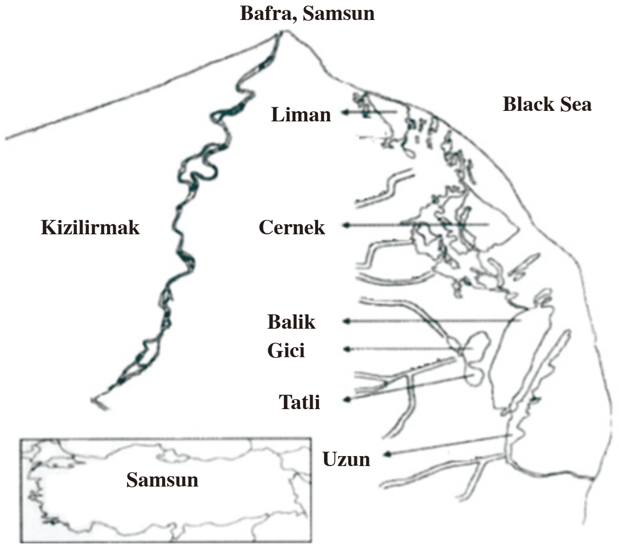

Figure 1. The study area.

2.2. Methods

Four fish species, *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca*, inhabiting waters of Balik Lake were analysed for heavy metal levels (Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe) in edible muscles and liver. Fishes were sampled with the help of local fishermen by using fishing net. Both muscle and liver tissues of the fish were prepared for analysis according to the method described by Bernhard[22]. For the heavy metal analysis, fish organ samples (muscles and liver) were placed in flasks, added 10 mL nitric acid to them, then covered the opening with a pear drop and sat still overnight. When the fish tissues were dissolved completely, the sample was placed on a hot plate and gradually heated to 200 °C until the vapour and the acid fluid inside the flask turned clear. When the vapour and the sample liquid was clear, the pear drop was removed and the flask was heated at 105 °C to evaporate the excess acid till the remaining digested fluid was at 1 mL. The fluid was cooled at room temperature and then 1 mol/L of nitric acid was added. Finally, the digests were quantified into 50 mL in a volumetric flask. The digested sample volume was filtered through 0.45 µm Whatman membrane filter. The heavy metal concentrations were determined by the flame atomic absorption spectrophotometer (UNICAM 929). All reagents used in the analysis were of the reagent grade. Double-deionized water was used for all dilutions. Chemicals were of high pure quality (Merck). The element standard solutions from Merck which were used for the calibrations were prepared by diluting stock solutions of 1 000 mg/L. The working standard values were used to plot a standard curve. The standards and blank were treated in the same way as the real samples to minimize matrix interferences during analysis. The laboratory glassware was kept overnight in a 10% v/v HNO_3 solution and then rinsed with deionised double distilled water[23,24]. All digested samples were analysed three times for each metal. The measured concentrations are expressed in mg/kg wet weight.

2.3. Statistical analysis

The statistical analysis of data was carried out by using statistica 7.0 statistical package program. All samples were collected and analysed in triplicate and the results are expressed as the mean ± SD. The statistical analysis was performed by the Student's *t*-test for significance and ANOVA with the multiple comparisons Turkey's test to determine the significant difference between treated means[25]. In all cases, the estimation was carried out at significant level of 0.05.

3. Results

Mean values of Cu, Co, Pb, Zn, Cd, Mn, Ni and Fe concentrations in muscle and liver tissues of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* are displayed in Figures 2–9. All heavy metal levels in liver of sampled fish species were higher than those in muscle tissues. The higher metal levels in liver represent storage capability of metals in the water[26,27]. Overall Fe levels were detected in higher concentrations followed by Zn, Mn, Cu, Ni, Co, Pb and Cd (Figures 2–9).

Fe is the essential element to all living organisms and has several vital functions to the body. In this study, the highest concentration of Fe was in *M. cephalus* (91 mg/kg in muscles and 105 mg/kg in liver) (Figure 9). The highest concentration of Co was also found in *M. cephalus* (0.43 mg/kg in muscles and 0.85 mg/kg in liver) (Figure 3).

Co is essential for red blood cell formation, the maintenance of nerve tissue. It is vital to many enzymatic systems and is useful for humans because of the formation of noble molecules, such as vitamin B12. Rich dietary sources of cobalt include fish meal, but high levels of cobalt can cause lung and heart diseases. Co level in *Leuciscus cephalus* was found as 0.62 mg/ kg in Kızılırmak Basin[28].

Zn and Cu are also essential elements for living organisms. Zn is an essential component of a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. In this study, the highest Zn levels were determined in *C. carpio* (38 mg/kg in muscles and 56 mg/kg in liver). In this study Zn levels in edible muscles of all fish specimens were lower

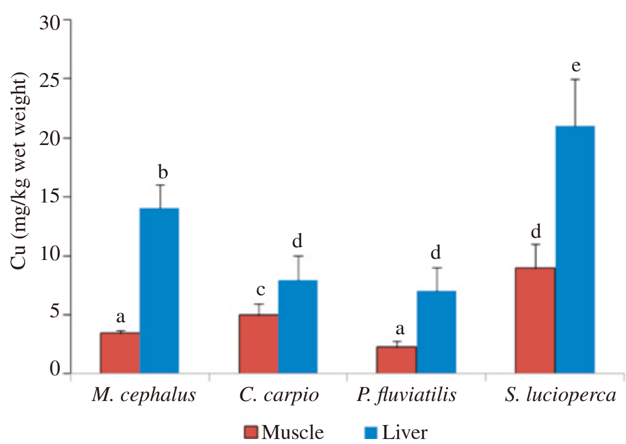

Figure 2. Cu concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

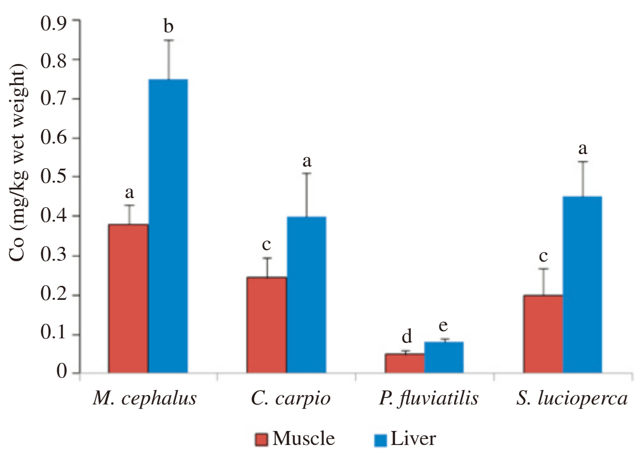

Figure 3. Co concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

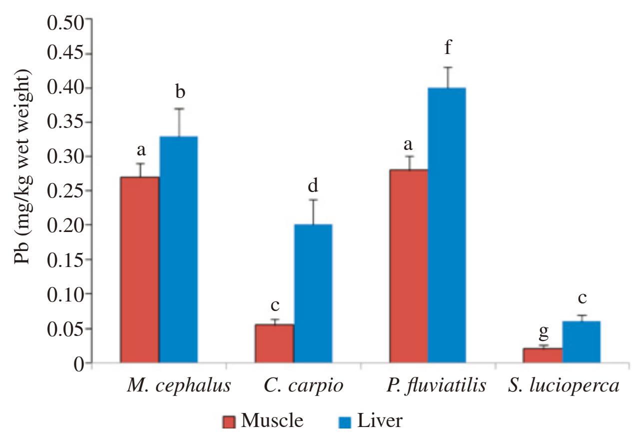

Figure 4. Pb concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

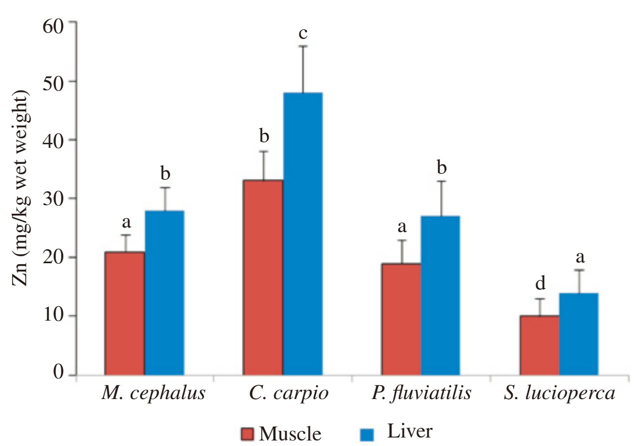

Figure 5. Zn concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis*, *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

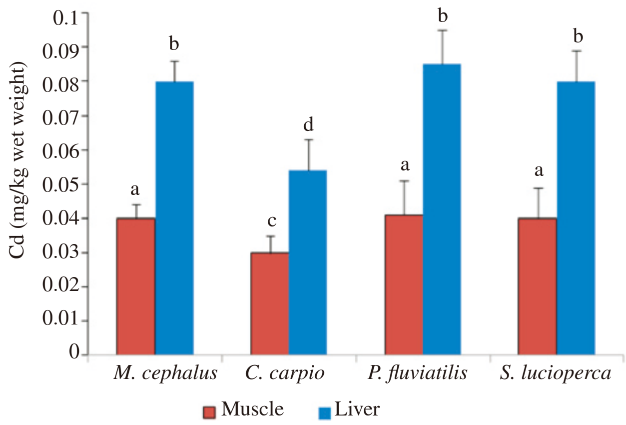

Figure 6. Cd concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

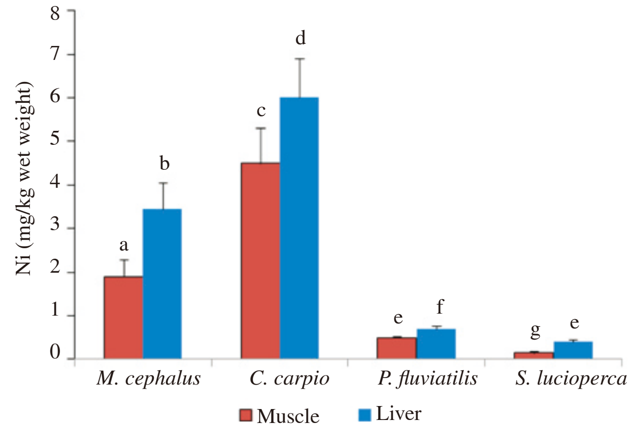

Figure 8. Ni concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

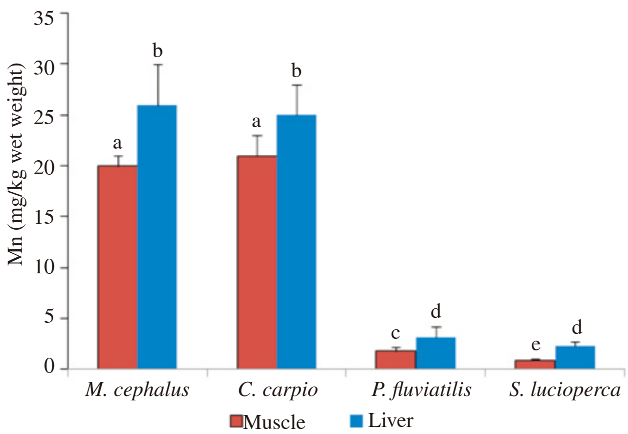

Figure 7. Mn concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

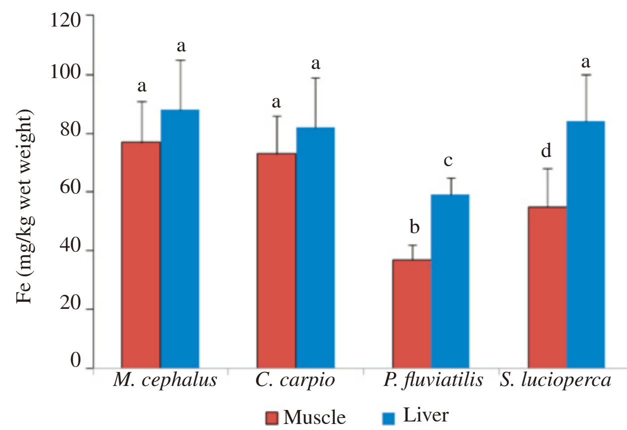

Figure 9. Fe concentrations in muscles and liver of *M. cephalus*, *C. carpio*, *P. fluviatilis* and *S. lucioperca* from Lake Balik, Kizilirmak in 2010.
Data are expressed as mean ± SD. The different letters beside the vertical bars in the graph indicate the values are significantly different ($P < 0.05$).

than guidelines limits established by the Food Safety of Fish Product (50 mg/kg wet weight)[29]. In this study, the highest Cu levels of edible muscle (11 mg/kg) and liver (25 mg/kg) were determined in *S. lucioperca* (Figure 2). Cu is an essential element. It makes health hazard and it is toxic to most forms of organisms when it presents in amounts exceeding the certain limits[11]. Cu levels [(9 ± 2) µg/g] in the edible muscle tissues did not exceed the critical limits set by the Turkish Food Codex in 2008 and the Food Safety of Fish Product in 1995 (20 µg/g wet weight)[29,30]. However, Zn and Cu levels in the liver tissues of *C. carpio* and *S. lucioperca*, respectively, exceeded the critical limits, but the livers of this fish were not consumed for human consumption (Figures 2 and 5).

Mn is an essential element with considerably maximum concentrations in liver (ranged from 0.8 to 23 mg/kg wet weight) than in muscle (ranged from 1.9 to 30 mg/kg wet weight) tissues due to its function as cofactor for the activation of a number of enzymes[31]. The highest level of Mn in the edible muscle (23 mg/kg wet weight) and liver (30 mg/kg wet weight) were detected in *C. carpio* and *M. cephalus*, respectively, (Figure 7). There is no information about the risk of cancer for Mn[32]. Ni is a trace element in fish and is essential for normal growth and reproduction of living organisms but shows carcinogenic effect if it is consumed in high quantity[33]. In this study, Ni showed low levels in general and ranged from 0.11 mg/kg in wet weight (muscle of *S. lucioperca*) to maximum 6.90 mg/kg in wet weight (liver of *C. carpio*) (Figure 8).

Cd and Pb belong to the group of non-essential and toxic metals and they have no known function in biochemical processes. Cd concentrations in the edible muscle and liver tissues ranged from 0.03 (*C. carpio*) to 0.04 (*M. cephalus* and *S. lucioperca*) and 0.054 (*M. cephalus*) to 0.085 (*P. fluviatilis*) mg/kg in wet weight, respectively, (Figure 6). Pb is also a toxic heavy metal. Pb concentrations in the edible muscle and liver tissues ranged from 0.02 (*S. lucioperca*) to maximum 0.31 (*P. fluviatilis* and *M. cephalus*) and minimum 0.066 (*S. lucioperca*) to maximum 0.43 (*P. fluviatilis*) mg/kg in wet weight, respectively, (Figure 4). The maximum Cd concentrations in liver tissues were considerably higher than the maximum level (0.05 µg/g wet weight) set by the Commission Regulation in 2006[34]. Similarly, the maximum Pb concentrations in liver tissues were slightly higher than the maximum level (0.30 µg/g wet weight) set by the Commission Regulation and Turkish Food Codex in 2008[30,34]. However, the Pb and Cd levels in the muscle tissues were below the guideline levels.

4. Discussion

Major threats in the Kızılırmak Delta are drainage and irrigation projects, pollution and filling of lakes with agricultural chemicals, illegal urbanisation on the coastal zone, sand extraction and uncontrolled hunting, degradation of the coastline due to the absence of alluvial flows from lower courses of the river after dam construction[20]. The wetland is polluted by agricultural run-off and untreated sewage from Bafra, which flows into Cernek Lake through the Badut channel, leading to eutrophication. In the eastern half of the delta, fish catches have declined drastically in recent years, from 500 tons a year in the 1970s to only 125 tons in 1995 as a result of eutrophication. Sand extraction, although illegal, is common. Reed-burning occurs throughout the year in order to improve grazing conditions[15,16].

Legal thresholds are not available for essential elements in Europe. The annual quantity of fish consumed is 6.3 kg/person[35], which is equivalent to 17.3 g/day for Turkey. The tolerable weekly intake of heavy metals as provisional tolerable weekly intake (PTWI), are set by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives. PTWI is the maximum amount of a contaminant that a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The estimated daily intake and estimated weekly intake in this study were calculated. Intake estimates were expressed as per unit body weight (mg/kg body, weight/weekly and daily).

The Joint FAO/WHO Expert Committee on Food Additives estab1ished a PTWI for Pb and Cd of 0.025 and 0.007 mg/kg body weight/week which was equivalent to 1.75 and 0.49 mg/week for a seventy-kilogram adult, respectively[36]. In this study, the estimated weekly intake and estimated daily intake for Pb and Cd minimum and maximum levels in edible muscles from consumption of Balik Lake were calculated as 0.0024–0.038 and 0.00035–0.0054 mg/kg wet weight for Pb and 0.0036–0.0048 and 0.0005–0.0007 mg/kg wet weight for Cd, respectively.

In general, metal levels in fish liver were higher than those in edible muscles at significant levels of metals. Results of the previous studies and the present study confirm that heavy metal concentrations in marine organisms have increased in liver tissues[37-42]. These findings are totally normal and will also be found in non-contaminated areas since the liver is a detoxifying organ which contains all the metals accumulated along the life-history of the fish[42]. Balik Lake has the biggest surface area and connects to the sea for irrigation in Bafra Fish Lakes in Kızılırmak Delta. Nutrients and different biological components are brought by canals into the lake. With the increasing water usage and growing population in the delta, ecosystem degradation occurs[15,16]. However, consumers do not routinely consume the liver of fish. Based on the above results, it can be concluded that metals bioaccumulation in the fish samples under study did not exceed the permissible limits set for metals by FAO/WHO. Therefore, the fishes from Balik Lake are healthy for consumption. However, it is necessary to continue the investigations on the heavy metal pollution effects on fish comparatively with other lakes in Kızılırmak Delta before reaching an exact conclusion.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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