УДК 57.022

BIOACCUMULATION OF HEAVY METALS IN TISSUES OF THE GIBEL CARP *CARASSIUS GIBELIO*: EXAMPLE OF MARMARA LAKE, TURKEY

© 2014 Murat Yabanli, Aykut Yozukmaz, Fatma Sel

Mugla Sitki Kocman University, Faculty of Aquaculture, Department of Hydrobiology
e-mail: muratyabanli@mu.edu.tr

Received September 16, 2013

Within the scope of this study, heavy metals presence (Al, Cr, Ni, Cu, As, Cd, Hg, Pb) in water samples and liver, gills and muscle tissue of gibel carp *Carassius gibelio* caught in Marmara Lake was analyzed seasonally. It was determined that there was not any difference seasonally with regard to heavy metals studied in lake waters ($p>0.05$). Mineralization of fish tissues was made by microwave-wet decomposition. To evaluate heavy metal concentrations in water and tissue samples, method of inductively coupled plasma – mass spectroscopy (ICP-MS) was applied. TORT 2 lobster hepatopancreas was used as certified reference matter in this study. According to obtained results, bioaccumulation rates for gills were found as Cu>Cd>Ni>Pb>Cr>Al>As>Hg, for liver and muscle tissues they were found as Cu>Cd>Ni>Cr>Pb>Al>As>Hg. It was proved that the most bioaccumulated heavy metal was Cu and the least bioaccumulated was Hg for all of the studied tissues.

Key words: *Carassius gibelio*, Marmara Lake, heavy metal, bioaccumulation.

Introduction

Environmental pollution first came up as a result of beginning of urban life and has increased in parallel to industrial improvement. Environmental pollution, which has accelerated depending upon population growth especially in the second half of the twentieth century, has caused life resources to be more polluted and as a result, corruption of ecosystem has gradually become a serious matter. Consequently, when aquatic ecosystem, which is a part of natural ecosystem, is used as a receiver and detractive zone for used waters and other wastes, it becomes a place, which is exposed to the densest pollution in proportion to air and earth within natural ecosystem [Yarsan et al., 2000].

The pollutant contaminants disturbing the ecological balance are some organic matters, industrial wastes, petroleum and its derivatives, agricultural chemicals, detergents, radioactivity, pesticides, inorganic salt, artificial organic chemical matters, heavy metals and waste heat. These matters are threatening factors effecting natural balance negatively. A lot of heavy metals are used in heavy metal industry and left to nature as waste. Particularly in the last twelve years, industrial improvements have proved that marine environment has been polluted by heavy metals and this pollution has affected the food chain. Heavy metals,released into ecosystem through water and foods, have the potential to change and damage all of the daily life activities by accumulating in the living beings [Hu, 2000].

The effect of heavy metals on environment and human health has recently been a focus of interest [Uluozlu et al., 2007]. Toxic elements are very harmful even if they are released in the environment at low amounts in a long period [Celik, Oehlenschlager, 2007].

Heavy metals can be in aquatic ecosystems and are bioaccumulated in food chain. Amount of heavy metals in water can change according to types and abundance of pollutant sources. Under

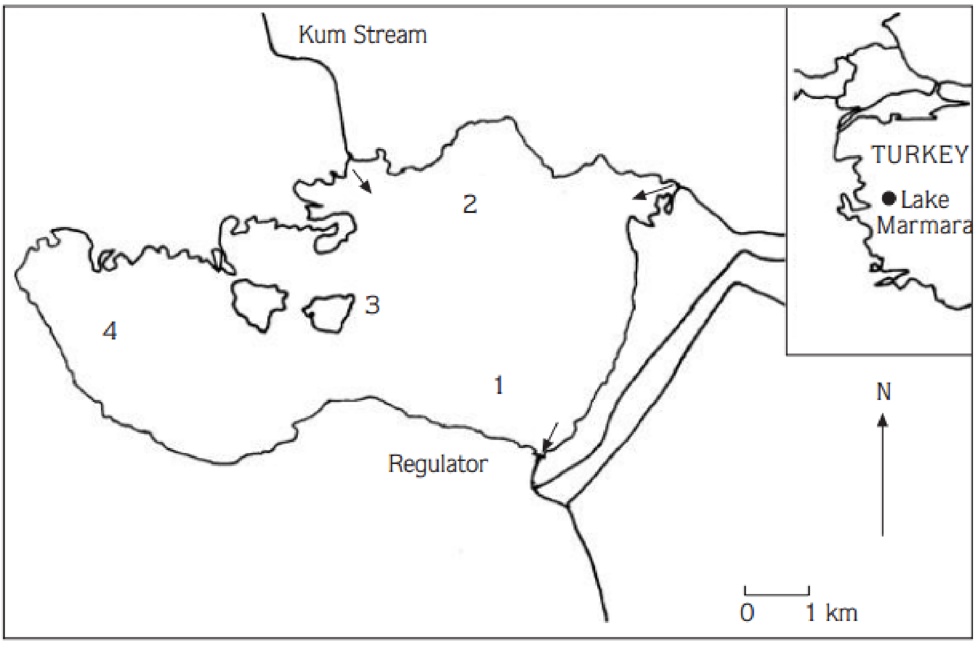

Figure 1. Lake Marmara and the 4 sampling stations used in this study. The arrows represent the outlets and inlets of the lake.

normal circumstances, ratio of heavy metals in nature is low [Kayhan et al., 2009]. Heavy metals in water can be toxic for fish, and humans consuming fish, according to their concentrations [Dural et al., 2007].

A lot of studies about heavy metal contamination in inland waters and freshwater fish are reported in literature from all around the world [Olaifa et al., 2004; Staniskiene et al., 2006; Begum et al., 2009; Klavins et al., 2009] and also from Turkey [Olaifa et al., 2004; Staniskiene et al., 2006; Begum et al., 2009; Klavins et al., 2009].

The control of natural fish stocks, which exist as a result of the sudden increase in world population, and fast consumption of natural resources can be performed with biological monitoring.

Fish is a healthy and cheap protein source for a bigger percentage of world population [Hajeb et al., 2009]. Fish is a qualified food in terms of energy and nutritious components (essential multi-unsaturated fatty acids, exogenic aminoacids, minerals and water-soluble vitamins) in human nutrition [Usydus et al., 2009]. In addition to its benefits in human nutrition, as fish accumulates toxic metals in its tissues, for public health its chemical monitoring has to be assess periodically [Cabañero et al., 2004].

The aim of this study was to determine the bioaccumulation of heavy metals (Al, Cr, Cu, Ni, As, Cd, Hg and Pb) in the tissues of gibel carp *Carassius gibelio*, an invasive fish species in Marmara Lake, and,on the basis of the results, to assess the presence of any potential risk for public health.

Materials and methods

Marmara Lake is located within the borders of Golmarmara and Salihli district in western Turkey. Marmara Lake (lat 38º 37′ N, long 28º 00′ E) is an alluvial dam lake at an altitude of 75 m. Its maximum depth is 3.5 m and it covers an area of 45 km^2 [Ustaoğlu, 1993]. It was formed as a result of tectonic collapse zone’s being filled with water and it is fed by the Demirköprü Dam in the east and the Kum River to the northwest, and it regularly drains by means of a regulator in the south end into the Gediz River (Figure 1). Agricultural activities are potently being performed around the lake.

Table 1. Microwave burning process

Phase	1	2	3	4
T (ºC)	155	200	50	50
P (pressure, psi)	30	30	0	0
Ta (run time, min)	2	10	1	1
Time (min)	15	30	15	1

Table 2. ICP-MS analysis parameters

Radio frequency power	1550 W
RF matching	2.1 V
Sample depth	8 mm
Carrier gas	0.85 L min^{-1}
Dilution gas	0.13 L min^{-1}
S/C heat	2ºC
Nebulizer type	MicroMist

Specimens of gibel carp were bought from fishermen in Marmara Lake every season and carried to laboratory in sterile polyethylene bags under cold conditions. Water samples were also collected seasonally and carried to laboratory under cold conditions after being soured with 1:3 (HNO_3:H_2O) depleted nitric acid (≥ %65 Puriss. p.a.) as pH<2. In laboratory watersamples were filtered through membrane filter of 0.45 µm. For each specimen of gibel carp, livers, muscle tissues and gills were collected and kept in Deepfreeze at -20ºC until they were analyzed. Containers in which samples were going to be put were placed in nitric acid for 15 minutes and then washed with deionized water before to be used for transportation of fish samples.

Method

Fish samples were burned in microwave burning unit (Berghofspeed wave MWS-3 and DAP-60 burning containers) and rendered liquid. Burning process was explained below.

500 mg sample was weighed and put into DAP-60 container system. 5 ml nitric acid (%65, suprapure) was added onto it. Then, covered container was put into microwave burning unit and microwave-burning program at the Table 1 was performed.

After the program ended, overall volume was completed to 25 ml with 4:1 HNO_3:H_2O_2 solution.

Samples, which were rendered liquid, were put into ICP-MS (Agilent 7700x) with the conditions shown at Table 2.

Before the analyses, mix stock solution was prepared from the multi-element Calibration solution for the elements of Al, Cr, Cu, Ni, As, Cd and Pb and standardization was made by doing measurements at the levels of 5, 10, 50, 100 and 200 ppb. For Hg, standardization was made by measuring stock solution, which was prepared with the Hg calibration solution as 0.5, 1, 5, 10 and 20 ppb.

The accuracy of microwave soaking and ICP-MS method was evaluated by using certified reference material (TORT-2 lobster hepatopancreas) for all of the elements except Al (Table 3). The accuracy of microwave soaking and ICP-MS method was evaluated by doing recovery study for Al and recovery rate as 87.98% was obtained.

Rates of bioaccumulation factors of fish were calculated by means of the formula below suggested by Gobas and Morrison [2000]:

$$BAF = K_B / K_S$$

where K_B represented concentration of chemical elements in fish and K_S

Table 3. Accuracy of the method

	TORT 2 (mg kg^{-1})	In this study (n=5, mg kg^{-1})	Recovery (%)
Cr	0.77 ± 0.03	0.75 ± 0.04	97.40
Ni	2.50 ± 0.19	2.13 ± 0.12	85.20
Cu	106.0 ± 10.0	92.36 ± 2.60	87.13
As	21.6 ± 1.8	19.65 ± 0.37	90.97
Cd	26.7 ± 0.6	24.84 ± 1.45	93.03
Hg	0.27 ± 0.06	0.28 ± 0.01	103.70
Pb	0.35 ± 0.13	0.36 ± 0.02	102.86

*Mean ± SD

Table 4. Heavy metal concentrations in gibel carp caught in Marmara Lake (mg kg^{-1})

	Gill	Liver	Muscle tissue
Al	1.31 ± 0.70	1.05 ± 0.39	1.22 ± 0.74
Cr	0.32 ± 0.33	0.36 ± 0.33	0.26 ± 0.30
Ni	0.50 ± 0.50	0.45 ± 0.53	0.35 ± 0.54
Cu	0.72 ± 1.36	0.62 ± 1.15	0.60 ± 1.32
As	0.49 ± 0.35	0.35 ± 0.20	0.28 ± 0.20
Cd	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Hg	0.04 ± 0.08	0.09 ± 0.13	0.05 ± 0.10
Pb	0.28 ± 0.09	0.16 ± 0.33	0.17 ± 0.05

*Mean ± SD

represented the concentration of chemical in water. In this study, the formula was accomplished considering each heavy metal separately.

Statistical Analyses

The statistical analysis were performed using IBM SPSS Statistics V.20 program. Summary statistics such as mean, standard deviation, minimum and maximum concentration, median, and skewness were calculated to characterize distribution of heavy metal contents. Skewness is a measure of the degree of asymmetry of a distribution in relation to a normal distribution. Given that a normal distribution has a skewness of zero, the skewness of a dataset indicates either a tail to the right (positive skewness) or to the left (negative skewness). One way-ANOVA, followed by Tukey’s test ($p < 0.05$) was used to evaluate differences among organs and in water samples.

Results

In this study, heavy metals (Al, Cr, Ni, Cu, As, Cd, Hg and Pb) analysis were made in the water samples and in the overall 96 tissues (liver, gill and muscles) of 32 fish caught in Marmara Lake.

Results of the analysis of gibel carp tissues were shown at Table 4.

The order of analyzed fish tissues in terms of heavy metal concentrations was shown below. The mean heavy metal concentrations in lake waters were shown in Table 5.

Also, dissolved oxygen, temperature, pH, TDS and salinity measurements in lake waters were made seasonally. The results were shown in Table 6.

Bioaccumulation rates calculated according to Gobas and Morrison (2000) was shown in Table 7. For gills, bioaccumulation rates were determined as Cu>Cd>Ni>Pb>Cr>Al>As>Hg and for liver and muscle tissues they were determined as Cu>Cd>Ni>Cr>Pb>Al>As>Hg.

Table 5. The mean heavy metal concentrations in lake waters

	µg L^{-1} (Mean ± SD**)**
Al	10.42 ± 2.00
Cr	1.35 ± 0.48
Ni	1.03 ± 0.32
Cu	0.33 ± 0.26
As	4.32 ± 1.04
Cd	0.02 ± 0.004
Hg	10.74 ± 4.99
Pb	1.06 ± 0.07

Table 6. Physicochemical parameters of lake water

	Summer	**Autumn**	**Winter**	**Spring**
Dissolved oxygen (mg L^{-1})	7.5	8.6	8.5	8.2
Temperature (°C)	22.78	12.9	8.1	13.6
pH	7.76	8.45	9.12	8.56
TDS (g L^{-1})	2.79	0.35	0.6	1.12
Salinity (‰)	0.24	0.26	0.29	0.25

Table 7. Heavy metal bioaccumulation rates of the tissues

	Gill	**Liver**	**Muscle tissue**
Al	125.72	100.77	117.08
Cr	237.04	266.67	192.59
Ni	485.44	436.89	339.81
Cu	2181.82	1878.79	1818.18
As	113.43	81.02	64.81
Cd	1500	1500	1500
Hg	3.72	8.38	4.66
Pb	264.15	150.94	160.38

Table 8. The mean difference between tissues in terms of bioaccumulation of different heavy metals

	Al	**Cr**	**Ni**	**Cu**	**As**	**Cd**	**Hg**	**Pb**
Muscle	0.09846	0.06615	0.09692	0.12154	0.21154^{a}	0.00231	0.01000	0.11000^{a}
Liver	0.26769	0.04077	0.12154	0.10154	0.13308^{ab}	0.00385	0.03923	0.11923^{a}
Gill	0.16923	0.10692	0.10154	0.02000	0.07846^{b}	0.00154	0.04923	0.00923^{b}

*The mean difference is significant at the 0.05 level.

The difference shown by tissues in terms of heavy metal bioaccumulation is given at Table 8. With regard to concentrations of metals Al, Cr, Ni, Cu, Cd and Hg, there was not found any difference. While there was a significant difference between concentration of metal As in muscles (a) and gills (b), the concentration in the liver (ab) was similar to both of the two tissues. With respect to Pb metal concentration, there was a similarity in muscles (a) and liver (b), it was different in gills (b).

Discussion

Aluminum: It is stated that 96-hour aluminum LC value shows a change between 95 µg L^{-1} and 235 mg L^{-1} for fish [Imray et al., 1998]. In this study, the mean aluminum concentration in lake water was determined as 10.42 µg L^{-1} and this amount was much lower than the minimum critical level. Stephens and Ingram [2006] researched aluminum level in water after deaths of *Carassius auratus* and *Cyprinus carpio* in a pool and determined aluminum concentration as 0.7 mg L^{-1}.

Chromium: As a metal found commonly and naturally, chromium mining is performed in the Philippines, South Africa, Turkey, Russia and Zimbabwe. It is used widely in the industry [Calamari, Solbé, 1994]. When fish is poisoned acutely with chromium compounds, its body surface is covered with mucus; its gill epithelium gets harmed and fish dies with suffocation symptoms. In the body cavities of fish poisoned by chromium compounds, an accumulation of orange-yellow liquid is observed. While Chromium III LD_{50} value observed in different fish species shows high toxicity with 2.0–7.5 mg L^{-1}, Chromium VI LD_{50} value shows average toxicity with 35–75 mg L^{-1} [Svobodova et al., 1993]. In this study, the mean chromium level in lake water was determined as 1.35 µg L^{-1}. Also, during the dissection of fish, yellow liquid accumulation was observed in the body cavities. Canli and Atli [2003] determined 2.22 mg kg^{-1} chromium in muscle tissues of European pilchard *Sardina pilchardus* in North Mediterranean. That value was almost nine times higher than the mean value of 0.26 mg kg^{-1}, which was obtained from muscle tissues in this study. Given that critical chromium amount for human health was 8.00 mg kg^{-1} [Tuzen, 2009], it was found in this study that there was not any threat to public health.

Copper: Copper which is an essential element, has the potential to be toxic for aquatic organisms when its amount is high in water [Martins et al., 2011]. The level of maximum copper remnant is given as 20 mg kg^{-1} in the Statement about Determination of Maximum Amounts of Certain Contaminants in Food Products-Turkish Food Codex that does not prevail now and published in September 23rd, 2002 in the 24885th issue of Official Journal [Anonymous, 2002a]. The mean copper level obtained from muscle tissues in this study was 0.60 mg/kg and this amount was 33 times lower than the maximum remnant limit. Safe copper concentration in water for fish is 1–100 µg L^{-1}. That said, this range could change as to fish species [Svobodova et al., 1993]. The mean copper concentration obtained from lake water in this study was 0.33 µg L^{-1} and this amount was much lower than safe level mentioned above. The highest mean copper amount obtained from muscle tissues of common carp, from Sakarya River by Barlas [1999] was 1.18 mg kg^{-1}. That amount was lower than the mean amount (0.60 mg kg^{-1}) found in this study. In another research on *Tilapia zillii*, copper amount in muscle tissues of control group was determined as 4.33 mg kg^{-1} [Ay et al., 1999] and it was seen that it did not exceed the limit value of 20 mg kg^{-1}.

Nickel: Nickel can be discharged from metal processing facilities to surface waters. Nickel compounds are average toxicity for fish. If fish is exposed to nickel compounds at the level of toxicity, gills are filled with mucus and lamellas turn into crimson [Svobodova et al., 1993]. 96-hour LC_{50} amounts for perch, American eel and common carp are 13.7 mg L^{-1}, 13.0 mg L^{-1} and 10.4 mg L^{-1} respectively [Calamari et al, 1994]. The mean nickel concentration obtained from lake water analyses in our study was 1.03 µg L^{-1}. It was seen that this nickel amount in water was not toxic for fish. Javed [2005] determined values of 3.74, 1.95 and 3.49 mg kg^{-1} nickel in the muscle tissues of *Catla catla*, *Oreochromis nilotica* and *Labeo rohita* respectively in Kolkata wetlands. In the study done by Barlas [1999], the highest nickel concentration found in common carp was stated as 3.06 mg kg^{-1}. In this research, the mean concentration revealed in the muscle tissues was 0.35mg kg^{-1} nickel. According to American Food and Drug

Administration, tolerable nickel concentration for Crustacea is 70 mg kg^{-1} and for bivalves it is 80 mg kg^{-1} [Anonymous, 2007]. According to Indian Official Export Supreme Audit Institution, the maximum amount of nickel remnant, which is permitted in fish and fishery products, is determined as 80 mg kg^{-1} [Anonymous, 2002b]. Nickel concentrations obtained from muscle tissues of fish in this study did not exceed the maximum levels of nickel remnant mentioned above.

Arsenic: It is an unessential metalloid, which can be found in nature plentifully. It is found in environment as a result of natural mobilization and mineralization processes such as erosion and biological activities of microorganisms [Duker et al., 2005]. Lethal arsenic concentration in water for fish is between 3–300 mg L^{-1} [Svobodova et al., 1993]. The mean arsenic concentration in the analyses made in lake waters in our study was determined as 4.32 µg L^{-1}. This level was 694 times lower than minimum lethal concentration value stated above. According to Australian standard, maximum arsenic concentration level allowed is 1 mg kg^{-1} [Anonymous, 1998]. As maximum mean arsenic concentration that we obtained from muscle tissues was 0.28 mg kg^{-1}, there was not any threat to public health. In a study done on ten freshwater fish species in the Manchar Lake (Pakistan), the highest arsenic amount obtained from muscle tissues of fish was found as 14.8 mg kg^{-1} in *Catla catla* and the lowest mean arsenic amount was found as 2.0 mg kg^{-1} in the species of *Labeo gonius* and *Cirrhinus mrigala* [Shah et al., 2009]. Also, it was seen that the mean arsenic concentrations in all of the fish exceeded the limit of Australian standard. Has-Schön et al. [2006] determined in the muscle tissues of common carp and grey mullet which they gathered from the River Neretva (Croatia) 0.038, 0.309 mg kg^{-1} arsenic respectively which was safe for public health.

Cadmium: It is a highly toxic element for all of the mammals and fish [Beširović et al., 2010]. Fish are the main source for people to be exposed to cadmium. Acute lethal concentration of cadmium in water for fish is 2–20 mg L^{-1} and acceptable cadmium concentration in water is 0.2 µg L^{-1} for salmonids and 1 µg L^{-1} for cyprinid [Svobodova et al., 1993]. In our study, the mean cadmium concentration obtained from lake water was 0.02 µg L^{-1}. This concentration was 10 times lower than critical level stated for salmonoids which were very sensitive to contaminants. According to European Union EC 1881/2006 commission regulation [Anonymous, 2006] and Contaminants' Regulation of Turkish Food Codex [Anonymous, 2011] maximum cadmium remnant level in muscle tissues of fish is 0.05 mg kg^{-1}. In this study, the mean cadmium level obtained from muscle tissues of fish was 0.03 mg kg^{-1}, so fish could be consumed safely. Barlas [1999] determined 0.181 mg kg^{-1} cadmium in the muscle tissues of common carp and this value exceeded the limit value in national and international regulations. While the mean cadmium concentrations were determined as 0.08, 0.05 mg kg^{-1} respectively in the muscle tissues of common carp and grey mullet from the Neretva Lake [Has-Schön et al., 2006], it was seen that cadmium amount found in common carp in the Neretva Lake exceeded the limit value (0.05 mg kg^{-1}). Jaffal et al. [2011] determined the highest mean cadmium concentration in the muscle tissues of *Salvelinus fontinalis* as 0.65 mg kg^{-1} and this value was very dangerous for public health.

Mercury: It is a toxicant, which is found as a result of anthropogenic activities and natural processes [Voegborlo, Adimado, 2010]. Fish accumulates the mercury in water within its body. So they undertake a main role in this heavy metal's infecting people [Voegborlo et al., 1999]. Acute lethal concentrations in inorganic mercury compounds in water are stated as 0.3–1.0 mg L^{-1} for salmonids and 0.2–4.0 mg L^{-1} for cyprinids [Svobodova et al., 1993]. In our study, the mean mercury concentration found in lake water was 10.74 µg L^{-1}. This concentration was almost 19 times lower than the lowest critical level determined for cyprinids. According to European Union EC 1881/2006 commission regulation [Anonymous, 2006] and Contaminants' Regulation of Turkish Food Codex [Anonymous, 2011], maximum mercury concentration in the muscle tissues of fish was 0.50 mg kg^{-1}. In our study, the mean mercury concentration found in the muscle tissues was 0.05 mg kg^{-1} and this value was 10 times lower than the safe level. In a study carried on in North Massachusetts, the highest mean cadmium concentrations found in *Micropterus salmoide* and *Perca flavescens* were 0.99, 0.48 mg kg^{-1} respectively [Hutcheson et al., 2008] and it was seen that mercury concentration found in the species of *Micropterus salmoides* exceeded the national and international mercury residual limits. Zhou and Wong [2000] studied on mercury concentrations in five freshwater fish species (*Tilapia*, common carp, bighead carp, silver carp, herbivorous grass carp) gathered from Pearl River Delta (Hong Kong) and determined the highest mercury concentration as 0.27 mg kg^{-1} in big head carp. This result matched up with the results of our study with regard to public health and there was not any threat.

Lead: It is an industrial pollutant found in environment and biological systems [Hsu, Guo, 2002]. General populations are exposed to lead with equal amount of air and food [Järup, 2003]. Acute toxic concentration of lead in water is 1–10 mg L^{-1} for *Salmonidae* and 10–100 mg L^{-1} for *Cyprinidae* [Svobodova et al., 1993]. According to the results of this project, the mean lead concentration in lake waters was determined as 1.06 µg L^{-1} and this value was 9433 times lower than the lowest limit determined for Cyprinids. According to European Union EC 1881/2006 commission regulation [Anonymous, 2006] and Contaminants' Regulation of Turkish Food Codex [Anonymous, 2011], maximum lead concentration in the muscle tissues of fish is 0.30 mg kg^{-1}. The mean lead amount found in the analyzed muscle tissues was determined as 0.17 mg kg^{-1} and this amount did not exceed national and international standards. Andreji et al. [2012] determined the mean lead amount as 0.10, 0.04, 0.04, 0.06 and 0.23 mg kg^{-1} in the muscle tissues of five Cyprinid species – *Gobio gobio*, *Leuciscus cephalus*, *Barbus barbus*, *Rutilus rutilus*, *Chondrostoma nasus* respectively – from the Nitra River (Slovakia). As in our study, there was not any threat to public health with regard to lead.

Conclusion

In the light of the results, it was observed that heavy metals were mostly found in gills because fish generally aspirated by filtering the water. In this study, it was seen that the most bioaccumulated heavy metal was copper and the least bioaccumulated heavy metal was mercury. When the muscle tissues of fish were analyzed in terms of mercury, cadmium and lead which was called toxic trio and were highly important for public health, it was determined that there was not any threat to public health as a result of comparison between obtained concentrations and Turkish Food Codex and EC 1881/2006 regulation. Also, when critical reference values in water, which could have toxic effect on fish and the obtained results, were compared, it was proved that there was not any toxicity danger for fish with regard to studied heavy metals. As water can be exposed to pollutants and contaminants easily and fish is well liked and consumed by people, it is very important that heavy metals in fish and water should be monitored periodically.

Acknowledgement

This article is extracted from the TAGEM/HS/11/09/02/192-numbered project supported by General Directorate of Agricultural Research and Policy and the Ministry of Food, Agriculture and Livestock.

References

Andreji J., Dvořák P., Líšková Z.D., Massányi P., Stráňai I., Naď P., Skalická M. Content of selected metals in muscle of cyprinid fish species from the Nitra River, Slovakia // *Neuroendocrinology Letters*, 2012. 33: 84–89.

Anonymous. Australia New Zealand Food Authority, Food Standards Code. Standard A12 Metals and Contaminant in Food, Issue 37. Canberra, Australia, 1998.

Anonymous. Regulation of Setting Maximum Levels for Certain Contaminants in Foodstuffs // Turkish Official Gazette, Issue 24885. Ankara, Turkey, 23 September 2002a.

Anonymous. Maksimum residual limits (MRLS) for pesticides, heavy metals and antibiotics and other pharmacologically active substances in fish and fishery products. India Export Inspection Council, Order S.O. 528 (E), 17 May 2002b.

Anonymous. Setting maximum levels for certain contaminants in food stuffs. Commission regulation (EC) No: 1881/2006.

Anonymous. Guide for the control of molluscan shellfish. US Food and Drug Administration, 2007, pp. 547.

Anonymous. Turkish Food Standards Codex // Turkish Official Gazette, Issue 28157, 2011.

Ay Ö., Kalay M., Tamer L., Canli M. Copper and lead accumulation in tissues of a freshwater fish *Tilapia zillii* and its effect on the branchial na, k-atpase activity // Bulletin of Environmental Contamination and Toxicology, 1999. 62: 160–168.

Barlas N. A pilot study of heavy metal concentration in various environments and fishes in the upper Sakarya river basin, Turkey // Environmental Toxicology, 1999. 14: 367–373.

Begum A., Harikrishna S., Irfanulla K. Analysis of heavy metals in water, sediments and fish samples of Madivala Lakes of Bangalore, Karnataka // International Journal of Chemtech Research, 2009. 1: 245–249.

Beširović H., Alić A., Prašović S., Drommer W. Histopathological effects of chronic exposure to cadmium and zinc on kidneys and gills of brown trout (*Salmo trutta* n. *fario*) // Turkish Journal Of Fisheries And Aquatic Sciences, 2010. 10: 255–262.

Cabañero A.I., Madrid Y., Cámara C. Selenium and mercury bio accessibility in fish samples: an in vitro digestion method. *Analytica chimica acta*, 2004, 526: 51–61.

Calamari D., Solbe J.F. Report on chromium and freshwater fish. Water quality for freshwater fish / Ed. Howells G. Gordon and Breach Science Publishers, 1994, pp: 223.

Calamari D., Lloyd R., Solbe J.F. Nickel And Freshwater Fish. Water Quality for Freshwater Fish / Ed. Howells G. Gordon And Breach Science Publishers, 1994, pp: 223.

Canli M., Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species // Environmental Pollution, 2003. 121: 129–136.

Celik U., Oehlenschlager J. High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets // Food Control, 2007. 18: 258–261.

Duker A.A., Carranza E.J.M., Hale M. Arsenic geochemistry and health // Environment International, 2005. 31: 631–641.

Dural M., Goksu M.Z.L., Ozak A.A. Investigation of heavy metal levels in economically important fish species captured from the Tuzla Lagoon // Food Chemistry, 2007. 102: 415–421.

Hajeb P., Jinap S., Ismail A., Fatimah A.B., Jamilah B., Rahim M.A. Assessment of mercury level in commonly consumed marine fishes in Malaysia // Food Control, 2009. 20: 79–84.

Has-Schön E., Bogut I., Strelec I. Heavy metal profile in five fish species included in human diet, domiciled in the end of flow of River Neretva (Croatia) // Arcvives Of Environmental Contamination And Toxicology, 2006. 50: 541–551.

Hsu P.-C., Guo Y.L. Antioxidant nutrients and lead toxicity // Toxicology, 2002. 180: 33–34.

Hu H. Exposure to metals // Occupational and Environmental Medicine, 2000. 27: 983–996.

Hutcheson M.S., Smith C.M., Wallace G.T., Rose J., Eddy B., Sullivan J., Pancorbo O., West C.R. Freshwater fish mercury concentrations in a regionally high mercury deposition area // Water Air Soil Pollution, 2008. 191: 15–31.

Gobas F.A.P.C., Morrison H.A. Handbook of property estimation methods for chemicals / Ed. Boethling R.S. Crc Press, 2000, pp: 192.

Imray P., Moore M.R., Callan P.W., Lock W. Aluminum. National environmental health monographs, Metal Series No.1, Public and Environmental Health Service, Australia, 1998, pp: 62.

Jaffal A., Paris-Palacios S., Jolly S., Thailly A.F., Delahaut L., Beall E., Roche H., Biagianti-Risbourg S., Betoulle S. Cadmium and copper contents in a freshwater fish species (brook trout, *salvelinusfontinalis*) from the subantarctic Kerguelen Islands // Polar Biology, 2011. 34: 397–409.

Järup L. Hazards of heavy metal contamination // British Medical Bulletin, 2003. 68: 167–182.

Javed M. Heavy metal contamination of freshwater fish and bed sediments in the river ravi stretch and related tributaries // Pakistan Journal of Biological Sciences, 2005. 8: 1337–1341.

Kayhan F.E., Muşlu M.N., Koç N.D. Bazı ağır metallerin sucul organizmalar üzerinde yarattığı stress ve biyolojik yanıtlar. (http://www.fisheriessciences.com/) 2009. 3: 153–162.

Klavins M., Potapovies O., Rodinov V. Heavy metals in fish from lakes in Latvia: concentrations and trends of changes // Bulletin of Environmental Contamination and Toxicology, 2009. 82: 96–100.

Martins C.M.G., Barcarolli I.F., Menezes E.J., Giacomin M.M., Wood C.M., Bianchini A. Acute toxicity, accumulation and tissue distribution of copper in the blue crab *Callinetes sapidus* acclimated to diffirent salinities: in vivo and in vitro studies // Aquatic Toxicology, 2011. 101: 88–99.

Olaifa F.E., Olaifa A.K., Adelaja A.A., Owolabi A.G. Heavy metal contamination of *Clarias gariepinus* from a lake and fish farm in Ibadan, Nigeria // African Journal of Biomedical Research, 2004. 7: 145–148.

Shah A.Q, Kazi T.G., Arain M.B., Jamali M.K., Afridi H.I., Jalbani N., Baig J.A., Kandhro G.A. Accumulation of arsenic in different fresh water species-potential contribution to high arsenic intakes // Food Chemistry, 2009. 112: 520–524.

Staniskiene B., Matusevicius P., Budreckiene R., Skibniewska K.A. Distiribution of heavy metals in tissues of freshwater fish in Lithuania // Polish Journal of Environmental Studies, 2006. 15: 585–591.

Stephens F.J., Ingram M. Two cases of fish mortality in low pH, aluminum rich water // Journal Of Fish Diseases, 2006. 29: 765–770.

Svobodova Z., Lloyd R., Machova J., Vykusova B. Water quality and fish health // Eifac Technical Paper, No 54, Rome, FAO, 1993, pp: 59.

Tuzen M. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey // Food and Chemical Toxicology, 2009. 47: 1785–1790.

Uluozlu Od., Tuzen M., Mendil D., Soylak M. Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey // Food Chemistry, 2007. 104: 835–840.

Ustaoğlu Mr. Zooplankton (*metazoa*) of the Lake Marmara (Turkey) // Biologia Gallo Hellenica, 1993. 20: 259–266.

Usydus Z., Szlinder-Richert J., Polak-Juszczak L., Komar K., Adamczyk M., Malesa-Ciecwierz M., Ruczynska W. Fish products available in polish market-assessment of the nutritive value and human exposure to dioxins and other contaminants // Chemosphere, 2009. 74: 1420–1428.

Voegborlo R.B., El-Methnani A.M., Abedin M.Z. Mercury, cadmium and lead content of canned tuna fish // Food Chemistry, 1999. 67: 341–345.

Voegborlo R.B., Adimado A.A. A simple classical wet digestion technique for the determination of total mercury in fish tissue by cold-vapour atomic absorption spectrometry in a low technology environment // Food Chemistry, 2010. 123: 936–940.

Yarsan E., Bilgili A., Türel İ. Van Gölü'nden toplanan midye (*unio stevenianuskrynicki*) örneklerindeki ağır metal düzeyleri // Turkish Journal of Veterinary and Animal Sciences, 2000. 24: 93–96.

Zhou H.Y., Wong M.H. Mercury accumulation in freshwater fish with emphasis on the dietary influence // Water Research, 2000. 34: 4234–4242.