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Original Research

Bioaccumulation of Trace Metals in Wastewater-Fed Aquaculture: A Case Study in Turkey

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Abstract

This study investigated the suitability of wastewater from secondary treatment used in breeding fishes of *Carassius gibelio* species, and using this fish for human food. For this purpose, metals (Fe, Mn, Cu, Ni, Zn, Cr, Pb, Cd, As and B) in treated effluent and muscle, gill and liver tissues of fish were examined seasonally. It was found that treated effluent was suitable for irrigation and partially available for aquaculture, and the concentrations in fish tissues were over standard values. The values in liver and gill were higher than muscles. The TF (transfer factor) values of all metals examined were observed as >1 in the three tissues and the metals caused bioaccumulation. Concentrations in muscles were found to be 10 to 1000 times higher than in water. Size order of TF and BCF (bio-concentration factor) values in eatable muscle tissue were the same and it was Zn>Fe>Pb>Cu>Ni>Cd>As>Cr>Mn>B. Concentration differences among the tissues for As, Cd, Pb, Ni and B were not significant (P>0.05). Because the correlations between Cd, Mn, Pb and Cu concentrations in tissues and treated effluent were found to be statistically significant, the metals caused bioaccumulation because of treated effluent. HQ (hazard quotient) and BCF values of Pb in muscle had carcinogenic risk levels.

Keywords: bioaccumulation, trace metals, wastewater-fed aquaculture

Introduction

Globally, more people live in urban areas than in rural areas, with 54 percent of the world's population residing in urban areas in 2014. In 1950, 30 percent of the world's population was urban, and by 2050, 66 percent of the world's population is projected to be urban. Africa and Asia are urbanizing faster than the other regions and are projected to become 56 and 64 per cent urban, respectively, by 2050. Urban population numbers and water use patterns mainly determine the management of water supply and wastewater disposal and thereby contribute to the ecological footprint of a city. For the reasons of population growth, urbanization and industrialization, serious water demand will be in Turkey and the world. In addition, it was forecasted that water demand in Turkey of 1586 L/person/day in 1997 will increase to 3375 L/person/day in 2030 [1].

Since domestic wastewater often intermixes with effluents from industries and agricultural runoff,

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multidimensional approaches have been made toward maximizing protein production through rational exploitation of available resources. Sewage-fed aquaculture is a unique system and has manifold advantages in developing countries acting as a major source of nutrients for crop farming and aquaculture, and it is economical for sustainable production and helps to combat environmental pollution. The subject of sewage-fed aquaculture is reviewed in terms of source, chemical nature, diversity pattern, recycling practices, production potential of aquaculture, environmental issues and safety measures for eco-friendly sustainable environmental management strategies [2]. Wastewater was used in agriculture and aquaculture in 10 countries of Latin America, North Africa, and west and southeast Asia [3]. The economic viability of an aquaculture system depends on many technical, social, and economic factors that are specific to a given area or country. However, if they facilitate more efficient excreta collection, they should lead to reductions in both environmental pollution and unsanitary disease, and augment food production. A cost-benefit analysis was made of the experimental culture of fish in the Quail Creek sewage lagoon system near Oklahoma City. Net return was estimated at $ 0.02/1,000 gallons raw of sewage [4]. Besides, if sewage water is treated, its use in aquaculture has some health disadvantages because of its toxic chemicals and microbiological pollution. Especiall, heavy metals are potentially harmful to most organisms at some level of exposure and absorption. Some heavy metals (cobalt, copper, iron, manganese, and zinc) are essential in trace concentrations to many organisms for enzyme function and are less toxic than non-essential heavy metals (cadmium, lead, mercury), which are toxic at the lowest concentrations [5].

It was found that wastewater-fed aquaculture in some of these studies could be used for human and animal food [6-10]. The major freshwater fish species used in this area include *Labeo rohita* (rohu), *Catla catla* (catla), *Cirrhinusmrigala* (mrigal), *Oreochromis mossambicus* (tilapia), and *C. carpio* (common carp) [10], black bass (*Micropterus salmoides*), big head (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*), tench *(tinca tinca),* kissing gouramy *(Helostoma tem,nincko),* silver barb *(Puntius gonionotus),* nilem carp *(Osteochilus hasseltil)* and giant gourami *(Osphronemusg oramy)*[4]. These species are warm-water fish of temperate origin and can survive year-round in climates with seasonally low temperatures [4].

In this study, trace metal concentrations in muscle, gill and liver tissues of the *Carassius gibelio* species fed with wastewater from Bursa Water and Sewage Administration East Treatment Place were investigated. In addition, their bioaccumulations and health risks (transfer factors – TF, bio-concentration factors – BCF and Hazard quotient – HQ) were computed and evaluated by comparison with metal concentrations in wastewater. *Carassius gibelio* is a species that can keep up with all kinds of climate and environmental conditions. Also, overbreeding of this species occurred as a result of the wrong inoculates in the past years in Turkish inland waters [11], and these fish must be evaluated economically. For this reason, this species was investigated in this study.

The objectives of this study were to investigate the suitability of fish for human food consumption in terms of metals, to provide a basis for the development of a standard on the concentration of heavy metals in reclaimed water used for fish aquaculture, and to search the possibilities of technical improvement of the system in terms of more efficient sewage treatment. This study was performed with the different fish species in the Marmara region, where the transition climate between the Mediterranean and Continent is dissimilar from the studies done so far that have contributed to the international literature and was significant. Also, this study included useful and valuable information for evaluating potential health risks in wastewater recovery as aquaculture feeding water.

Material and Methods

Study Locations

The East Wastewater Treatment Plant occupies 250000 m^2 in the Küçük Balıklı area and treats domestic wastewater of Bursa City East Basin. It appeals to a population equal to about 1550000 and the project volume of flow is 240000 m^3/day for 2017 and 320000 m^3/day for 2030. Its discharge area is Deliçay Creek, which is in the Susurluk River Basin. According to sensitive and less sensitive water areas notification, the discharge place is normal water area. The process type is the five-stage Bardenpho, which is an advanced biological treatment method, and nitrogen and phosphorus removal is performed. There is not a chemical treatment established. The flow diagram of the East Wastewater Treatment Plant is given in Fig. 1 and the influent-effluent wastewater characteristic of Treatment Plant of 2011 and removal performance data are presented in Table 1.

Carassius gibelio-type fish grown in wastewater-fed ponds were caught seasonally and metal concentrations in muscles, gills and livers were examined.

Sample Handling and Analysis of Water and Fish Tissues

The samples were examined for the presence of 10 elements (Fe, Mn, Cu, Zn, Cr, Pb, Cd, Ni, As and B) that were widely found in contaminated water and fishes.

The water samples were taken by using an Aquacell P2-COMPACT portable composite sampler, then transferred to dark polyethylene (PE) bottles washed with HNO_3 and deionized water [12]. All the samples were taken monthly in one year from January 2011 to

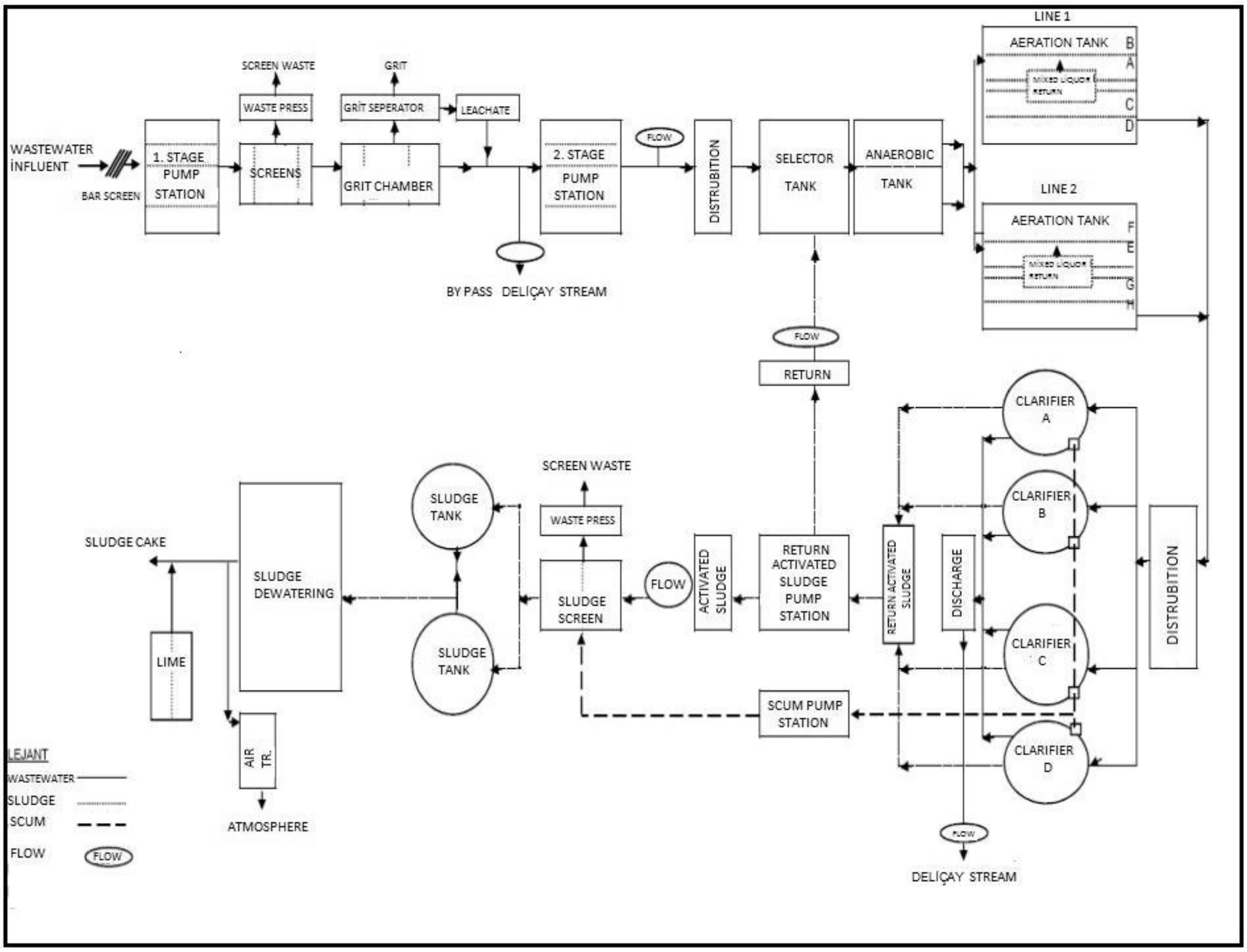

Fig. 1. Flow diagram of East Wastewater Treatment Plant.

2012 in order to see the seasonal changes. Water samples were filtered through a Milipore filter paper with pre-weighed 0.45 µm pore-size. The filtered water samples were acidified with 0.2% (v/v) concentrated nitric acid as for the precipitation samples and kept in glass bottles cleaned with detergent, water, nitric acid and Mili-Q water [13].

Twelve fish samples of *Carassius gibelio* specie were caught in the same year (2011-2012, 3 samples per season) from the fish breeding pool after the last clarifier.

Table 1. Influent-effluent wastewater characteristic of treatment plant (2011) and removal performance data.

Seasons	Influent							Effluent						Efficiency (%)				
	pH	T	AKM	BOI	KOI	TP	TN	pH	AKM	BOI	KOI	TP	TN	AKM	BOI	KOI	TP	TN
Winter	7.64	14.9	195	211	478	8.6	49	.56	10.8	14	39	2.3	12.5	94	93	92	73	74
Spring	7.71	16.7	169	185	410	7.8	48	7.56	10.2	14	37	2.1	11.2	94	92	91	73	77
Summer	7.72	23.9	202	218	496	8.5	55	7.73	11.5	14	39	2.6	7.4	94	94	92	69	87
Autumn	7.66	21.7	208	233	499	8.1	51	7.76	10.4	14	41	1.7	4.9	95	94	92	79	91
Annual Average	7.68	19.3	193.5	211.8	470.8	8.25	50.8	7.65	10.73	14	39	2.18	9	94.3	93.3	91.8	73.5	82.3
Max	7.72	23.9	208	233	499	8.6	55	7.76	11.5	14	41	2.6	12.5	95	94	92	79	91
Min	7.64	14.9	169	185	410	7.8	48	7.56	10.2	14	37	1.7	4.9	94	92	91	69	74
Std Dev.	0.04	4.24	17.18	20.06	41.55	0.37	3.1	0.11	0.573	0	1.63	0.38	3.49	0.5	0.96	0.5	4.12	8.06

The fish were taken to a laboratory in polyethylene caps and their sizes were recorded. The fish were cut from their backing using a stainless steel knife, and muscle, liver, and gill tissues were removed. These tissues were then homogenized and 0.5 g (wet weight) were weighed and placed in constantly weighed petri dishes to dry for 24 hours in a drying oven. Afterward, the tissue samples fixed to dry weight were placed in temperature- and pressure-compensated HP500 Teflon caps. 7 ml nitric acid (HNO_3) and 1 ml hydrogen peroxide (H_2O_2) were added as reagents and the samples were digested using a CEM Mars 5 Model microwave instrument [14]. Microwave decomposition operations were programmed as a 3-phase process. The device operated at 5 psi (1 psi = 6.89 kPa) for 1 minute in the 1st stage, at 25 psi for 5 minutes in the 2nd stage and at 120 psi for 60 minutes in the 3rd stage [13]. After the samples were cooled to room temperature they were diluted to 50 ml using ultra-pure water. The chemicals used in the digestion were analytically pure and ultra-pure water was used throughout the study [14]. For 10 years, microwave digestion systems have been used for sample extractions [15-17]. This technique is preferred because of its advantages such as rapid digestion, less contamination probability, and less acid consumption [16]. Blanks and European standard reference materials (ERMBB422) were included in each digestion batch to verify the accuracy of the calibration and standardization method, and all analyses were done in duplicate. The device was calibrated using a solution of 5 mg L^{-1} prepared from a Merck Mn solution of 500 mg L^{-1}. An ambient temperature of 25ºC was maintained to prevent possible expansion during calibration [13].

Heavy metals and trace elements in water and fish tissues were determined using the VISTA-MPX model of the VARIAN brand ICP-OES device. Also in water samples, dissolved oxygen (DO), temperature (T), pH and electrical conductivity (EC) parameters of water were measured using a HACH Sension 156 device [12]. Suspended solid matter (SS), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) parameters were measured according to the standard methods [12]. Total nitrogen (TN) parameters were measured by water vapors distillation and, finally, total phosphorus (TP) was measured using the ascorbic acid method [12].

Selection of the Fish Species

In the last 100 years, intentional or unintentional entrance of invading fish species increased remarkably the destruction on aquatic habitats, and the success of these species over the world was been worrisome for all aquatic forms [18]. The *Carrassius gibelio* species that was assumed to be the most dangerous invading species by the Ministry of Agriculture was recorded in the harmful species with the 37/2 numbered circular and sportive fishing, and it was unleashed during 12 months in Turkey [19]. In this research, this species that causes environmental pollution was studied in order to examine metal accumulation and to investigate the availability of the usage of this species for different purposes (e.g., food, bait etc.) and to make it beneficial for decreasing this species.

Determining Metal Bioaccumulations and Statistical Analysis

Evaluating Metals in Fish and Wastewater

Metal concentrations in the effluent wastewater of a treatment plant from which the fish was fed were compared with national and international toxic values for potable water, irrigation water, surface water and wastewater discharge standards [20-27]. Metal concentrations found in muscle, gill and liver tissues of fish samples were evaluated separately for dry and wet weight, and toxic levels were compared according to national and international standards [28-33].

Metal Bioaccumulations and Risk Assessment

The transfer and bio-concentration factors were calculated in order to determine metal bioaccumulation in fish tissues. The transfer factor in fish tissues from the aquatic ecosystem, which include water and sediments, was calculated according to Rashed (2001) [34] as follows:

$$TF = M_{tissue}\,(\text{mg/kg dry weight}) / M_{sediment\ or\ water}\,(\text{mg/L}) \quad (1)$$

and also bio-concentration factor as follows [35]:

$$BCF = M_{tissue}\,(\text{mg/kg wet weight}) / M_{water}\,(\text{mg/L}) \quad (2)$$

…where M_{tissue} is the metal concentration in fish tissue and $M_{sediment}$ is metal concentration in sediment. The concentrations in TF sediments were not used in this study because only the effect of water was examined.

BCF is based on water-only exposures (lab data). BCF and TF are inversely related to exposure concentrations. BCF >1000 and TF >1 have been used to signify hazards in many national regulatory schemes. However, hazard and the potential for chronic effects cannot be just evaluated by magnitude of BCF [36]. TF, BCF and standard concentration values should be evaluated together. BCF has different values for each metal [35].

The risk for human health as a result of eating *Carassius gibelio* was evaluated by calculating estimated daily intake (EDI) using the following equation [37, 38]:

$$EDI = \frac{\text{Cfish}*\text{Dfish}}{BW} \quad (3)$$

…where C_{fish}= the average trace element concentration in fish muscle (µg/g dry weight), D_{fish}= global average daily fish consumption (g/day) (which was only 1.7 g/day for Turkey) [39], and BW= average body weight (kg).

U.S. EPA risk analysis considers an adult average body weight of 70 kg [40]. The hazard quotient (HQ) was calculated by dividing the estimated daily intake (EDI) by the established RfD (reference dose) to assess the health risk from fish consumption. There would be no obvious risk if the HQ were less than 1 [37].

Statistical Analysis

Statistical analysis of metals in treated effluent wastewater and fish tissues was performed. Correlation coefficients (r) determined the correlations between the parameters, and p values showed the significance levels and factorial ANOVA analysis carried out by using the general linear model method to be able to see if there were any variations in metal concentrations between tissues and seasons. The significance level was detected as $p \leq 0.05$. Statistical calculations were done with a Minitab 17.0 Program [41]. The results were presented as the mean of triplicate determination.

Results and Discussion

Metal Concentrations in Fishes and Treated Effluent Wastewater (TEW)

Metal (Fe, Mn, Cu, Ni, Zn, Cr, Pb, Cd, As and B) concentrations in muscle, gill and liver tissues of *Carassius gibelio* species were determined seasonally and their average values were compared with national and international standards. Concentrations were evaluated according to FAO [28, 30, 31, 38, 42], WHO [29], USFDA (U.S. Food and Drug Administration) [38, 43], TFC (Turkish Food Codecs) [33], England [32], the European Union (EU) [25] and Turkey [33] aquaculture standards.

The order of the metals according to their concentrations was: Fe>Zn>B>Pb>Ni>Mn>Cu>Cr>Cd>As. According to FAO and WHO standards, it was found that Mn, Cr, Pb, Cd, and Zn were higher and Cu, Ni, Fe and As were lower. Different from the FAO and WHO values, according to English and Turkish standards Mn and Zn were found to be much lower. Metal concentrations in muscle tissues were compared with the results of other research examining *Carassius gibelio* and different fish species. As concentrations were found between the same and different fish species, B was found to be higher than other fish species. Fe and Ni were higher than *Carassius gibelio,* and lower than *Cyprinus carpio* in other research. Zn, Cr and Pb were higher than *Carassius gibelio* and other species. Mn and Cd were in the range of concentrations of *Carassius gibelio* and higher than other species. Cu was in the concentration range determined for *Carassius gibelio* and lower than *Cyprinus carpio.*

The order of the metals in gill tissue according to their concentrations was: Zn>Fe>Mn>B>Pb>Ni>Cu>Cr>Cd>As. When the concentrations were compared with standard values, Fe, Mn, Zn, Cr, Pb, Cd and As were found to be higher than FAO and WHO standards, but Cu and Ni were found to be lower than these standards. According to Turkish standards, Mn was lower as well, and other metals were the same as evaluating FAO and WHO standards. When it was compared with other research, Fe was found to be lower than other *Carassius gibelio* and *Cyprinus carpio* species. Mn was found to be lower than other *Carassius gibelio* and higher than *Cyprinus carpio* species. Cu and As were found in the range determined for *Carassius gibelio* species and lower than *Cyprinus carpio* species. Zn and Ni were higher than the concentrations of *Carassius gibelio* species, and lower than *Cyprinus carpio*. Cr, Pb and Cd were higher than the values determined for other *Carassius gibelio* and *Cyprinus carpio* species. B was found to be higher than the concentrations found in other fish species.

The order of the trace elements in livers tissue according to their concentrations was: Fe>Zn>B>Pb>Cu>Ni>Mn>Cr>Cd>As. According to FAO and WHO standards, Fe, Mn, Zn, Cr, Pb and Cd were found to be higher, and Cu, Ni, and As were found to be lower. While Mn, according to Turkish standards, and Pb, according to English standards, were found to be lower, other metals were evaluated as the same as evaluating of FAO/WHO standards. When compared with other research, Fe, Cu and Cr were in the range determined for *Carassius gibelio*, and higher than *Cyprinus carpio* and other species. Mn was in the concentration range determined for similar species – *Carassius gibelio*, and Pb and Zn were found to be higher than fish in both the same and different species. Cd was higher than the other concentrations of *Carassius gibelio* species, and in the range determined for other fish species. Ni was higher than the concentrations of Carassius *gibelio* species and lower than the other species. As was lower than the concentrations of the same species, and in the range of values determined for other species. B was found to be higher than other species [14, 44].

For B element there were no standard values found in regulations. Muscle, gill and livers were determined respectively as 8.525, 8.388 and 21.013 $mg.kg^{-1}$ (dw). As value was found for the limit value in each three tissues. Comparison of the metals found in muscle, gill and liver tissues and national-international standard values are presented in Tables 2-5.

The most harmful heavy metals investigated in this research were Pb and Cd. These metals were hazardous in every concentration, they did not have biological functions and they were in the primary contaminant list [60]. The As, which was in the second group metals, was not biochemically necessary for the human body. Only its trace amounts could be tolerated. The third group of

Table 2. Comparison of the metals found in muscle tissues and national-international standard values.

Element	References	Muscle (mg kg^{-1})	FAO, 1983/ WHO (dw mg kg^{-1})	Turkish Guidelines / TFC (ww mg kg-^{1})	England (ww mg kg^{-1})
Fe	Özparlak, et al. 2012 (Beyşehir Lake, *Carassius gibelio*, (dw)) [58]	2.61 ±2.43			
	Köse and Uysal 2008 (Enne Dam Lake, *Cyprinus carpio,*(ww)) [59]	16.98±0.02			
	Milosˇkovic´, et al. 2013 (Gruzˇa Reservoir, Serbia, Carassius gibelio, (dw /ww)[60]	18.1±9.65 / 7.53±4.02	100		
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw))[61]	48.47±10.43			
	This study (ww / dw)	15.273±7.30 / 82.686±39.55			
Mn	Özparlak, et al. 2012 (Beyşehir Lake, *Carassius gibelio*, (dw))[58]	9.34± 0.36			
	Uysal and Atalay 2007 (DPÜ Lake, *Cyprinus carpio*, (ww)) [62]	0.14±0.05			
	Iraida et al. 2012 (Kadi Lake, *Carassius auratus gibelio*, (ww))[63]	0.64±1.174			
	Milosˇkovic´, et al. 2013 (Gruzˇa Reservoir, Serbia, Carassius gibelio, (dw ww)[60]	1.23±0.59 / 0.512±0.244	1	20	
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw))[64]	0.67±0.29			
	This study (ww / dw)	0.855±1.316 / 4.628±7.123			
Cu	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,*(dw))[64]	1.01±1.22			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw))[65]	7.4±1.1			
	Uysal and Atalay 2007 (DPÜ Göleti, *Cyprinus carpio* (ww))[62]	2.55±0.6			
	Wang et al, 2014 (Huainan Coal Fields , Carassius Gibelio, (dw))[66]	1.14±0.22	30	20	20
	Milosˇkovic´, et al. 2013 (Gruzˇa Reservoir, Serbia, Carassius gibelio, (dw/ww)[60]	0.877±0.286 / 0.365±0.119			
	Yabanli, et al. 2013 (Marmara Lake, Carassius gibelio, (dw))[67]	0.60±1.32			
	This study (ww / dw)	0.777± 0.563 / 4.205±3.047			
Zn	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw))[64]	33.86±8.70			
	Sapozhnikova et al. 2005 (Dniester River, *C. auratus gibelio*, (ww))[68]	6.90-9.95			
	Özparlak, et al. 2012 (Beyşehir Lake, *Carassius gibelio*, (dw))[58]	13.98±2.70			
	Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww))[69]	6.71	50/100	50	50
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw))[61]	46.39±16.12			
	This study (ww / dw)	9.453± 3.102 / 51.169±16.791			

Table 2. Continued.

Cr	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw))[65]	0.7±0.2			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,* (dw))[64]	<Detection Limit			
	Sapozhnikova et al. 2005 (Dniester River, *C. auratus gibelio*, (ww))[68]	0.15	1		
	Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw))[67]	0.26±0.30			
	This study (ww / dw)	0.420±0.399 / 2.273±2.159			
Pb	Çiçek, et al. 2008 (Uluabat Lake, *Carassius gibelio* (dw))[70]	0.64± 0.65			
	Mazej et al. 2010 (Velenjsko jezero, *Cyprinus carpio,* (ww))[69]	0.01	0.5	1 / 0.3	2
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw))[65]	1.3±0.5			
	Wang et al, 2014 (Huainan Coal Fields , Carassius Gibelio, (dw))[66]	0.26±0.06			
	Milosˇkovic´, et al. 2013 (Gruzˇa Reservoir, Serbia, Carassius gibelio, (dw/ww)[60]	0.437±0.287 / 0.182±0.119			
	This study (ww / dw)	1.046±0.784 / 5.661±4.243			

metals that were needed biochemically were Fe, Mn, Cu, Zn, Ni, and Cr. However, these metals might be toxic above a specific amount. These group elements like Ni, Cr and Cu were carcinogenic due to their interaction with nucleic acids [61]. In addition, heavy metals caused structural malfunctions and DNA breakage at the cellular and molecular level in aquacultures [62]. The reason for concentrations of Fe, Zn, Mn and B were found to be higher in order of magnitude is that they were essential elements.

When the metal concentrations in tissues were compared, it was seen that national and international

Table 3. Comparison of the metals found in gill tissues and national-international standard values.

Elements	References	Gill (mg kg^{-1})	FAO, 1983/ WHO (dw mg kg^{-1})	Turkish Guidelines / TFC (ww mg kg^{-1})	England (ww mg kg^{-1})
Fe	Öztürk et. al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	203.7±106.9			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	913.75±360.27			
	Köse and Uysal 2008 (Enne Dam Lake, *Cyprinus carpio*, (ww)) [59]	87.19±11.88	100		
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	315.27±84.56			
	This study (ww / dw)	54.322±20.051 / 287.309±106.049			
Mn	Uysal and Atalay 2007 (DPÜ Lake, *Cyprinus carpio*, (ww)) [62]	3.08±0.78			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	41.57±14.35			
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	33.86±9.42	1	20	
	This study (ww / dw)	4.088±1.410 / 21.621±7.404			

Table 3. Continued.

Cu	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	11.9±4.1			
	Uysal and Atalay 2007 (DPÜ Lake, *Cyprinus carpio*, (ww)) [62]	3.815±0.12			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,*(dw)) [64]	2.26±1.16			
	Wang et al, 2014 (Huainan Coal Fields , Carassius Gibelio, (dw)) [66]	1.7±0.16	30	20	20
	Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw)) [67]	0.72±1.36			
	This study (ww / dw)	0.800±0.350 / 4.231±1.851			
Zn	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	191.89±29.67			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	31.3± 10			
	Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww)) [69]	58.6			
	Uysal and Atalay 2007 (DPÜ Lake, *Cyprinus carpio*, (ww)) [62]	218.96±14.46	50/100	50	50
	Wang et al, 2014 (Huainan Coal Fields , Carassius Gibelio, (dw)) [66]	14.44 ±0.75			
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	286.11±103.65			
	This study (ww / dw)	131.520 ±45.916 / 695.609±242.849			
Cr	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	1.4±0.6			
	Uysal and Atalay 2007 (DPÜ Lake, *Cyprinus carpio*, (ww)) [62]	0.51±0.02	1		
	Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw)) [67]	0.32±0.33			
	This study (ww / dw)	0.604±0.377 / 3.194±1.993			
Pb	Çiçek, et al. 2008 (Uluabat Lake, *Carassius gibelio (dw)*) [70]	1.28±136			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	5.95±2.52			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	3.1± 1.6			
	Mazej et al. 2010 (Velenjsko jezero, *Cyprinus carpio,* (ww)) [69]	0.06	0.5	1 / 0.3	2
	Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww)) [69]	0.48			
	Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw)) [67]	0.28±0.09			
	This study (ww / dw)	1.481±0.628 / 7.833±3.321			

Table 4. Comparison of the metals found in liver tissues and national-international standard values.

Elements	References	Liver (mg kg^{-1})	FAO, 1983/ WHO (dw mg kg^{-1})	Turkish Guidelines / TFC (ww mg kg-^{1})	England (ww mg kg^{-1})
Fe	Öztürk et. al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	94.27±54.85			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	<Detection Limit	100		
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	7926.08±4648.01			
	This study (ww / dw)	202.25±153.018 / 1104.576±835.63			
Mn	Iraida et al. 2012 (Bol'shoi Ussuriiskii Island, *C. auratus gibelio*, (ww)) [63]	2.37±1.852			
	Iraida et al. 2012 (Sindinskaya Passage, *Carassius auratus gibelio*, (ww))	9.22±8.121			
	Iraida et al. 2012 (Kadi Lake, *Carassius auratus gibelio*, (ww))	0.64±0.266	1	20	
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	8.13±5.98			
	This study (ww / dw)	0.961±0.707 / 5.248±3.860			
Cu	Iraida et al. 2012 (Bol'shoi Ussuriiskii Island, *C. auratus gibelio*, (ww)) [63]	4.71±1.717			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	20.5±3.8			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,*(dw)) [64]	8.34±5.76	30	20	20
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	36.197 ±54.185			
	Yabanli, et al. 2013 (Marmara Lake, Carassius gibelio, (dw)) [67]	0.62±1.15			
	This study (ww / dw)	1.270±0.683 / 6.935±3.729			
Zn	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	108.12±81.18			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	27.4±3.0			
	Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww)) [69]	38.6	50/100	50	50
	Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	119.96±129.65			
	This study (ww / dw)	97.523±65.213 / 532.573±356.128			
Cr	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	2.7±0.9			
	Öztürk et. al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	0.83±0.53			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,* (dw)) [64]	<Detection Limit	1		
	Yabanli, et al. 2013 (Marmara Lake, Carassius gibelio, (dw)) [67]	0.36±0.33			
	This study (ww / dw)	0.438±0.139 / 2.391±0.759			

Table 4. Continued.

Pb	Çiçek, et al. 2008 (Uluabat Lake, *Carassius gibelio, (dw)*) [70]	8.1±18.52			
	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio*, (dw)) [64]	0.55±0.96			
	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	3.1±0.6			
	Mazej et al. 2010 (Velenjsko jezero, Cyprinus carpio, (ww)) [69]	0.06	0.5	1 / 0.3	2
	Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww)) [69]	0.05			
	Yabanli, et al. 2013 (Marmara Lake, Carassius gibelio, (dw)) [67]	0.16±0.33			
	This study (ww / dw)	1.613±0.839 / 8.808±4.581			

limit values are different. Six of 10 metals examined conformed to national standards, but did not conform to international standards. For example, while Mn and Cr concentrations were found in each three tissue, in terms of wet weight, were under Turkish standard values, their concentrations, in terms of dry weight, were above international limit values. There was too much difference between standard values. The difference between concentration levels of countries and institutions, in terms of wet and dry weight, was also insignificant. Because of possible analysis mistakes stemming from moisture loss while determining concentrations in terms of wet weight, determining concentrations in terms of dry weight would help to

Table 5. Comparison of Cd, Ni, As and B elements found in muscle, gill and liver tissues and national-international standard values.

Element	Tissues	References	Concentration (mg kg^{-1})	FAO, 1983/ WHO (dw mg kg^{-1})	Turkish Guidelines / TFC (ww mg kg-^{1})	England (ww mg kg^{-1})
Cd	Muscle	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	0.29±0.19			
		Özparlak, et al. 2012 (Beyşehir Lake, *Carassius gibelio*, (dw)) [58]	2.29±0.06			
		Sapozhnikova et al. 2005 (Dniester River, *C. auratus gibelio*, (ww)) [68]	0.04	0.5/1	0.1 / 0.05	0.2
		This study (ww / dw)	0.229±0.264 / 1.239±1.429			
	Gill	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	0.45±0.29			
		Öztürk et al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	0.15±0.14			
		Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw)) [67]	0.03±0.02			
		This study (ww / dw)	0.248±0.306 / 1.311±1.618			
	Liver	Ebrahimpour et al. 2011 (Anzali Wetland, *Carassius gibelio,* (dw)) [65]	1.05± 0.34			
		Mazej et al. 2010 (Velenjsko jezero, *Carassius auratus gibelio*, (ww)) [69]	0.02			
		Öztürk et. al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	0.79±0.33			
		This study (ww / dw)	0.289±0.275 / 1.578±1.501			

Table 5. Continued.

Ni	Muscle	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,* (dw)) [64]	0.77±1.26			
		Özparlak, et al. 2012 (Beyşehir Lake, *Carassius gibelio*, (dw)) [58]	1.71±0.29			
		Öztürk, et. al. 2009 (Avsar Dam Lake, Cyprinus carpio, (ww) [71]	1.27±1.18	10		
		This study (ww / dw)	0.966±0.945 / 5.228±5.115			
	Gill	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,* (dw)) [64]	1.55±1.16			
		Öztürk et al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	3.52±3.33			
		This study (ww / dw)	1.004±0.694 / 5.310±3.670			
	Liver	Çiçek et. al. 2009 (Manyas Lake, *Carassius gibelio,* (dw)) [64]	2.46±1.50			
		Öztürk et. al. 2009 (Avşar Dam Lake, *Cyprinus carpio*, (ww)) [71]	7.0±1.94			
		This study (ww / dw)	0.977±0.645 / 5.335±3.522			
As	Muscle	Iraida, et. al. 2012 (Kadi Lake, *C. auratus gibelio*, (ww)) [63]	0.04±0.023			
		Iraida, et. al. 2012 (Sindinskaya Passage, *C. auratus gibelio*, (ww)) [63]	0.06±0.042			
		Milosˇkovic´, et al. 2013 (Gruzˇa Reservoir, Serbia, Carassius gibelio, (dw/ww) [60]	0.255±0.150 / 0.106±0.062	0.27		
		This study (ww / dw)	0.042±0.0236 / 0.227±0.1277			
	Gill	Has-Schön et al. 2008 (Hutovo Blato, *Cyprinus carpio*, (ww)) [72]	0.093±0.005			
		Yabanli, et al. 2013 (Marmara Lake, *Carassius gibelio*, (dw)) [67]	0.49±0.35			
		This study (ww / dw)	0.0585±0.0267 / 0.309±0.141			
	Liver	Has-Schön et al. 2008 (Hutovo Blato, *Cyprinus carpio*, (ww)) [72]	0.071±0.007			
		Iraida, et. al. 2012 (Kadi Lake, *C. auratus gibelio*, (ww)) [63]	0.03±0.016			
		Skoric, et al. 2012 (Ecka Fish Farm, *Carassius gibelio*, (dw)) [61]	0.69±1.36			
		This study (ww / dw)	0.0443±0.0296 / 0.241±0.161			
B	Muscle	Emiroğlu et. al. 2010 (Seydi Stream, *Leiscus cephalus*, (dw)) [57]	7.34±0.44			
		Uysal et. al. 2009 (Enne Dame Lake, *Carassius carassius*,(ww)) [23]	<Detection Limit			
		This study (ww / dw)	1.575±1.457 / 8.525±7.886			

prevent evaluation mistakes. Since there were no guideline values or provisional limits for metal intake (g/day/body weight), the results obtained in this study could be used to derive such guideline values. However, this needs to be further examined in future studies [38]. Heavy metals not only influence living bodies depending on their concentrations. The influence depends on species of organisms and ionic structure

Table 6. ANOVA concentration differences between tissues and months .

	As	Cr	Cd	Pb	Cu	Ni	B	Fe	Mn	Zn
P values (Tissue)	0.207	**0.015**	0.829	0.123	**0.027**	0.979	0.159	**0**	**0**	**0**
P values (Months)	**0.01**	**0**	**0.008**	**0.02**	**0.003**	**0**	0.496	0.117	**0**	**0.025**

*Dark Colored p<0.05

of metal (solubility value, chemical structure, redox and ability of forming complex), intake type to body, presence frequency in the environment and pH value of water. Therefore, the water used in aquaculture was limited by legal institutions with limit values and their control was an obligation [63].

The wastewater that the fish fed from TFE was evaluated by national and international regulations. According to this, it is determined that most of the metals were above the drinking water standards [24, 25, 27] and the USEPA's toxic evaluation values of surface water [26], Zn and Cu is not available according to Turkish Aquaculture Regulation [22], but other metals were available, and all metals were appropriate with the standards for Irrigation Water of Technical Methods Notification of Wastewater Treatment Plants [23]. In addition, according to Aquaculture Regulation of the People's Republic of China-GB 8978, all metals were determined as convenient except for Cd [21]. Annual average and standard deviation values of metals in treated feed wastewater (TFE) were presented in the chapter "Metal Bioaccumulations and Risk Assessment," according to which wastewater might be available for irrigation water and partially available for aquaculture.

Statistical Analysis

In order to determine whether the concentration difference between tissues and months were important or not, variance analysis and factorial ANOVA table with general linear method were made and calculated by using the Minitab 16 program [41]. Significance of the results was evaluated at a significance level of $p<0.05$.

In general, different heavy metal accumulation capacities were observed among different tissues. While the high metal concentrations were found in livers and gills, the lowest metal accumulation was observed in muscles. The high metal concentrations in the gills were the first target for pollutants that could be due to the formation of complex ions with mucus, which virtually could not be completely removed from the gill Lamellae before preparation for analysis [64]. Also, liver is a detoxification organ accumulating toxic elements. The reason for low levels of heavy metals could be the low levels of binding protein in muscles [38]. In Table 6, p values of the ANOVA table, which were calculated for concentration differences between tissues and months, were presented.

According to the table, concentration differences between tissues for Cr, Cu, Fe, Mn and Zn were significant ($p<0.05$), but the differences for As, Cd, Pb, Ni and B elements in the tissues were not significant ($p>0.05$). According to annual concentration averages, the highest values for Cr, Mn and Zn were found in gills and the highest values for Cu and Fe were found in livers. The metal concentration differences among the months were examined significantly ($p<0.05$), except for B and Fe. When the concentrations in all tissues were

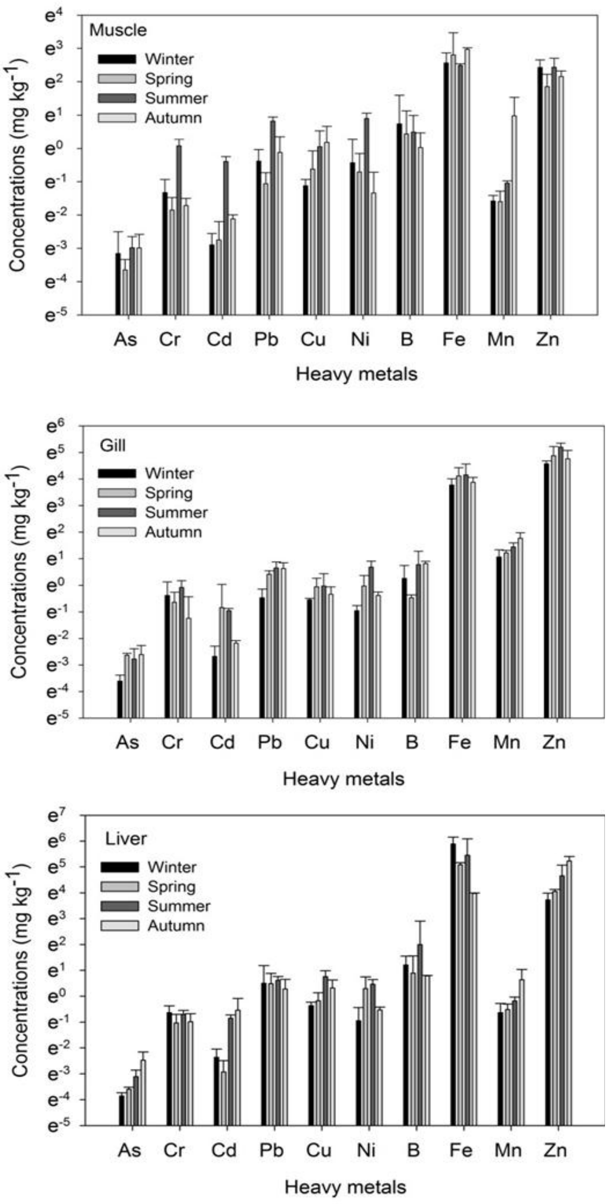

Fig. 2. Seasonal changes of metal concentrations found in tissues.

Table 7. Seasonal trace element concentrations of treatment plant effluent.

Elements	Winter (mg L^{-1})	Spring (mg L^{-1})	Summer (mg L^{-1})	Autumn (mg L^{-1})	Annual Mean (mg L^{-1})
As	0.0026±0.0006	0.0041±0.0008	0.0026±0.0001	0.0037±0.0017	0.0033±0.0011
Cr	0.0197±0.0072	0.0139±0.0064	0.0528±0.0117	0.0536±0.0222	0.035±0.0224
Cd	0.0046±0.0006	0.0028±0.0007	0.0429±0.0011	0.011±0.0033	0.0153±0.0168
Pb	0.0235±0.0045	0.0180±0.0029	0.0516±0.0125	0.0585±0.0180	0.0379±0.0206
Cu	0.0044±0.0039	0.0073±0.0029	0.0422±0.0081	0.0587±0.0109	0.0282±0.0247
Ni	0.0353±0.0069	0.0390±0.0108	0.0533±0.0245	0.0337±0.0392	0.0403±0.0229
B	0.3727±0.0430	0.3684±0.1307	0.2469±0.1683	0.3690±0.0169	0.3393±0.0970
Fe	0.2696±0.2083	0.3654±0.1307	0.257±0.1236	0.8098±0.2879	0.4254±0.2935
Mn	0.0881±0.0323	0.0706±0.0194	0.0773±0.0444	0.1593±0.0556	0.0988±0.0513
Zn	0.1174±0.0359	0.0933±0.0209	0.2006±0.1186	0.0966±0.0291	0.1270±0.0731

evaluated, the high values were found in summer and autumn months. However, Cr and Fe concentrations in liver were high in January. Seasonal changes of metal concentrations found in tissues are presented in Fig. 2.

Seasonal changes of trace element concentrations of the pool water fed by effluent from the Sewage and Water Company of Bursa (BUSKİ) East Wastewater Treatment Plant was investigated [65]. So, similar to concentrations in fish, it was observed that trace elements were higher levels in summer and autumn months except for arsenic and boron. Seasonal trace element concentrations of treatment plant effluent are presented in Table 7.

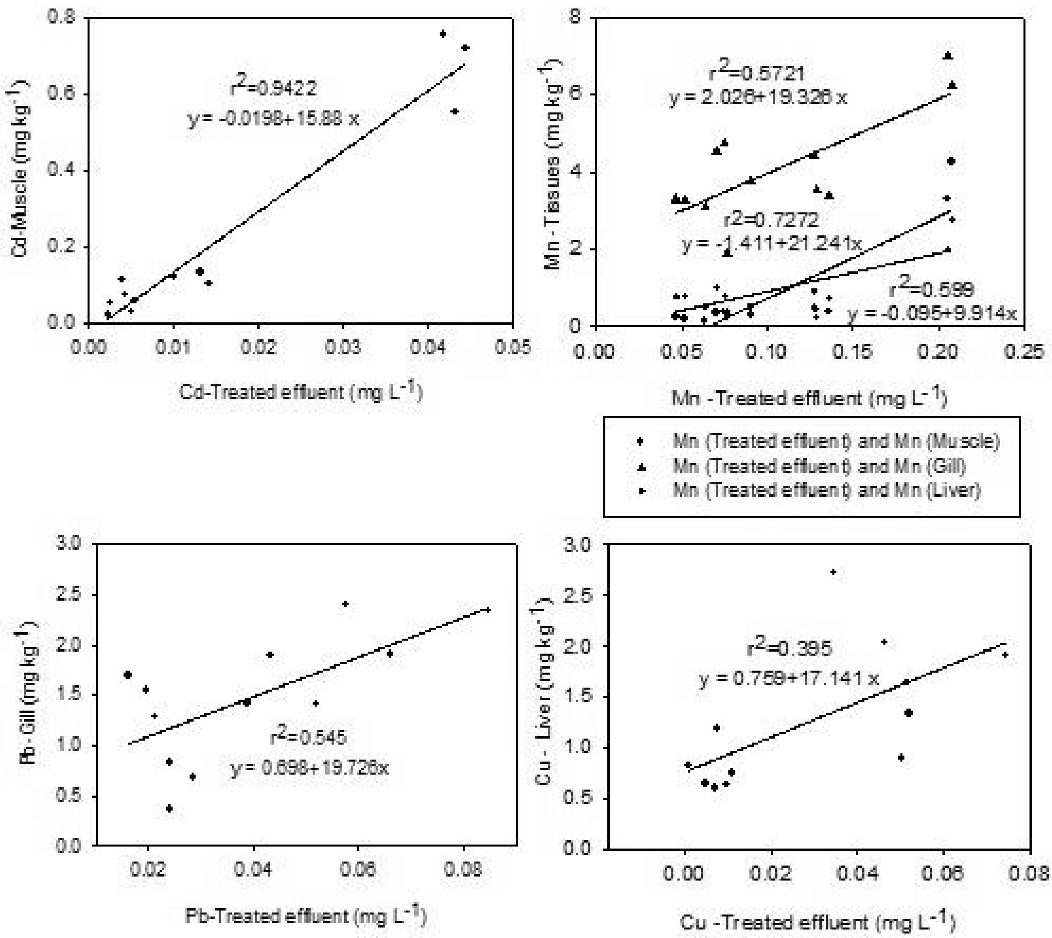

Fig. 3. Correlations between fish tissues and treated effluent.

Correlations between effluent concentrations and concentrations in fish tissues were calculated. In addition, all of the trace element concentrations in fish tissues were found to be 10 to 1000 times higher than the values in effluent. The correlation coefficients (r) between Cd in effluent and Cd in muscle tissue, Mn in effluent and Mn in all three tissues, Pb in effluent and Pb in gills, Cu in effluent and Cu in liver were found to be statistically significant ($p<0.05$). The correlations between fish tissues and effluent were presented in Fig. 3.

Metal Bioaccumulations and Risk Assessment

Annual and seasonal averages of transfer factors (TF), bio-concentration factors (BCF) and estimated daily intake values (EDI) were calculated by using metal concentrations in treated effluent, muscle, gill and liver tissues. In this way, bioaccumulation of metals in fish and the health risks in case of consuming were evaluated. TF, BCF, EDI, HQ values and the annual mean metal concentrations of treated effluent are given in Table 8.

According to the calculated values, TF values of all tissues for each metal were found to be higher than 1. We found that according to BCF values, only the Pb element was higher than U.S. EPA limit values, and the other elements were lower than the limit values.

TF values are more reliable than BCF values. Because big BCF do not indicate a hazard, it is not an intrinsic property for metals. Larger values indicate low exposure and low potential for chronic effects or secondary poisoning. There is no one value above which a hazard can be ascribed. Nearly all metals (including iron) have BCF >1000 in natural ecosystems that are deemed to be healthy and with aqueous concentrations in the background. Extremely clean systems have even larger BCF. Metal regulation systems operate in most organisms. Metals frequently occur as charged ions in aqueous solutions and require active transport to facilitate uptake for both essential and non-essential elements. Active transport mechanisms exhibit saturable kinetics (i.e., rate limited and uptake rates decline as exposure increases). Neutral lipophilic organics uptake substances via passive diffusion across lipid bilayer. There is an inverse relationship between tissue concentration and exposure level for several metals. Hazards and the potential for chronic effects cannot be evaluated by magnitude of BCF [36]. For this reason, in this study, transfer factors of 10 metals from computed treated effluent indicated that they were all above 1, which means that bioaccumulation of all metals is a concern for potential health effects [34].

When the annual and seasonal averages of TF and BCF values in all three tissues were examined, Zn and Fe were the high values, and B and Mn were the low values. Annual averages of TF and BCF values of metals were in the same order. It was found as

Table 8. TF, BCF, EDI, and HQ values and the annual mean metal concentrations of treated effluent

Metals	Treated Effluent Concentrations (mg L^{-1})	Muscle		Gill		Liver		US EPA (1999)	EDI (µg/kg b.w/day)	RfD µg/kg b.w/day USEPA, 2005	Hazard Quotient (EDI/RfD)
		TF(L/kg)	BCF(L/kg)	TF(L/kg)	BCF(L/kg)	TF(L/kg)	BCF(L/kg)	BCF(L/kg ww)			
Fe	0.4254±0.2935	194.372	35.903	675.386	127.696	2596.560	475.456	-	2.008	700	0.0028
Mn	0.0988±0.0513	47.224	8.724	220.622	41.715	53.551	9.806	-	0.112	140	0.0008
Cu	0.0282±0.0247	149.113	27.553	150.035	28.370	245.922	45.035	710	0.102	40	0.0025
Zn	0.1270±0.0731	402.906	74.433	5477.236	1035.590	4193.490	767.900	2059	1.242	300	0.0004
Cr	0.035±0.0224	64.943	12.514	91.257	17.257	68.314	12.514	19	0.055	3	0.0183
Pb	0.0379±0.0206	149.367	27.599	206.675	39.076	232.401	42.560	0.09	0.137	0.05	2.74
Cd	0.0153±0.0168	80.392	14.967	85.686	16.209	103.137	18.890	907	0.030	1	0.030
Ni	0.0403±0.0229	129.727	23.970	131.762	24.913	132.382	24.243	78	0.126	1.5	0.084
As	0.0033±0.0011	68.788	12.727	93.636	17.727	73.030	13.424	114	0.005	0.3	0.0166
B	0.3393±0.0970	25.125	4.642	24.721	4.674	61.930	11.340	-	0.207	-	

*EDI values were calculated only for muscle tissue because it was eatable.

Zn>Fe>Pb>Cu>Ni>Cd>As>Cr>Mn>B in muscle, as Zn>Fe>Mn>Pb>Cu>Ni>As>Cr>Cd>B in gill, and as Zn>Fe>Cu>Pb>Ni>Cd>As>Cr>B>Mn in liver. When the seasonal changes in TF and BCF values were compared, some similarities were observed. Fe, Zn and Cu elements were found to be higher in magnitude order. In both factors, Cr, Pb, Ni and B elements in muscle tissue were found to be higher in summer months, Cd was higher in spring, Mn was higher in autumn and Zn was higher in winter. However, BCF values of As, Fe and Cu were higher in autumn, TF values of As and Fe were higher in summer months, and TF value of Cu was higher in spring. For both factors, Cr, Cd and Zn values in gill tissues were found to be higher in spring, while Ni and Fe were higher in summer and Cu was found to be higher in winter. However, while BCF values of As, Pb, B and Mn were higher in autumn, TF values of As, B and Mn were higher in summer months, and TF value of Pb was higher in spring. For both factors, As, Cd, Mn and Zn values in liver tissues were found to be higher in autumn, while Cr, Cu and and Fe were higher in winter and Pb and Ni were found to be higher in spring. However, TF value of B element was higher in summer; BCF value was higher in autumn. In Figure 4, Seasonal changes of TF values determined for muscle, gill and liver tissues are presented.

Although the magnitude order of annual averages of metal concentrations found in fish tissues were different, according to the standards of international institutions (FAO and WHO) the same elements in all three tissue were found to be high (Mn, Zn, Cr, Cd and Pb) and were low (Cu and Ni). In all three tissues, Ni element was higher than *Carassius gibelio* species living in different areas, and Zn, Cr and Pb were higher than the other fish species. When the magnitude order of concentrations in tissues and the magnitude order of TF and BCF of bioaccumulation factors were compared, although similar, some differences were observed. While the concentrations of B and Mn elements were found to be high in all tissues, magnitude orders of this element's bioaccumulation factors were lower, and while the concentrations of Cd and As elements were lower, their biological accumulations were found to be higher in all tissues. However, according to TF values, all elements bio-accumulated in all three tissues. When the seasonal changes in TF and BCF values were compared, Fe, Zn and Cu elements were found at the highest values in all three tissues. In general, concentrations in the tissues and effluent water were found to be higher in summer and autumn. However, seasonal changes of bioaccumulation factors were found to be different.

Seasonal changes of metal concentrations in tissues and bioaccumulation factors were found to be different. Except for B and Fe, while other element concentrations were found to be higher in all tissues in summer, TF and BCF values were found to be higher in different seasons. Similar to concentrations in fish other than As and B, the elements in effluent water were found to be higher in summer and autumn. However, the correlations between Cd, Mn, Pb and Cu concentrations in effluent and in fish tissues were found to be statistically significant. It was considered that these elements caused bioaccumulation because of effluent. In terms of other elements, there was a difference between seasonal changes of biological accumulation factors and seasonal differences of metal concentrations in the effluent. For this reason, the bioaccumulations in the fish were thought to be influential by the baits outside of the water and the sediment layer of the feeding pool.

As was seen in Table 8, EDI and HQ values that were calculated by using annual average concentrations had carcinogenic risk only in terms of Pb and did not have any carcinogenic risk in terms of other metals. HQ value of Pb was found to be greater than 1. In addition to being carcinogenic, the lead element is poisonous and causes brain damage because it imitates the metabolic behavior of calcium in many ways and prevents many enzyme systems from functioning [61].

Conclusions

The treated effluent water used in this research was considered as partially available for aquaculture and completely available for irrigation water. Metal concentrations in the fish were found to be higher than the standards, and their seasonal changes (higher in summer and autumn) were statistically significant. While the concentrations were higher in liver and gill, less metal accumulation was observed in muscle. However, all metals examined (Fe, Mn, Cu, Ni, Zn, Cr, Pb, Cd, As and B) were biologically accumulated in each of the three tissues. Because the correlations between Cd, Mn, Pb and Cu in tissues and these elements in effluent water were significant, it was considered that Cd, Mn, Pb and Cu caused bioaccumulation stemming from treated effluent water, and other metals might have caused bioaccumulation stemming from baits and sediment layer in the feeding pool. Concentrations of Cd, Mn and Pb in each of three tissues that had high correlation with treated effluent water were found to be higher than the international standards. In addition, according to calculated HQ values, Pb element in muscle had carcinogenic risk and BCF value of only Pb among all elements was higher than limit values. In addition, all metals (except As and Cu) in muscle were higher than *Carassius gibelio* species living in different surface water and other fish species. Therefore, the fish were not available for human consumption and animal feed. The fact that the most dangerous metals, Pb and Cd, were higher than the international standards in all three tissues and that the concentration differences between tissues were not statistically significant indicated that the use of the part of the fish (muscle) and other organs as animal bait was not appropriate. This research proved that treated effluent water might cause biological accumulation even if it was suitable for aquaculture. For this reason, attempts should be retried by cultivating the fish in the treated water using "advanced treatment" methods. Add to this, in later studies investigating concentrations in baits and sediment layers would be useful. In addition, comparative studies of different fish species and different micro-pollutants would provide more comprehensive information on the use of treated wastewater in aquaculture. Also, there are no guideline values or provisional limits for metal intake (g/day/body weight), the results obtained in this study could be used to derive such guideline values. However, this needs to be further examined in future studies.

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