Chapter

Investigation of Trace Metal Bioaccumulation in Wastewater-Fed Fish: A Case Study

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Abstract

It was stated that the use of urban wastewater in food production in the 1970s and 1980s may lead to the development of alternative farming systems in the future. Fish fed with wastewater are grown in Asian countries. However, due to the mixing of domestic wastewater with industrial wastewater, many toxic micro-polluting wastewaters affect fish farming even more. 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 wastewater treatment. This study will be useful in terms of precautions and disadvantages that can be taken against food shortages that may be experienced with the effect of climate change.

Keywords: bioaccumulation, fish, trace metals, transfer factor, wastewater

1. Introduction

It is estimated that one billion people depend upon freshwater fish as the prime source of protein [1]. Fish consumption makes a major contribution to nutrition, especially for the poorest (e.g., in Cambodia, Laos, and China). Therefore, it is useful to look briefly at the conclusions of the IPCC AR4 on fisheries. Fisheries will come under pressure from increased temperature stress and rising Ph associated with global warming. The frequency of extreme droughts and floods will have a disproportionate effect on fish habitat and populations, and the incidence of diseases is expected to rise. This will result in species extinctions at the margins of their current habitats (e.g., salmon and sturgeon), and fish yields in places like Lake Tanganyika are expected to fall by around 30 percent [2]. Cities will generate increasingly large amounts of effluent that will be recycled for agriculture, subject to water quality and health and safety considerations.

“Water reuse” refers to the production of water through water treatment processes, which introduces a feedback loop in the water cycle. Water reuse presents environmental, economic, and social benefits but also potential drawbacks. Treated wastewater was used in urban uses (green area irrigation, vehicle washing, fire extinguishing, urban pools and toilet water, etc.), industrial (cooling, boiler feeding, process water, etc.), agricultural irrigation, groundwater feeding, direct or indirect drinking water. Also, it could be used for feeding and improving surface waters and for fish production [3]. The reuse of wastewater for different purposes is even more important these days when there is a danger of drought [4].

1.1 Treatment and advanced treatment applications that could be used for wastewater feeding fishes

Point and diffuse pollutants are converted into end products such as CO_2, N_2, H_2S, and biomass by being mineralized (decomposed) by natural treatment processes (with the cooperation of bacteria/archae and algae) in rivers, wetlands, estuaries, and seas. A similar separation occurs in wastewater treatment plants [5]. Considering highly treated wastewater as a new water source will become more important in the future, as river flows are predicted to decrease by 20–30% due to global climate change [5]. After the water consumed as drinking/utility water is transformed into wastewater, it can be brought to suitable water quality for different reuse alternatives with the second, third, or advanced treatment stages [6].

Wastewater-fed fish culture has a history of more than a century in Germany. First, it receives well-treated wastewater from wastewater treatment systems. The latter is designed to treat raw wastewater that has been mechanically pretreated only. Net fish yield from wastewater-fed fish ponds is 500 kg/ha/7 months on average (estimated as 860 kg/ha/year), and loading rates are equal to 2000 persons/ha/day [7].

There are still serious psychological, social, and etic hesitations in front of the use of domestic wastewater with advanced treatment, even if it is brought to the quality of tap water, directly as drinking and utility water. It is known that water of this nature is given to aquifers and then drawn by wells and distributed to cities from a separate network (purple network) and used as B quality/class water at 50% lower cost for irrigation, WC flushing water, or industrial process water supply. The most courageous application in which treated wastewater of this quality was used as drinking water in pet bottles, called new water (NEWater), was made in Singapore [8]. The current legislation on the reuse of wastewater in Turkey was published in 2010. "Wastewater Treatment Plants Technical Procedures Communiqué" (Official Gazette no: 27527). In this communique, the selection of treatment technology, design criteria, and technical procedures for reuse of wastewater originating from settlements were given. According to the Communiqué, the main areas of use of treated wastewater were agriculture, industry, aquifer feeding, indirect firewater, use in toilets, and direct drinking water [9].

However, among the areas where treated wastewater can be reused, the most accepted ones are irrigation for agriculture and landscape purposes. The lowest accepted usage areas are direct use in the kitchen and bathroom [10]. Therefore, it is of great importance to increase the low rate of acceptance of the public in using this water, despite the reuse of wastewater by using appropriate engineering techniques [3].

In this case, as the dilution capacity of the streams will decrease, it may be necessary to apply "Ozone Oxidation + Granular Activated Carbon Filtration" at the Advanced Biological WWTP outlet [5]. In the removal of viruses, ultrafiltration membrane application and maturation pools and UV applications for the removal of other pathogenic microorganisms have been determined to provide the desired purification efficiency to a large extent [11]. Smin >3 (1 unit wastewater +2 units river/lake water) criterion can be taken as a measure for the minimum dilution in the discharge of low pollution (gray water or equivalent pollutant) used or treated wastewater into surface waters (streams and lakes). Absolute water should be more than two times of treated wastewater. It is thought that the domestic wastewater that has undergone advanced treatment and disinfection can be mixed with more than two times of clean water and used in the production of aquaculture.

In this study, trace metal concentrations in muscle, gill, and liver tissues of *Carassius gibelio* specie fed with wastewater from Bursa Water and Sewage Administration East Treatment Place were investigated. 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. This study was ensured useful and valuable information for evaluating potential health risks in wastewater recovery as aquaculture feeding water.

2. Materials and methods

2.1 Study locations

East Wastewater Treatment Plant treats the wastewater of the eastern part of Bursa City. It covers an area of approximately 250,000 m2 and wastewater of about 1,550,000 inhabitants is mixed with the facility. The 2017 flow rate of the treatment plant is 240,000 m^3/day. It is designed as 320,000 m^3/day for the year 2030. The Wastewater Treatment Plant is discharged into Deliçay Stream, which is a tributary of Nilüfer Stream, in the Susurluk River Basin. The Biological Treatment Plant is a five-stage Bardenphod that removes nitrogen and phosphorus [12].

2.2 Sample handling and analysis of water and fish tissues

The species *Carrassius gibelio* examined in this study has been recognized as an invasive species by the Republic of Turkey, and its prey has been released throughout the year [13]. The metal concentrations in the muscles, gills, and livers of fish fed with the effluent of the wastewater treatment plant were investigated seasonally. The investigated metals were chosen among the most common ones (Fe, Mn, Cu, Zn, Cr, Pb, Cd, Ni, As, and B) in wastewaters and fish.

Measurements were made by taking three fish samples (*Carrassius gibelio*) in each season in 2011–2012. The sizes of the fishes taken in polyethylene caps were measured in the laboratory. The tissues of muscle, liver, and gill were spared with stainless steel and homogenized. The tissue samples of 0.5 g (wet weight) in petri dishes were dried 24 hours in a drying oven. The samples in which dry weights were obtained were decomposed in a CEM Mars 5 Model microwave device by placing them in HP500 Teflon containers and adding 7 ml of nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2) [14]. After filtering, the water samples were acidified with 0.2% (v/v) nitric acid and stored in glass bottles [15]. Water and fish samples were taken and prepared simultaneously.

Trace elements in water and all fish tissues were measured with the ICP-OES device (VISTA-MPX model-VARIAN brand) [16].

2.3 Determination of metal bioaccumulations and risk assessment

Metal concentrations (based on wet and dry weight) in muscle, gill, and liver tissues were evaluated with national and international standards [17–22].

Transfer and bio-concentration factors (TF and BCF) were calculated to determine the level of bioaccumulation in fish tissues. Transfer factor was used to determine the amount of metal transferred from water or sediment to fish tissues [23]. TF and BCF formulations were given below [23, 24]:

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

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

where M_{tissue} is the metal concentration in fish tissue; $M_{sediment}$, 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 calculated to see the effect of concentrations in water. BCF and TF are inversely proportional to exposure concentrations in the aquatic environment. In international references, it was stated that bioaccumulation was dangerous when BCF was >1000 and TF was >1. BCF and TF should be evaluated together to accurately determine the chronic effects [24, 25].

The consumption of *C. gibelio*, the fish species examined in this study, as the food was determined by the estimated daily intake (EDI) value [26, 27]:

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

where C_{fish} = the average trace element concentration in fish muscle (μg/g dry weight), D_{fish} = the global average daily fish consumption (g/day) which was only 1.7 g/day for Turkey [28], and BW = average body weight (kg).

The USEPA was stated that the average body weight for an adult human for risk analysis was 70 kg [29]. 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. It was stated that there was no significant risk when the HQ value was less than 1 [26].

3. Results and discussion

3.1 Muscle

Order of magnitude of the metal concentrations in muscle was as follows: Fe > Zn > B > Pb > Ni > Mn > Cu > Cr > Cd > As. It was determined that Mn, Cr, Pb, Cd, and Zn were higher and Cu, Ni, Fe, and As were lower than FAO and WHO standard values. Mn and Zn were determined in lower concentrations compared to Turkish and British standards. Metal concentrations determined in muscle, gill and liver tissues and national-international standard values are given in **Tables 1**–**3**, respectively.

3.2 Gill

Order of magnitude of the metal concentrations in gill tissue was as follows: Zn > Fe > Mn > B > Pb > Ni > Cu > Cr > Cd > As. Fe, Mn, Zn, Cr, Pb, Cd, and As were determined higher and Cu and Ni were lower than FAO and WHO standards. Mn was lower than Turkish standards. According to Turkish standards, other metals were evaluated similar to FAO/WHO standards.

3.3 Liver

Order of magnitude of the metal concentrations in liver tissue was as follows: Fe > Zn > B > Pb > Cu > Ni > Mn > Cr > Cd > As. Fe, Mn, Zn, Cr, Pb, and Cd were determined higher and Cu, Ni, As were lower than FAO and WHO standards.

Comparing the Turkish and English standards, Mn and Pb were determined as lower, and other metals were determined similar evaluating FAO/WHO standards.

Element (ww/dw)	Muscle (mg kg^{-1})	FAO, 1983/ WHO (dw mg kg^{-1})	Turkish Guidelines/ TFC (ww mg kg-^{1})	England (ww mg kg^{-1})
Fe	15.273 ± 7.30 / 82.686 ± 39.55	100		
Mn	0.855 ± 1.316 / 4.628 ± 7.123	1	20	
Cu	0.777 ± 0.563 / 4.205 ± 3.047	30	20	20
Zn	9.453 ± 3.102 / 51.169 ± 16.791	50/100	50	50
Cr	0.420 ± 0.399 / 2.273 ± 2.159	1		
Pb	1.046 ± 0.784 / 5.661 ± 4.243	0.5	1 / 0.3	2

Table 1.
Metals concentrations determined in muscle tissues and national-international standard values.

Elements (ww/dw)	Gill (mg kg^{-1})	FAO, 1983/WHO (dw mg kg-1)	Turkish Guidelines/ TFC (ww mg kg^{-1})	England (ww mg kg-1)
Fe	54.322 ± 20.051 / 287.309 ± 106.049	100		
Mn	4.088 ± 1.410 / 21.621 ± 7.404	1	20	
Cu	0.800 ± 0.350 / 4.231 ± 1.851	30	20	20
Zn	131.520 ± 45.916 / 695.609 ± 242.849	50/100	50	50
Cr	0.604 ± 0.377 / 3.194 ± 1.993	1		
Pb	1.481 ± 0.628 / 7.833 ± 3.321	0.5	1 / 0.3	2

Table 2.
Metal concentrations determined in gill tissues and national-international standard values.

Elements (ww/dw)	Liver (mg kg^{-1})	FAO, 1983/WHO (dw mg kg-1)	Turkish Guidelines/ TFC (ww mg kg^{-1})	England (ww mg kg-1)
Fe	202.25 ± 153.018 / 1104.576 ± 835.63	100		
Mn	0.961 ± 0.707 / 5.248 ± 3.860	1	20	
Cu	1.270 ± 0.683 / 6.935 ± 3.729	30	20	20
Zn	97.523 ± 65.213 / 532.573 ± 356.128	50/100	50	50
Cr	0.438 ± 0.139 / 2.391 ± 0.759	1		
Pb	1.613 ± 0.839 / 8.808 ± 4.581	0.5	1 / 0.3	2

Table 3.
Metals concentrations determined in liver tissues and national-international standard values.

Cd, Ni, As, and B elements determined in muscle, gill and liver tissues, and national-international standard values are given in **Table 4**.

The metal concentrations and accumulation amounts (g/day/body weight) obtained in this study could be used to form a guide value for metal intake. Similar

Element (ww/dw)	Tissue	Concentration (mg kg^{-1})	FAO, 1983/WHO (dw mg kg^{-1})	Turkish Guidelines/ TFC (ww mg kg^{-1})	England (ww mg kg^{-1})
	Muscle	0.229 ± 0.264 / 1.239 ± 1.429	0.5/1	0.1 / 0.05	0.2
Cd	Gill	0.248 ± 0.306 / 1.311 ± 1.618			
	Liver	0.289 ± 0.275 / 1.578 ± 1.501			
	Muscle	0.966 ± 0.945 / 5.228 ± 5.115	10		
Ni	Gill	1.004 ± 0.694 / 5.310 ± 3.670			
	Liver	0.977 ± 0.645 / 5.335 ± 3.522			
	Muscle	0.042 ± 0.0236 / 0.227 ± 0.1277	0.27		
As	Gill	0.0585 ± 0.0267 / 0.309 ± 0.141			
	Liver	0.0443 ± 0.0296 / 0.241 ± 0.161			
B	Muscle	1.575 ± 1.457 / 8.525 ± 7.886			

Table 4.
Cd, Ni, As, and B elements determined in muscle, gill and liver tissues and national-international standard values.

studies should be done with different fish species [27]. The effects of heavy metals on alive changes depending on their concentrations, type of organism, ionic properties of metals (solubility value, chemical structure, ability to form redox and complexes) , tissue in which they are taken into the body and way of intake. Also, other minerals in the ambience and chemical properties of water effect the metal bioaccumulations. Because of these reasons, the physicochemical properties of the water used in aquaculture should be investigated and limited by legal values [30].

3.4 Metal bioaccumulations and risk assessment

The treated feed wastewater (TFE) was improved with national and international standard values. It was determined that most of the metals examined were above portable water standards [31–33] and USEPA surface water standards for toxic commentating [34]. There is no standard value for Zn and Cu in the Turkish Fisheries Regulation [35]. However, all metals were below the "Irrigation Water of Technical Methods Notification of Wastewater Treatment Plants" [36].

Except for Cd, all the other metals were found below the standard values of the People's Republic of China Fisheries Regulation-GB 8978 [37]. In light of these evaluations, it was been determined that the wastewater fed by the fishes was suitable for irrigation standards but not suitable for some parameters for aquaculture.

Annual and seasonal averages of transfer factors (TF), bio-concentration factors (BCF), and estimated daily intake values (EDI) were computed by using metal concentrations in treated wastewater effluent and examined fish tissues. The calculated factors and values provided a better assessment of the accumulation levels of metals in fish and the health risks that may occur when consumed by humans as

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 for only muscle tissue due to human consumption [12].*

Table 5.
The annual averages of metal concentrations in treated effluent and calculated TF, BCF, EDI, HQ values.

food. The values of TF, BCF, EDI, HQ, and the annual average metal concentrations of treated effluent are presented in **Table 5**. Computed TF values of all metals in all tissues were determined above the 1. Except for Pb, all other elements were found to be lower than the USEPA BCF limit values. It is known that the TF value gives more realistic results than the BCF values. Large BCF values indicate low chronic effects and low potential for secondary poisoning. In other words, large BCF indicates that there is no high danger. No value can show the hazardous status for BCF values. BCF values of most metals (such as iron) are above 1000 in healthy aquatic ecosystems. Metals have a greater BCF value in systems without contamination. Transfer factors were evaluated since the possibility of coronal effect and the danger status could not be evaluated with BCF [23, 25]. The TF values of all metals examined in this study were found to be above 1. This value shows that metals could bioaccumulate and had potential health effects.

Annual and seasonal averages of TF and BCF values in fish tissues showed that Zn and Fe were high and B and Mn were low values. The order of the annual mean TF and BCF values calculated in the tissues was the same. It was found as 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. Fe, Zn, and Cu were found to be higher according to the seasonal means of TF and BCF values for the three tissues. Similarities were found in seasonal changes in tissues. According to the calculations for both factors, Cr, Pb, Ni, and B values in muscle were determined as higher in the summer season, Cd was raised in spring, Mn was raised in autumn, and Zn was raised in winter.

Nevertheless, seasonal differences of As, Fe, and Cu elements were found for both factors. 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 higher in spring, Ni and Fe were higher in summer, and Cu was found 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 higher in autumn, Cr, Cu, and Fe were higher in winter, and Pb and Ni were found higher in spring. However, TF value of B element was higher in summer; BCF value of B was higher in autumn. The annual means of metal concentrations in the tissues were found to differ in the order of magnitude, but according to FAO and WHO standards, the same elements were found to be high (Mn, Zn, Cr, Cd, and Pb) and low (Cu and Ni) in all three tissues. Ni concentrations was over than *C. gibelio* species exist in other water resources. Also, Zn, Cr, and Pb were over than the different fish types. There were differences between the order of magnitude of the concentrations in tissues and the order of magnitude of TF and BCF. While B and Mn concentrations were high in all tissues, the order of bioaccumulation factors of these elements was lower than other elements. Also, the concentrations of Cd and As were lower than other elements; however, their bioaccumulation factors were found higher than the others in all tissues.

It was determined that all elements bioaccumulated in the three tissues according to TF values. TF and BCF values of Fe, Zn, and Cu elements had the highest values in all tissues. The metal concentrations in summer and autumn were higher than in the other seasons. Nevertheless, seasonal differences of bioaccumulation factors were determined distinct from concentration alteration.

The element concentrations apart from B and Fe were determined higher in all tissues in summer, and TF and BCF calculations were determined higher in different seasons. Metal concentrations other than As and B in effluent water were higher in summer and autumn than in the other seasons like concentrations in fish. Nevertheless, the correlations among the Cd, Mn, Pb, and Cu concentrations in all tissues and effluent water were determined statistically important. The correlations calculated for Cd, Mn, Pb, and Cu elements were found to be significant, indicating that the bioaccumulation was due to effluent. Seasonal changes of other elements' biological accumulation factors and their concentrations in the effluent were found different. Due to these reasons, it was considered that baits and sediment layer of the feeding pool could affect the bioaccumulations in the fishes.

According to EDI and HQ values (**Table 5**), it was observed that there is only a carcinogenic risk in terms of Pb among all metals. Finding the HQ value of Pb greater than 1 indicates a carcinogenic risk. In addition, lead prevents the enzyme systems from working because it imitates the metabolic behavior of the calcium element. Pb is toxic and causes brain damage [29].

4. Conclusions

The results of these studies showed that the treated wastewater used in fish feeding is suitable for irrigation water, but not for aquaculture. Metal concentrations in the fish tissues were determined as over than the standards. The concentrations of liver and gill were higher than muscle. It was determined that investigated metals (Fe, Mn, Cu, Ni, Zn, Cr, Pb, Cd, As, and B) were bioaccumulated in all tissues. HQ values of Pb element in muscle tissue had carcinogenic risk and BCF value of only Pb among all elements was higher than limit values. It was determined that Pb and Cd, which were the most hazardous metals, were higher than the international regulations. For this reason, it has been determined that the examined fish were not suitable for human and animal edible.

Suggestions for the improvement of this study were presented below:

- In the future, the effects of sediment layer and feed on metal accumulation in fish fed in wastewater-fed ponds should be investigated.

- The effects of different pollutants on different wastewater-fed fish species should be investigated.

- These studies could be used in future studies to establish a guide value for metal accumulation that should be taken per day according to body weight (g/day/body weight).

- Re-use of water in the industry must be done. Thus, an approach compatible with the circular economy approach is followed. There might be health and ethical risks in its use for food production. To feed aquatic products in wastewater, projects that include advanced toxicology experiments should be carried out to reveal the toxic effects that may occur in the long term.

- In-depth research on primary pollutants (pharmaceutical residues, personal care products, industrial chemicals, endocrine disruptors, etc.) in wastewater was required. In addition to the benefits, the risks also need to be evaluated correctly. Considering all these issues, it was concluded that it would be more appropriate to work with advanced treated domestic sewage treatment water that does not mix with industrial water.

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