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ARTICLE

Health Risks of Trace Metals in Wastewater-Fed Fishes: A Case Study

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ABSTRACT

In this study, the usage wastewater from secondary treatment in feeding fishes of *Carassius gibelio* species and suitability of the fishes for human food were evaluated. The metals (Ag, Al, As and B) in treated effluent and skeleton, skin, eyes and brain tissues of fishes were examined seasonally. It was found that treated effluent was not suitable for irrigation and aquaculture in terms of Al according to the Turkish standard values. According to annual averages the size order of Ag and B concentrations were skeleton> skin> eyes>brain and skeleton> skin>brain>eye respectively. Also, skin>brain>eyes>skeleton was for As and Al.   TF (Transfer Factor) values of all metals examined were determined as >1 in the four tissues and the metals caused bioaccumulation because of treated effluent. Concentrations in muscles were found 7 to 6227 times higher than in water. The size order of TF and BCF (Bio-concentration Factor) values in skin and eye tissue were the same and it was Ag>Al>As>B. It was Ag>Al>As>B in skeleton, Al>As>Ag>B in brain. HQ (Hazard Quotient) of Al in all tissues had carcinogenic risk level.

1. Introduction

Serious water demand caused by population growth, urbanization and industrialization have been in the world [1]. Many countries are seeking reuse of waste waters for meeting their national water requirement [2].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, economical for sustainable production and helps to combat environmental pollution [3]. In the urban swage wastewater is mixed with industrial wastewater. Even if sewage water is treated, its use in aquaculture has some health disadvantages because of its toxic chemicals and microbiological pollution. Especially, heavy metals are potentially harmful to most organisms at some level of exposure and absorption [4]. Most of the metals are absorbed into the body through respiration, digestion and skin. Metals could be found in high concentrations in the skeleton and skin tissues and can cross the brain wall [5, 6, 7] Brain tissues accumulated more heavy metal than edible muscle tissues.

The aims of this study were to evaluate the suitability of treated wastewater-fed fishes to health risk for human food consumption in terms of the metals of Ag, Al, As and B. This study may contribute valuable information for evaluating potential health risks of wastewater reuse as aquaculture feeding water were exist. This study was important to see the accumulation of metals in fish tissues due to feed with wastewater and the level of their cancer risks.

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2. Material and Methods

2.1 Experimental Studies

In this study *Carassius gibelio* type fish grown in wastewater-fed ponds were caught seasonally and Ag, Al, As and B concentrations in skeleton, skin, eye and brain tissues were determined.

The fish ponds were fed with the effluent of East Wastewater Treatment Plant of Bursa City has the advanced biological treatment (five-stage Bardenpho). The volumetric flow is 240000 m^3/day for the year 2017 [8].

12 fish samples *of Carassius gibelio specie* were catched along the same year (2011-2012, 3 samples per season) from the fish breeding pool after last clarifier. The fishes were taken to laboratory in the polyethylene caps and their sizes were recorded. The fishes were cut from its backing with the help of 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 drying oven. Afterward, the tissue samples fixed to dry weight placed in to 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 by using CEM Mars 5 Model microwave instrument [9]. 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 [10]. After the samples were cooled to room temperature and the samples were diluted to 50 ml by using ultra-pure water. 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 water samples were taken by using with Aquacell P2-COMPACT portable composite sampler then they were transferred to the dark polyethylene (PE) bottles washed with HNO_3 and deionized water [11]. All the samples were taken monthly along 1 year from January 2011 to 2012 in order to see the seasonal changes. Water samples were filtered through a Millipore filter paper with preweighed 0.45 µm pore-size. The filtered water samples were acidified with 0.2% (v/v) concentrated nitric acid as the precipitation samples and kept in glass bottles cleaned with detergent, water, nitric acid and Milli-Q water respectively [10]. Heavy metals and trace elements in water and fish tissues were determined using the VISTA-MPX model of the VARIAN brand ICP-OES device.

2.2 Metal Bioaccumulations and Assessment of Health Risk

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) [12] as follows:

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

And also bio-concentration factor as follows [13]:

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

Where, M_{tissue} is the metal concentration in fish tissue; $M_{sediment}$, 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 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 hazard in many national regulatory schemes. However hazard and potential for chronic effects cannot be just evaluated by magnitude of BCF [14]. TF, BCF and standard concentration values should be evaluated together. BCF has different values for each metal [13].

The risk for human health as a result of eating *Carassius gibelio* was evaluated by calculating estimated daily intake (EDI) using the following equation [15, 16]:

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

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 [17], and BW= average body weight (kg).

US-EPA risk analysis, considering an adult average body weight of 70 kg [18]. 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 [15].

3. Results and Discussion

Metal (Ag, Al, As and B) concentrations in skeleton, skin, eye and brain tissues of *Carassius gibelio* species were determined in summer and winter. In skeleton and brain tissues, summer concentrations of Ag and As were higher than winter values. Winter concentrations of Al and B were higher than summer values in these tissues. In winter Ag, Al and As concentrations of eye tissues higher

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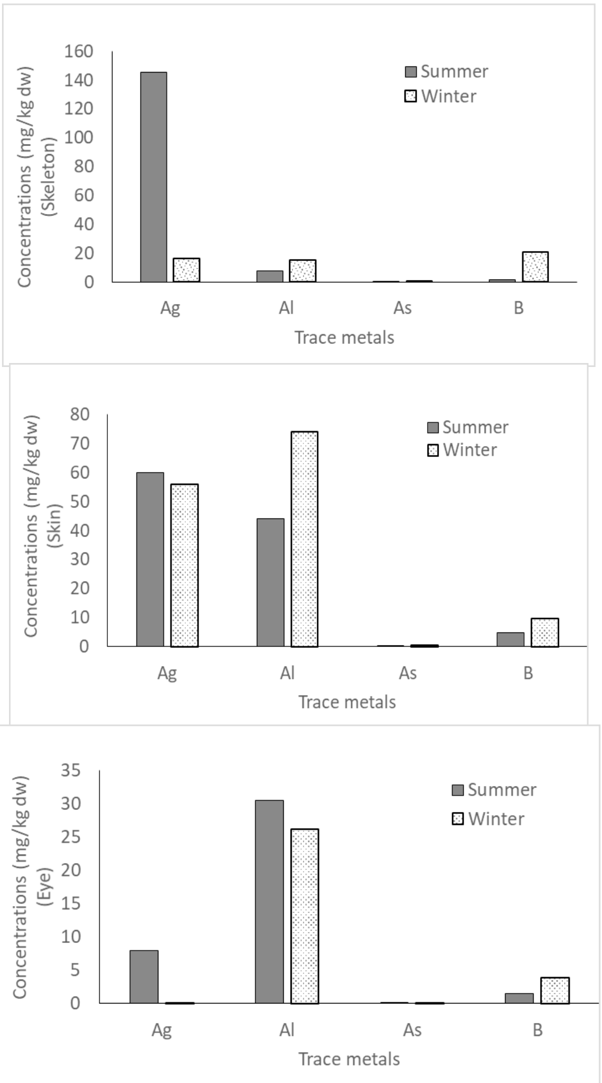
than winter values but B concentrations of eye in winter higher than summer values. In skin tissues, winter concentrations of As, Al and B higher than summer, also Ag in summer higher than winter concentrations. The concentrations in the skeleton and brain showed similar seasonal changes.The seasonal metal concentrations in skeleton, skin, eye and brain tissues were shown in Figure 1.



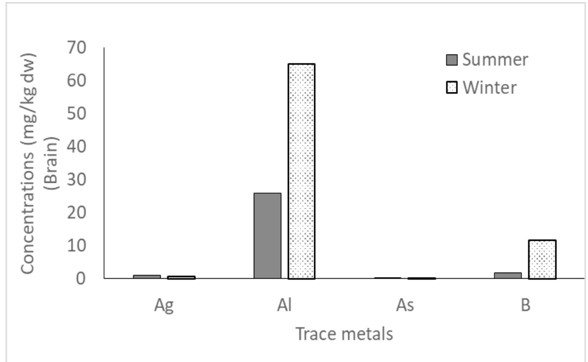


Figure 1. The seasonal metal concentrations in skeleton, skin, eye and brain tissues

According to annual averages the size order of Ag and B concentrations were skeleton> skin> eye>brain and skeleton> skin>brain>eye respectively. Also skin>brain>eye>skeleton was for As and Al. Higher concentrations were found in skeletal and skin tissue. Higher concentrations in skeletal and skin tissue were found than the other tissues. It was determined that metal accumulation was found in nervous system.In different studies, B concentrations in fish head were higher than muscle, liver and gill [19]. It was determined that Cd concentration in skeleton was higher than skin, muscle and gill tissues and Ni concentrations in skeleton, brain and skin tissues were found close to each other [5]. Adebayo, et. al., 2017 was found that Zn, Pb and Fe in brain higher than eye [6]. Cd, Pb, Cu, Zn and Mn in skeleton were higher than skin concentrations [20]. Yin, et. al., 2018 was studied with 8 different fish species and metal concentrations in internal organs were determined higher than edible muscle tissue [21]. The annual averages of the metal concentrations in the tissues were given in Table 1.

According to the Turkish food codex annual averages of As concentration in all tissue were above the standard value [22]. National and international standard concentration values of other metals are not exist for fishes. There are daily intake doses of these metals [15, 19]. For this rea-

Table 1. Annual average of the metal concentrations in the tissues

Metals	Skeleton (mg/kg)		Skin (mg/kg)		Eye (mg/kg)		Brain (mg/kg)	
	Wet weight	Dry wight	Wet weight	Dry wight	Wet weight	Dry wight	Wet weight	Dry wight
As	0.04245±0.0078	0.0604± 0.011	0.07975± 0.010	0.3065± 0.0415	0.02015± 0.015	0.077± 0.061	0.05255± 0.025	0.2625± 0.129
B	7.73285±6.697	11.0465± 9.567	1.889± 0.642	7.265± 2.469	0.687± 0.305	2.642± 1.173	1.317± 0.987	6.585± 4.935
Al	7.872±2.683	11.245± 3.833	15.3605± 3.926	59.078± 15.102	7.3635± 0.566	28.321± 2.179	9.075± 3.914	45.375± 19.57
Ag	56.66± 45.254	80.9545± 64.649	15.0825± 0.505	58.009± 1.944	1.0585± 1.023	4.0705± 3.936	0.16± 0.044	0.8±0.22

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son, health risk was determined by calculating the values of the hazard quotient, transfer and bio-concentration factors.

Annual mean of Ag, Al, As and B concentrations of treated effluent water were 0.013, 0.3383, 0.0033 and 0.3393 mg/L respectively. TF (Transfer Factor) values of all metals examined were observed as >1 in the four tissues and the metals caused bioaccumulation because of treated effluent. Concentrations in muscles were found 7 to 6227 times higher than in water. Size order of TF and BCF (Bio-concentration Factor) values in skin and eye tissue were the same and Ag>Al>As>B. They were Ag>Al>As>B in skeleton and Al>As>Ag>B in brain. HQ (Hazard Quotient) of Al in all tissues had carcinogenic risk level. Because Ag, As and Al except than Boron are not essential elements [19] excess of this element's concentrations caused toxic effect for fishes and humans. Calculated hazard quotient, transfer and bio-concentration factors were given in Table 2. Although treated wastewater was used in aquaculture and irrigation in many countries [23, 24, 25], it was observed that Bursa urban wastewater cannot be used for aquaculture.

Table 2. Calculated hazard quotient, transfer and bio-concentration factors

Metals	US EPA (1999) BCF (L/kg)	RfD	Skeleton				Skin				Eye				Brain			
			TF(L/kg)	BCF (L/kg)	EDI (µg/kg b.w/gün)	HQ	TF(L/kg)	BCF (L/kg)	EDI (µg/kg b.w/gün)	HQ	TF(L/kg)	BCF (L/kg)	EDI (µg/kg b.w/gün)	HQ	TF(L/kg)	BCF (L/kg)	EDI (µg/kg b.w/gün)	HQ
As	114	0,3	18	12	0,001	0.003	92	24	0.007	0.023	23	6	0.001	0.003	79	15	0.006	0.02
B	–	–	32	22	0.268	–	21	5	0.176	–	7	2	0.064	–	19	3	0.159	–
Al	**76**	0.025	33	23	0.273	10.920	174	45	1.434	57.36	83	21	0.687	27.48	134	26	1.11	44.04
Ag	**–**	5	6227	4359	1.966	0.393	4462	1160	1.408	0.281	313	81	0.098	0.019	61	12	0.019	0.003

4. Conclusions

This study showed that wastewater treated with advanced purification may not be appropriate with regard to aquaculture for metal parameters. It was determined that Ag, Al, As and B caused the bioaccumulation in skeleton, skin, eye and brain tissues in this study. TF and BCF (Bio-concentration Factor) values in skin and eye tissue showed a similar trend and Al in all tissues had carcinogenic risk level. These results were important to see the levels of metal accumulation in fish tissues and to show that the usage of treated urban wastewater for aquaculture could be harmful.

In the future bioaccumulations of different micro-pollutants in varied fish species must be research. 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. Reuse of wastewater will become more important in the future due to water scarcity.

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