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Some Heavy Metal Ratios in Tissues of *Squalius Cephalus*, *Cyprinus Carpio* (Culture), *Cyprinus Carpio* (Carp) and *Chondrostoma Nanus*

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Abstract:

Pollutants that disrupt the natural balance in aquatic habitats cause serious economic, ecological and sociological problems. The risk posed by humans in terms of heavy metal toxicity is tried to be determined by measuring the metal concentrations of the most consumed aquatic species with high economic value. Therefore, in our study, it was aimed to determine the heavy metal content of some fish in the dam used as drinking water. Cd, Pb, Cu and Zn concentrations in the visceral organs, gill and muscle tissue of the fish species (*Chondrostoma nanus, Squalius cephalus, Cyprinus carpio) Cyprinus carpio* (Culture), from Gelingüllü Dam Lake after homogenization were identified by Flame Atomic Absorption Spectrometry (FAAS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) methods in this study. The highest and lowest values of heavy metals were detected as 0.51-0.11 mg/kg for Cu, 64.44-3.23 mg/kg for Zn, 0.29-0.05 mg/kg 0.19-0.05 mg/kg for Cd. The recoveries were approximately (95%) and standard deviations for the elements which were studied were determined as 10%. Most heavy metals can accumulate in cells and tissues at high rates. even this accumulation causes toxic effects in the organ. Measures should be increased for pollutants entering water resources.

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Keywords **— Gelingullu Dam Lake, Heavy metals, Atomic absorption spectroscopy, fish tissues**

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I. INTRODUCTION

Heavy metal pollution is a global severe problem. Heavy metal pollution has been considered as a World public health condition. Modern economic developments have threatened the life and environment and accelerated environmental pollution. Several activities in the sectors of industry and agriculture have increased the heavy metal concentrations in the water. Several industrial companies cause to serious pollution that is generated by heavy metals. There might be positive or negative effects on humans, animals and plants according to the amounts of heavy metals. Radioactive wastes, domestic and industrial wastes in the atmosphere and chemical pollution is spread to the water systems such as oceans, rivers and lakes. As a result, they lead to the aggregation of toxic substances in the bodies of aquatic organisms.

Heavy metals are melted, relocated by water and received by fishes and other living beings. Fish are significant source of protein in aquatic ecosystems and retreated with heavy metal pollution due to excessive pollution. Heavy metals might be accumulated in fish tissues that are on top of food chain due to the pollution in the water. Hence, fish are generally used as bioindicators to investigate the metal concentrations, ecological and health risks in the water environment. Heavy metals which are aggregated in marine organisms could be passed to human beings by way of the food chain. Some heavy metals which are fundamental nutrients are required for metabolism and biological systems. They have toxic and lethal effects if they are taken in a high concentration (Morton, 1976; Tuncer, 1980; Peng et al., 2016). Heavy metals are absorbed by aquatic organisms in a easy way and bind to the organisms' proteins in a strong way (Kalay et al., 2004; Ale et al., 2016) and that leads to several environmental and health problems (Heath,1995; Montero et al., 2005; Eroğlu et al., 2016).

Gelingüllü Dam Lake is approximately 40 km Yozgat province center in the Central Anatolian Region. The Dam was founded on the Delice River. The volume of the lake is 1.362 million m^3 and the area of the lake is 23,20 km^2 (Kırankaya,1999). Several villages, towns and agricultural areas throughout the river are available. Gelingüllü Dam Lake is influenced by agricultural activities, industrial activities and other similar wastes in a indirect or direct ways. Gelingüllü Dam Lake contains valuable fish species such as *Cyprinus carpio, Capoeta tinca, Capoeta capoeta sieboldi, Squalius cephalus, Chondrostoma nanus, Alburnus orontis, Barbus tauricus, Orthrias sp.* These fish species are significant for the food of the residents and also for amateur fishing.
Identification of the concentrations of heavy metals (copper, zinc, lead and cadmium) in Chondrostoma nanus, Squalius cephalus, Cyprinus carpio, Cyprinus carpio (Culture) tissues from Gelingüllü Dam Lake was aimed in this study.

II. MATERIAL AND METHODS

Samples

In this study, 28 fish samples of 4 different species (Cyprinus carpio (Culture), Cyprinus carpio, Squalius cephalus, Chondrostoma nanus) were collected from Gelingüllü Dam Lake in Yozgat between January 2013 and December 2013 (figure 1).

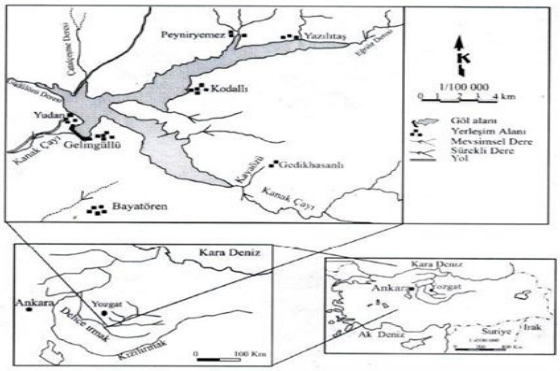

Figure 1. Working Area

The samples that were collected were sent to the laboratory. After that, they were washed with double-distilled water. Gills, muscle tissues and visceral organs of the wet fish were dissected and dried for 24 hours at 105 ° C'de (Elektro-mag, m5040p, Turkey). The dried samples were homogenized by the use of an agate muller (Turkey) and placed into the small polyehylene bags (made in Turkey) and kept at -30 ° C until the analysis.

Instrumentation and Chemical

Perkin Elmer Analyst 700 AAS which is Deuterium background corrector (Norwalk, CT, USA), electro-mag, m5040p (Istanbul, Turkey), model oven, Milli-Q Millipore (Millipore Corporation, USA), deionized water system, and hot plate (IKA, KS501D model, Germany) were used in this study.
All reagents which were used in the experiments in this study had the analytical purity. Bidistille deionized water (Milli-Q system, MΩcm-1 resistance, Millipore Corporation, USA) was applied to prepare all the aqueous solutions. Suprapure HNO3 and H202 were provided from E.

Merck, Darmstadt, Germany. All the plastic and glassware were made clean by keeping overnight in 10% (w/v) nitric acid solution, then washed with deionized water. Standard solutions of the investigated element (1000 mg/L) were prepared by the dissolution of pure metal or their salts (E. Merck) and they were diluted before use. Matrix modifiers ($NH_4H_2P0_4$, Pd and Mg $(NO_3)_2$) were purchased from Sigma Chem (St Louis, USA). Standard reference material (NRCC-DORM-2 Dogfish Muscle) was used to determine the accuracy of the method.

Analytical Procedure

Pb and Cd elements in the fish tissues were designated by HGA graphite furnace using argon as an inert gas. Platformed pyrolytic coated graphite tubes were applied for lead and cadmium. The other evaluations were performed in an air/acetylene flame. The conditions and instrumental parameters for the investigated elements were adjusted according to the instructions of manufacturer. 200 µg $NH_4H_2P0_4$ and 15 µg Pd + 10 µg Mg (NO_3) were added to the matrix modifiers for Pb and Cd. The limit of determination was designated as the concentration corresponding to three times the standard deviation of 10 blanks. Metal determination limits were described as 0.10 µg / g for Cu in flame AAS, 0.15 mg / kg for Zn. Pb and Cd were found to be under the flame AAS detection limit. The detection limits for Pb and Cd by the graphite furnace AAS were 0.03 and 0.02 µg / kg, respectively.

Microwave Digestion Procedures

Milestone Ethos D microwave closed system (maximum pressure 1450 psi, maximum temperature 300 ºC) (Milestone, İtaly) was applied for the digestion. Samples of 0.5 g were digested in the microwave digestive system for 23 minutes with 4 mL $HN0_3$ (% 65) and 2 mL H_20_2 (% 30), then diluted to 10 mL volume with deionized water. An empty digestion was performed in a similar way. The digestive conditions of the microwave system were as the follows: 2 minutes for 250 W, 2 minutes for 0 W, 6 minutes for 250 W, 5 minutes for 400 W, 8 minutes for 550 W, ventilation for 8 minutes (Mendil,2005).

III. RESULTS AND DISCUSSION

Amounts of the metals were identified as mg/kg according to the dry weight. The recovery values were found to be approximately (95%) for the wet powdery method. Relative standard deviations were less then 10% for all the underresearched elements. The factualty of the method was estimated by the use of reference material (NRCC-DORM-2 Dogfish Muscle). The results which were obtained from the study are in consistent with certified values.

Heavy metal concentrations (mean ± standard deviation) in the tissues of fish species are seen in table 1 and figure 2-5. Copper is essential for human health. On the other hand, high copper intake may cause to several health conditions such as injury of kidney and liver (Ikem and Egiebor,2005; Said et al.,2014)

The Cu concentration was in the range of 0.51-0.11 mg/kg. Cu levels in Iskenderun Gulf have been declared as 0.97-0.11 mg/kg by Çelik and 5.43-0.04mg / kg by Turkmen (Celik and Oehlenschlager, 2004; Turkmen et al., 2005)

It is known that zinc takes a role in several biological pathways in human beings and its deficiency may cause to growth retardation, immunological abnormalities appetite loss, and skin alterations. The highest and lowest Zn concentrations were 64.44 mg/kg and 3.23 mg/kg. It has been reported that the zinc content of many fish in the Black Sea in Turkey was in the range of 93.4-38.8 mg / kg by Tuzen (Tuzen, 2009). Turkmen et al. has determined that Zn levels of the fish from Iskenderun Bay (Turkey) were in the range of 11.57-0.60 mg / kg (Turkmen et al., 2005).

Lead is an insignificant substance and it may cause to neurotoxicity, nephrotoxicity, and several other negative health effects (Garcia-Leston et al., 2010). The highest and lowest Pb levels were 0.29 mg/kg and 0.05 mg/kg. Morgana et al. has reported that Pb concentrations were in the range of 0.481-0.026 mg / kg [17]. Turkmen et al. has reported that lead amounts were 6.95-0.09 mg / kg (Garcia-Leston et al., 2010). Furthermore, in an another study, it has

been declared that lead concentrations were in the range of 0.54-0.28 mg / kg (Mendil et al.,2005) Cadmium acts as a toxic substance. Cadmium might be stored in the body of human beings and it can affect renal system, pulmonary system, hepatic system, skeletal system, reproductive system and cancer (Zhu et. al., 2011). The highest and lowest cadmium levels were in the range of 0.19-0.05 mg / kg. Tuzen et al. has declared that Cd concentrations of fish were 0.05 mg/kg (Tuzen, 2009). In the literatüre, it has been reported that cadmium levels of samples of fish species from the Dhaleshwari River in Bangladesh were 0.73-0.51 mg / kg (Ahmed et al., 2009)

The concentrations of lead, copper, cadmium, and zinc in internal organs were 0.51-0.34 mg / kg, 64.36-7.65 mg / kg, 0.29-0.06 mg / kg and 0.19-0.10 mg / kg. On the other hand, copper, cadmium, and lead amounts are low in the internal organs and the zinc average is high as Mendil et al. [10] and Kucuksezgin et al. has reported (Kücüksezgin et al., 2002).

The determined concentrations of Zn, Cu, Pb, Cd in muscle tissue were 0.16-0.10 mg / kg, 6.40-5.35 mg / kg, 0.11-0.10 mg / kg and in the gills were 0.36-0.12 mg / kg, 64.44-3.23 mg / kg, 0.19-0.05 mg / kg, 0.06 mg / kg. Lead concentrations in muscle and gill tissues have been reported by Mormemede and Devis in the range of 0.027-0.002 mg / kg and 0.093-0.012 mg / kg (Mormede and Davies, 2001)

The concentrations of Cd, Pb, Cu, Zn in muscle tissue were lower than the results which were previously declared (Moiseenko and Kudryavtseva, 2001; Astorga-Espana et al., 1999; Cid et al., 2001). The concentrations of Cu, Pb, Cd in the gills were lower than these reported in the literature (Zhu et al., 2011) Zinc concentration in the gill was determined as higher/lower than reported in the literature (Mormede and Davies,2003) The amounts of copper, zinc, and lead were 0.51-0.11 mg / kg, 64.44-3.23 mg / kg, 0.29-0.05 mg / kg and 0.19-0.05 mg / kg, respectively.

The zinc concentrations of the fish samples were higher in this study. It has been determined that high amounts of Cu accumulation in *Cyprinus carpio* (culture) and high amounts of Zn, Pb and Cd accumulation in *Cyprinus carpio*. Cadmium, lead, zinc, and copper levels in visceral organs were higher than in muscle and gill tissues.

Table 1. Levels of Trace elements as µg/g in fish tissues (mg/kg)

Fish species	Tisues	Cu	Zn	Pb	Cd
Cyprinus carpio(common carp)	Visceral organs	0.39±0.03	64.36±4.8	0.29±0.02	0.19±0.01
	Muscle	0.10±0.01	5.35±0.41	0.11±0.01	BDL
	Gill	0.12±0.01	3.23±0.29	0.12±0.01	0.06±0.003
Cyprinus carpio (culture)	Visceral organs	0.51±0.05	48.63±4.2	0.13±0.01	0.18±0.02
	Muscle	0.11±0.01	6.40±0.61	0.11±0.01	BDL
	Gill	0.13±0.01	64.44±5.8	0.17±0.01	BDL
Chondrostoma nanus	Visceral organs	0.34±0.03	7.65±0.74	0.17±0.01	0.10±0.01
	Muscle	0.15±0.01	5.91±0.5.3	0.10±0.01	BDL
	Gill	0.36±0.03	6.91±0.63	0.19±0.02	0.05±0.003
Squalius cephalus	Visceral organs	0.44±0.04	17.64±1.5	0.06±0.005	0.13±0.01
	Muscle	0.16±0.01	5.46±0.52	BDL	BDL
	Gill	0.20±0.02	16.36±1.3	0.05±0.004	0.06±0.003

BDL: Blow Detection Limit

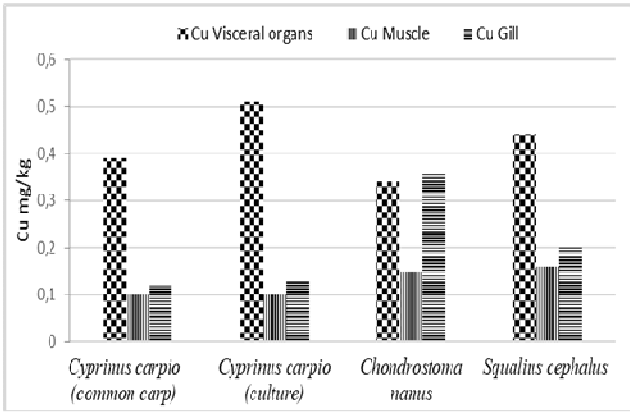

Fig. 2. Levels of Cu concentrations in fishes tissues

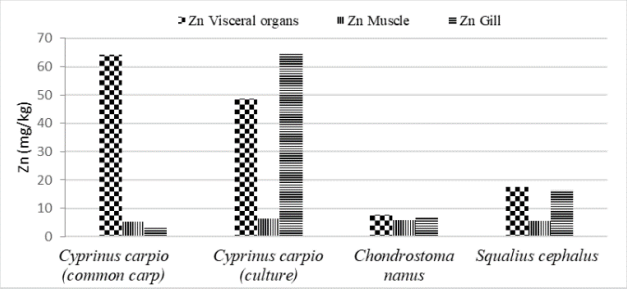

Fig. 3. Levels of Zn concentrations in fishes tissues

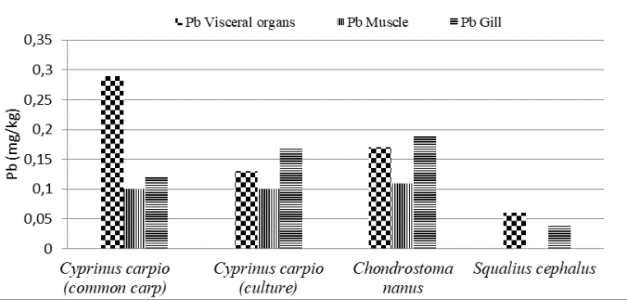

Fig. 4. Levels of Pb concentrations in fishes tissues

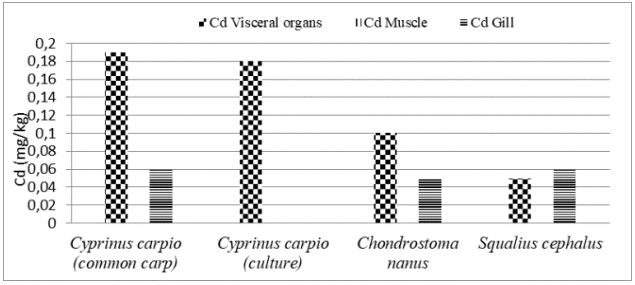

Fig. 5. Levels of Cd concentrations in fishes tissues

It is known that zinc takes a role in several biological pathways in human beings and its deficiency may cause to growth retardation, immunological abnormalities appetite loss, and skin alterations [15]. The highest and lowest Zn concentrations were 64.44 mg/kg and 3.23 mg/kg. It has been reported that the zinc content of many fish in the Black Sea in Turkey was in the range of 93.4-38.8 mg / kg by Tuzen [15]. Turkmen et al. has determined that Zn levels of the fish from Iskenderun Bay (Turkey) were in the range of 11.57-0.60 mg / kg [14]. Lead is an insignificant substance and it may cause to neurotoxicity, nephrotoxicity, and several other negative health effects [16]. The highest and lowest Pb levels were 0.29 mg/kg and 0.05 mg/kg. Morgana et al. has reported that Pb concentrations were in the range of 0.481-0.026 mg / kg [17]. Turkmen et al. has reported that lead amounts were 6.95-0.09 mg / kg [14]. Furthermore, in an another study, it has been declared that lead concentrations were in the range of 0.54-0.28 mg / kg [10]. Cadmium acts as a toxic substance. Cadmium might be stored in the body of human beings and it can affect renal system, pulmonary system, hepatic system, skeletal system, reproductive system and cancer [18]. The highest and lowest cadmium levels were in the range of 0.19-0.05 mg / kg. Tuzen et al. has declared that Cd concentrations of fish were 0.05 mg/kg [15]. In the literatüre, it has been reported that cadmium levels of samples of fish species from the Dhaleshwari River in Bangladesh were 0.73-0.51 mg / kg [19].

The concentrations of lead, copper, cadmium, and zinc in internal organs were 0.51-0.34 mg / kg, 64.36-7.65 mg / kg, 0.29-0.06 mg / kg and 0.19-0.10 mg / kg. On the other hand, copper, cadmium, and lead amounts are low in the internal organs and the zinc average is high as Mendil et al. [10] and Kucuksezgin et al. [20] has reported.

The determined concentrations of Zn, Cu, Pb, Cd in muscle tissue were 0.16-0.10 mg / kg, 6.40-5.35 mg / kg, 0.11-0.10 mg / kg and in the gills were 0.36-0.12 mg / kg, 64.44-3.23 mg / kg, 0.19-0.05 mg / kg, 0.06 mg / kg. Lead concentrations in muscle and gill tissues have been reported by Mormemede and Devis in the range of 0.027-0.002 mg / kg and 0.093-0.012 mg / kg [21].

The concentrations of Cd, Pb, Cu, Zn in muscle tissue were lower than the results which were previously declared [22, 23, 24]. The concentrations of Cu, Pb, Cd in the gills were lower than these reported in the literature [18]. Zinc concentration in the gill was determined as higher/lower than reported in the literature [25]. The amounts of copper, zinc, and lead were 0.51-0.11 mg / kg, 64.44-3.23 mg / kg, 0.29-0.05 mg / kg and 0.19-0.05 mg / kg, respectively.

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