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Determination of Copper, Iron and Zinc in the Muscles of Freshwater Fish from Beheshtabad River

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Abstract: The present study was conducted with the aim of measuring the levels of zinc, copper and iron in the muscles of 4 species of fishes caught off the Beheshtabad River, Iran. Fish samples included *Cyprinus carpio*, *Capoeta aculeata*, *Carassius auratus gibelio* and *Alburnus alburnus*. The results revealed that mean concentration of zinc, copper and iron was 67.3±26.00 μg kg^{-1}, 55±0.74 μg $kg^{-1}$and 177.34±65.45 μg kg^{-1}, respectively. The highest concentrations of copper, zinc and iron were found in *Alburnus alburnus* and *Cyprinus carpio*. A significant difference was observed in copper level in meat of different fish species ($P<0.05$). Seasonal varieties were observed for all examined metals with a significant difference for copper ($P<0.05$). Presence of metals in fish meat is probably due to miss using of fertilizers in agriculture activities and entry of agricultural discharges into the river which constitutes a serious health risk to human.

Key words: Copper · Zinc · Iron · Atomic Absorption Spectrometry · Fish muscles

INTRODUCTION

Contamination of the aquatic environment by heavy metals has risen in recent years due to the industrial development and global population increase. Accumulation of metals in fish or other aquatic organisms constitutes health problems for both fish and human. Pollutants such as heavy metals in sediments or water can be harmful to exposed fish and can also be carried higher in the food chain, including to humans through their consumption of fish [1].

Essential metals such as iron, copper and zinc play an important role in biological systems, whereas, non-essential metals such as lead and cadmium are toxic, even in traces. The essential metals can also produce toxic effects when the metal intake is excessively elevated [1, 2]. Zinc is essential element acting as structural component and having specific properties [3]. The danger of zinc is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically but are only transformed from oxidation state or organic complex to another. Zinc is a potential toxicant to fish [4], which causes disturbances of acid-base and ion regulation, disruption of gill tissue and hypoxia [5]. Accumulation of zinc has attained a serious dimension causing a pathogenic stage like Alzheimer's disease. [6] Iron is also one of the important trace metals that play major roles in many biochemical process including electron transfer, gen regulation, binding and transport of oxygen and regulation of cell growth and differentiation [7].

The Beheshtabad River is located in Chaharmahal va Bakhtyari province in the West of Iran. The River is about 150 km long and is one of the main branches of the Karun River, the longest river of Iran which flows into the Persian Gulf [8]. The purpose of this study was to determine the metals concentration (Zn, Cu and Fe) in the muscles of 4 fish species from the Beheshtabad River.

MATERIALS AND METHODS

Ninety fish including *Cyprinus carpio*, *Capoeta aculeata*, *Carassius auratus gibelio* and *Alburnus alburnus* were caught from the main channel of Beheshtabad River. The collected samples were immediately transported to the laboratory in clean plastic containers filled with crushed ice. Edible muscles were dissected from fishes and stored at -18°C before the analysis.

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A Perkin Elmer Model 4100 atomic absorption spectrometer and deuterium background corrector was used. Samples were injected into the graphite furnace using Perkin Elmer AS-800 autosampler. The atomic absorption signal was measured as a peak height mode against an analytical curve. Perkin Elmer Analyst 4100 model AAS was used for metal determination. The recoveries of the metals were determined by adding increasing amounts of the metals to the samples and taking them through the digestion procedure. All the plastic and glassware were soaked in nitric acid for 15 min and rinsed with deionized water before use. All reagents and solvents were of analytical reagent grade (Merck, Germany). Double distilled water was used for the preparation of solution. The stock solutions of mercury (1000 mg/L) were obtained by dissolving appropriate metal salts (Merck, Germany). The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solutions. The solution was boiled for about 5 min, cooled and nitrogen bubbled through it to expel any metal impurities.

The moisture content of the tissue samples was determined according to AOAC method in triplicate. Samples were digested using the wet digestion techniques [9] and then metals were determined against aqueous standards. Heavy metals were determined in all the digests using Atomic Absorption Spectrophotometer (FIAS 4100, Perkin Elmer).

RESULTS

A total of 90 meat samples for 4 species of fishes include *Cyprinus carpio*, *Capoeta aculeata*, *Carassius auratus gibelio* and *Alburnus alburnus* caught off the Beheshtabad River, Iran, from summer 2007 to spring 2008, were used for determination of zinc, copper and iron by atomic absorption spectrometry after wet digestion. Biometric characteristics of studied fishes are shown in Table 1.

The results revealed that the mean concentration of zinc, copper and iron were 67.3±26.00 μg kg^{-1}, 55±0.74 μg kg^{-1} and 177.34±65.45 μg kg^{-1}, respectively. Heavy metals contents in fish ranged from 32.7 to 151 μg kg^{-1} for zinc, 0.62 to 3.67 μg kg^{-1} for copper and 72.2 to 368.4 for iron.

The highest concentrations of the studied metals were found in *Alburnus alburnus* and *Cyprinus carpio* (Table 2) and a significant difference was observed between copper levels in meat of different fish species ($P<0.05$). Seasonal varieties of heavy metals were also studied and the highest concentration of copper, zinc and iron were observed in autumn 2007 (1.65 μg kg^{-1}), spring 2008 (71.25 μg kg^{-1}) and winter 2007 (182.55 μg kg^{-1}) respectively, with a significant difference for copper ($P<0.05$). Comparing the metals contents in fishes with different age indicate that the level of copper in one year fishes is statistically higher than other fishes ($P<0.05$).

Table 1: Biometric characteristics of studied fishes

	Age		Length (cm)		Weight (g)	
Fish species	Mean	SD	Mean	SD	Mean	SD
Capoeta aculeata	2.1	1-3	22.8	18.5-22.5	199.3	98-311
Carassius auratus gibelio	2.2	1-3	23.7	21.5-37	240.3	147-788
Alburnus alburnus	2.2	1-3	15.7	11-26	176	75-342
Cyprinus carpio	2.1	1-3	23.4	12-43	299.9	147-1562

Table 2: Mean concentrations of zinc, copper and iron in different fishes (μg kg^{-1})

	Zinc		Copper		Iron	
Fish species	Mean	SD	Mean	SD	Mean	SD
Capoeta aculeata	54.4	28.4	133.5	76.25	152.3	65.9
Carassius auratus gibelio	66.4	11.1	138.6	40.8	174.8	28.8
Alburnus alburnus	68.5	27.27	211.6*	77.18	183.3	75.4
Cyprinus carpio	75.2	26.44	125.5	55	189.4	67.21

*: There were significant differences ($P<0.05$) in the levels of zinc, copper and iron with fish species

Table 3: Mean concentrations of zinc, copper and iron in fishes with different age (μg kg^{-1})

	Zinc		Copper		Iron	
Age	Mean	SD	Mean	SD	Mean	SD
One year	69.1	30.11	195.6*	75.96	181.8	71.65
Two years	66.2	26.2	143.6	72.52	175,6	60.6
Three years	67.6	32	128.2	74.35	175.1	70.41

*: There were significant differences ($P<0.05$) in the levels of zinc, copper and iron with age

DISCUSSION

Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain [10]. The contamination of fresh waters with heavy metals has become a matter of concern over the last decade. The natural aquatic system may extensively be contaminated with heavy metal released from domestic, agricultural, industrial and other activities.

Iron, copper and zinc are essential metals which play important roles in biological systems. The essential metals can also produce toxic effects when the metal intake is excessively elevated. Copper is one of a relatively small group of metallic elements which are essential to human health. These elements, along with amino and fatty acids as well as vitamins, are required for normal metabolic processes. Iron is both an essential nutrient and a potential toxicant to cells; as such, it requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation (7). The amount of iron is reported in this study is lower than Acar *et al*., 2010 [11] who reported that iron ranged from 1.14 to1.63 mg kg^{-1} in *Sciaena umbra* from Marmara Sea. This amount is also lower than the amount of iron reported by [12] (9.77 mg kg^{-1}) in freshwater fish from Cadiz Bay.

In this study the mean copper value in the studied fishes was 55±0.74 µg kg^{-1}. Copper levels have been reported 1.57 mg kg^{-1} in muscles of fish from Iskenderun Bay [13], 0.7-27 mg kg^{-1} in muscles and 3.1-323 mg kg^{-1} in livers of fish from Lake Budi, Chile [14], <3.17 mg kg^{-1} in muscles and 305 mg kg^{-1} in livers of fish from Esmoriz-Paramos coastal lagoon[15], 0.57 mg kg^{-1} in muscles of *Pagrus pagrus* [16], 0.32-6.48 mg kg^{-1} for muscles and 5.29-14.9 for livers of fish from Turkish seas [17]. Our results were higher than those in other researches. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for copper of 3.5 mg kg^{-1} body weight/week [18] which was equivalent to 245 mg/week for a 70 kg adult. According to the results, the estimated PTWI of zinc in this study is far below the established PTWI.

Iron contents in the literature have been reported in the range of 8.87-18.8 mg kg^{-1} in muscles of fish from Iskenderun Bay [19], 7.16-16.5 mg kg^{-1} in muscles and 48.1-384 mg kg^{-1} in livers offish from Tuzla Lagoon, Mediterranean Sea region, 1.49-3.69 mg kg^{-1} in muscles of fish from Mediterranean Sea region [20]. The iron concentrations in this study (mean=177 mg kg^{-1}) were lower that other researches. PTWI of 5.6 mg/kg/week for iron has been established by The Joint FAO/WHO Expert Committee on Food Additives.

Zinc contents in other studies have been reported in the range of 4.71-23.1 mg/kg for muscles of fish from Ria de Averio, Portugal [21], 10.7 mg kg^{-1} for muscles of fish from Esmoriz-Paramos coastal lagoon, Portugal [15], 4.36 mg kg^{-1} for muscles of fish from Iskenderun bay, Turkey [13]. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for zinc of 7 mg kg^{-1} body weight/week [18] which was equivalent to 490 mg/week for a 70 kg adult. The mean content of zinc in this study (67 µg kg^{-1}) was quite lower that other researches.

A statistical difference was found in heavy metal contents in different seasons, these variations depend on fish feeding, physiology and behavior in different seasons and also agricultural activities around the river [22, 23]. According to the results, metal content of fish is also highly dependent on fish species and age as there was a significant relation between heavy metal content and age or species of fish.

As a result this amount of zinc, copper and iron in fish from Beheshtabad River in lower that maximum permissible amount according to international standards and is safe for human consumption.

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