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ORIGINAL ARTICLE

Assessment of Heavy Metals (Cu, Pb and Zn) in Different Tissues of Common Carp (*Cyprinus carpio*) Caught from Shirinsu Wetland, Western Iran

Soheil Sobhan Ardakani[1], Seyed Milad Jafari[2*]

[1]Department of the Environment, College of Basic Sciences, Hamedan Branch, Islamic Azad University, Hamedan, Iran

[2]Young Researchers and Elite Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran

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ABSTRACT: Determination of metal levels in muscle, liver and gill of common carp (*Cyprinus carpio*) species caught from Shirinsu Wetland was done using ICP-OES. The mean level of Cu, Pb and Zn in muscle, liver and gill of fish were 0.01±0.002, 0.015±0.003, 0.007±0.001; 0.13±0.005, 0.006±0.001, 0.62±0.11 and 0.26±0.008, 0.009±0.002, 0.53±0.10 μg g^{-1}, respectively. Metal levels in muscle were generally lower than those in liver and gill. Metal concentrations in the edible parts of fish were assessed for human uses according to maximum permissible level. The estimated values of all metals in muscle, liver and gill of fish species in this study were below the permissible levels. Therefore, it can be concluded that these metals in edible parts of the examined species should not pose health problems for consumers.

INTRODUCTION

Because the dietary habits are among the major concerns of human, and are increasing in importance in almost all countries, ingestion, both in amount and quality, of the nutrients for good functioning of the human organism is fundamental. Among the products available, fish is currently considered as one of the most interesting foods [1, 2]. Fish is known to be nutritious, with essential proteins, polyunsaturated fatty acids and liposoluble vitamins. Fish, generally accumulate contaminants from aquatic environments, and have been largely used in food safety studies. Due to their toxicity and accumulative behavior, heavy metals discharged into the marine environment can damage both marine species diversity and ecosystems [3]. Low or high trace element unbalances can be considered as a risk factor for several diseases. Metals, such as Cu and Zn are essential metals since they play important roles in

* Corresponding author: miladjafari2013@yahoo.com (M.Jafari).

biological systems, whereas Pb is toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals with proven hazardous nature are to be completely excluded in food for human consumption. Metal absorption in fish is carried out via two uptake routes: digestive tract (diet exposure) and gill surface (water exposure) [4]. Therefore, we selected muscle as a primary site of metal uptake and liver and gill as tissues specialised in metal storage and detoxification. Due to the deleterious effects of metals on aquatic ecosystems, it is necessary to monitor their bioaccumulation in key species, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health [5, 6]. Shirinsu Wetland is a fresh water lake located in 35° 30' to 35° 45' North and 28° 25' to 40° 48' East in the Northwest of Hamedan province, Iran. Area of Shirinsu Wetland is about 300 ha. Shirinsu wetland survival is mostly dependent upon the water quantity entrance through the natural springs and seasonal river near the mentioned lake. Shirinsu Wetland ecosystem has a great biodiversity and aesthetic value. Every year in winter, many aquatic and wading birds migrate to this lake such as *Gelochelidon nilotica*, *Anas platyrhnchos*, *Ciconia ciconia*, *Phalacrocorax carbo*. Also *Cyprinus carpio* are most commonly fish species founded in this wetland [7]. Because the metal pollution in aquatic environments can be harmful to human health, it is necessary to understand and control the hazard levels of pollution in fish and seafood. Therefore, this study aimed to determine the levels of Cu, Pb and Zn in the muscle, liver and gill of the different species from Shirinsu Wetland, and to assess the public health risks associated with consuming fish harvested from this area.

MATERIALS AND METHODS

Sampling

28 Fish samples (*Cyprinus carpio*) were caught from Shirinsu Wetland, western Iran from April to May 2012. Specimens were frozen in prewashed polyethylene bags and frozen samples brought to the laboratory in ice chests. Approximately 1 g sample of muscle, liver and gill from each fish was dissected, washed with distilled water, weighed, packed in polyethylene bags and stored at -18 °C until the performance of chemical analysis.

Chemical analyses

Tissues were homogenised and digested with 10ml of 1N HNO_3 in Teflon vessels in microwave oven (CEM MARS-5 closed vessel microwave digestion system) using the following microwave digestion programme; pressure 200 psi, ramp time 25 min, temperature 210°C, maximum power 300 W, hold time 10 min. Then, hydrogen peroxide (1.5ml, 30%) was added to each digest to breakdown organic matter that may be undigested during the acid digestion and again the same heating programme was applied. After cooling, residue was transferred to 25ml volumetric flasks and diluted to level with deionized water. The residue was transferred into 10 ml volumetric flasks and diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45μm membrane filter. Sample blanks were prepared in the laboratory in a similar manner to the field samples. All metal concentrations were determined on wet weight basis as $\mu g\ g^{-1}$. All samples were analyzed three times for Cu, Pb and Zn by ICP-OES (Optima 2100 DV, Perkin Elmer). Standard solutions were prepared from stock solutions (Merck, multi element standard, Germany) [6].

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) and Tukey test were used to determine whether Cu, Pb and Zn concentrations varied significantly between tissues, with values less than 0.05 ($p<0.05$) considered statistically significant. The statistical calculations were done using

SPSS 15.0 version (SPSS Inc., Chicago, IL, USA) statistical package.

RESULTS AND DISCUSSION

One group of elements known to be essential to life (Cu and Zn) and non-essential (Pb), reflect an exogenous influence that may be related to environmental pollution [1]. These elements are referred to as toxic elements and their concentrations for the samples studied are displayed in Table 1 along with the statistical parameters. Statistical analysis of the data for common carp showed significant differences among all of the tissues. Figure 1 shows the comparative levels of evaluated metals in the various tissues of common carp. It can be seen that the average concentrations of Cu in gill is more than 2 and 26 times higher than common liver and muscle respectively. Similarly the Zn in liver is higher than muscle. On the other hand, the average Pb content in liver was lower than muscle. Statistical grouping of the concentrations of each element in the different tissues by ANOVA and the Tukey test are shown in table 1. The results indicated that there were significant differences within and between all of the evaluated brands ($p<0.05$).

Table 1. Mean metal concentrations (µg g^{-1} wet wt) in the muscle, liverand gill tissues of the common carp from Shirinsu Wetland, Iran.

	Mean±SD		
Tissue	**Cu**	**Pb**	**Zn**
muscle	0.01±0.002[a]	0.015±0.003[c]	0.007±0.001[a]
liver	0.13±0.005[b]	0.006±0.001[a]	0.62±0.11[c]
Gill	0.26±0.008[c]	0.009±0.002[b]	0.53±0.10[b]

Vertically, letters a, b and c show statistically significant differences ($p<0.05$).

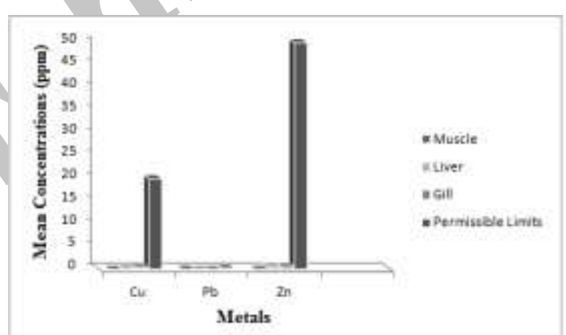

Figure 1. Compare mean concentrations of metals in fish tissues with permissible limits.

Mean Cu concentrations ranged from 0.01 to 0.26 µg g^{-1} in the evaluated tissues (Table 1). The concentrations in all of tissues were below the toxic limit of Cu 30 µg g^{-1} [8]. As Cu is an essential part of several enzymes and necessary for the synthesis of haemoglobin, most marine organisms have evolved mechanisms to regulate concentrations of this metal in their tissues in the presence of variable concentrations in the ambient water, sediments, and food. The richest sources of Cu are shellfish, especially oysters and crustaceans [9].

Sobhanardakani and colleagues reported that mean Cu concentration in the muscle, liver and gill tissues of five fish species (*Otolithes ruber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss)* ranged from 0.007-0.23 μg g^{-1}; 0.009-0.44 μg $g^{-1}$and 0.01-0.34 μg g^{-1}**,** respectively [10]. Also, in Tuzla Lagoon, Turkey, ranging between 0.26 and 0.82 μg g^{-1} [11], and in the Black and Aegean Sea, ranging between 0.73 and 1.83 μg g^{-1} [12], in the Black Sea, ranging between 1.28 and 2.93 μg g^{-1}, 0.65 and 2.78 μg g^{-1} and 1.4 and 2.4 μg g^{-1} [13, 14, 15], in Turkey in the northeast Mediterranean Sea, ranging from 0.65 to 4.36 μg g^{-1} [16] and from 0.639 to 2.976 μg g^{-1} [17], in Marmara Sea, ranging from 0.23 to 9.49 μg g^{-1} [18] and in the lakes in Tokrat ranging from 1.0 to 4.1 μg g^{-1} [19], in mackerel muscle measured in the Aegean and Mediterranean Seas ranged in different locations from 0.51 to 6.22 μg g^{-1} [6]. In six fish species caught in the northeast Mediterranean Sea, Cu level in muscle tissue ranged from 2.19 to 4.41 μg g^{-1} [20]. Comparison of the results of this study with other studies is shown in Table 2.

Highest Pb concentrations were found in the muscle of *Cyprinus carpio* (μg g^{-1} wet wt) while lowest Pb concentrations (0.006 μg g^{-1} wet wt) were always found in liver tissues of fish. All samples contained Pb below 0.4 and 0.5 μg g^{-1} EC [26] and FAO [8], respectively. Masoud and colleagues found that gills exhibited a high accumulation of Pb [27]. Previous researchers concluded that this could be attributed to the similarity of Pb in their deposition and mobilization from gills. Sobhanardakani and colleagues reported that mean Pb concentration in the muscle, liver and gill tissues of five fish species (*Otolithes ruber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss)* ranged from 0.007-0.09 μg g^{-1}; 0.005-0.10μg $g^{-1}$and 0.031-0.11μg g^{-1} respectively [28]. Also Pb levels reported in fish from the eastern Adriatic in the range 0.049-0.141 μg g^{-1} in hake and 0.057-0.158 μg g^{-1} in red mullet [29]. In six fish species collected along the coast of the Adriatic Sea, the highest Pb values were observed in the central area of the Adriatic Sea, for anchovy 0.046μg g^{-1}, red mullet 0.036 μg g^{-1} and mackerel 0.011 μg g^{-1} [30]. Pb levels found in fish from Catalonia, Spain, ranged (μg g^{-1}) between 0.002 and 0.07 in red mullet, 0.01 and 0.02 in anchovy, 0.01 and 0.02 in mackerel [31]. However, Pb concentrations measured in Turkey, Northern Mediterranean Sea, ranged from 0.09 to 6.95μg g^{-1} [17], 0.22 to 0.85, 0.28 to 0.87 and 0.28 to 0.64 μg g^{-1} in fish samples from the Black Sea [13, 14, 15], 0.068 to 0.874μg g^{-1} [16] and 0.33 to 0.93μg g^{-1} in the Black and Aegean Seas [12], 0.49 to 2.44μg g^{-1} from the Tuzla Lagoon [11], 0.14 to 1.28μg g^{-1} in the Aegean and Mediterranean Seas [6], and 0.7 to 2.4μg g^{-1} in the lakes in Tokrat [19], in muscle tissue of three fish sampled form Southern Caspian Sea, Iran 53.7 to 168.9 μg g^{-1} [32]. However, in six fish from the northeast Mediterranean Sea, Pb levels were much higher and ranged from 2.98 to 6.12 μg g^{-1} [20]. Comparison of the results of this study with other studies is shown in Table 2.

Zinc, as an essential micronutrient for both animals and humans, has been a cofactor in nearly 300 enzymes in all marine organisms. Therefore, marine animals are able to regulate tissue Zn at the concentrations in sea water and sediment from normal ambient levels to incipient lethal levels [33]. Zinc toxicity is rare, yet it can be toxic above the limit of 50 μg g^{-1} in muscle. It appears to have a protective effect against the toxicities of both Cd and Pb [34]. The higher levels of Zn content have shown in liver tissue (0.62 μg g^{-1} wet wt), as a constituent of many enzymes, Zn is responsible for certain biological functions, for which a relatively high level is required to maintain them [2]. Analysis of variance revealed Zn concentrations to differ significantly among species with more affinity for sandy/muddy bottoms and pelagic species. Cronin and colleagues suggested that concentrations of this element in fish muscle increased with depth [35]. Sobhanardakani and colleagues reported that mean Zn concentration in the muscle, liver and gill tissues of five fish species (*Otolithes ruber, Pampus argenteus*, *Parastromateus niger, Scomberomorus commerson*, *Onchorynchus mykiss)* ranged from 0.005-0.40 µg g^{-1}; 0.012-0.45 µg $g^{-1}$and 0.03-0.062 µg g^{-1}, respectively [10]. Also Zn levels in muscle tissue of 469 fish sampled form middle and lower reaches of the Yangtze River basin, China reported in the range of 0.8 to 50.8 µg g^{-1} [36]. Comparison of the results of this study with other studies is shown in Table 2.

Table 2. Comparison of present mean values in fish tissues with other studies result

Fish Species	Area	Tissue	Cu (µg g^{-1})	Pb (µg g^{-1})	Zn (µg g^{-1})
		muscle	0.01±0.002	0.015±0.003	0.007±0.001
Common Carp (*Cyprinus carpio*)	Present Study	Liver	0.13±0.005	0.006±0.001	0.62±0.11
		gill	0.26±0.008	0.009±0.002	0.53±0.10
		muscle	0.157-1.21	0.035-0.058	0.23-0.32
Milk fish (*Chanos chanos*)	India[1]	Liver	1.41-1.91	0.122-0.127	1.06-3.88
		gill	0.01-0.124	0.127-0.139	0.31-0.49
Triglia lucerna		muscle	4.19±3.65	0.14±0.15	28.2±9.39
		Liver	38.1±24.6	2.48±1.21	26. 8±11.3
Lophius budegassa	Turkey[2]	muscle	6.24±2.34	0.17±0.02	20.8±11.3
		Liver	31.1±12.9	1.77±1.05	38.2±15.4
Solea lascaris		muscle	5.64±3.92	0.39±0.05	27.5±5.22
		Liver	22.9±9.95	2.98±0.75	32.0±17.6
Anchovy			0.001-6.29	0.001-0.34	-
Red Mullet			0.001-57.3	0.001-0.27	-
Mackerel	Croatia[3]		0.001-15.9	0.002-0.24	-
Picarel			0.08-32.9	0.001-0.46	-
Scyliorhinus caniculus, Scomber scombrus	Northeast Atlantic[4]	muscle	0.11-0.97	-	-
Solea vulgaris, Anguilla Anguilla, Liza aurata	Spain[5]		0.40-1.50	-	-
Leuciscus cephalus, Lepomis gibbosus	Turkey[6]		0.193-2.611	-	-
Mullus barbatus	Mediterranean Sea[7]			2.6-478.0	2157-3822

[1]Rajeshkumar and Munuswamy [21], [2] Yilmaz et al. [2], [3]Bilandzic et al. [22], [4]Celik & Oehlenschlager [23], [5]Usero et al. [24], [6]Yilmaz et al. [16],

[7]Kucuksezgin et al. [25]

CONCLUSION

The results from this study show that the mean level of Cu, Pb and Zn in muscle, liver and gill of common carp caught from Shirinsu Wetland were 0.01±0.002, 0.015±0.003, 0.007±0.001; 0.13±0.005, 0.006±0.001, 0.62±0.11 and 0.26±0.008, 0.009±0.002, 0.53±0.10 μg g^{-1}, respectively. Also metal levels in muscle were generally lower than those in liver and gill. On the other hand the results suggested that significant differences existed in the metal concentrations across three different tissues of analyzed fish. Analytical data obtained from this study shows that the mean concentrations of metal for all of the tissues were generally within the FAO/WHO, U.S. FDA and U.S. EPA recommended limits for fish (table 1 and Figure 1). Therefore, there is no serious health risk associated with the consumption of the fishes analyzed.

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