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Determination of lead and zinc in *Cyprinus carpio* and *Oncorhynchus mykiss* from Iran in 2001 and 2011

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Abstract

The present study was carried out to investigate contamination of heavy metals Pb and Zn in liver and muscle in *Cyprinus carpio* and *Oncorhynchus mykiss* from Iran in 2001 and 2011. Heavy metal levels in fish samples were analyzed by Perkin Elmer 4100 zl atomic absorption. Sampling was done completely random and 48 number fishes were prepared of farmed fishes from the ahvaz and shehrekrod. The results show that the The highest concentration of Pb in muscle of *Oncorhynchus mykiss* 0.73 ± 0.05 mgKg⁻¹dw (2001). Also determined lowest concentration of Pb was in liver this fish 0.16 ± 0.01 mgKg⁻¹dw (2001). The highest concentration of Zn in the liver of *Cyprinus carpio* 62 ± 5.81 mgKg⁻¹dw (2001) and lowest concentration of Zn in muscle of *Oncorhynchus mykiss* 2.78 ± 0.13 mgKg⁻¹dw (2001). This study concentration of heavy metals Zn and Pb were in the liver and muscle of *Cyprinus carpio* and *Oncorhynchus mykiss* significant difference between 2001 and 2011 ($P < 0.05$), except Pb in muscle and liver *Cyprinus carpio* ($P \geq 0.05$). The mean estimated concentrations for Zn in the present study were higher than International Standards for these metals as declare by the UKMAFF and FAO. Concentrations of Pb in this study were lowest than WHO, UKMAFF, FAO, FDA and NHMRC.

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Introduction

Fish are a major part of the human diet and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish. In recent years, fish lipids have also assumed great nutritional significance, because of their high polyunsaturated fatty acid levels and good source of digestible protein, vitamins, minerals (Prudente *et al.*, 1997; Puwastien *et al.*, 1999; Kucuksezgin *et al.*, 2001; Lewis *et al.*, 2002; Ikem and Egiebor, 2005).

Heavy metal pollution of aquatic environment has become a great concern in recent years. HMs can have toxic effects on organs (Macfarlane and Burchett, 2000). Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards. Iron, copper, zinc and manganese are essential metals while, mercury, lead and cadmium are toxic metals (Canli and Alti, 2003). Heavy metals still play an important role as pollutants affecting aquatic systems (Merian, 1991). Some of the metals found in the fish might be essential as they play important role in biological system of the fish as well as in human being, some of them may also be toxic as might cause a serious damage in human health even in trace amount at a certain limit. The common heavy metals that are found in fish include copper, iron, copper, zinc and manganese, mercury, lead and cadmium (Rashed, 2001; Munoz-Olivas and Camara, 2001; Canli and Alti, 2003; Fernandes *et al.*, 2008). Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Celik and Oehlenschlager, 2007).

Lead finds its way in waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries etc. Pb also enters the rivers both from terrestrial sources and atmosphere and the atmospheric input of Pb aerosols can be substantial (Mitra *et al.*, 2010).

Lead enters into the body with gill cells and especially is accumulated in gills and the later aim organs are liver and muscle (Sadeghi-Rad, 1997). Although, Zinc usually is accumulated in bone, skin, liver, gill and kidney are accumulated the great amount of them (Celik and Oehlenschlager, 2004).

This matter that, importance of the heavy metals measuring relate to two important subjects which are aquatics ecosystem management and human health, the present study was carried out to determine the level of Lead and zinc in liver and muscle samples of *Cyprinus carpio* and *Oncorhynchus mykiss* from Iran. The fish and fish products for the people in those ports are generally catch and carried by local vehicles from the Ahvaz and Shahrekord cites. It should be noted that fish species are considered to be a heavy metals part of the diet in the region. No data exist on Zinc and Lead levels in this fish from mentioned areas.

The main objective of this study was to determinate the contents of zinc and lead in the muscle and liver of *Cyprinus carpio* and *Oncorhynchus mykiss* in from Iran, in order to assess fish quality and to assess the health risk for humans.

Material and methods

Collection of samples

The *Cyprinus carpio* and *Oncorhynchus mykiss* in this study were collected 48 samples of farmed fishes from the Ahvaz and Shahrekord cites took place twice in 2001 and 2011. After capture, fishes were placed in plastic bags and transported to the laboratory in freezer bags with ice. Samples were cut into pieces and labeled, and then all sampling procedures were carried out according to internationally recognized guidelines (UNEP, 1991). Total fish weight and length were measured to the milometer and gram (Table 1).

Sample analysis

A Perkin-Elmer, model 4100 ZL atomic absorption spectrophotometer, equipped with a GTA Graphite furnace, was used. Pyrolytic-coated graphite tubes with a platform were used and signals were measured

as peak areas. The instrument setting and furnace programmers for analysis of Zinc and Lead metals are described in table 2. All reagents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solution. All the plastic and glass ware were soaked in nitric acid for 15 min and rinsed with deionized water before use. The stock solutions of metals (1000 mg l^{-1}) were obtained by dissolving appropriate salts of the corresponding metals (E. merk) and further diluted prior to use. High purity Argon was used as inert gusted prior to use. The samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1gr) was placed in to Teflon container and 5 ml of concentrated HNO_3 was added. The system was heated to 130°C for 90 min and finally diluted to 25 ml with deionized water. The sample solution was clear. A blank digest was carried out in the same way. Zinc and Lead metals were determined against aqueous standards.

Statistical analysis

Analysis of variance (ANOVA) was run for all the collected data for fish samples different using SPSS (16 version) computer programs. Mean values of each parameter were compared using Fisher's protected least tests with significance levels of 5% were conducted on each metal to test for significant differences between sites (Table 3 and 4). All

statistical analyses were conducted using the Office Excel 2003 software package.

Results and discussion

Determination metals

Concentration levels of metals Zn and Pb in muscle and liver of *Cyprinus carpio* and *Oncorhynchus mykiss* were measured and presented in table 3 and 4. Concentrations of metals are presented in mg Kg^{-1} dry weight unless otherwise mentioned. The highest and lowest concentration of Pb in tissues was done in muscle and liver *Oncorhynchus mykiss* in 2001. The distribution patterns of Pb some of samples in muscle were higher than in liver (table 3). The highest and lowest concentration of Zn in tissues was done in liver *Cyprinus carpio* in 2001 and muscle *Oncorhynchus mykiss* in 2011. The distribution patterns of Zn in tissues of *Cyprinus carpio* and *Oncorhynchus mykiss* follows the order: liver > muscle (table 4). Heavy metal concentrations were higher in the gill and liver, when compared with muscle. livers were chosen as target organs for assessing metal accumulation (Mohammadi *et al.* 2011; Askary sary and Velayatzadeh, 2012). This study concentration of heavy metals Zn and Pb were in the liver and muscle of *Cyprinus carpio* and *Oncorhynchus mykiss* significant difference between 2001 and 2011 ($P < 0.05$), except Pb in muscle and liver *Cyprinus carpio* ($P \geq 0.05$).

Table 1. Mean length and weight of the species examined in present study.

Year	species	The number of samples	Length \pm SD (mm)	Weight \pm SD (g)
2001	<i>Cyprinus carpio</i>	12	480.6 \pm 23.52	2250 \pm 86.60
	<i>Oncorhynchus mykiss</i>	12	290 \pm 11.14	273.34 \pm 7.55
2011	<i>Cyprinus carpio</i>	12	32.88 \pm 1.78	738.12 \pm 26.39
	<i>Oncorhynchus mykiss</i>	12	31.67 \pm 1.52	498.58 \pm 18.92

Comparison Zn and Pb

Estimation of the levels of various elements in different fish species as a measure of environmental pollution has been of great concern over decades. A variable range of different metal concentrations has been observed by various researchers worldwide (Ashraf *et al.*, 2006). The absorption of metals on to

the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the liver (Heath, 1987). Distribution patterns of metal concentrations in liver and muscle of *Cyprinus carpio* and *Oncorhynchus mykiss* follows the sequence: Zn>Pb. There are various studies on the heavy metal levels in fish from

different waters. Oymak *et al.* (2009) studied the heavy metal levels in kidney, liver, gill and muscle of *Tor grypupus* and Maaboodi *et al.*, (2011) studied the concentration of Zn and Pb in liver of Carrassius, *Cyprinus carpio*, *C. aculeate* and *C. damasciana* which concentration of Zn were higher than Pb. Also, Turkmen *et al.*, (2010) studied the heavy metal levels

in muscle, liver, gonad, and gill of gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and keeled mullet (*Liza carinata*) which concentration of Zn were higher than Pb. The levels of Zn in all tissues were higher than the Pb levels, as Zn is present in many enzymes throughout the fish's body (Oymak *et al.*, 2009).

Table 2. The instrument setting and furnace programmes for analysis of Zn and Pb by Perkin-Elmer, model 4100 ZL.

Working conditions	Pb	Zn
Wavelength (nm)	283.3	307.5
Slit width	0.5	0.7
Lamp current (MA)	8	15
Ar Flow (ml/min)	250	250
Injection Volume (µl)	25	20
Heating programme temprture ^o C [ramp time (5), hold time (5)]		
Drying 1	125(1.20)	115(1.20)
Drying 2	150(5.30)	150(5.30)
Pyrolysis	900(15.10)	1250(15.10)
Atomization	2150(0.5)	1900(0.5)
Cleaning	2400(1.2)	2400(1.2)

Table 3. The concentrations and comparison of Pb in muscle and liver *Cyprinus carpio* and *Oncorhynchus mykiss* (mgKg⁻¹).

Sample	Location	2001	2011
<i>Cyprinus carpio</i>	muscle	0.23±0.01 ^a	0.30±0.02 ^a
	liver	0.26±0.01 ^a	0.34±0.04 ^a
<i>Oncorhynchus mykiss</i>	muscle	0.73±0.05 ^b	0.36±0.03 ^b
	liver	0.16±0.01 ^b	0.42±0.02 ^b

a: non-significant differences at p<0.05

b: significant differences at p<0.05.

Lead

It is known that arsenic, mercury, lead and cadmium are the most commonly distributed environmental metal poisons (Castro-Gonzalez and Mendez-Armenta, 2008). They are accumulated in human tissues and may be the cause of some diseases (Rodriguez *et al.*, 2003; Yilmaz *et al.*, 2007). In this study minimum Pb levels 0.16 mgKg⁻¹dw and maximum concentrations of this metals 0.73 mgKg⁻¹dw. Agah *et al.* 2009 that among Pb in five fishes from Persian Gulf, which concentration of Grunt

(*Pomadasy sp.*), Flathead (*Platycephalus sp.*), Greasy grouper (*Epinephelus tauvina*), Tiger-tooth (*Otolithes rubber*) and Silver pomfret (*Pampus argenteus*) were 2-25, 0.2-17, 2-9, 1-9 and 3-33 ngg⁻¹. In other study concentration of Pb in muscle Indo-Pacific king mackerel and Tigertooth croaker were 0.625±0.517 and 0.31±0.258 mgKg⁻¹ (Dobaradaran *et al.* 2010). The concentrations of Pb in liver and muscle some of samples in 2011 higher than were 2001, except in muscle *Oncorhynchus mykiss*. The Pb values in fish species were found to be in range of

0.068–0.874 mgg⁻¹. These values were lower than those reported earlier in fish species of different lakes (Aucoin *et al.*, 1999; Mendil *et al.*, 2005). Turkish acceptable limits and EU limits were 0.4 mgg⁻¹. The range of international standards for Pb in fish is 0.5–10 mgg⁻¹ (EU, 2001; TFC, 2002; Yamazaki *et al.*, 1996). The concentrations of Pb in liver *Cyprinus*

carpio in 2001 and 2011 were higher than muscle. Muscle tissue is the main edible fish part and can directly influence human health. Lead enters into the body with gill cells and especially is accumulated in gills and the later aim organs are liver and muscle (Sadeghi-Rad, 1997; Alonso *et al.*, 2004; Castro-Gonzalez and Mendez-Armenta, 2008).

Table 4. The concentrations and comparison of Zn in muscle and liver *Cyprinus carpio* and *Oncorhynchus mykiss* (mgKg⁻¹).

Sample	Location	2001	2011
<i>Cyprinus carpio</i>	muscle	14±0.92 ^b	5.33±0.33 ^b
	liver	62±5.81 ^b	6.07±0.38 ^b
<i>Oncorhynchus mykiss</i>	muscle	9±0.62 ^b	2.78±0.13 ^b
	liver	60±5.63 ^b	3.40±0.24 ^b

a: non-significant differences at p<0.05

b: significant differences at p<0.05.

Table 5. the tolerable values of some heavy metals in the fish (mgkg⁻¹).

Standards	Pb	Zn	References
WHO ¹	2	1000	WHO 1996
FDA ²	0.5	-	Tuzen 2009
UK(MAFF) ³	2	50	MAFF 1995
NHMRC ⁴	1.5	150	Tuzen 2009
FAO ⁵	0.5	30	FAO 1983
This study	0.16-0.73	2.78-62	-

1- World Health Organization

2- U.S. Food and Drug Administration

3- Ministry of Agriculture, Fisheries & Food (UK)

4- National Health & Medical Research Council (Australia)

5- Food and Agriculture Organization.

Zinc

In this study minimum and maximum of Zn 2.78 and 62 mgKg⁻¹dw. Yilmaz *et al.*, 2007 reported that among Zn concentration were 6.350–28.550 mgKg⁻¹ in tissues of *Leucis cephalus* and 6.540–16.064 mgKg⁻¹ in tissues of *Lepomis gibbosus* (Yilmaz *et al.*, 2007). Level of Zn in muscle and liver of *Sciaena umbra* were 11.6 and 28.3 mgKg⁻¹ (Turkmen *et al.*, 2008). Also Abu Hilal and Ismail (2008) reported that among Zn concentration was the highest and lowest 1.9–35 mgKg⁻¹ in muscles, livers, gills, gonads, and stomachs of eleven common fish species collected

at three sites in the northern Gulf of Aqaba. The concentration of Zn in muscle and liver of Garfish (*Belone belone*) and the Bluefish (*Pomatomus saltatrix*) were 3.85–15.9 mg kg⁻¹ (Turkmen *et al.*, 2009). The concentrations of Zn in liver were higher than muscle of *Cyprinus carpio* and *Oncorhynchus mykiss*. Concentrations of Pb and Zn in liver were higher than muscle of *Scomberomorus guttatus*, *Scomberomorus commerson* and *Otolithes ruber* from Persian Gulf (Askary sary and Velayatzadeh, 2012). In other study such as *Sciaena umbra* (Turkmen *et al.*, 2008), *Sparus auratus*, *Trigla*

cuculus, *Sardina pilchardus*, *Mugil cephalus*, *Atherina hepsetus*, *Scomberesox saurus* (Canli and Altı, 2003), *Serranus scriba*, *Epinephelus costae*, *Cephalopholis nigri* and *Pseudupenaeus prayensis* (Romeo *et al.*, 1999) concentrations of Zn in liver was higher than muscle. The observed variability of heavy metal levels such as Zn and Pb in different species depends on feeding habits (Romeo *et al.*, 1999), ecological needs, metabolism (Canli and Furness 1993), age, size and length of the fish (Linde *et al.*, 1998) and their habitats (Canli and Atli, 2003; Tuzen and Soylak, 2007).

Comparison of International Standards

The mean estimated concentrations for Zn in the present study were higher than International Standards for these metals as declare by the UKMAFF and FAO. Concentrations of Pb in this study were lowest than WHO, UKMAFF, FAO, FDA and NHMRC (Table 5).

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