Study of the heavy metals (Cd and Pb) content in the tissues of rainbow trouts from Hamedan coldwater fish farms

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Received: July 2014 Accepted: December 2015

Abstract

The aim of this research was to determine the concentration of cadmium (Cd) and lead (Pb) in the muscle and liver tissues of rainbow trout (*Oncorhynchus mykiss*) in Hamedan Province (west of Iran) cold water fish farms, and compare the results with the FAO/WHO guidelines. Heavy metal concentrations were determined from the three randomly selected fish farms by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method. Concentration of heavy metals were detected to be high in rainbow trout during dry season. Concentration of Cd and Pb in the muscle and liver tissues were 3.67µg/kg and 12.82µg/kg, respectively. However concentrations of these two heavy metals were within the standard limits set and recommended by FAO/WHO, indicating that the rainbow trout meat raised from this region is safe for human consumption.

Keyword: Bioaccumulation, Consumer, Environment, Fish farm, Heavy metals, permissible limit

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Introduction

Fish products are widely consumed in many parts of the world because it has high protein content, low saturated fatty acids, calcium, phosphorus, iron, and trace elements such as copper as wellas a fair proportion of the group B-vitamins to support good health (Nkpaa *et al*., 2013). Fish products now account for 30% of the human protein supply in Asia, 20% in Africa, and 15% in Latin America and the Caribbean. Because they are neutrally buoyant, most fishes have less need for a supporting skeleton, and consequently have a higher ratio of muscle to bone than land animals. This characteristic as well as having high levels of protein, essential fatty acids, minerals and vitamins makes them a very valuable and healthy alternative to other meats (Bone and Moore, 2008).

The aquaculture industry, which has been growing rapidly for many years, is still projected to continue growing faster than most other industries for the foreseeable future (Lekang, 2007). According to the reports published by the Iranian Fisheries Organization, the Iranian fisheries and aquaculture production reached 522,000 metric tons (MT) in 2005, of which 75% was originated from capture fishery and 25% from aquaculture activities. The production was 7.3% higher compared to the previous year, indicating a clear increase in the production and consumption of seafood products in Iran (Rajaby, 2008).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most valuable salmonid fishes worldwide, because of its high meat quality, well advanced artificial propagation and farming techniques, and being prized for sport fishing. After Atlantic salmon, this speciesis the most important farmed fish among the *Salmonidae* family (Adeli and Baghaei, 2013). In Iran, rainbow trout is the sole species for cold water aquaculture practices (Hassanpour *et al*., 2010). The total production of farmed rainbow trout in Iran, increased from 835 tons in 1993 to 91519 tons in 2010, with an average annual increase of 31.8% during this period (Adeli and Baghaei, 2013).
Among the wide range of organic and inorganic substances released into aquatic ecosystems, heavy metals have received much attention due to their different toxicity and potential bioaccumulation in many aquatic organisms, which are eventually transferred to human beings via the food chain.

Cadmium (Cd) is one of the principal heavy metals responsible for causing kidney damage, renal disorder, high blood pressure, bone fracture and destruction of red blood Cells. Human beings have reported to have nausea and vomiting at a level of 15 mg/L of cadmium (Senthil Kumar *et al*., 2010).

Lead (Pb) is one of the most dangerous pollutants in our environment which accumulates in the body due to its low rate of elimination.

Lead enters aquatic systems from urban, mining and agricultural runoff, atmospheric precipitation, plating processes, and gasoline containing lead that leaks from fishery boats and a variety of natural sources. Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb (Mahmoud *et al*., 2013).

There have been various studies on the heavy metals levels in rainbow trout from different freshwater ecosystems (Sloman *et al*, 2003; Amundsen *et al*, 2007; Oja and Wood, 2007; Tekman *et al*., 2008; Fallah *et al*., 2011). The objective of the work discussed in this paper was to determine the concentrations of Cd and Pb in sthe muscles and liver tissues of rainbow trout (*O. mykiss*) from some cold water fish farms in Hamedan Province (west of Iran) and compare the results with the FAO/WHO guidelines.

Materials and methods

The study area

This research has been conducted in Hamedan Province during 2012 and 2013.

Hamedan Township, cowering 4084 square kilometers, is located between 34º,35',00" and 35º,10',00" northern latitudes and between 48º,20',00" and 49º,27',00"eastern longitudes (Fig.1). Alvand Mountain with an altitude of 3584 meters has the highest peak in Hamedan Province, and lies as the natural boundary between Hamedan and Tuyserkan Townships. The lowest part is Amr-Abad with an altitude of 1600 meters where the Gharah-Chai River flows out of the province. Due to the low investments in industrial activities, the development of the area is built upon the improvement of agriculture and aquaculture (Reyahi Khoram *et al*., 2005).

Sampling and sample collection

Three fish farms were randomly selected from several fish farms in the area. Thirty six rainbow trout fishes were caught using a scoop net during March 2013 to August 2013. The body weight and length of each sample were measured with a precision of 0.1 g and 0.1 cm. The overall mean weight and length of the fishes were calculated as 547.5 g 341.1 mm, respectively. Fish samples were washed and packed in clean polyethylene bags and transferred to the lab in an ice box.

Sample preparation and chemical analysis

Once in the laboratory, the specimens were dissected and the muscle and liver tissue samples were taken and cut into small pieces and washed several times separately with distilled water and placed in two labelled laboratory dishes. All samples were air-dried for 2 days at ambient temperature and pressure. After that, all samples were placed in the oven for 2 hours at a constant temperature of 65°C and then were ground to a fine powder with the aid of an electric milling machine.

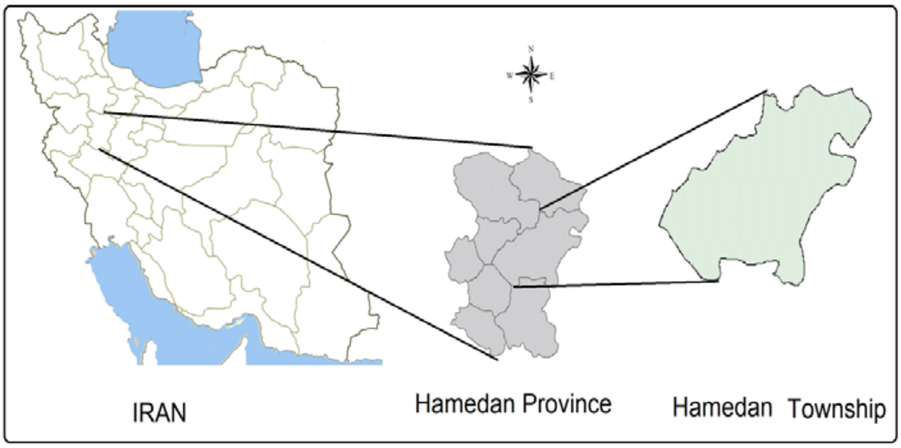

Figure 1: Location of the study area.

Then exactly 3 g of muscle tissue and 1 g of liver tissue were placed in porcelain crucibles and heated in an air circulating oven at 450 °C for 2 hours to give it a dark grey colour.

After that the samples were digested with concentrated nitric acid and perchloric acid until a clear and transparent solution was obtained. Heavy metal concentrations were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES); Varian 710-ES. To digest the tissue samples, 3 and 1 grams of muscle and liver samples were placed in two separate test tubes (polyethylene tubes), respectively. Then, 4.2 g of freshly prepared nitric acid (HNO_3, Merck, %65) and 4.8 g perchloric acid ($HClO_4$, Merck, %60) were added to the muscle sample, and 0.8 g of nitric acid (HNO_3, Merck, %65) and 1.6 g perchloric acid ($HClO_4$, Merck, %60) were added to the liver sample and left overnight at room temperature. The digests were filtered through a Whatman 42 filter paper to remove the insoluble particles and brought to a final volume of 15ml with de-ionized water. The samples preparations and analysis were carried out according to the procedure described by Erdogrul and Ates (2006) and Schmitt *et al*. (2006).

Statistical analysis

Statistical Analysis of data was carried out by using SPSS statistical package. The Kolmogorov-Smirnov test (KS-test) is one of the useful and general nonparametric methods for comparing two samplesK-S test was used to measure the goodness-of-fit of the distribution. One-way analysis of variance (ANOVA) was performed, followed by Scheffe post hoc comparisons for the source of statistically significant differences. Additionally, the simple linear regression test was used to check significant relationships between trace metal concentrations in fish muscle and liver tissues.

Results

Evaluation of normal distribution of samples

According to Table 1, it is shown that significance level of the test in all the variables is above 0.05, so the data is normal and parametric tests used in this study have been suitable.

The results of the metal concentrations in samples taken from three selected fish farms are given in Tables 2, 3 and 4. In these Tables, the obtained data are classified based on different criteria such as, fish farm, fish tissue and season.

The mean concentrations of heavy metals in tissues of rainbow trout (*O. mykiss*) from various fish farms are summarized in Table 2. Mean Cd concentrations in fish tissues ranged from 0.17 to 13.74 μg/kg dw. The present results show that the metal concentrations in rainbow trout are evaluated in the following order: Pb>Cd.

The mean concentrations of heavy metals in muscle and liver of rainbow trout and the average results are summarized in Table 3. Concentration of heavy metals detected in the liver tissues and muscle tissues samples showed a different accumulation pattern of heavy metals in various tissues of rainbow trout.

Based on obtained results it seems that the seasons have an influence on the concentrations of the Cd and Pb in fishes captured from the Farms (Table 4). The results indicated that the bioaccumulations of heavy metals (Cd and Pb) in rainbow trout tissues were high during dry season compared to the monsoon season.

Comparison between organs

Two heavy metals (Cd and Pb) have been measured in rainbow trout tissues collected from the farms during seasons. Mean concentrations of Cd and Pb in different tissues of the selected fishes are given in Table 5. The results of this study did not show any significant differences between the two organs of the fish (muscle and liver) for Cd (p>0.05) and Pb (p>0.05).

Comparison between seasons

Sampling seasons were evaluated and presented in Table 6. There were significant differences between the monsoon season and dry seasons in the concentrations of Cd (p<0.05) and Pb (p<0.05). The levels of Cd and Pb could have been affected by rainfall variations. In the present study, accumulation of Cd and Pb in rainbow trout were found to be high during dry season than monsoon season. The seasonal variation of heavy metals in the fish species have been affected by rainfall variations, physico-chemical related to environmental conditions and increased metabolism at summer temperatures, which influence the bioavailability of metals.

Table 1: Results of Kolmogorov-Smirnov-Test for normality of original data as (μ g/kg dw).

Parameters	A		B		C		Muscle		Liver		Monsoon season		Dry Season	
Metal	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
N	10	10	11	11	10	10	18	18	13	13	16	16	15	15
Mean (μ g/g)	3.71	8.14	5.26	11.58	2.11	22.74	3.67	12.82	3.84	15.81	2.18	11.20	5.41	17.13
Std. Deviation	4.89	7.19	4.87	11.85	2.39	19.82	4.68	19.84	3.90	10.24	1.95	18.14	5.47	9.89
K-S Z	1.10	0.62	0.73	0.67	0.89	0.68	0.90	0.97	1.43	0.78	1.03	1.35	1.10	0.52
p value[a]	0.17	0.82	0.62	0.75	0.39	0.74	0.38	0.29	0.03	0.57	0.23	0.05	0.17	0.94

a.Test distribution is Normal (p>0.05).

Table 2: Mean heavy metals concentration (μ g/kg dw) in rainbow trout captured from three farms .

Element	Fish farm	n	M±SD	Max	Min
Cd	A	10	3.71±4.89	13.56	0.17
	B	11	5.26±4.88	13.74	0.17
	C	10	2.11±2.39	6.97	0.18
	Total	31	3.74±4.24	13.74	0.17
Pb	A	10	8.14±7.19	20.98	0.34
	B	11	11.59±11.85	33.4	0.88
	C	10	22.74±19.82	70.17	2.56
	Total	31	14.07±14.56	70.17	0.34

Table 3: Mean heavy metals concentration (μ g/kg dw) in different tissues of rainbow trout examined.

Heavy metal	Tissue	n	M±SD	Max	Min
Cd	Muscle	18	3.67±4.68	11.88	0.17
	Liver	13	3.84±3.90	13.74	0.17
	Ave.	31	3.74±4.31	13.74	0.17
Pb	Muscle	18	12.82±10.24	35.19	0.34
	Liver	13	15.81±19.84	70.17	1.29
	Ave.	31	14.07±14.80	70.17	0.34

Table 4: Mean heavy metals concentration (μ g/kg dw) in rainbow trout captured from three farms during the investigation period.

Heavy metal	Season	n	M±SD	Max	Min
Cd	Monsoon season	18	2.19±1.95	6.97	0.17
	Dry season	13	5.41±5.47	13.74	0.17
	Total	31	3.74±4.23	13.74	0.17
Pb	Monsoon season	18	11.21±18.14	70.17	0.34
	Dry season	13	17.13±9.89	33.42	1.92
	Total	31	14.07±14.56	70.17	0.34

Table 5: Results of T test analysis comparing the mean concentration (μg/kg dw) of heavy metals in muscle and liver of rainbow trout captured from the study area.

Tissue	Cd (X ±SD)	Pb (X ±SD)
Muscle	(3.67±4.68)	(12.82±10.24)
liver	(3.84±3.90)	(15.81±19.84)
df	29	29
t	0.101	0.548
p-value	0.91*	0.58*

**:Not significant, p>0.05.*

Comparison between fish farms

In this study, one-way ANOVA were performed for statistical comparisons. The results of the one-way ANOVA showed that there were no significant differences in the level of Cd (p>0.05) and Pb (p>0.05) between the fishes taken from the three selected fish farms (Table 7).

Correlation between tissues

Statistical correlations between Tissues was calculated. Based on the data analysis, There is a significant correlation (p<0.01) between Pb concentration in muscel tissue and liver tissue. But there is not any significant correlation (p>0.01) between Cd concentration in muscel tissue and liver tissue (Table 8).

Comparison with FAO/WHO guidelines

The mean concentration of the heavy metals (Cd and Pb) was statistically compared with FAO/WHO guidelines levels (FAO/WHO, 2011; FAO/WHO, 1984; Eman El-Wazzan et al, 2014). The mentioned recommendation for Cd and Pb is 1000 and 300 μg/kg dw, respectively (Tables 9 and 10). Cd and Pb levels were found in fish samples at mean concentrations below the permissible limits values. Therefore, it can be accepted that these rainbow trout could be safe for human consumption.

Table 6: Results of T test analysis comparing the mean concentration (μg/kg dw) of heavy metals in rainbow trout collected from the three farms during investigation period.

Heavy Metal	Cd (X ±SD)	Pb (X ±SD)
monsoon season	2.19±1.95	11.21±18.14
df	29	29
t	2210	1118
Dry season	5.41±5.47	17.13±9.89
p-value	0.04*	0.03*

**:significant, p<0.05*

Table 7: Two-way analysis of variance of data on metal concentration (μ g/kg dw) of selected rainbow trout from different farms of Hamedan district.

Metal	Cd	Pb
F- ratio	1.438	3.03
p- value	0.254*	0.06*

**:Not significant, p>0.05*

Table 8: Correlations between metal concentration (μ g/kg dw) in muscel and liver of rainbow trout collected from study area.

Correlation between	R value	P value
Pb concentration in muscles and liver of the selected fishes	0.86	0.01*
Cd concentration in muscles and liver of the selected fishes	0.47	0.10**

**:significant, p<0.05*
***:Not significant, p>0.05*

Table 9: Results of one sample t-test analysis comparing the mean concentration (μ g/kg dw) of heavy metals in rainbow trout collected from the study area with FAO/WHO recommended levels.

Metal	n	Mean±SD	FAO/WHO guidelines	P-Value
Cd	31	3.67±4.68	1000	0.001*
Pb	31	12.82±10.24	300	0.001*

**:significant, p<0.05*

Table 10: Heavy metal concentration in guidelines.

Metal	Measured Value	FAO/WHO guidelines	TEG*	EC**	SA***	MFR****	ISIRI*****
Cd (μ g/kg dw)	3.67	1000	100	50	500	1000	50
Pb (μ g/kg dw)	12.82	300	1000	500	2000	2000	300

*: Turkish Environmental Guidelines (Safahieh *et al*, 2011).
**: European Communities (Safahieh *et al*, 2011).
***: Saudi Arabia (Safahieh *et al*, 2011),
****:Malaysian Food regulation (Fathi Alhashmi *et al*., 2012).
*****: Institute of Standards and Industrial Research of Iran (ISIRI, 2010).

Discussion

High standard deviation shows that the data are spread out over a large range of values and it may be because of the variation in weight and/or length of fish samples (Table 1).

Most of fish organs are sensitive to toxicity of heavy metals. In this research, Fish muscle was chosen as target organ for assessing metal accumulation that reflects the concentration of metals in water where the fish species live, and because of its importance for human diet as a basic nutrition and the necessity for health care consumption. Liver tissue was chosen; because it is the main organ for body and primary tissue for metabolism of toxic oil constituents.

Based on the results of the study, the concentrations of heavy metals in liver tissues were higher than that in muscle tissues. Heavy metals are first deposited in various organs (liver, kidneys, brain), and then may penetrate from blood to milk, while a mammary gland itself is a kind of biological barrier (Dobrzański *et al*. 2009). Some of researchers attributed the high accumulation of heavy metals in liver and gills tissues to the metallothionein proteins which are synthesized in liver and gills tissues when fishes are exposed to heavy metals and detoxify them. These proteins are thought to play an important role in protecting them from damage by heavy metal toxicants (Saeed and Shaker, 2008). Also, other researchers claim that heavy metal bioaccumulation in fish is species-dependent (Su *et al*., 2009; Malik *et al*., 2013).

It is reported that, the variation in the level of heavy metals in some species of fish depends upon its feeding habit, age, size, length of the fish and their habitats. The metal concentration in muscle is important because it is the chief edible part of the fish. However the affinity for metal absorption from contaminated water and food may differ in relation to ecological needs, metabolism and the contamination gradients of water, food and sediments as well as other factors such as salinity, temperature and increasing agents (Shanthi and Ramanibai, 2011). Saeed and Shaker (2008) in their research which was focused on the pollutants levels including the accumulation of some heavy metals in the water, sediments and fish organs of Nile tilapia in northern Delta Lakes, reported that the concentration of heavy metals in fish gills and liver were much higher than what was measured in muscles.

Some researchers indicate that the target tissues of heavy metals in fish are those which are metabolically more active such as the liver, kidneys and gills.The liver serves as a storage site for heavy metals and it plays an important role in detoxication and elimination of many harmful substances from the body (Amundsen *et al*., 2007; Al-Kahtani, 2009; Fallah *et al*., 2011; Brázová *et al*., 2012; Stancheva *et al*, 2013).

In the present study, the concentrations of Cd and Pb in muscle tissues of rainbow trout fish were 3.67 μg/kg and 12.82 μg/kg, respectively, as shown in Table 5. Therefore, heavy metal concentrations (Cd and Pb) in rainbow trout fish of the studied area were well within the limits set by the recommendations of FAO/WHO standards and indicate that rainbow trout from the studied region are safe for consumers. Heavy metals concentrations measured in this study are compared with some guidelines in Table 10.

Comparable results were obtained by Taghavi Jelodar *et al.* (2011). They found that the mean concentrations of Cd and Pb in the muscle of *Liza aurata* from southern part of the Caspian Sea, were 1070 and 2600 μg/kg dw respectively. In another study was performed in Persian Gulf ,near the Iran coast, by Hossein Khezri *et al.* (2014), Cd and Pb concentrations in the fish muscle were observed between 36- 107 and 264- 1188 μg/kg dw, respectively. Based on the literature review discussed in this study, it may be assumed that fish species, caught from inland waters, had lower elemental concentrations than those caught from coastal waters.

Acknowledgements

This research is carried out as a Master Thesis at the department of environment, which was financially and technically supported by Hamedan Branch, Islamic Azad University, to which the authors' thanks are due.

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