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Bioaccumulation of Lead and Cadmium in liver and muscle tissues of Kutum fish (Teleostei: Cyprinidae) in the southern Caspian Sea

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Abstract: The current study is focused on determination of the accumulation rate of Cadmium (Cd) and Lead (Pb) in the liver and muscle tissues of the Caspian Kutum fish. Specimens were collected randomly from twelve stations in Mazandaran Province, the Caspian Sea Basin, between October 2009 and March 2010. Fork length, weight and length-weight relationship of samples were measured. One-way analysis of variance (ANOVA) showed that there is a significant difference for Pb and Cd content in liver and muscle (P<0.05). The highest level of bioaccumulation obtained for Pb in liver tissue (0.809 µg/g) from Fereidounkenar and the lowest level obtained for Cd in muscle tissue (0.038 µg/g) belongs to Koliver station. The results of linear regression analysis showed that, there were significant relationships between the heavy metal contents in both liver and muscle tissues and the fish weight (P<0.05). Comparison of heavy metal contents in both tissues revealed that the bioaccumulation of heavy metals in liver is higher than muscle. Comparisons showed that the range of Cd and Pb content in fish body is below of the international limited amounts given for these hazardous substances.

Keywords: Cd, Pb, Heavy metals, Hazardous substances.

Introduction

Nowadays, marine products play a great role in human's food regime. There is an up-growing tendency towards consumption of sea foods due to increasing public awareness about their preponderance to other protein products (Rezaei et al. 2005). Heavy metal pollution in aquatic ecosystems and consequently, their accumulation in aquatic organism tissues is one of recent concerns in seafood industries. Presence of pollutants in aquatic ecosystems is the result of atmospheric deposition, or from anthropogenic sources, such as industrial discharge, sewage, agricultural waste, and mining wastes (Mansouri et al. 2013). Although small amounts of some heavy metals such as Cu, Zn and Fe are necessary for the metabolism of aquatic organisms as well as human bodies, some others such as cadmium, lead and mercury are harmful for living organisms even in small amounts (Canli & Atli 2002). So far, researches have shown that zinc, cadmium, mercury and lead are known as pollutants for aquatic ecosystems and harmful for human's health (Mendil et al. 2005; Ikram et al. 2010; Reichmuth et al. 2010).

Caspian Sea is the world's largest closed water body. It is a rich source of various living aquatic organisms such as fishes and crustaceans. However, lack of any natural linkage with other water bodies has made it very susceptible to external factors such as climatic conditions and human activities (Aladin & Plotnikov 2004). Over the past decade, Caspian Sea has been the center of attentions due to its geographical and political position. Since the Caspian Sea, more specifically the southern part, is one of the major resources of oil and gas stock, extraction and transportation of oil through the sea is

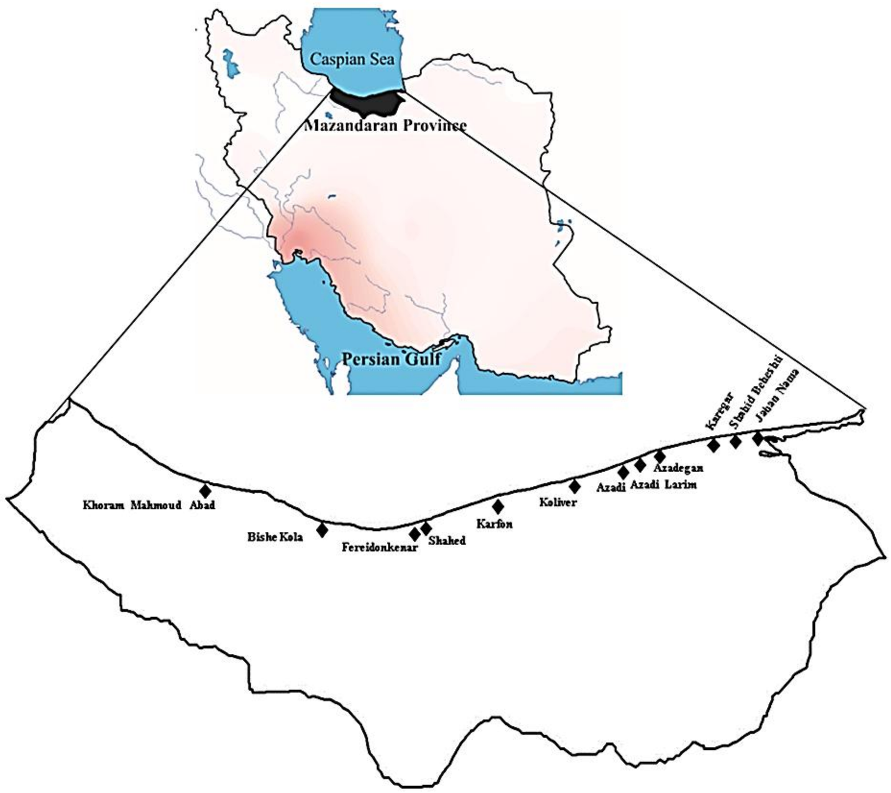

Fig.1. Sampling sites of Kutum fish from Southern Caspian Sea Basin

considered as the main source of pollution in Caspian Sea coastal waters. Subsequently, a specified amount of oil will be spilled into the sea during extraction (e.g., 1 million tons leaked oil from 100 million tons of extracted oil during 10 years from 2000-2010). It is also one of the main seaways for shipping (Boraei 2010). Another source of pollution is the number of cities and industries which surrounded the Caspian Sea. Pollution from these cities and industries enter the sea either directly or through rivers (Ganjidoust 2001). Therefore, a considerable proportion of natural habitats have been deteriorated remarkably and this has affected fish populations (Boraei 2010). It has been proved that contamination of water by oil patches will transport a high amount of heavy metals including cadmium and lead into the water (Prego & Cobelo-García 2004).

The Kutum fish, *Rutilus kutum* which is considered as *Rutilus frisii* (Nordmann, 1840) (see Freyhof & Kottelat 2008; Parin et al. 2014; Eschmeyer 2015) or *Rutilus kutum* (Kamensky, 1901) (see Naseka & Bogutskaya 2009; Esmaeili et al. 2010, 2014) is a migratory anadromous fish, Kutum is an edible carp and there are numerous researches on its economic benefits including those of Salehi (2003, 2008, 2010). Moreover, Caspian Sea products supply almost the whole seafood protein sources of the people in northern and northwestern regions of Iran (Aladin & Plotnikov 2004). According to Food and Agriculture Organization (FAO), fish consumption in Iran is about 6400g per capita (FAO 2012). This means that daily consumption of fish products is about 17.5g per person. Consequently, the amount of heavy metals that can be absorbed through fish consumption, per week per person, would be considerable. Therefore focusing on the content of heavy metals in the fish body tissues which is reflecting water pollution on the one hand, and its impacts on consumers and the fish population itself, on the other hand, is noteworthy. There are little knowledge about bioaccumulation of heavy metals in Caspian Kutum, so this study is important for consumers' health especially coastal dwellers.

Table 1. r values and average of fork length and weight of *Rutilus kutum* in Iranian waters of the Southern Caspian Sea Basin.

		Fork Length (cm)			Weight (g.)			
Stations	N	Mean	Max	Min	Mean	Max	Min	*r* Value
Jahannama	30	35.0	39.7	25.0	583.9	604.0	396.0	0.95
Karegar	30	38.1	43.4	33.0	759.5	973.0	525.5	0.96
Azadegan	30	33.6	37.2	26.7	521.2	734.4	362.3	0.95
Shahid beheshty	30	43.5	47.1	33.4	865.9	1100	398.7	0.98
Koliver	30	44.2	46.0	30.3	1004	925.1	693.7	0.97
Azadi	30	39.4	44.7	33.8	695.0	770.1	464.2	0.94
Azadilarim	30	43.8	48.1	33.0	845.5	1023.0	760.0	0.96
Karfon	30	40.26	47.3	38.5	881.6	983.5	628.3	0.98
Khoram Mahmoodabad	30	41.2	42.9	36.6	783.2	876.0	542.5	0.92
Freidonkenar	30	38.9	45.6	32.1	754.5	953.0	336.0	0.95
Bishekola	30	40.8	44.6	35.3	920.0	1146.0	524.5	0.98
Shahed	30	40.9	46.0	29.0	1068.1	1124.0	632.0	0.97

Materials and methods

Sampling and collection sites: Samples were purchased from fishers in twelve stations of Mazandaran Province randomly during October 2009 till March 2010 (Fig. 1). The collected samples were transferred to laboratory and washed with distilled water, dried in filter paper, homogenized and stored below -20°C before analysis (Tüzen 2003). Fork length and weight of specimens were measured to the nearest 0.1cm and 0.1gr, respectively.

Determination of the heavy metal content in fish tissues: Liver and muscle tissues were detached and preserved under 25˚C temperature. Laboratory dishes were washed with 10% Nitric acid and then sterilized in 60ºC for 24 hours. Tissues were washed with distilled water and then dried in 60º C temperature for 48 hours. In order to chemical lyses, 6ml of nitric acid (HNO_3) 65% and 2ml of H_2O_2 (30%) was added to 0.5gr of each dried tissues. Solutions were heated up to 100°C temperature for 31 minutes. Furthermore, 4ml perchloric acid was added after cooling. The preparation procedure is performed based on Honda (1983). Digested specimens were filtered by filter paper and prepared for measuring their atomic absorption. Measurement of atomic absorption was performed by flame atomic absorption spectrophotometer model THERMO M5. Statistical analysis of data was carried out with the Statistical Package for the Social Sciences (SPSS) and the linear model of regression with the maximum likelihood of 95% was calculated.

Results

The mean length and weight of Kutum in twelve stations in Mazandaran Province are shown separately by station in Table 1. The mean length ranged from 25 to 48.1cm and the mean weight ranged from 336 to1146g (Table 1). The collected samples from various stations were nearly in the same size range but the highest size can be seen for Shahed station (length=40.9cm and weight= 1068.1gr). Analyzing length-weight relationship showed a high significant correlation between the length and weight of Kutum (r values ranged 0.92 to 0.98) (P<0.05).

The mean values of Pb in liver and muscle tissues and Cd in liver and muscle tissues were 0.126-0.809, 0.068-0.415 and 0.154-0.364, 0.038-0.211, respectively (Table 2). The highest concentration was obtained for Pb in liver (0.809 µg/g) from Fereidounkenar and the lowest concentration for Cd in muscle from Koliver (0.38 µg/g) (Table 2).

Generally, concentration of heavy metals in liver were significantly higher than their concentration in musculature tissue (P<0.05). The results of linear regression analysis showed a significant negative relationships between the heavy metal contents and the fish weight except for the relationship between cadmium content in muscle and weight which was positive (Table 3).

Table 2. Heavy metals analysis in liver and muscle tissue of Kutum fish (µg/g.dw) from 12 stations in Mazandaran Province, Southern Caspian Sea Basin.

Stations & Heavy metals	Liver concentration		Muscle Concentration	
	Mean	S.D.	Mean	S.D.
Jahannama				
Pb	0.29	±0.11	0.11	±0.07
Cd	0.35[a]	±0.15	0.21[a]	±0.08
Karegar				
Pb	0.17	±0.14	0.12	±0.06
Cd	0.21	±0.17	0.07	±0.04
Azadegan				
Pb	0.16	±0.09	0.06	±0.05
Cd	0.23	±0.14	0.11	±0.07
Shahid Beheshty				
Pb	0.19	±0.08	0.26	±0.33
Cd	0.22	±0.09	0.16	±0.03
Koliver				
Pb	0.26	±0.07	0.16	±0.09
Cd	0.20	±0.21	0.03[a]	±0.06
Azadi				
Pb	0.57	±0.14	0.22	±0.08
Cd	0.31	±0.15	0.19	±0.07
Azadilarim				
Pb	0.66	±0.17	0.27	±0.11
Cd	0.18	±0.11	0.14	±0.04
Karfon				
Pb	0.71	±0.13	0.23	±0.06
Cd	0.24	±0.19	0.06	±0.07
Khoram Mahmoodabad				
Pb	0.39	±0.12	0.15	±0.08
Cd	0.21	±0.08	0.07	±0.10
Freidounkenar				
Pb	0.80[a]	±0.11	0.41[a]	±0.16
Cd	0.36[a]	±0.23	0.05	±0.07
Bishe kola				
Pb	0.21	±0.15	0.16	±0.12
Cd	0.27	±0.11	0.06	±0.05
Shahed				
Pb	0.12	±0.08	0.11	±0.07
Cd	0.15	±0.09	0.05	±0.08

a: concentration of metals in different tissues are statistically significant from some stations $P<0.005$.

Table 3. The relationships between heavy metal concentrations and total fish lengths and weights in the liver and muscle tissues of the Kutum fish from Mazandaran Province, Southern Caspian Sea Basin.

		Liver		Muscle	
Tissue	**Data**	**Cadmium**	**Lead**	**Cadmium**	**Lead**
Length	Df	122	122	122	122
	Equation	Y=39.406-1.32)X	Y=38.59+1.272 X	Y=39.648-4.93)X	Y=38.957+0.314X
	P value	0.64	0.45	0.31	0.16
Weight	Df	122	122	122	122
	Equation	Y=812-262.)Z	Y=743.91-7.972Z	Y=829.632+714.1)Z	Y=747.714-1.98)Z
	P value	0.02	0.04	0.001	0.01

Discussion

The bioaccumulation of Pb is higher than Cd in tissues. Bioaccumulation of heavy metals in liver and muscle tissues did not significantly depend on the sampling stations except for the content of Cd in liver from Jahan Nama and Freidounkenar, the content of

Table 4. Legal limits of hazardous substances in fish and fishery products (FAO 1993).

Location	Lead (ppm)	Cadmium (ppm)
Canada	0.5	-
Australia	2.5	2
Hong Kong	6.0	2.0
India	5.0	-

Cd in muscle from Jahan Nama and Koliver and content of Pb in liver and muscle from Fereidounkenar. This may be due to pouring industrial wastes or sewage into the water body in these regions. Mean Cd and Pb contents was 0.2µg/g and 0.3µg/g, respectively, compared with the limited amounts cited for some countries given in Table 4 (FAO 1993). Comparison of data indicates that, despite that Kutum is a benthic feeder, the average content of Cd and Pb in muscle and liver from all stations is still less than standard amounts given in Table 4. This result is comparable with other studies on Caspian Kutum such as; Hosseini et al. (2011) who reported the mean concentration of lead in Kutum muscle to be 0.112µg/g, Froghi et al. (2007) who reported the mean concentration of lead in Kutum muscle to be 0.894µg/g and other fish species (Chattopadhyay et al. 2002; Papagiannis et al. 2004; Hayat et al. 2007; Raja et al. 2009).

The variation in heavy metal contents in the tissues of fishes might be a result of their capacity to induce metal-binding proteins such as Metallothioneins that may be related to the differences in ecological needs, swimming behaviors and the metabolic activities among different fish species. Also, differences in metal concentrations and chemical characteristics of water from which fish were sampled, metabolism and feeding patterns of fish and the season in which studies were carried out might be considerable as reasons for these differences (Rauf et al. 2009). Comparison of heavy metal contents in both liver and muscle tissues show that the bioaccumulation of heavy metals in liver tissue is higher than muscle. Higher levels of trace elements such as lead in liver relative to other tissues may be attributed to the affinity or strong coordination of Metallothionein protein with this element (Ikem et al. 2003). This fact is reported by other authors (Farkas et al. 2002; Shukla et al. 2007; Vinodhini & Narayanan 2008).

The results showed a negative relationships between fish weight and the concentration of heavy metals except for the relationship of cd in the muscle and fish weight which was positive. There are many reports of positive relationship between the concentration of heavy metals and fish size (Hayat et al. 2007; Parveen & Javed 2010; Naeem et al. 2011) as well as the negative relationships between heavy metal levels in the tissues and fish sizes (Papagiannis et al. 2004; Hussain et al. 2010). Widianarko et al. (2000) also investigated the relationship between metal (Pb, Zn, Cu) concentration and fish (*Poecilia reticulata*) size and found that there is a significant decline in lead concentrations with the increase in size. Hosseini et al. (2011) studied the effects of heavy metals on Caspian Sea Kutum and its influence on the survival rate and growth performance of this species and also consumers' health. According to Abel's theory (Abel 1989), heavy metals are not only vulnerable for fish populations but also for the sea food consumers.

Heavy metals studied here are hazardous substances in the environment. In addition, lead is the heaviest and the most toxic element in the environment and can be found in aquatic systems in high amounts (Esmaeili 2002). According to the World Health Organization, every level of lead can be harmful for the human being and thus the amount of consuming Kutum can be hazardous for pregnant women and children due to high sensitivity of infants and children below 10 years old to various lead levels. Esmaeili (2002) also concluded that intake of this substance by consumers will lead to nervous system and behavioral malfunction. Cadmium is another hazardous substance, which may be absorbed in a high amount by food and may affect the host body to skeletal problems, bronchitis, Emphysema, anemia, and the kidney stone (Esmaeili 2002). Therefore, it is necessary to determine a standard amount of fish consuming (daily or weekly) based on their heavy metal contents (Kojadinovic et al. 2006). Also, a scientific detoxification method is essential to improve the health of this economic fish in stressed environmental conditions.

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