Iranian Journal of Fisheries Sciences 11(**4**) 825-839 2012

Concentration of heavy and toxic metals Cu, Zn, Cd, Pb and Hg in liver and muscles of *Rutilus frisii kutum* during spawning season with respect to growth parameters

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Received: May 2012 Accepted: September 2012

Abstract

Concentration of heavy and toxic metals Cu, Zn, Cd, Pb and Hg were determined in liver and muscles of *Rutilus frisii kutum* and their relationships with growth parameters (length, age, condition factor) and hepatosomatic index were examined. Thirty-six fish samples were collected from February through March 2009 caught by beach seine in the southwest parts of the Caspian Sea. Atomic absorption and Hg determined concentrations of Cd, Pb, Zn and Cu by vapor method. Cadmium was recorded only in liver samples. Range of other metals in muscle tissue were ND -0.591, 0.001-0.013, 11- 26 and 0.729 -7.261 $\mu g.g^{-1}$dw for Pb, Hg, Zn and Cu respectively. Highest levels of Pb, Zn, and Cu were recorded in muscles Hg and Cd in liver samples. Growth parameters showed a significant relationship with Zn and Cd concentrations in liver samples and only Zn concentrations in muscle samples. There was a positive significant correlation between concentration of Cd in liver and physiological indices ($p<0.05$). Although higher concentration of Pb was recorded in this study in comparison to previous studies, based on Provisional Tolerable Weekly and daily Intake of fish for human health, kutum is considered safe for human consumption. Considering the results of this study it seems reproductive status of the fish influences heavy metals concentration in liver and muscles of kutum and therefore concentrations of some metals such as Zn and Cu in liver samples may not be a reliable bioindicator for environmental pollution.

Keywords: *Rutilus frisii kutum*, Caspian Sea, Reproduction, Growth, Condition factor, HSI

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[DOR: 20.1001.1.15622916.2012.11.4.10.2]

Introduction

Caspian Sea is the largest continental water body and contains over 40% of inland waters of the world. Main sources of pollution of the Caspian Sea are petroleum products, phenols, organic substances, metals, nitrogen compounds and etc. Russia, Azerbaijan and Iran release highest amounts of pollutants into the Caspian Sea (UNEP, 2006). Discharge of pollutants such as heavy metals threatens both marine species diversity and ecosystems through toxic effects ; reduction in growth rate and fecundity (Ebrahimi and Taherianfard, 2011; Heidary et al., 2012) and cumulative behavior (Newman et al., 2008; Türkmen et al., 2009). Certain heavy metals like zinc and copper are essential for normal metabolism of aquatic organisms in low concentrations (Clark, 2001), while cadmium, lead and mercury are nonessential with no recognized role in biological systems (Canli et al., 2003). Even essential metals could be toxic for biological activities of organisms in high concentration (Kucuksezgin et al., 2006). Among aquatic organisms, fishes are generally capable of accumulating high levels of contaminants from their surrounding environment (Türkmen et al., 2009).

Stocks of *Rutilus frisii kutum* has declined in the past decades (Abdoli, 1999) and the species is in the “Conservation dependent organisms” list of the IUCN due to habitat limitation and decrease in population size (Naderi et al., 2005) overfishing , degradation of spawning grounds in rivers fluctuation of the Caspian Sea level and heavy pollution loads (Abdolmaleki, 2006). Therefore we decided to study the current situation of metal pollution in this species. Several studies on metal concentration in fish have addressed their potential risks for human consumption (Anan et al., 2005; Agusa et al., 2007; Ploetz et al., 2007) while less attention has been given to the effects of metal accumulation on fish health and effects of fish health on accumulation patterns of metals in various tissues. Considering the importance of kutum and strange synchrony of fishing and reproduction seasons of the species, we decided to determine concentration of Pb, Cd, Hg, Zn and Cu in liver and muscles of kutum during its spawning season and study their functions with regard to the growth parameters including length, age, hepatosomatic index (HSI) and condition factor (CF). Liver tissue was selected as the target organ for assessing metal accumulation and muscles as potential risk indicator of kutum consumption for human health. Public health risks associated with consumption of fish were estimated based on PTDI, PTWI and tested with values recommended by globally recognized institutions.

Materials and methods

Sampling

Kutum specimens were collected from beach seines active in the southwest Caspian Sea (located between the longitudes 48˚53´-50˚34´E and latitudes 36˚34´-38˚27´N) from February through March 2009 (Figure 1) and were transported to the laboratory in ice box. Total length (cm) and weight (g) of all

[DOR: 20.1001.1.15622916.2012.11.4.10.2]

samples were measured. Age was determined by examining annual ring structures of scale samples and Arabic numerals were used to designate fish age (Farkas et al., 2003). The mid-dorsal muscle samples (filleted and skinned) and liver tissue were dissected, washed in deionized water, weighed, packed in polyethylene bags and stored at -20 ^{o}C for further analyses.

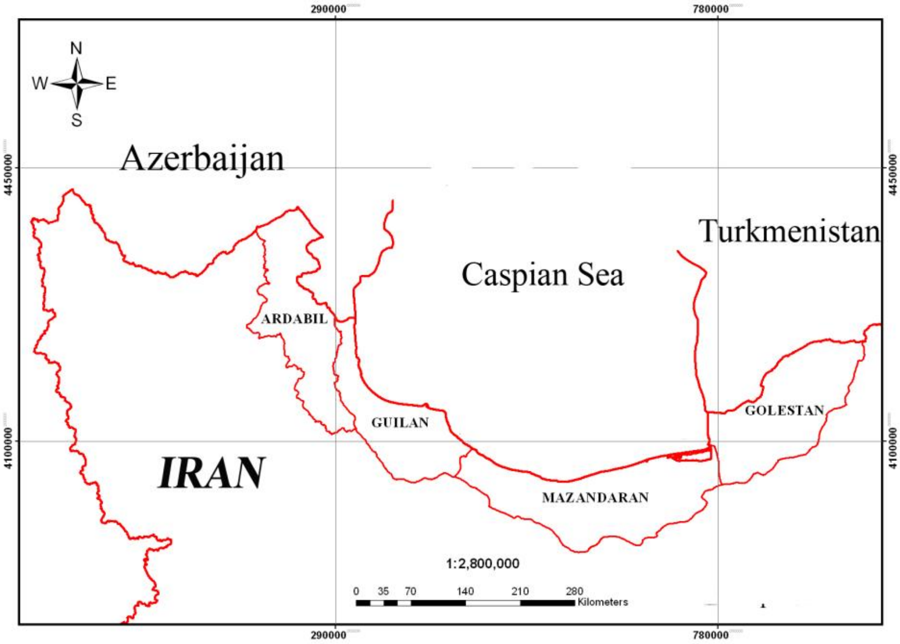

Figure 1: Sampling location in Caspian Sea

Chemical analyses

Samples were dried to a constant weight at 70 ^{o}C for 48 h. To determine Cd, Pb, Cu and Zn concentrations, tissue samples were digested with a mixture of nitric acid (HNO3, Merck, %65) and perchloric acid (HClO4, Merck, %60) (2:1 v/v)(Muramoto, 1983; Canli and Atli, 2003). To assess mercury concentrations, samples were decomposed with a mixture of nitric acid (HNO3, Merck, %65) and perchloric acid (HClO4, Merck, %60) (7:3 v/v) (Reeve et al., 1994). Glass and plastic containers used for tissue analysis were cleaned with 5% solution of nitric acid and rinsed with deionized water to minimize the possibility of contamination (Gašpić et al., 2002). The Cd, Pb, Cu and Zn concentrations were measured by Perkin-Elmer-5100 Atomic Absorption Spectrophotometer and mercury samples were read by cold-vapor technique using SnC_{l2} as reluctant. In this study metal concentrations were calculated on dry weight basis and expressed in terms of µg.g^{-1}. Moisture content of the muscles was 75.16 ± 0.9%. To compare our data with published values reported on a wet weight basis, the latter was converted to dry weight basis using a conversion factor of 4.

Statistical analyses

Data normality was tested by Kolmogorov–Smirnov test. Since some data were not normally distributed, they

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were logarithmically transformed before parametric analysis. To determine whether metal concentrations of organs differed in male and female kutum, analysis of covariance was used, with metal concentrations as a dependent variable, sex as an independent variable and total body length as the covariate. The analysis showed no significant differences in metal concentrations between males and females, therefore all data were pooled within sampled groups and tested by One-way ANOVA. To determine correlation between metal concentrations and growth parameters, Pearson's correlation was performed. To examine the effect of size on metal contents of organs, fish length was considered as the basic measure, since it is less likely to be subject to major fluctuations as compared to weight which is highly influenced by changes in proximate composition of muscle tissue, especially lipid percentage (Farkas et al., 2003). To understand the effect of condition factor on metal loads of kutum, condition factor of each fish was calculated based on the following formula [body weight / (length) ^{3}] ×100 (Fernandes et al., 2008) and to determine the effect of metal on fish health, HSI was calculated as [liver weight / body weight]×100 (Fernandes et al., 2008). All statistical analyses were performed with SPSS 15.0 for windows where significance level was set at 0.05.

Results

Fish specimens examined in this study were 1-7 years in age with a sex ratio of M:F=1:1 (Table 1).

Table1: Morphometric data of kutum from the southwest Caspian Sea

No	Age (mean± SE)	Length(cm) (mean± SE)	Weight(gr) (mean± SE)	CF(g/cm^3) (mean± SE)	HSI (%) (mean± SE)	Sex M	Sex F
36	4.17 ± 0.22	43.93 ± 0.71	847.64 ± 43.51	0.97 ± 0.01	1.26 ± 0.15	18	18

The highest concentrations of Hg and Cd were observed in liver samples (Cd was not detected in muscle samples) while Pb, Zn, Cu were significantly higher ($P<0.05$) in muscle tissues than in liver (Fig. 2). There was no correlation between muscle levels with liver levels for any metal ($p>0.05$). Analyses of metal contents of liver and fish size revealed a positive correlation between concentration of Zn and Cd with fish length and age ($p<0.05$) (Table 2) while in muscles the relationship was significant only for Zn ($p<0.05$)(Table 2). A significant positive correlation was observed also between Cd values with CF and HSI ($p<0.05$; Table 2).

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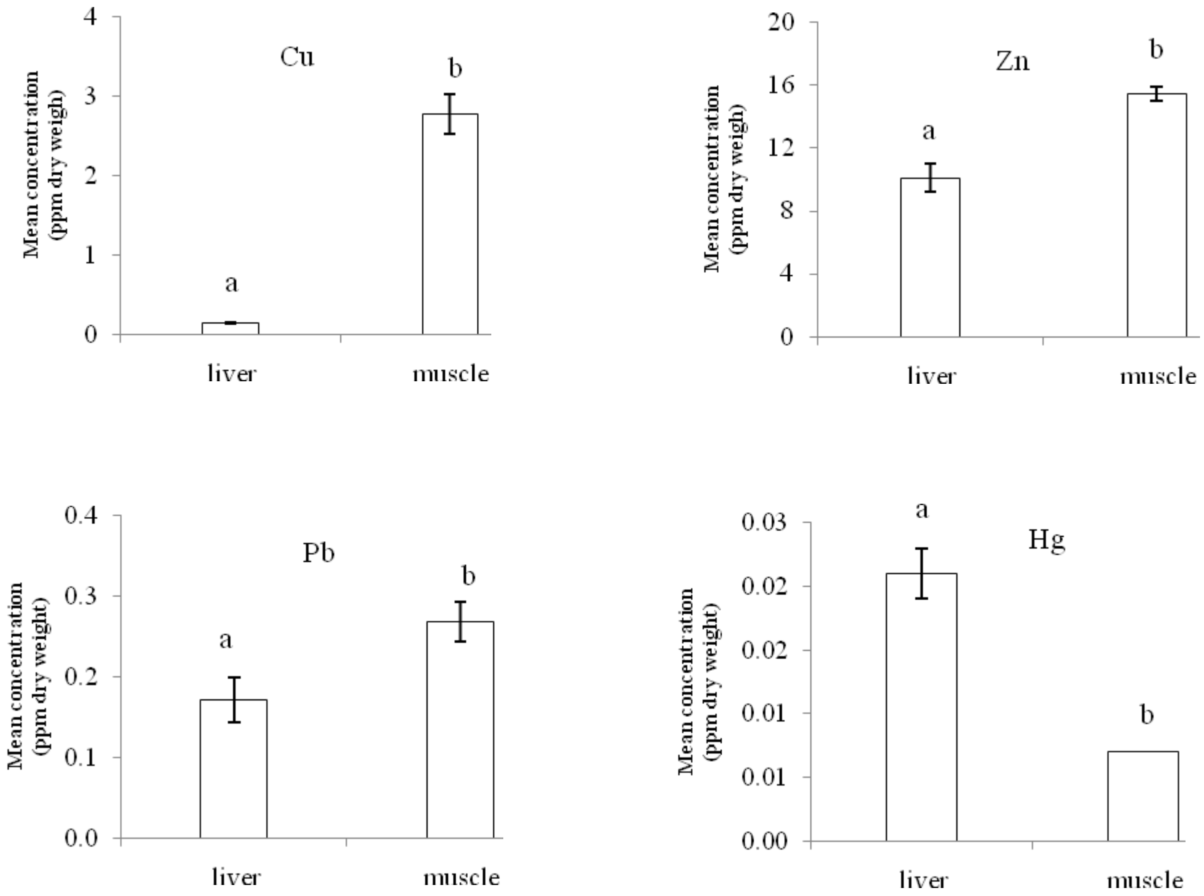

Figure 2: Mean (± SE) Pb, Hg, Zn and Cu concentrations in tissues of kutum from the southern Caspian Sea during fishing season in February- March 2009

Different letters indicate significant differences between tissues ($p < 0.05$)

Table 2: Pearson correlation coefficients (r) and levels of significance (p) for the relationships between metal concentrations of kutum with growth parameters and HSI

Variable	Element	Muscle		Liver	
		r	*p*	*r*	*p*
Length	Cd	-	-	0.38	0.01
	Zn	0.416	0.01	0.2	ns
Age	Cd	-	-	0.39	0.01
	Zn	0.471	0.004	0.5	0.004
CF	Cd	-	-	0.34	0.04
HSI	Cd	-	-	0.39	0.01

Table 3: Mean concentration of metals (µg/g dry weight) in fish muscle tissues in different ecosystems

Location (Species)	Cu	Zn	Cd	Pb	Hg	References
Caspian sea						
Rutilus frisii kutum	2.763	15.45	0.000	0.270	0.007	this study
*Rutilus frisii kutum**	0.689	3.85	0.000	0.067	0.002	this study
Rutilus frisii kutum	1.010	17.20	0.001	0.010	0.190	Anan et al(2005)
Rutilus rutilus caspicus	0.260	18.80	0.010	0.010	0.200	Anan et al(2005)
Mugil auratus	3.140	43.46	-	-	-	Zeynali et al(2009)
Acipenser persicus	1.740	21.70	0.002	0.006	0.330	Agusa et al(2004)
Lake Balaton						
Abramis brama	1.995	12.70	0.515	1.035	0.145	Farkas et al(2003)
Vitoria, Australia						
*Pagruss auratus**	0.300	5.15	0.020	0.050	0.170	Fabris et al(2006)
Hutovo Blato, Bosnia						
Tinca tinca	-	-	0.010	0.100	0.125	Has-Schön et al(2008)
Beymelek Lagoon, Turkey						
*Mugil cephalus**	1.030	5.52	-	-	-	Usyal et al(2008)
Mediterranean sea						
*Scomber japonicus**	0.630	7.21	0.080	0.250	-	Türkmen et al(2009)
WHO[1]	10	1000	0.200	-	-	Pourang et al (2005)
MAFF*[2]	20	50	0.200	2	-	Anan et al(2005)

*: µg/g wet weight
[1] World Health Organization
[2] Ministry of Agriculture, Fisheries and Food (UK)

Table 4: Estimated daily and weekly intake of metals in a mature man on kutum consumption

Metal	PTWI[a]	PTWI[b]	PTDI[c]	EWI[f]	EDI[j]
Cd	7[d]	490	70	0	0
Pb	25[d]	1750	250	8.44	1.21
Hg	5[b]	350	50	0.21	0.03
Zn	7000[e]	490000	70000	485.47	69.35
Cu	3500[e]	245000	35000	86.85	12.41

a: Provisional Permissible Tolerable weekly intake in µg/week/kg body weight
b: PTWI for 70 kg adult person (µg/week/70 kg body weight)
c: PTDI, provisional permissible tolerable daily intake (µg/week/70 kg body weight)
d: (FAO/WHO. 2006)
e: (Türkmen et al., 2009)
f: EWI = average concentration (µg/g) × consumption [126 g/w/bw (70 kg)]
j: calculated from EWI

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Discussion

Comparison of metals content in tissues

In this study the highest concentration of Pb, Zn and Cu were observed in muscles and Hg in liver samples. Several studies e.g. Farkas et al., 2003; Henry et al., 2004; Agusa et al., 2005 Fernandes et al., 2007; Yılmaz et al., 2007; have reported lowest concentrations of Pb, Zn and Cu and highest concentrations of Hg in muscles. The reason could be presence of metalothionin proteins in liver with high tendency to bind with Cu, Zn and the specific function of liver as accumulation and transportation source for metals (Fernandes et al., 2007) and high tendency of Hg to bind with muscle proteins (Golovanova, 2008). Metalothionin has high tendency to adsorb Hg (Sigel et al., 2009) so that several studies have reported higher concentrations of Hg in liver than muscles (Régine et al., 2006; Ruelas-Inzunza et al., 2008; Has-Schön et al., 2008). The time of the year in which fish is exposed to Hg plays a significant role in its distribution and accumulation in tissues. Accumulation of Hg in liver is a sign of fish exposure to metals in the past while concentration of Hg in muscle reflects very recent exposure to metal, however many factors which influence fish metabolism can alter this pattern (Ruelas-Inzunza et al., 2008). Régine et al., 2006 reported that fish's feeding habit can influence Hg concentration in liver and in benthivorous fish, liver and kidney are the main storage sites for Hg. Lower levels of Zn in livers of females in our study could be due to the release of E2(17β-estradiol). Studies have shown that elevated release of this hormone in spawning season reduces Zn concentration in liver (Thompson et al., 2001) which coincides with increase in concentration Zn in plasma and ovaries (Thompson et al., 2001, 2003).

Increase in E2 hormone release in females in their spawning season could justify lower Zn deposits in liver. On the other hand migration of lipid from liver to gonads and decline of metalothionin in liver along with reduced efficiency of metalothionin to absorb trace metals e.g Zn and Cu may result in lower concentration of these metals in liver in comparison to muscle. Higher concentrations of Pb in muscles relative to livers in present study comply with Ploetz et al., 2007 who studied *Scomberomorus cavalla* Cuvier and low tendency of Pb to bind with metalothionin is responsible for this result. Metalothionin shows low propensity to bind with Pb and since Pb is not in direct contact with metalothionin in cell (Sigel et al., 2009) Pb deposition in liver is lower than in muscles. Cadmium was detected only in liver which is similar to results of Rezaei (2007) on *Liza aurata*. Several authors have reported higher concentrations of Cd in livers than in muscle (Rashed, 2001; Gašpic et al., 2002; Meador et al., 2005; Karaytug et al., 2007). Cadmium deposition in liver is a result of strong bond with cistein of metalothionin though this bond may appear with other proteins as well. Higher propensity of Cd to bind with sulfid groups and its potential to make co-valence bonds results in its increased deposition and toxicity. Lower level of Cd in muscles is because of lower density of cistein and methionin in muscle tissues (Newman et al., 2003).

Metals and growth parameters

Increase in age and length, resulted in increased concentrations of metals in liver, and while in muscle samples only Zn showed similar increase. Our results for liver are in agreement with Al- Yousuf et al., 2000 in their study on *Lethrinus lentjan*. Farkas et al.(2003) studied *Abramis brama* and reported positive relationship between Cd and fish length and negative with Zn. Anan et al.(2005) studied bony fishes of the Caspian Sea and reported that increase in body size reduces metal concentration in muscles. According to Anan et al. (2005) the metabolic ratio and dilution of metal during growth period is responsible for this relationship. When growth rate is faster than metal deposition ratio, increase in age and weight decrease metal concentration in tissues even in polluted environments (Gašpić et al., 2002).

Changes in feeding behavior with increase in age towards benthopelagic strategy could be a reason for increase in metals concentration with age in our study. Some organisms e.g. crustaceans and mollusks possess a high potential to accumulate metals and other pollutants, they can act as carriers of metal to fish (Al- Weher et al., 2008) and kutum feeds generally on mollusks. We observed no correlation between fish size and Hg concentration which is similar to Foroughi et al.(2007), though other studies have reported positive correlations (Storelli et al., 2002; Storelli et al., 2005; Burger et al., 2007). Lack of correlation between Cu and Pb with fish size in this study is similar to studies conducted by Henry et al., 2004 and Ploetz et al., 2007. In general in fish species of small or medium body size increase in size often has no influence on metal deposition and accumulation in tissues (Hugett et al., 2001; Gašpić et al., 2002).

Metals concentration and physiological indices

There was a slight positive correlation between Cd contents of liver and CF in present study while Farkas et al. (2003) observed negative relationship between metals and CF in *Abramis brama*. According to these authors lipid concentrations of tissue and dilution effects of lipid on metals were responsible for their results. Giguère et al.(2004) also reported results similar to Farkas et al. (year?) stressing on environmental factors as the main drivers. Since our samples were captured in winter they were poor in fat reserves in comparison to fish caught in active feeding seasons. Gonad development during active feeding season results in higher CF in mature fish (Sattari, 2002). Low fat content of tissues and lower growth and development of gonads may result in positive correlation between metals and CF. Fernendes (2008) studied two commercial fish species (*Trisopterus luscus, Lepidorhumbos boscii*) and reported that increase in CF resulted in decreased HSI and concluded that reduction in liver size due to loss of glycogen or fat resources is a response to toxic effects of this metal. The nature of response to toxic metals in different species varies depending upon pollutant level, metal type and duration of exposure to pollutants. Van Dyk (2007) reported

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that exposure to Cd increases cell volume due to malfunction of ATPase enzyme and changes in ion regulation activities. Increase in Cd causes increase in liver weight which is reflected in the positive relationship between Cd and HSI in this study.

We compared metal concentration of muscles of kutum with previous studies on same species and other species in different ecosystems, and found that Cd and Zn were comparable to previous studies, while Pb, Hg and Zn concentrations were significantly higher in the present study. Anan et al.(2005) reported highest values of Pb in fish captured in southwest Caspian Sea. Since we also collected our fish from the same area of the Caspian Sea, the significant increase in Pb levels observed in muscles of fish in this study reflects increased pollutant inputs into the Caspian Sea specially oil pollution, because according to Novan Magsoudi (2007) Pb is one of the oil derivatives. We also noticed a decrease in Hg and increase in Cu levels in this study. Agusa et al.(2004) and Anan et al.(2005) reported the highest concentration of Hg in fish from southeast parts (Golestan Province) of the Caspian Sea. Hence lower levels of Hg in the present study compared to previous studies may be due to effects of location and sampling sites. On the other hand higher levels of Cu reflected higher concentration of Cu in sediments in southwest parts. Although Cu distribution in sediments of the Caspian Sea does not follow any regular pattern, highest levels have been reported from the southwest parts (de Mora et al., 2004) where our samples were caught. Studies show that metal concentrations in fish tissues is influenced by their concentration in water, sediment and also ecological factors like DO, salinity, hardness, nutrient levels, pH…etc (Dušek et al., 2005) and behavior of the target species (Vicente- Martorell et al., 2009). Differences in metals concentration in various species of the Caspian Sea may be related to their habitats and migratory behavior. *Clupeonella delicatula* is a pelagic species, while kutum is a benthopelagic and anadromous species and therefore accumulation of metals in these species follow different patterns (Anan et al., 2005). Differences in metal concentration in fish collected from different ecosystems basically depend upon pollution type and their density in the area (Andres et al., 2000), interspecies differences (Dalman et al., 2006) and differences in ecological behavior, metabolic characteristics and food requirements (Canli et al., 2003).

Fish health assessment

Marine feed consumption is one of the major routes of metal accumulation and contamination in human (Gašpić et al., 2002; Agusa et al., 2007) therefore studies on metal contamination in fish are in fact addressing their health for human consumption (Cheung et al., 2008). According to Food and Agriculture Organization (FAO) the per capita fish consumption in Iran is 6400 g (FAO, 2009) which is 18 g/day and 126 g/week. Based on these data and metal concentration of fish muscles (edible part), the estimated daily and weekly intake

(EDI, EWI) calculated for an adult person with mean weight of 70 kg are presented in Table 4. EDI and EWI values show that metal intake in an adult person consuming kutum caught in the southwest parts of the Caspian Sea is much lower than the recommended values for human consumption (FAO/WHO, 2006; Türkmen et al., 2009), thus kutum consumption is not dangerous for human health for the date.

Based on obtained results it seems Cu and Zn contents (essential elements) in livers of kutum are influenced by reproduction conditions of the fish therefore concentration of these metals in liver during spawning season may not be an accurate indicator of the fish environment. Moreover concentration s of Pb, Cd, Hg, Zn, Cu in fish muscles in southwest parts of the Caspian Sea is much lower than the standard values recommended for human consumption and calculated values for PTDI and PTWI confirms healthy status of kutum though Pb is much higher than the previous studies and requires special attention.

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