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Toxic heavy metal concentration in soft tissues of gray mullet *Liza aurata* (Mugilidae: Perciformes) during the sexual maturity and sexual rest

Norouzi M.[1*]; Bagheri Tavani M.[2]; Ghodrati Sh.[2]; Amirjanati A.[2]

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Abstract

This study was conducted to determine the concentration of five heavy metals, including lead, cadmium, mercury, arsenic, and chromium in muscle, liver and gills of the gray mullet, *Liza aurata* in southern part of the Caspian Sea. The samples were collected during sexual maturity (in the fall) and sexual rest (in the spring). The mean concentration of lead, cadmium, mercury, arsenic, and chromium in the muscle tissue were 1.40, 0.43, 0.16, 0.07 and 0.54 µg/g, respectively during sexual maturity and 1.90, 0.93, 0.24, 0.12 and 0.61 µg/g, respectively during sexual rest. Generally, the uptake of heavy metals during sexual rest was higher (Pb>Cd>Cr>Hg>As) than that (Pb>Cr>Cd>Hg>As) during sexual maturity. Pollutants are effective in the accumulation of heavy and toxic metals in *L. aurata* in the Caspian Sea during different seasons. The concentration pattern of the metals in the three tissues was as follows: liver>gill>muscle. The difference between the concentrations of the metals in studied tissues was significant. A highly significant correlation between the elements in tissues was observed in both sexual periods. The comparison of the data obtained for muscle tissue with the WHO and NHMRC guidelines showed that the concentrations of all the five heavy metals (Pb, Cr, Cd, Hg, As) were higher than the global standard levels for these metals.

Keywords: Heavy metals, Bioaccumulation, Sexual cycle, *Liza aurata,* Caspian Sea.

1-Department of Marine Biology and Fisheries Sciences, Islamic Azad University, Tonekabon Branch, Tonekabon, 46817, Iran.

2 -Young Researchers and Elite Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

*Corresponding author's Email: mnoroozi@toniau.ac.ir

Introduction

The Caspian Sea is the largest lake in the world located in the north of Iran, where small and large rivers enter. The lake is exposed to high levels of industrial, agricultural and oil pollutants. Rivers and human activities are the main sources for heavy metals in the Caspian Sea. Fish species are the ultimate consumers in the food pyramid in aquatic ecosystems (Amini Ranjbar and Sotudehnia, 2005). Among non-Inorganic pollutants, heavy metals are of particular importance because they are not naturally degraded, accumulate in the food chain and have adverse biological effects. Elements such as lead, cadmium, mercury, chromium and arsenic which are not used biologically can disrupt the normal function of fish even in small amounts. Since fish comprise a large part of the human diet, these heavy metals can enter the human body through the consumption of contaminated fish (Lakshmanan *et al.,* 2009). The food chain is one of the ways for heavy metals to enter the body. Many mollusks can store a lot of heavy metals in their soft tissues (Esmaili Sari, 2002).

Studies by Amini Ranjbar and Sotudehnia (2005), Fazeli *et al.,* (2005), Taghavi jelodar *et al.* (2011), and Solgi and Esfandi Sarafraz (2015) are among the studies that measured heavy metal concentrations in *L. aurata* in the southern coasts of the Caspian Sea and other studies on Mugilidae in other parts of the world can be noted (Filazi *et al.,* 2003; Bahnasawy *et al.,* 2009). Other studies in the Caspian Sea, Nasrolahi , *et al.,* (2017), Mirzajani, *et al.,* (2016) and Taghavi Jelodar , *et al.,* (2016), can be noted.

Earlier studies show that the reproduction season affects heavy metals accumulation in different tissues of the fish body (Hamed, 1998; Ibrahim *et al.,* 1999; Kalay *et al.,* 1999). However, there is little information about the absorption of heavy metals in commercial fish, such as *L. aurata*, due to their reproduction period in the southern coasts of the Caspian Sea. This study aimed to evaluate the concentration of five toxic heavy metals (Pb, Cd, Cr, Hg, As), among elements that the World Health Organization and Food and Agriculture Organization of the United Nations have mandated their measurement in edible fish, in the tissues of muscle, liver and gills in *L. aurata* in two periods of sexual maturity (fall) and sexual rest (spring) and compare them in these two periods, and also to compare their amounts with guidelines of World Health Organization, Australian National Health and Medical Research Council, as well as Food and Drug Administration.

Materials and methods

Sample collection and bioassay index

The samples were randomly collected from 100 adult *L. aurata* from 10 stations in the south coasts of the Caspian Sea, during sexual rest (SR), April 2014 and sexual maturity (SM) November 2014 (Fig. 1).

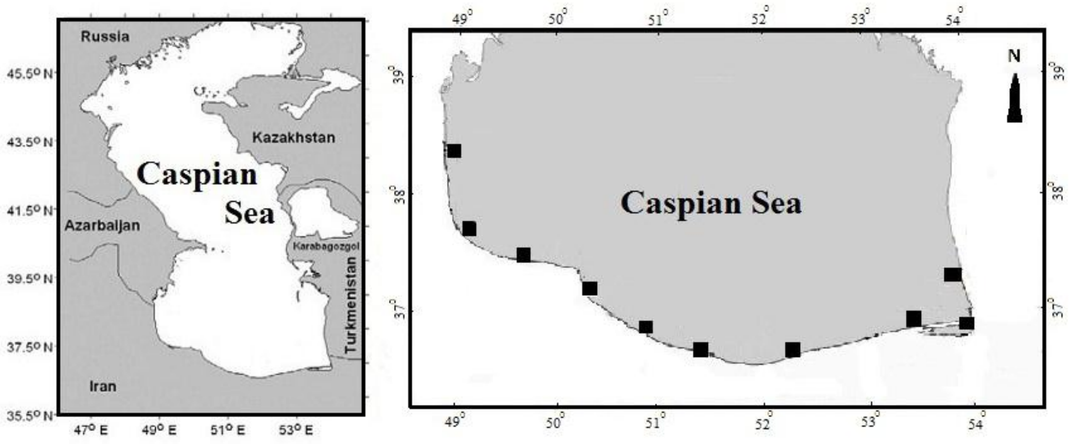

Figure 1: Map of sampling stations of fish in the south coasts of Caspian Sea.

Ten (10) adult fish were collected from each station (5 fish during the sexual rest and 5 other fish during the sexual maturity). The samples were taken to the Fisheries Research Laboratory, University Branch in an ice box. Once the fish were washed, the bioassay indexes, including weight (W) and total length (TL), were measured using a scale of ±2 g precision (a digital scale, SE-62 DY-888, Iran) and a Biometric board of ±1 mm precision, respectively.

Chemical analyses

Fish samples were washed with water to remove the surface and skin pollution. Then 10 g of each tissue (muscle, liver and gills) of fish was separated by dissection instruments (scalpel, scissors and forceps), for chemical digestion and were weighed by a digital scale (a digital scale, SE-62 DY-888, Iran). Each tissue was placed separately in a round-bottom flask and then 50 mL distilled water H_2O_2 and 50 mL nitric acid were added. Samples were filtered through Whatman filter paper #1 and then deionized water was added to the filtered solution to make up the volume of 5 mL and the solutions were separately poured into digestion tubes (Lakshmanan *et al.*, 2009). Then, flame atomic absorption spectrophotometer AAS4 Zeiss) was used to measure heavy metal concentrations (Standard methods, 2005).

Statistical analyses

Data are expressed as Mean±SD Significant differences between groups were determined by a One-way analysis of variance (ANOVA) followed by Duncan test. Statistical analysis such as Pearson correlation analyses to understand inter relation among the measured elements was performed using the SPSS for Windows software, version 18 (SPSS Inc., Chicago IL, USA). Mean values are considered significantly different at $p<0.05$.

Results

Bioassay for both sexual periods

Table 1 provides the mean bioassay results of the different stations for the sexual rest (SR) and the sexual maturity (SM) period. The biomarkers showed that both weight and length were higher in the fall than in the spring. According to the ANOVA results, the weight and total length differed significantly in both sexual maturation and sexual rest periods ($p<0.05$).

Heavy metals accumulation in three tissues

The results showed that the accumulation of five heavy metals was different in muscle, liver and gills. According to Table 2, metal accumulation pattern in the three tissues was liver>gill>muscle. The results of the ANOVA test showed that this difference was statistically significant ($p<0.05$). The result was similar in both sexual maturation and sexual rest periods.

Heavy metals accumulation in sexual maturation and sexual rest

The results showed that the uptake of heavy metals; lead, cadmium, mercury, chromium and arsenic was higher in sexual rest than in sexual maturity in muscle, liver and gills.

The results of the t-test showed that this difference was statistically significant ($p<0.05$). Also the accumulation of lead, cadmium and mercury in three tissues were different in the sexual periods of *L. aurata* based on the results of t-test (Table 2). However, there was a significant difference for chromium accumulation only in the liver tissue in the two sexual periods ($p<0.05$). No significant difference was observed for arsenic accumulation in the three tissues in the two sexual periods of *L. aurata* ($p>0.05$). Metal accumulation during sexual maturation was as Pb>Cr>Cd>Hg>As and during sexual rest was as Pb> Cd> Cr>Hg>As. As can be seen in Figs. 2 to 6, the highest heavy metal accumulation in both sexual periods was in the liver and the lowest was in muscle tissue.

Relationship between the heavy metals accumulation and tissues

The significant positive correlation (r = 0.924, $p<0.05$) was observed between weight and length of *L. aura*. The Pearson correlation test was used to determine the correlation between the tissues and the heavy metal accumulation of the golden gray mullet in both sexual periods and shown in Table 3.

Table 1: Mean bioassay results (± standard deviation) in (L. aurata) for both sexual periods.

Period	Weight (g)	Range	Total Length (cm)	Range
SR	821.10±197.02	384-1252	48.45±3.78	40-58
SM	934.56±331.33	388-1844	50.11±5.68	37.5-61
p Value	*		*	

* Significant at 0.05 level.

Table 2: Mean (± SD) heavy metal concentration (µg/g) for both sexual periods.

Period	Tissue	Parameters	Pb	Cd	Hg	Cr	As
SR	Muscle	M ± SD	$1.90^{b*}\pm0.361$	$0.93^{c}\pm0.23$	$0.24^{c}\pm0.07$	$0.61^{c}\pm0.13$	$0.12^{c}\pm0.04$
		Range	1.33-2.60	0.60-1.50	0.10-0.39	0.40-0.89	0.03-0.20
	Liver	M ± SD	$2.63^{a}\pm0.38$	$1.55^{a}\pm0.29$	$0.68^{a}\pm0.14$	$1.53^{a}\pm0.18$	$0.28^{a}\pm0.11$
		Range	2.10-3.38	1.20-2.30	0.42-0.90	1.21-1.86	0.10-0.58
	Gills	M ± SD	$1.88^{b}\pm0.36$	$1.23^{b}\pm0.24$	$0.54^{b}\pm0.13$	$1.03^{b}\pm0.22$	$0.19^{b}\pm0.07$
		Range	1.44-2.71	0.77-1.63	0.28-0.75	0.55-1.39	0.06-0.37
SM	Muscle	M ± SD	$1.40^{b}\pm0.25$	$0.43^{c}\pm0.18$	$0.16^{c}\pm0.05$	$0.54^{c}\pm0.15$	$0.07^{c}\pm0.03$
		Range	1-1.82	0.18-0.95	0.06-0.26	0.3-0.8	0.02-0.16
	Liver	M ± SD	$2.03^{a}\pm0.30$	$0.90^{a}\pm0.21$	$0.58^{a}\pm0.11$	$1.35^{a}\pm0.12$	$0.21^{a}\pm0.10$
		Range	1.7-2.85	0.45-1.5	0.38-0.79	1.11-1.6	0.09-0.5
	Gills	M ± SD	$1.42^{b}\pm0.22$	$0.79^{b}\pm0.15$	$0.41^{b}\pm0.09$	$0.94^{b}\pm0.26$	$0.13^{b}\pm0.05$
		Range	1.1-1.84	0.4-1	0.25-0.61	0.1-1.35	0.04-0.28
	p Value		**	**	**	**	**

* Significant at 0.05 level, ** Significant at 0.01 level.

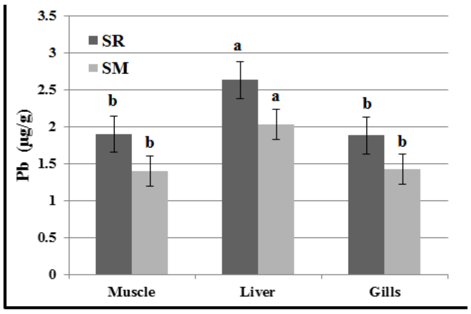

Figure 2: The concentration of Pb during SM and SR.

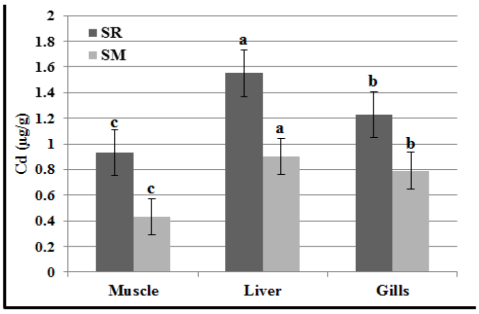

Figure 3: The concentration of Cd during SM and SR.

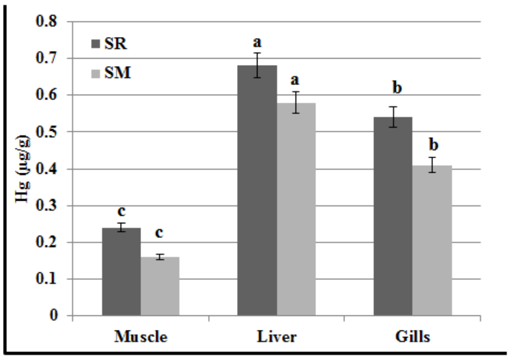

Figure 4: The concentration of Hg during SM and SR.

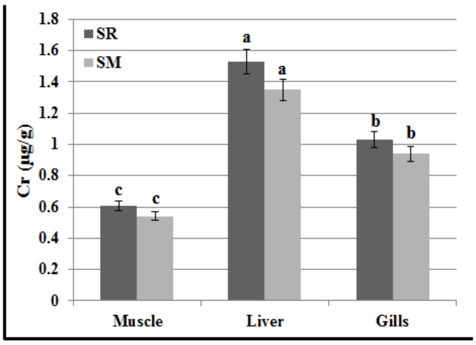

Figure 5: The concentration of Cr during **SM and SR.**

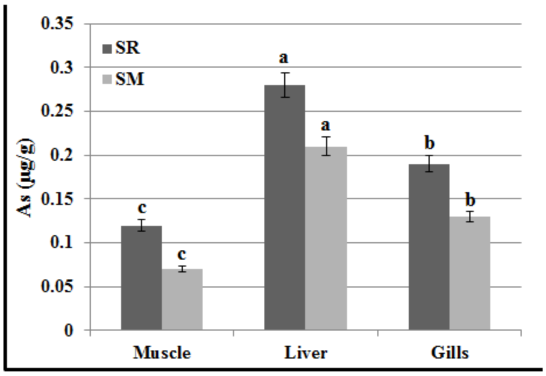

Figure 6: The concentration of As during SM and SR

Table 3: Pearson correlation coefficients and levels of significance for the relationships between metal concentrations of *Liza aurata*.

		SR			SM		
	Parameters	**Muscle**	**Liver**	**Gills**	**Muscle**	**Liver**	**Gills**
Lead Vs	Cadmium	0.918**	0.819**	0.763**	0 .567**	0.455*	0.289*
	Mercury	0.255	-0.384*	-0.383*	-0.194	-0.492**	-0.117
	Chromium	-0.300	-0.639**	-0.641**	-0.035	0.051	-0.479**
	Arsenic	0.617**	0.655**	0.321	0.136	0.723**	0.631**
Cadmium Vs	Mercury	0.104	-0.585**	-0.543**	0.087	-0.355	-0.667**
	Chromium	-0.245	-0.629**	-0.536**	-0.047	0.131	0.315
	Arsenic	0.517**	0.662**	0.176	0.575**	0.450*	0.033
Mercury Vs	Chromium	0.053	-0.443*	0.246	-0.302	0.088	-0.261
	Arsenic	0.354	-0.413*	-0.194	0.304	-0.275	0.032
Chromium Vs	Arsenic	-0.183	-0.581**	-0.505**	-0.228	-0.147	-0.465**

* Significant is 0.05 level, ** Significant is 0.01 level.

There was a positive relationship ($p<0.01$) between lead, cadmium and arsenic concentrations. According to the results, there was a negative relationship ($p<0.01$) between the lead, mercury and chromium concentrations. Moreover, there was a positive relationship ($p<0.01$) between cadmium and arsenic concentrations. But a negative relationship ($p<0.01$) was observed between the concentrations of cadmium and mercury and chromium. The results showed a significant negative relationship ($p<0.01$) between the concentrations of arsenic and mercury and chromium. There was no clear difference in the levels of the metals tested when factors such as length and weight were compared except for arsenic during sexual rest. The length and weight negatively correlated with concentration of arsenic.

Discussion

Comparison of bioassay factors

In the comparison of the two sampling occasions, the highest levels of the biomarkers were observed in the fall, i.e. the spawning season for this particular fish. This might be due to increase in gonad weight. The golden gray mullet breeds near the coast in the fall (Rehbein and Oehlenschlager, 2009). The fish gonads are full of gametocytes (the oocyte and spermatozoa) during the fall, and a higher mean weight compared to that in the spring is not surprising. Gonads are completely discharged of sex cells in the spring and therefore lose weight.

Comparison of heavy metals accumulation in three tissues

Contaminants usually enter fish tissues from five main ways: food or non-food particles, gills, water entering through the mouth and absorption through the skin. These pollutants are transferred to storage sites or the liver through blood vessels after absorption (Nussey *et al*., 2000). Absorption of metals by the gills is the first sign for water pollution, so gills can play an important role in revealing their total level in water (Varanasi and Markey, 1978). Also the liver is considered as an environmental indicator for assessment of water pollution because of the tendency to accumulate high levels of various contaminants compared to other tissues (Yilmaz, 2009) and muscle tissue is used for evaluating heavy metal concentration to ensure the health of fish.

In general, concentration of various metals in the tissues depends on their physiological role. Also, behavioral and dietary habits are another factor in the difference of accumulation in different organs of the body (Al-Yousuf *et al.,* 2000; Carvalho *et al.,* 2005). The results showed that concentration of lead, cadmium, mercury, chromium and arsenic in three tissues of *L. aurata* was as liver>gill>muscle, with the highest concentration in the liver and the lowest in muscle tissue. The element, the kind of aquatic animal, tissue, physiological characteristics of fish, ecological characteristics and environmental conditions, the chemical and physical properties of the environment, such as water hardness, pH, temperature, nutrients and fish growth time are effective in metal accumulation in body tissues (Dixon *et al.*, 1996; Fuhrer *et al.*, 1996). The difference between heavy metal concentrations in different fish tissues can be attributed to variable power of heavy metals in overcoming metal bonds in proteins such as metallothioneins. Also the differences between ecological requirements and metabolic activities of fish can be considered as another important factor (Canli and Atli, 2003). Heavy metals choose their target organ based on its metabolic activity and this explains the reason why metals accumulate more in tissues such as the liver and gills compared to muscle tissue (with low metabolic activity) (Filazi *et al.,* 2003). Metals concentration in muscle are lower than those in liver because muscle tissue is not the first storage place for these metals; heavy metals are first stored in the liver and then transferred to the muscle (Beheshti, 2011). In this study, the highest heavy metal concentration was in the liver, and then in gills and the lowest was in muscle tissue. Target organs, such as liver and gills are metabolically active tissues, and metal accumulations have been reported in higher levels in these tissues compared to muscle tissue in many species of mullet in several regions. Studies on gray mullet in the Northeast Mediterranean (Filazi *et al.,* 2003), *Liza abu* mullet in Turkey Ataturk Dam Lake (Fuhrer *et al.,* 1996), *Liza dussumieri* (Askary Sary, 2010), *L. aurata* in the Caspian Sea (Filazi *et al.,* 2003; Amini Ranjbar and Sotudehnia, 2005; Fazeli *et al.*, 2005; Taghavi jelodar *et al.,* 2011; Solgiand Esfandi Sarafraz, 2015) and other mullets (Bahnasawy *et al*., 2009) show similar results.

According to the recorded data and compared to the permissible standard limit (Table 4), the level of all five heavy metals; lead, cadmium, mercury, chromium and arsenic were higher than the permissible limit in *L. aurata* which is harmful to human health.

Table 4: Comparison of heavy metal concentrations (µg/g) in muscle tissue of *Liza aurata* with other organs.

	Pb	Cd	Hg	As	Cr
WHO (WHO, 1985-1989-1996)	0.05	0.005	-	-	0.2
UK (MAFF, 1995)	1.5-2	0.05-0.2	0.05	-	-
FEPA (FEPA, 1999) Specification	<1.0	<1.0	-	-	-
USEPA (USEPA, 1987) Specification	0.0058	0.008	-	-	0.05
FAO (Nauen, 1983; EFSA, 2004; Codex, 2005)	-	-	0.5	0.5	-
FAO, 2011	0.3	0.5	0.1	0.5	-
NHMRC (Tuzen, 2009)	0.02	0.02	0.05	-	-
Amini Ranjbar and Sotudehnia, 2005	0.321	2.337	-	-	-
Fazeli *et al.,* 2005	3.01	-	-	-	-
Pazooki *et al.* 2009	-	0.282	-	-	0.607
Taghavi jelodar *et al.,* 2011	1.5	0.35	-	-	0.74
Solgi and Esfandi Sarafraz, 2015	0.072	0.67	-	-	-
This study	1.40-1.90	0.43-0.93	0.16-0.24	0.07-0.12	0.54-0.61

The Caspian Sea pollution with heavy metals is due to the geological structure of the area or pollutant sources resulting from human activities, such as municipal, industrial and agricultural wastewater discharge on the beaches, fishing, recreation, trading boat traffic, entering of organic and also inorganic substances from the northern coast to the southern shores of the Caspian Sea. This high concentration of heavy elements definitely reveals the reason for high pollution of the Caspian Sea. The accumulation of cadmium and mercury in the human body can cause nervous system impairment and kidney dysfunction and high levels of lead can cause anemia and accumulation of arsenic is a risk factor for cancer. Also chromium can cause respiratory problems, reduce body resistance to diseases, and cause infertility and tumor formation, so the importance of heavy metal pollution in these fish should be attended.

Comparison of heavy metals accumulation in sexual maturation and sexual rest

According to this study, absorption of heavy metals in the surveyed tissues is higher during sexual rest in spring than during sexual maturation in fall (Table 2). *L. aurata* spawns in autumn and uses body reserves (fat and protein) for the formation of sexual products and at this time consumes little food due to the physiological state of the body (Rehbein and Oehlenschlager, 2009). Fat is stored in the fish body again due to active feeding in spring after the reproduction period, when the gonads are inactive. It should be noted that many metals enter the fish body through food (Nussey *et al.,* 2000). It seems that lower feeding rate during the reproduction period in fall is the main factor for reducing heavy metal concentrations compared to spring. In addition, the fish physiological status can be effective in the bioaccumulation of each metal (Kotze *et al.,* 1999). On the other hand, the amount of bioaccumulation of a metal is influenced by environmental, genetic and biological factors leading to the differences in the bioaccumulation of the metal between different individuals, tissue types, seasons and regions (Sadeghi Rad *et al.,* 2005). Obasohan and Eguavoen (2008) in a study on freshwater fish *Erpetoichthys calabaricus* reported that the accumulation of manganese, cadmium, nickel and lead in the fish body follows the seasonal pattern of these metals in water. This shows that there is a close relationship between concentration of metals in water and fish body. Similar to the above study, Nasehi *et al.* (2013) also investigated heavy metal concentration in carp in Aras River and found that heavy metal concentration does not have the same pattern in fish tissue in the four seasons, and declared that a decrease in summer rainfall can lead to increased metal absorption in the fish.

Bahnasawy *et al.* (2009) studied the absorption of lead and cadmium in different seasons in two species of mullet and reported that the highest metal concentration is in summer and the lowest is in winter. This is due to the fact that in the warm season the temperature of water increases which leads to increased fish activity and ventilation and increased temperature ultimately reduces blood oxygen leading to increased metal concentration (Grobler, 1988). Family members of mullet fish are widely spread in tropical and subtropical waters. Given that the Caspian Sea waters get warm in the spring, the temperature and salinity are in the physiological range of mullet, so its growth increases due to suitable environmental and biological conditions (Helfman *et al.,* 1997). Increased metabolic rate forces the fish to increase feeding frequency that can cause the entry of many metals in the fish body through feeding (Nussey *et al.,* 2000). Finally, it should be noted that season, reproduction, environmental conditions, and food access are important factors in increased metals concentration during the sexual rest in mullet.

The examination of the relationship between the heavy metals accumulation and the biological characteristics

A highly significant correlation between the elements in the tissues analyzed was observed statistically in both sexual periods. Specially, the correlation between Pb and Cd was higher than that among other metals. These inter-elemental relationships may be attributable to similar physicochemical properties of the metals involved; also it has been regarded as indicative of similar biochemical pathways or, at its simplest, as demonstrating that the binding of certain metals in animals indicates the occurrence of particular ligands (Pourang *et al.,* 2005). Maybe, high levels of correlation coefficient between the metal represents the source of these elements is identical.

An increase in weight and total length of the body, with accumulation of heavy metals in the tissues showed no significant association except with arsenic. That is arsenic concentration decreases gradually as weight and length increases, while lead, cadmium, chromium and mercury did not present significant correlation with length and weight. These findings are similar to those in previous reports (Gey, 1988; Henry *et al.,* 2004; Pourang *et al.,* 2005; Demirak *et al.,* 2006; Agtas *et al.,* 2007; Ploetz *et al.,* 2007; Monsefrad *et al.,* 2012). 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).

The absence of a relationship between the metal concentrations and the weight and total length of fish may be explained in part due to the body capacity to regulate the concentrations of these metals and the fact that body size and biochemical factors associated have a small or null influence on variability (Pourang *et al.,* 2005). The relationships between heavy metals levels in the tissues and the biological characteristics have been documented by several investigators. Sometimes even contradictory results have been obtained from different researches. For example, Amini Ranjbar and Sotudehnia (2005) found a positive linear relationship between accumulation of Cd with weight and standard length and a negative linear relationship between accumulation of Pb in muscle with weight and standard length of *L. aurata*. Pazooki *et al.* (2009) reported a significant negative relationship between fish size and the Cr levels in muscle of *L. aurata*. Farkas *et al.* (2003) studied *Abramis brama* and reported positive relationship between Cd and fish length. Anan *et al*. (2005) studied bony fishes of the Caspian Sea and reported that increase in body size reduces metal concentration in muscles.

In general, the results of this investigation showed that heavy metal concentrations in *L. aurata* from the southern part of the Caspian Sea were altered by sexual periods, as the sexual rest concentrations were significantly higher than the sexual maturity concentrations. The results indicate that factors such as season, reproductive cycle and fish feeding affect the concentrations of heavy and toxic metals. The fact that during both sexual rest and sexual maturity, mean levels of Pb, Cd, Cr and Hg exceeded WHO (1985), UK (MAFF, 1995), FAO (2011), and NHMRC (Tuzen, 2009) guidelines in food, suggested that the *L. aurata* from the southern part of the Caspian Sea might be unfit for human consumption. These metals could be passed on to humans through the food chain and thus predispose the consumers to possible health hazards. Based on the above findings, monitoring of these and other heavy metals in the fishes from the southern part of the Caspian Sea is strongly advocated, in view of the possible health implication to consumers of the fishes of the Caspian Sea.

Acknowledgments

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