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Research Article

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Seawater, Sediment and Fish Tissue Heavy Metal Assessment in Southern Coast of Caspian Sea

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ABSTRACT

To assess the health condition of table fishes of Caspian Sea and any possible relavancy with pollutants, the concentrations of heavy metals such as copper (Cu), Zinc (Zn) and lead (Pb) in the seawater, sediment and fish tissues (liver and muscle) were measured in three stations situated at southeast coastal regions of Caspian Sea including Chamkhaleh (St1), Kiashahr (St2) and Anzali (St3). The fish samples (golden mullet, Liza aurata) were selected from beach seine catches along with water and sediment every month during a year round. The levels of heavy metals were measured by atomic absorption spectrophotometry. Stations showed significant differences in sea water contents of Pb but not for Cu and Zn, where Pb in Kiashahr and Anzali stations were higher than FAO/WHO standard. Cu, Zn and Pb concentrations in the sediments did not exceed the sediment quality standards (ERL and TEC) in study areas and thus there is no pollution risk due to these metals. Liver and muscle samples contained Zn, Cu and Pb above standard values. Such occurrence might be due to high tendency of liver cells to bind with Cu, Zn and its detoxifying function. It is strongly recommended to discard visceral organs of fishes caught at Guilan province shore line. Accordingly anthropogenic activities such as industrial, agricultural and domestic discharges into rivers which are led to the Caspian Sea were the most probable sources of metal pollution. A sustained assessment of pollutants involving commercially important organisms was insisted.

Key words: *Pollution, Health, Fish, Aquatic Organisms.*

INTRODUCTION

The essential heavy metals such as zinc (Zn) and copper (Cu) play important roles in biochemical and physiological processes of both plants and animals, although essential metals could be toxic for organisms in high concentration [1]. Non-essential heavy metals like cadmium (Cd), lead (Pb) and mercury (Hg) have no role in biological processes [2] and basically impair biological activities. The accumulation of heavy metals in sediments [3] and in organisms living in or on it make susceptible sources to be evaluated either from sediment by itself or from organisms which are fed there or are food for others. In aquatic ecosystems heavy metals are very persistent substances [4] and except natural processes, sources of heavy metals in the environment originates mostly from anthropogenic activities which includes broadly various types of industries, agriculture and domestic applications [5]. As heavy metals could be concentrated in sediments, the measurements are suitable for pollution monitoring in aquatic environments and possible effects on organisms such as fish and shellfish [3].
Various anthropogenic activities increase many sorts of pollutants including heavy metals in the Caspian Sea. Heavy metals are discharged into the sea through rivers flowing through the surrounding countries namely Azerbaijan, Iran, Kazakhstan, Russia and Turkmenistan [6]. In the southern part of Caspian Sea i.e. Iranian coast much attention have been paid to investigate the concentration of heavy metals in water, sediment and also fish tissues such as *Rutilus*

frisii kutum, Acipenser persicus, Rutilus rutilus caspicus and Cyprinus carpio [7-9] to assess fish health and people’s food.

Golden mullet (*Liza aurata*) is well known worldwide as favorable commercial species and has now been well established in the Caspian Sea since it was introduced from Black Sea in 1930. *L. aurata* otherwise called *Chelon aurata* is a well-studied euryhaline fish species residing Caspian marine waters but visiting estuaries and coastal waters for dense feeding. [10].

Present investigation was carried out to determinate the concentration of heavy metals such as Zn, Cu and Pb contamination in seawater, surface sediments of the Caspian Sea and fish tissue. The investigation also aimed to understand possible link between the rate of environmental pollution and fish tissue heavy metal concentration caught at the same locality.

MATERIALS AND METHODS

Study area

The Caspian Sea is located in the north part of Iran having a coastal line of over 1000 km [11]. In the province of Guilan three stations were selected including Chamkhaleh (St1 - 37°13'44.8"N 50°18'58.4"E), Kiashahr (St2 at 37°28'8.6"N 49°59'34.4"E) and Anzali (St3 at 37°29'16.5"N 49°28'35.2"E) (Fig. 1). It has to be suggested Anzali sampling site was situated beyond the wave breaker and port activities.

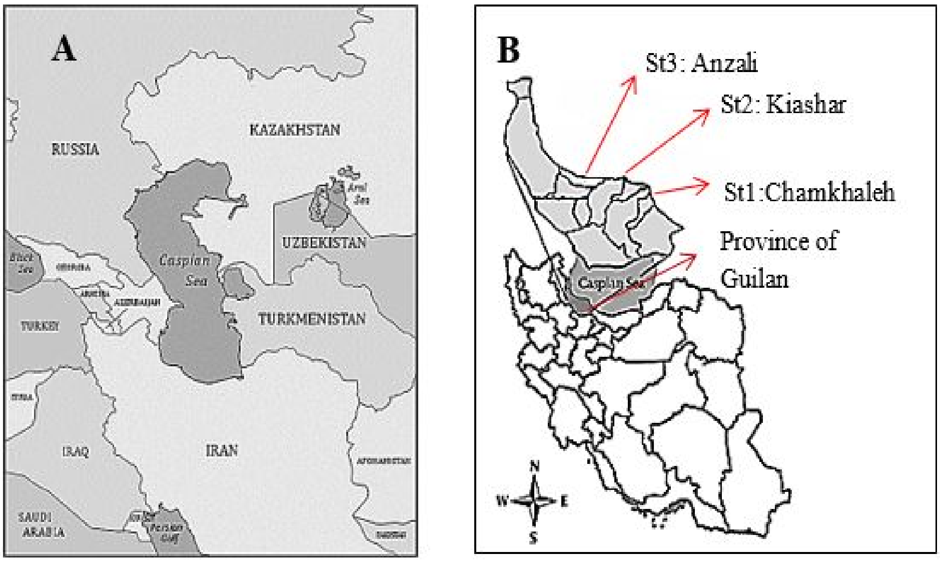

Figure 1. A) Showing the location of Iran and surrounding countries of Caspian Sea. **B)** Sampling stations in province of Guilan at southern coastal region of the Caspian Sea.

The sampling stations were selected based on its pollution background, the amount of river runoff into the sea and access feasibility to the sites.

Water and sediment sampling

Sampling locations was resumed by Garmin GPS device. The water samples were collected every month from the depth of approximately 5 m (about 3 to 4 km from shore line) by Niskin sampling cylinder (Hydro-Bios) for a period of one year. Water variables including temperature (Cº), salinity (ppt ‰) and pH were determined on spot by Consort Multipara meter Analyzer. For metals analysis, water samples were transported to polyethylene (PE) bottles which were pre-cleaned by nitric acid 10% and rinsed three times by distilled water. Then water samples were immediately acidified by acid nitric to reach the pH of 2 and maintained at 4Cº in cool box for analysis [12, 13].

The Sea bottom sediments at the depth of approximately 5 m were collected by means of Van Veen (2000 cm^3) Grab and stored frozen in zippered plastic bags.

Fish sampling

Newly perished golden mullet specimens, *Liza aurata* were obtained from local fishermen by means of beach seine and gill net. Ice cooled fish specimens were transferred to laboratory and a piece of muscle tissues from the area behind head and a small part of liver were stored in polypropylene bottles which were pre-cleaned by 10% nitric acid and rinsed thrice by distilled water and then frozen at -20 Cº until analysis.

Determination of metals concentration in water, sediment and fish tissue

Metals concentration in water was directly determined by acidified solutions. Prior to analysis, sediment samples were dried at temperature of ≤ 60°C to constant weight and passed through a 63 µ sieve to remove large particles. [14].

Preparation of sediment samples were carried out by Aqua regia method [15], so that 1gr *of dried sediment was digested in 5 ml nitric acid (69% Suprapur) and 15 ml hydrochloric acid* (1 :3, v : v). The mixture was boiled on a hotplate until complete digestion [16].

Liver and muscle tissues were freeze-dried for 24 hours. Later 1 gr of every dried tissue sample was digested separately in 10 ml of concentrated nitric acid (69%). Digesting tissues were first placed on a hot plate at low temperature for 1 hour but were then fully digested at higher temperature (140º C) for at least 3 hours [17]. All digested tissue samples were diluted to 50 ml volume with double distilled water (DDW). They were then filtered through No.42 filter paper and stored at 4º C until analysis were performed for Cu, Zn and Pb using atomic absorption spectrophotometer (Younglin AAS 8020).

Data analysis

Kolmogorov-Smirnov test was employed to analyze normality of data distribution. Mean environmental parameters among stations were tested by One-way analysis of variance (ANOVA). Tukey post hoc comparisons test was applied to determine significance among various data. The relationship between metal concentration (in water, sediment and tissues) and water variables (temperature, pH and salinity) and relationships between the heavy metals in water, sediment and tissues were analyzed by Pearson correlation analysis. Results were considered significant at the 95% level ($p<0.05$). To determine more information about the relationships between the heavy metals, Principal Component Analysis (PCA) was carried out. Components with factor loadings above 0.75, between 0.5 and 0.75, and between 0.3 and 0.5 were considered to be strong, moderate and weak, respectively [18]. Statistical analysis was carried out using SPSS 22 software.

RESULTS

Environmental variables

The environmental parameters determined in three stations are summarized in Table 1. Pearson correlation results showed that in all regions water temperature showed significant relationship with salinity and pH respectively ($p<0.05$). Statistical analysis showed that there was no significant difference between stations in all of the environmental parameters.

Table 1. The mean environmental parameters examined in the present study.

Sampling stations	Water Temp. (Ċ)	pH	Salinity (‰)
Chamkhaleh (St1)	18.35± 6.72	8.1±0.13	11.9±0.41
Kiashahr (St2)	18.47±6.79	8.12±0.19	11.98±0.48
Anzali (St3)	18.54±6.64	8.06±0.22	12± 0.52

Metal concentration in water and sediment

The results for concentration levels of heavy metals in the water (µg/L) and sediment (µg/g dry weight) samples are summarized in tables 2 and 3. Table 2 showed Pb content of water was highest and lowest at St3 and St1 respectively ($p < 0.05$). There was no significant difference in the Zn content of water among three stations. The ranking order of the heavy metal concentration at St1 was as Zn > Cu > Pb ($p < 0.05$). According to table 2, Pb and Zn concentrations in both St2 and St3 stations were significantly higher than that of Cu concentration.

Table 2. The mean concentrations of Cu, Zn and Pb (µg/L ± standard deviation) in the water samples from three stations.

Sampling station	Zn	Cu	Pb	Ranking
Chamkhaleh (St1)	15.79±8.59	11.62±12.68	5.62±5.1	Zn>Cu>Pb
Kiashahr (St2)	14.99±6.42	6.59±7	18.47±18.7	Pb>Zn>Cu
Anzali (St3)	18.77±9.44	5.62±4.03	19.21±16.91	Pb>Zn<Cu

Table 3 showed Cu content in the sediment was significantly highest at St1 and lowest at St2 ($p < 0.05$). There was no significant difference in the concentrations of Zn in all stations and Pb in St2 and St3 when they were compared. On the other hand Zn, Cu concentrations in the sediment at St1 were significantly higher than that of Pb ($p < 0.05$). Also, Zn, Pb concentration at St2 and St3 were significantly higher than that of Cu of the same station ($p < 0.05$).

Table 3. The mean concentrations of Cu, Zn and Pb (µg/g dw ± standard deviation) in the sediment sampled from three stations.

Sampling stations	Zn	Cu	Pb	Ranking
Chamkhaleh (St1)	50.14±36.09	26.76±20.38	13.91±19.19	Zn>Cu>Pb
Kiashahr (St2)	50.69±35.04	10.61±7.72	25.84±19.77	Zn>Pb>Cu
Anzali (St3)	46.93±12.65	13.53±10.78	22.57±11.77	Zn>Pb>Cu

The correlation coefficients between heavy metals in the seawater and sediment of three stations are summarized in Tables 4. Results obtained from the Principal Component Analysis (PCA) in the three stations are shown in Table 5. St1 revealed three significant components accounting for 81.931% of the cumulative variance. The first component (F1), explaining 40.06% of the total variance, had positive loadings of Zn content in water and sediment and moderate negative loading of Pb in the water. In addition those two heavy metals were correlated (Table 4, $p<0.05$, $p<0.01$). The second component (F2), explaining 23.31% of the total variance had strong positive loadings of Cu in the water and Pb in sediment. The Pearson correlation between heavy metal concentration in the water and the sediment showed relationships between Cu and Pb.

For St2, the first component (F1) explaining 42.54% of total variance, showed strong positive loadings of Pb in both water and sediment. The second component (F2), explaining 22.61% of total variance, had a high positive loading of Zn in water and Cu in sediment. Also positive correlations were found between these heavy metals (Table 5, $p<0.05$, $p<0.01$).

For St3, the first component (F1), explaining 27.84% of total variance showed strong negative loading of Zn in the sediment and Pb in the water. The second component (F2) had a high positive loading of Zn in water and Cu in sediment. Zn and Pb were correlated (Table 5, $p<0.05$).

Table 4. Correlation coefficients between heavy metals of water (w) and sediment (s) for three stations.

		Zn.s	Zn.w	Cu.s	Cu.w	Pb.s	Pb.w
Zn.s		1					
Zn.w	St1	.752**					
	St2	.611**	1				
	St3	.254					
Cu. s	St1	-.202	-.209				
	St2	.071-.031	.504*	1			
	St3		.362				
Cu. w	St1	.477*	.206	-.071			
	St2	.334	.171	.213	1		
	St3	-.078	.040	.316			
Pb.s	St1	.104	-.188	-.178	.449*		
	St2	-.218	-.303	-.279	-.377	1	
	St3	-.176	-.042	.062	.126		
Pb.w	St1	-.473*	-.477	-.252	-.227	.024	
	St2	-.399	-.192	.167	-.320	.672**	1
	St3	-.535*	-.065	.308	-.071	-.097	

Levels of significance : *$p<0.05$; **$p<0.01$.

Table 5. Principal Component Analysis (PCA) for heavy metals in the water and sediment from the three stations.

	St1			St2		St3		
	F_1	F_2	F_3	F_1	F_2	F_1	F_2	F_3
Zn.s	0.882			-0.577		-0.837		
Zn.w	0.925				0.815		0.778	
Cu.s			0.930		0.863		0.827	
Cu.w		0.728		-0.579				
Pb.s		0.884		0.781				0.783
Pb.w	-0.666		-0.552	0.913		0.892		

Variance (%)	40.055	23.311	18.565	42.543	22.612	27.835	25.390	19.501
Cumulative (%)	40.055	63.366	81.931	42.543	65.155	27.835	53.225	72.726

Factor loadings smaller than 0.5 have been removed.

Metal concentrations in fish tissues

The results of the concentration of the heavy metals in the fish liver and muscle samples are presented in Table 6. The order of the heavy metals concentration in liver presented for all three stations was as Cu > Zn > Pb ($p < 0.05$). The content of Cu and Zn in liver was significantly higher than muscle in all stations ($p < 0.05$).

Table 6. The lowest, highest and mean concentrations of Cu, Zn and Pb (µg g-1 dw ± standard deviation) in *Liza aurata* sampled from three stations.

Sampling stations	Tissue	Zn	Cu	Pb	Ranking
		Lowest- Highest Mean±SD	Lowest- Highest Mean±SD	Lowest- Highest Mean±SD	
Chamkhaleh (St1)	Liver	2-111.67 46.55±37.46	640-2625 1192.91±582.92	7-32 13.25±11.45	Cu>Zn>Pb
	muscle	2-80 18.96±28.79	2-115 44.24±38.93	9.5-51 19.7±14.8	Cu>Pb>Zn
Kiashahr (St2)	Liver	25-236 127.68±48.12	108-910 472.63±249.65	7-41.51 18.15±13.4	Cu>Zn>Pb
	muscle	0.8-33 4.64±7.24	4-57 24.33±17.42	10.2-95 38.82±26.51	Pb>Cu>Zn
Anzali (St3)	Liver	65-242 129.62±82.46	996-2430 1437.05±776.71	32.5-152 76.82±53.04	Cu>Zn>Pb
	muscle	28.8-78 17.79±27.95	5-274 77.62±86.79	10-62.3 24.78±16.68	Cu>Pb>zn

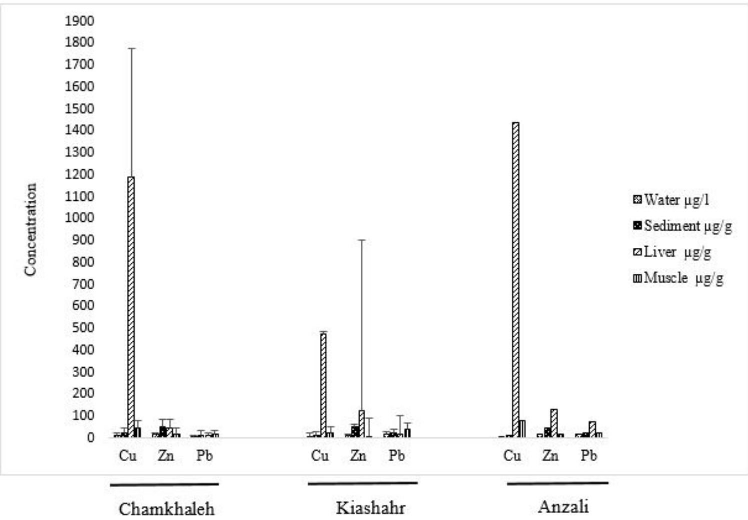

Figure 2. The mean concentration of Cu, Zn and Pb in seawater, sediment and L. aurata tissues (liver and muscle) from three sampling stations.

The correlation coefficients among heavy metals in the fish liver and muscle from three stations are summarized in table 7.

Table 7. Correlation coefficients between heavy metals in liver (l) and muscle (m) of *L. aurata* from three stations.

		Cu.l	Cu.m	Zn.l	Zn.m	Pb.s	Pb.w
Cu.l		1					
Cu.m	St1	-.200					
	St2	.123	1				
	St3	.629**					
Zn.l	St1	.213	-.385				
	St2	-.430*	-.110	1			
	St3	.523**	.325				

Zn.m	St1	.213	-.295	.600*			
	St2	-.479*	.354	.593**	1		
	St3	.215	.096	.121			
	St1	-.325	-.602	.095	-.032		
Pb.l	St2	-.550**	-.285	.226	.114	1	
	St3	.296	.019	-.072	-.421		
	St1	.124	-.060	.163	.100	.361	
Pb.m	St2	-.371	-.328	.247	-.079	.521**	1
	St3	-.111	.043	-.302	-.046	.088	

Levels of significance : $^{*}p<0.05$

Results obtained from the Principal Component Analysis (PCA) in the three stations are shown in Table 8. For St1, two principal components obtained from the concentration of elements in liver and muscle. Factor 1 (expressing 32.23% of total variance) includes strong positive association of Zn in the liver and muscle and moderately related to Cu of liver. Cu in muscle showed quit strong inverse relation. Factor 2 (expressing 23.96% of total variance) included well established Pb content of liver and muscle. The results obtained from the PCA, Pearson correlation matrix (Table 8) showed that Zn in the liver and muscle had positive correlation which was in the first component.

Table 8. Principal Component Analysis (PCA) for heavy metals in the liver (l) and muscle (m) of *Liza aurata* from the three stations of Chamkhaleh (St1), Kiashahr (St2) and Anzali (St3).

	St1		St2		St3	
	F_1	F_2	F_1	F_2	F_1	F_2
Cu.l	0.574		-0.714		0.915	
Cu.m	-0.670			-0.734	0.839	
Zn.l	0.753		0.768			
Zn.m	0.713		0.906			0.822
Pb.l		0.895		0.734		-0.859
Pb.m		0.649		0.789		
Variance (%)	32.23	23.96	41.02	27.86	34.99	24.39
Cumulative (%)	32.23	56.19	41.02	68.88	34.99	59.38

Factor loadings smaller than 0.5 have been removed

For St2, the first component (F1), explaining 41.02% of the total variance, had strong positive presence of Zn in the liver, muscle but moderate inverse relation to Cu in liver. Factor 2 (explaining 27.86 % of total variance) included strong positive loadings of Pb in liver and muscle and negatively related to Cu of muscle. In addition, these heavy metals were correlated (Table 8).

For St3, the first component (F1), explaining 34.99% of the total variance, had strong positive loadings of Cu in the liver, muscle. The second component (F2), explaining 24.39% of the total variance so that F2 includes strong positive association of Zn in the muscle and negative Pb in the liver.

DISCUSSION

Caspian Sea is a enclosed body of water without any connection to open oceans and in fact is lower than open sea level. In spite of constant subsurface seasonal water circulation which affects Caspian Sea by overall pollutant load imposed through surrounding countries, the nature and the intensity of pollution in surrounding shoreline depends on the local anthropological activities.

In present study, to evaluate the intensity of pollution and its consequent effects on regional fishes of eastern part of Guilan province, the concentration of heavy metals including Zn, Cu and Pb in seawater, sediments and tissues (liver and muscle) of *Liza aurata* obtained from three stations in the southern coasts of Caspian Sea were assessed. *Liza aurata* otherwise known as *Chelon aurata* is a grazer found in shallow waters particularly during spring and summer which is time for their intensive feeding and growing. Therefore they are subjected to polluted coastal waters and the food they ingest. All three stations were close to important cities for tourism and high anthropologic activities. The domestic wastewater, the run-off by close paddy fields, local industries such as boat and small vessel workshops and freight transport are serious pollution sources of Cu, Zn and Pb released into seawater as it was explained by Alloway (2013). The three metals i.e. Pb, Cu and Zn behaved differently at above mentioned stations in water, sediment and fish tissues. Though the concentration of heavy metals showed certain relationships between water and sediment it did not seem to have permanency. For example table2 showed in both St2 and St3 the sea water concentrations of Pb and Cu were highest and lowest of three metals respectively. St1 showed differently as Zn and Pb were highest and lowest of all respect to each of them. Sediment concentration of metals exhibited a more stable pattern as Zn possessed highest mean concentration in all three stations but lowest one was Cu for St2 and St3 and Pb for St1 (table 3). There was not a logical reasoning for chaotic presence of metals in water and sediment. Meanwhile higher concentration of metals in all samples of sediment when compared with values obtained from water samples bring the analytical results into order up to certain extend. However Pb values in the water samples from St2 and St3 were higher than the recommended standards by FAO and WHO (2011) (Table 2, 9).

Table 9. International standards for Zn, Cu and Pb in the water samples. (µg /L)

Standards	Zn	Cu	Pb	Reference
WHO	3000	2000	10	FAO/WHO, 2011

Sediment higher content of metals compared to surrounding water is a natural process and has been mentioned very often. Ochieng et al. (2007) stated that absorption and accumulation of heavy metals in sediments is several times higher than the water [19]. Therefore sediments are the final sink of metals and humic substances contained in sediments producing metal compounds [3, 20]. Davies et al. (2006) reasoned that the transition of metals from water into sediments depends on environmental parameters such as pH, salinity and conductivity [21]. Another cause to find higher level of metals in sediment is increased temperature which could be correlated to reduced water volume and evaporation [22, 23]. According to our results, heavy metals accumulations in the sediments were higher than dissolved metals in seawater and though a significant positive correlation between Zn concentration in the water and sediment, particularly in St1 and St2 was present this aspect could not be generalized for all studied metals and for all three stations. Moreover narrow difference in water temperature, salinity and pH among stations (table 1) would rule out the impressibility of metal values by mentioned factors. Such erratic situations bring about perplexity and specificity of each station based on pollutant load and environment. Surprisingly table 2 present an adverse gradient between Pb and Cu both in water and sediment. Wherever Cu is high Pb concentration is lower and vice versa. Zn was independent from Cu and Pb and dominant at least in sediment contents as far as concentration was concerned and exhibited moderate concentrations in water samples (table 2 and 3). Correlations among heavy metals such as Ag, Cu and Fe has been studied in water sediment system southwest coast of Pakistan by Tariq et al. (1994) [24] and among Cu, Zn and Pb in coastal area of Tuaran of Malaysia by Tan et al. (2016) [25]. Tan et al (2016) also detected Zn as dominant metal in sediment and correlation was established only in certain metals and not all.

Our results revealed that all study locations showed some degrees of pollution (e.g. Cu, Zn and Pb) and the main contribution to the high level of metals could be attributed to domestic wastewaters from urban areas and the run-off by agricultural farms. In the present study, the Sediment Quality Guidline (SQGs) and the National Oceanic and Atmospheric Administration (NOAA) are major standard sources to compare the results SQGs standard wwere presented as TEC and PEC and NOAA standards were as ERL and ERM. Cu, Zn and Pb concentrations in the sediments (table 3) did not exceed the sediment quality standards in study areas and thus there is no pollution risk due to these metals.

Table 10. Values of heavy metals in the sediments according to international standards (µg /g dw)

Standards	Zn	Cu	Pb	Reference
ERL	-	34	47	Long and Mac Donald, 1998 [26]
ERM	-	270	220	Long and Mac Donald, 1998 [26]
TEC	121	31.6	35.8	Hongyi et al., 2009 [27]
PEC	459	149	128	Hongyi et al., 2009 [27]
ERL : Effect Range Low EMR : Effect Range Medium TEC: Threshold Effect Concentration PEC: Probable Effect Concentration				

Heavy metals accumulate in both soft and hard fish tissues such as liver, muscles and bone are serious threats for organisms and human, therefore the heavy metals are categorized as the most dangerous of pollutants [28]. In present study, the concentrations of Cu, ZN and Pb were estimated in liver and muscle of *Liza aurata*. Table 6 obviously exhibits livers to be more contaminated rather than muscles. Cu is shown to be dominant metal in fish liver tissues caught in all stations of which St3 fishes were most contaminated. All stations Zn as second order metal and Pb as the third order one proved to be highest in St3 fishes. St2 fishes exhibited less contamination for Cu and Zn as far as liver was concerned. Metal ranking for muscle tissues alienate a different perception. Fishes of St1 and St3 possessed most contaminated muscle tissue for Cu and St2 for Pb. In contrary the least metal values in muscle tissues belonged to Zn for all stations. It was obvious that bioaccumulation of Cu and Zn was much more in liver tissue compared to muscle but muscle showed higher contamination by Pb rather than liver. It seems metallothionein proteins in liver have high tendency for binding with Cu, Zn and function specifically as detoxification site, thereby happen accumulation and transportation of metals [29-31]. Also, the muscle is an important organ for metals accumulation. It seems the metals have high tendency for binding with sulphate groups in methionine and cysteine proteins that are abundant in the muscle [32]. The increased Pb levels observed in muscles of *L. aurata* might be the results oil production activities in the Caspian Sea as Pb is one of the oil derivatives.

Table 11 contains Permissible limits on heavy metals for food safety. Cu levels in liver of sampled fishes from St1 and St3 were higher than National Health and Medical Research Council (NHMRC) (1987) values which indicates food chain could be at risk [33].

Table 11. Permissible limits on heavy metals for food safety set by international standards (µg /g dw)

Standards	Zn	Cu	Pb	Reference
FAO/WHO	100	-	4	FAO,1983; WHO, 1989
NHMRC	750	350	-	NHMRC,1987
USFDA	-	-	11.5	USFDA, 1990
BOE	-	-	25	BOE, 1991

FAO: Food and Agriculture Organization [34] WHO: World Health Organization [35]
NHMRC: National Health and Medical Research Council
ISFDA: U S Food and Drug Administration
BOA: Boletin Official del Estado [36]

Zn in liver and Pb concentration in both liver and muscle of fishes of three stations were higher than standards (table11). Also Pb values in the muscle of St2 fishes and liver of St3 fishes were higher than BOE (1991) standard. The difference in metal concentration of liver and muscle tissue illustrated that these tissues are susceptible for bioaccumulation of harmful elements. It is fortunate that fish viscera is not a favorite edible part of fishes except for certain species like Caspian kutum (*Rutilus frisii kutum*). As precautionary measures it is strongly recommended to avoid consuming fish visceral organs.

Principal Component Analysis (PCA) for heavy metals in liver and muscle (table 8) showed positive relation for Cu present in liver and muscle of St3 fishes meanwhile St1 fishes were adversely affected. Zn and Pb were very well established addressing positive association for liver and muscle in St1 and St2 fishes.

The correlations among different metals found in single type tissue indicated homologous accumulating action and their interactions in an organism. Totally fishes are exposed to metal complexes which are usually more toxic than an individual metal [37]. Metals accumulation in fish tissues depend on biotic and abiotic factors, competition or cooperation between metals, chemical nature of the metal, the tissue type, exposure concentrations and duration of exposure [38]. There are no clear patterns for heavy metals interaction. For example, interactions between Cd and Zn varied from antagonistic to synergistic in different studies [39-41]. Our results showed positive significant correlation between Zn and Pb concentration in the liver and muscle (Tables 7 and 8). Also the same results were observed for Cu concentration in both tissues (Table 7).

Metals such as Zn and Cu have chemical and physical similarity, thus they can have a competitive effect at binding sites assisted by binding proteins such as metallothionein (MT). It may result into a kind of Cu-Zn complex or mixtures. It was observed that Cu concentrations in gills of rainbow trout enhanced in Cu-Zn mixtures [42]. Such finding may explain unexpected and uncommon presence of heavy metals in environment and organisms.

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