

Studying the accumulation of heavy metals (Fe, Zn, Cu and Cd) in the tissue (muscle, skin, gill and gonad) and its relation with fish (*Alosa braschinkowi*) length and weight in Caspian Sea coasts

Abstract

This research is done with aim of determining the accumulation of heavy metals (Fe, Zn, Cu and Cd) in different tissues of Caspian Marin shad (*Alosa braschinkowi*) in south area of Caspian Sea (Babolsar). Then 22 species of fishes were collected from commercial fishing, randomly. The density of samples' heavy metals after biometry (length and weight) and tissue isolation and chemical digestion was measured by the Flame atomic absorption. The concentration of Fe in the tissues was such as, gonad > gills > skin > muscle, the Zn is such as muscle < gills < skin < gonad, the Cd is such as gonad > skin > gills > muscle and Cu was such as Muscle < gonad < gills < skin. The results of spearman correlation test have shown that in the tissue of gills between weight and the amount of Cd absorption ($p < 0.05$), in the tissue of gonad between fork length and the amount of Cu absorption, there is a negative and strong correlation ($p < 0.01$). However, in the tissue of muscles there isn't any meaningful relationship. Also, the concentration of Zn in the skin and gonad was higher than FAO and UK (MAFF) standards and in all tissues the concentration of Cd was higher than all standards.

Keywords: caspian marin shad; fork long weight; heavy metals; caspian sea; *alosa braschinkowi*

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Introduction

Fish is an important source of animal protein, which is cheap for humans that have very significant importance because of economic value and sensitivity against pollutants. Metals, exists in the environment normally and some of them play an important role in biologic processes.¹ Natural processes (Volcanoes, weathering and stone erosion, sediments, firing) and industrial progresses and technology causes the increasing the pollution of different natural resources in the environment like water and soil.

Pollutants with influencing on aquatic life create significant losses for them and subsequent consumers.²⁻⁵ Heavy metals because of toxic effects and high accumulation power in many species of aquatics is considered significant.⁶⁻⁷ Among Zinc (Zn) copper (Cu) and Iron (Fe) are essential for body a little, but high amount of them create deleterious effects for creatures.

There are other groups such as cadmium (Cd), lead (Pb) and Mercury (Hg) which is detrimental for body even a little.^{3,8,9}

The most important bad effect of cadmium consumption in human is decease of Itay-Itay or the disease of joints and bonds that consequently leads to death.^{3,10} Cadmium in the fish, affects the enzymatic routes of ascorbic acid synthesis.¹⁰

In addition, accumulation of cadmium causes decreasing the size of liver and losing the glycogen resources of liver and muscle in the fishes.¹⁰

Fishes in their environment cannot escape from these destructive harms of pollution and lead to polluting the food chain.¹¹⁻¹³ Age, length, weight, sexuality, food habits, ecologic requirements, the concentration of heavy metals in the water and sediment period of fish shelf life in aquatic environment, fishing season, water chemistry (salinity, intensity and temperature) are the effective reason in accumulation of heavy metals in different organs of fish.¹⁴⁻¹⁶

Fishes are more sensitive to available pollutions in the water rather than humans. As a result, the sample of accumulation trend and elements accumulation in the fish tissues can be used as a biological bio indicator to the changes in aquatic environments.¹⁷⁻¹⁹ Caspian Marin shad (*A. braschinkowi*) from the family of clupeid that its length is 30 to 50cm. It is one of the bony fishes that because of having suitable length and weight is a commercial and economic species in the south area of Caspian Sea.^{20,21} Several studies by researchers are done about amount of heavy metals in the fishes. In a research which was done by Yonesipour et al. on the *Cyprinus* fish of Caspian sea (*Cyprinus arpio*), the average amount of density in (SD) Fe, Cu, Zn, Ni, Co and Mn in the muscles of this fish is 77/91±11, 12/11±17, 63/45±14, 121±14/3, 323/5±0/54µgr/gram of net weight respectively.

Another research which was done by Akan et al.,²² the amount of Cu, Zn, Co, Mn, Fe, Cr, Cd, Ni and Pb maintained from 4 species of Fishes (*Clarias anguillaris*, *Tilapia zilli*, *Synodontis budgetti* and *Oreochromis niloticus*) from Beneu river of Nigeria. The highest amount of density was related to Fe in the gills of every 4 fishes and the lowest amount of Concentration of metals was maintained in the

tissue of gills>liver>Viscera> kidney> bones >Muscle respectively. In a research by Taghavi et al.,²³ the concentration of heavy metals such as Fe,Cu, Zn, Cd, Ni, Cr, Hg and Zn in the tissue of liver, gills and muscles of golden mullet (*liza aurata*) which was collected from south of Caspian Sea were measured.

The concentration of Cu, Cd, Fe and Zn was done in the muscle<gills< Liver while, the density of Ni, Cr, Hg in the muscle<Liver<gills were maintained.

The aim of this study is analyzing the relationship between fork length and weight with the amount of absorption of heavy metals in the tissues of Caspian marine shad.

Materials and methods

Sampling in Babolsar is done with the geographic location of length and latitude 52.704365 and 36.719673 respectively 22 fishes were provided randomly in autumn and winter of 2014 (each season 11 pieces) during fishing in specified area. The samples after conveying to laboratory first were washed with double distilled water and were biometry. Fork length was measured by biometry board with 1 mm accuracy and samples weight with digital scale with 1gr accuracy. Then, from each fish, a sample of scale, girls, gonad and skin was isolated and provided in special package for digestion.

Sample digestion

Sample digestion was done by dry digestion, in order to digesting the samples, first 2 to 5gr of samples were put in Oven with 55°C for 5 hours to samples become ash. Then, 10^{cc} Hcl, 6 molar was added to each sample and put on the heater until complete drying, after boiling, samples were passed from 42Micron filter paper with HNO₃, 0/1 Normal in volumetric flask 50^{cc}. The ready solution for measurement was collected in a container special for sterile.

For preparing the standard solutions of mentioned metals, pure Nitrate salts, which are related to Merc Company of Germany, were used, that after drawing the calibration curve, was maintained each metal's density by flame atomic absorption system made in Analytikjena company by model AA400. For analyzing the data, Excel software SPSS 19 was used, in order to studying the statistical tests Mann–Whitney U and determining the relationship between metal's concentration in the tissues and biological characteristics, spearman correlation test was used.

Results

The average (±SD) of general weight and fork length and standard length in under samples was 484.77±34.74 gr, 36.37±6.59, 32.33±7.07 and 30.54±7.07 cm that were in the ages between 3 to 5 years old (Table 1).

The amount of different heavy metals density in different tissues have shown that the maximum (63.49±16.73 gr) and minimum (1.47±0.44gr) respectively were related to Zinc and cadmium in the tissue of gonad and muscle. In addition, the arrangement concentration of heavy metals in several tissues was different. However the arrangement of Feconcentration in the tissues was such as gonad> gills > skin > muscle, Zn was such as muscle < gills < skin < gonad<Cd was such as gonad> skin > gills > muscle and Cu was such as muscle < gonad< gills< skin.

The metric relationship of spearman correlation test has shown that, in the skin tissues there is a negative correction between weight

and the amount of cadmium absorption, and also there is a negative correlation between fork length and the amount of Cu and Cd absorption (p <0.05), (Table 3). There is positive correlation between the amount of Zn and Cd absorption and between length and weight in the muscle tissue (p<0.05) (Table 4).

In the gills tissues, there was a negative correlation between weight and the amount of cadmium absorption and between fork length and the amount of cadmium absorption. (p<0.05). However, there was a high and positive correlation between the amount of Zinc and copper absorption (p<0.01)(Table 5).

In the gonad tissues there was a high negative correlation between weight and the amount of Cd absorption (p<0.01). But between fork length and the amount of Cd absorption there was a negative correlation (p<0.05). Similarly between fork length and weight with the amount of Cu absorption, there was a high negative correlation (p<0.01).

However; between the amount of Cd and Cu absorption in gonad tissues there was positive correlation (p<0.05) (Table 6).

Table 1 Biometry indexes in 22 pieces of Caspian marine shad (*Alosa braschnikowi*)

Biological index	Average (±SD)	Min	Max
Total Length(cm)	36.37±6.59	28.0	44.5
Fork length(cm)	32.33±7.07	28.0	39.5
Standard length(cm)	30.54±7.07	24.5	37.0
Weight(gr)	484.77±34.74	234.34	1022.34
Age (year)	4.16±0.57	3	5

Table 2 The average of (±SD) heavy metals in different tissues of *Alosa braschnikowi*

Tissues	Cd	Cu	Zn	Fe
Skin	2.29±1.24	3.70±2.95	59.77±28.87	22.38±11.31
Muscle	1.47±0.44	1.93±1.19	7.02±6.53	14.26±15.23
Gill	2.11±0.57	3.04±2.55	20.12±8.46	28.15±7.23
Gonad	2.96±2.18	2.98±1.41	63.49±16.73	35.99±8.59
Total	2.21±1.11	2.91±2.02	33.10±15.14	25.19±10.59

Table 3 Metrics relationship resulted by spearman correlation in the sample's skin tissues of *Alosa braschnikowi*

	weight	Fork length	Cu	Cd	Zn	Fe
Fe	-0.392	-0.2	0.196	0.441	0.021	1.000
Zn	0.336	0.399	0.142	-0.105	1.000	
Cd	-0.664*	-0.669*	0.266	1.000		
Cu	-0.517	-0.634*	1.000			
Fork length	0.918**	1.000				
weight	1.000					

*Correlation in confidence level, %95 **Correlation in confidence level, %99

Table 4 Matrices relationships maintained by spearman correlation in the sample muscle tissue of *Alosa braschnikowi*

	Weight	Fork length	Cu	Cd	Zn	Fe
Fe	-0.035	0.182	0.000	0.049	0.224	1.000
Zn	-0.245	-0.060	-0.280	0.601*	1.000	
Cd	-0.280	-0.109	-0.287	1.000		
Cu	-0.021	-0.095	1.000			
Fork length	0.918**	1.000				
weight	1.000					

*Correlation in confidence level, %95 **Correlation in confidence level, %99

Table 5 Metrics relationship maintained by spearman correlation in gills tissues of *Alosa braschnikowi*

	Weight	Fork length	Cu	Cd	Zn	Fe
Fe	-0.329	-0.203	0.168	-0.035	0.517	1.000
Zn	-0.399	-0.511	0.846**	0.014	1.000	
Cd	-0.699*	-0.627*	0.028	1.000		
Cu	-0.273	-0.399	1.000			
Fork length	0.918**	1.000				
weight	1.000					

*Correlation in confidence level, %95 **Correlation in confidence level, %99

Table 6 Metrics relationship maintained by spearman correlation in sample gonad tissues of *Alosa braschnikowi*

	weight	Fork length	Cu	Cd	Zn	Fe
Fe	-0.559	-0.417	0.098	0.455	0.413	1.000
Zn	-0.329	-0.273	-0.056	0.538	1.000	
Cd	-0.804**	-0.708*	0.587*	1.000		
Cu	-0.720**	-0.792**	1.000			
Fork length	0.918**	1.000				
weight	1.000					

*Correlation in confidence level, %95 **Correlation in confidence level, %99

Table 7 International standards for heavy metals (g.gu⁻¹)

Organization	CU	Zn	Fe	CD
WHO	10	100	-	0.2
FAO	20	50	-	0.3
UK(MAFF)	20	50	-	0.2

Discussion

Nowadays, many researches are done about the heavy metals absorption in aquatic creatures that represent the increasing trend of these metals in the aquatic environment because of human activities. Then, extra increasing the normal concentration of these metals damages the ecosystem.

The results of this research have shown that the concentration of heavy metals in different tissues of fish is different with each other. This difference may be (1) because of some reasons such as food diet, body length, age, habitation and tissue type in metals distribution between different tissues is influential^{24,25} (2) Heavy metals, select their target organs based on its metabolic activity.^{22,26} (3) Result in being variable the power of heavy metals in terms of overcoming on proteins metallic bonds like metalotionins (4) Because of difference in ecologic requirements and fishes' metabolic activities.²⁷ (5) Difference of organs 'physiologic performances.

With respect to the important role of muscle in nutrition and healthy can be selected as target organ. In the present study muscle as target organ, has shown the lowest concentration of metals rather than other tissues. Being low the concentration of heavy metals in the muscle by other researchers²⁸ is reported that its reason is the presence of metalotion in proteins responsible for removing and neutralizing the heavy elements and their toxic traces. In addition, being low the concentration of heavy metals in the muscle rather than other tissues is because of this tissues low metabolic activity. This study, there was a positive correlation between the amount of Zn and Cd absorption in the muscle which is parallel with Zauke et al result in 1999(p<0.05).

The average of Fe, Zn and Cd in the gonad is more than other tissues which is correspondence with Wang, et al. The reason of high metal's accumulation in the gonad is because of fish's defensive mechanism in moderating the metals and pollutants. In this process, some of organic and mineral pollutants in order to rebuffing is transferred from body to eggs than evacuate from body. The results maintained from Yilmaz et al.,³⁰ on the *Penaeus semisulcatus* tissues, have shown that; the concentration of Cu in gonad is more than other tissues. It may, being high the essential metal in gonad is because if it's important role in cellular division and growth process.³¹

In the study there was a meaningful and negative relationship between Cd and Cu density with weight and length which is correspond with Altı and Canlı²⁷ results that was maintained in Mediterranean Sea on *Scorpaenopsis saurus*. Its reason is that with increasing the length and weight, the concentration of metals will be reduced.³²

Gills and skin are the first tissues that are in direct contact with available pollutants in water. Gill tissue has an important role in transmitting and receiving oxygen from the surrounding environment. Gills play an important role in oxygen transmission and reception from around environment, needs to high density of essential blood and metals, and can absorb the materials in direct contact of

water and indirect contact of foodstuffs. In addition, because of its role was selected in breathing and osmosis balance as target organ. In the present study, the amount of metals in gills is such as $Fe > Zn > Cu > Cd$. Which is correspondence whit Nwani & Ozturk et al⁹ results.

This study has shown that, gills have the highest Fe concentration rather than skin and muscle. Being high the concentration of metal in the gills can be related to its settling and sediment on this gills. He emphasizes that iron sedimentation on gills can lead to iron bacteria's growth on it, then useful level of gills breathing will be reduced. In addition, one of indirect harmful influences of Fe is producing the sediment of Hydroxide Iron II. This compound causes fishes and fetus-bearing eggs of other aqueous suffocation.

Based on the reports of some researchers, skin is an organ, which is influential in removing the superfluous materials resulted in body metabolism and harmful chemical material. As a result, it can be considered as one of target organs. In this study, Cd has a negative relationship with length and weight but Cu has a negative relationship only with fork length ($p < 0.05$). A negative and meaningful relationship between heavy metals with length in some fishes of Caspian Sea is registered by pouring et al.,³³

On the basis of comparing the present results with international standards (Table 7) it is specified that the amount of Zn density in the tissue of skin and gonad is more than FAO standard and UK (MAFF) but less than WHO standards. The amount of Fe, Cu and Zn concentration in muscle and gills was less than international standards but the amount of Cd concentration in under study tissues was higher than all standards. Heavy metals accumulation in different tissues can be affect by some processes such as detoxification and fish different tissues need to metals.³⁴⁻⁴⁶

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Conflict of interest

Author declares that there is no conflict of interest

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