# Bioaccumulation of heavy metals Cu, Zn, and Hg in muscles and liver of the stellate sturgeon (*Acipenser stellatus*) in the Caspian Sea and their correlation with growth parameters

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**Abstract**

Bioaccumulation of Cu, Zn, Hg in muscles and liver tissues of stellate sturgeon (*Acipenser stellatus*) from the southern Caspian Sea was measured. Effects of condition factor (CF), age and hepatosomatic index (HSI) on metal accumulation were also determined. Some 40 stellate sturgeons were collected using gill nets in winter and spring 2007-2008 from the southern shores of the Caspian Sea. Total (TL) and fork (FL) length and weight of all specimens were measured. Cross sections of first ray of pectoral fins were used for age determination. Muscle and liver samples were taken and transported to the laboratory in ice packs and kept in -20 $^{o}$C prior to analyses. In the laboratory samples were oven dried, digested with $HClO_3$ and $HNO_3$ and diluted with dionized water to 50cc to measure Cu, Zn, and Hg concentration with a flame spectrophotometer. Sequence of metals in *Acipenser stellatus* was Zn>Hg> Cu in muscle tissues and Zn> Cu> Hg in liver samples. Concentration of Zn was slightly in correlation with age (R= 0.3). A negative correlation was observed between Zn concentrations with condition factor (R= -0.3). Zn concentrations were negatively correlated in muscle tissues (R=-0.3). The results were compared to international standards proposed by MAAF, EEC and NHMRC. Our study showed that accumulation of heavy metals in sturgeon tissues was influenced by concentration of metals in sediment and the physiological state of fish.

**Keywords:** *Acipenser stellatus*, Heavy metals, Condition factor, Hepatosomatic index, Liver, Muscle

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## Introduction

The Caspian Sea is the largest inland water body on earth and is surrounded by Russia, Kazakhstan, Turkmenistan, Iran and Azerbaijan. It serves as a main habitat for four important commercial sturgeon species which in general live exclusively in the northern hemisphere. Stellate sturgeon (*Acipenser stellatus*) is typical in northern parts of the Caspian Sea however; it may regularly be encountered in southern parts of this water body as well (Khodorevskaya et al., 1997). Based on available data the annual catch of sturgeon in the Azov Sea and the Caspian Sea, which is almost 90% of the world landings was approximately 24000–25000 tons in 1970–1985 but these figures have dropped below 2000t in 1999 (Billard and Lecointre, 2001). The main cause of this drastic decline is claimed to be over-fishing, environmental degradation (river fragmentation, dam construction across the rivers, water and sediment pollution) which in fact disturbs the migratory routs and consequently reproduction potential of the sturgeons (Billard and Lecointre, 2001). Among these, heavy metals have long been recognized as serious pollutants of the aquatic environment. They impose serious damage to metabolic, physiological and structural systems of organisms when present in high concentrations in the environment. Metals such as Zinc and Copper are essential elements for normal metabolism of aquatic organisms in low concentrations, while mercury is nonessential without any recognized role in aquatic systems (Canli and Atli, 2003). They may have direct effects on organisms by accumulating in their body or indirectly through food web to the next trophic level. One of the most serious consequences of this transfer is biological amplification through the food chain (Unlu et al., 1993). In an aquatic environment, heavy metals are easily taken up in dissolved form by organisms. These metals are then strongly bound with sulfhydril groups of proteins and accumulate in tissues of the organism (Hadson, 1988; Kargin, 1996) which may result in chronic illness damage to the population (Barlas, 1999).

Concentrations of heavy metals in water samples, sediments and benthic organism from Volga River has increased to a great extent (Karpinsky, 1992). Several studies have reported high concentrations of heavy metals in fish species (Karpinsky, 1992; Watanabe and Tanabe, 2003) and seals (*Pusa caspica* ) of the Caspian Sea (Anan et al., 2002; Watanabe et al., 2002; Ikemoto et al., 2004). However, status of multi elemental contamination in sturgeons of the Caspian Sea is not well known. In the present study, we measured concentration of Cu, Zn and Hg in muscles and liver of stellate sturgeons and discussed the results in relation to location, age, fish health, hepatosomatic index (HSI) and condition factor (CF). Public health risks associated with consumption of fish were estimated based on PTDI and PTWI and tested with values recommended by globally recognized institutions.

## Materials and methods

The study was conducted in winter and spring of 2007 to 2008 in the southern part of the Caspian Sea. The study area and

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sampling sites are shown in figure 1. Two sampling sites (1 and 2) were selected in south- west and 2 sampling sites (3 and 4) in south east Caspian Sea. Sampling sites were located between Talesh (37° and 46" north latitude and 48°and 59" east longitude) and Torkaman (36° and 50" north latitude and 53°and 34" east longitude).

A total of 40 stellate sturgeons were collected using gill nets from various depths in the Caspian Sea.

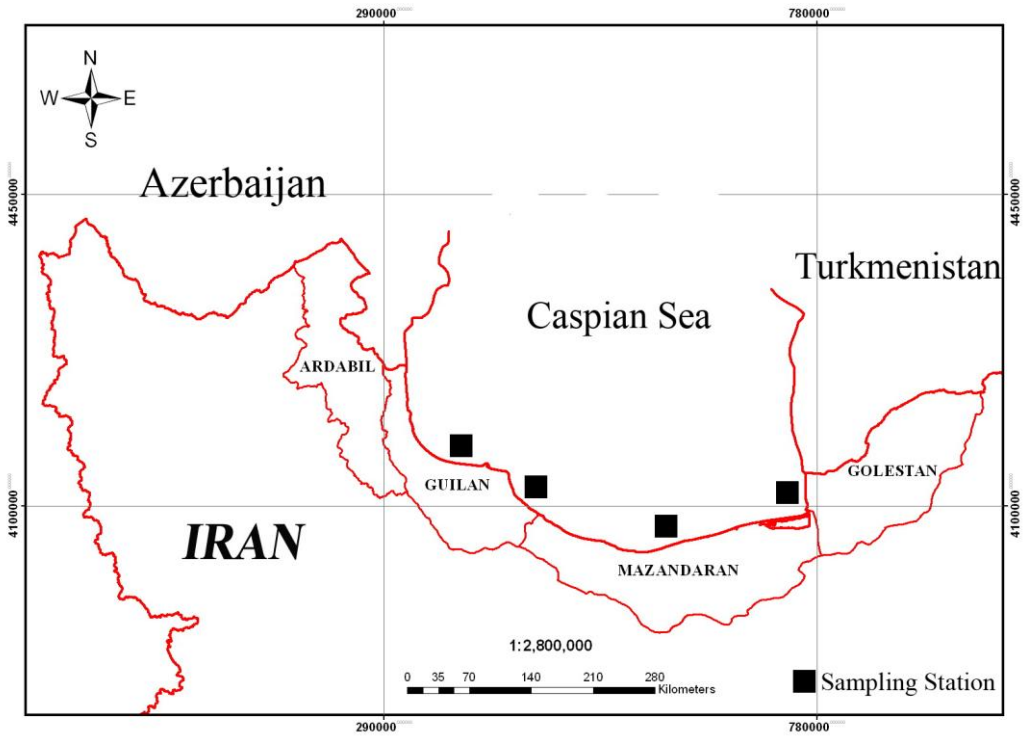



**Figure 1: Sampling sites of stellate sturgeon in the Caspian Sea. Fish samples were collected from the locations with squares**

Cross sections (0.4 mm thickness) of first ray of pectoral fins were used for age determination. Muscle (250g) and liver samples (number of liver samples was 20) were taken and stored at -20 °C for further analyses. The procedure used to determine metal concentration has been described by Anan et al., 2001. Muscle and liver samples were dried at 80°C for 24h and homogenized. The homogenized samples were then digested in an oven with $HNO_3$ and $HClO_3$ acids. Thermo Electron Corporation Flame Spectrophotometer was used to determine Cu and Zn values and mercury samples were read by cold-vapor technique using $SnCl_2$ as a reluctant. Accuracy of the measurement was tested against DORM2 (National Research Council Canada). Values were presented on a dry weight basis.

Moisture content of the muscle samples was 76.5 ± 2.3%. A conversion factor of 4.25 was used for conversion of dry weight results to wet weight basis. Condition factor (CF) and hepatosomatic

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index (HSI) were calculated using the following formulas:

$CF= W/L^3 \times 100$

Where W= fish weight (g) and L= total length (cm) (Hung et al., 2002)**;**

$HSI= w/W \times 100$

Where w= liver weight (g) and W= fish weight (g) (Wahli et al., 2002).

*Statistical analysis*

Condition factor (CF) and hepatosomatic index (HSI) and Relative weight ($W_r$) were calculated using the following formulas:

$CF= W/L^3 * 100$

Where W= fish weight (g) and L= total length (cm) (Hung et al., 2002)**;**

$HSI= w/W * 100$

Where w= liver weight (g) and W= fish weight (g) (Wahli et al., 2002).

Normal distribution of data was examined with Kolmogorov- Smirnov's test. Log normals were used where data were not distributed normally. Pearson's correlation was used to examine the relationship between fish length, weight, CF and HSI. One- way ANOVA was used to examine metal concentration in fish tissues. Effect of location on metal concentration was examined by ANCOVA with body length as covariate. All analyses were performed in SPSS (Ver. 14) at 95% confidence level and graphics in excel (2003).

## Results

Sturgeon specimens were in the range of 3-17 years in age. Their mean weight was 730- 13000 g and mean length 54- 143 cm FL (62- 163cm TL). The majority of fish were sexually mature and male/female ratio was 1.3: 1. The sequence of metal accumulation was Zn> Hg>Cu in muscle and Zn> Cu>Hg in liver samples. The condition factor (CF) and Hepatosomatic index were 0.56± 0.06 and 0.7± 0.06 in south-west Caspian Sea **(**Guilan Province) respectively. The condition factor (CF) was 0.27± 0.009 in south-east Caspian Sea (Golestan Province). Table 1 shows biological data of specimens and table 2 metal concentrations (Min, Max and Mean ± SD) in muscle and liver samples. Lead and Zinc showed the highest and lowest accumulation in both muscle and liver.

**Table 1: Biological data of examined fish specimens in south-west and south-east Caspian Sea (Dec. 2007- June 2008)**

| **Guilan** | | **Golestan** | |
|---|---|---|---|
| **Length TL. (cm)** | **Weight (gr)** | **Length TL. (cm)** | **Weight (gr)** |
| **n=20** | **n= 20** | **n=20** | **n= 20** |
| **Max= 154** | **Max= 13000** | **Max= 163** | **Max= 11000** |
| **Min= 62** | **Min= 730** | **Min= 120** | **Min= 3800** |
| **Mean± SD= 130.35± 27.9** | **Mean± SD= 7793± 3612** | **Mean± SD= 137.9 ± 12.7** | **Mean± Sd= 7100 ± 2099.3** |

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**Table 2: Trace element concentrations (µg/g dry wt.) in muscle and liver of stellate sturgeons from the Caspian Sea (Guilan and Golestan Provinces, Dec.2007-June 2008)**

| Muscle samples | Zn | Cu | Hg |
|---|---|---|---|
| **Guilan** | | | |
| n=20 | | | |
| Min | 1.075 | 0.007 | 0.241 |
| Max | 5.857 | 0.819 | 3.48 |
| Geometric mean | 3.818 | 0.554 | 2.18 |
| SD | 1.075 | 0.201 | 0.35 |
| | | | |
| **Liver samples** | | | |
| n=20 | | | |
| Min | 0.293 | 0.324 | 0.096 |
| Max | 7.128 | 3.641 | 2.221 |
| Geometric mean | 5.456 | 1.166 | 1.13 |
| SD | 1.359 | 1.166 | 0.24 |
| | | | |
| **Golestan** | | | |
| **Muscle samples** | | | |
| n=20 | | | |
| Min | 0.04 | 0.159 | 0.074 |
| Max | 60.233 | 0.476 | 7.853 |
| Geometric mean | 7.612 | 0.384 | 1.78 |
| SD | 12.61 | 0.07 | 0.48 |

The highest concentration of Cu (3.641± 1.166 ppm) was observed in liver samples in Guilan Province while the highest concentrations of Zn with 60.233 ±12.61 ppm and Hg with 7.853± 0.48 ppm were observed in muscles of fish caught in Golestan Province. Significant differences were observed in concentrations of Cu in two provinces ($p<0.05$).

There were no significant differences in metal concentrations between Talesh and Anzali in liver samples. Regardless of sampling sites and fish sex significant negative correlation was observed with age and Cu accumulation in muscles ($p<0.05$; $r= -0.4$; Figure 2) while in liver samples Cu accumulation positively correlated with age ($p<0.05$; $r= 0.4$; Figure 3). Zn accumulation in muscle samples also showed positive correlation with age ($p<0.05$; $r=0.3$; Figure 4).

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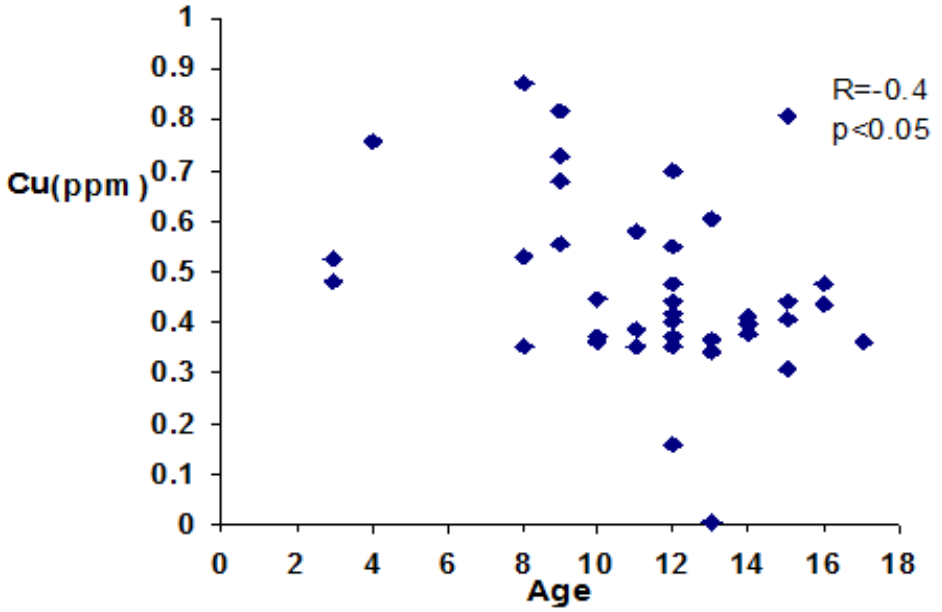



**Figure2: Concentration of Cu in muscle samples of *A. stellatus* in different ages (Dec.2007-June.2008)**

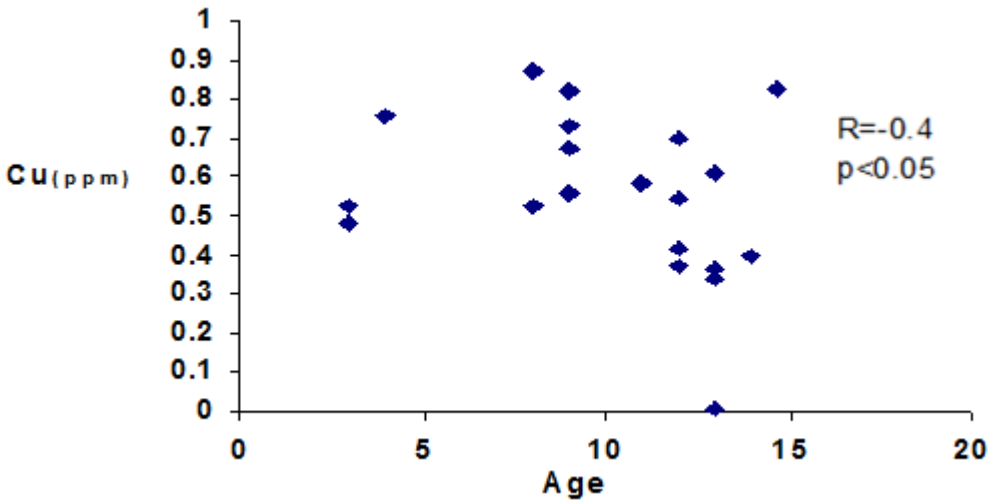



**Figure 3: Concentration of Cu in liver samples of *A. stellatus* in different ages (Dec.2007-June.2008)**

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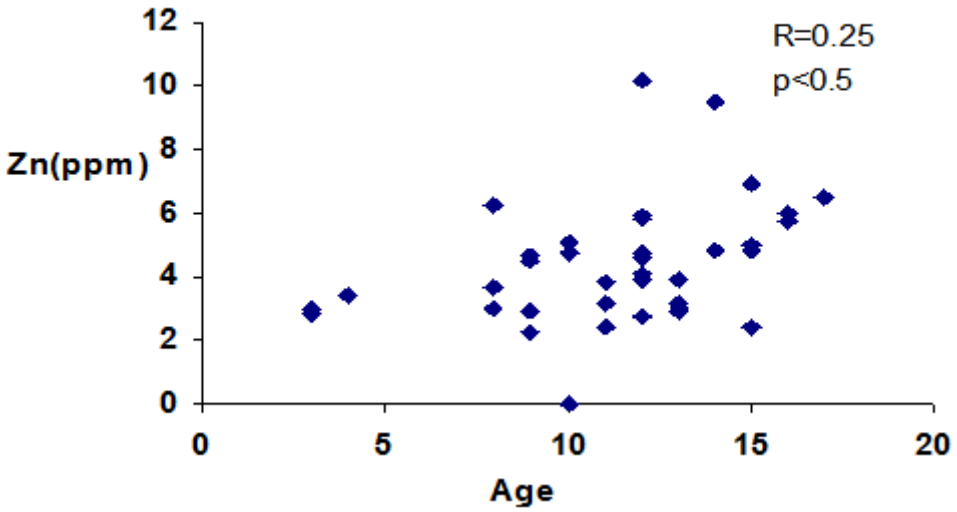



**Figure 4: Concentration of Zn in muscles samples of *A. stellatus* in different ages (Dec.2007-June.2008)**

A positive correlation was observed between concentration of Cu and condition factor (R=+0.2, p<0.05). Regardless of fish sex there was a very strong and negative significant interaction between muscle concentration of Zn (R=-0.9, p<0.05). A positive interaction between Cu and Zn (r=0.40) and negative interaction between Cu (r= -0.3) were also observed. There was no correlation between concentration of metals in liver and hepatosomatic index.

## Discussion

The present study showed higher accumulation of Zn and Cu in liver as compared to muscle tissues. It is generally related to physiological activities of organs, for instance Cu and Zn are involved in enzyme activities in liver, consequently higher quantities of these elements are accumulated in liver (Sobolev,. 2005; Henry et al., 2004). On the other hand Hg shows high tendency to bind with muscle proteins such -OH, -COOH, -$NH_2$, - SH (Golovanova, 2008). It is well documented that levels of heavy metals vary in fish depending on factors such as habitat and migration (Romeo et al., 1999; Andres et al., 2000; Canli and Atli, 2003). Stellate sturgeon feeds more on benthos as compared to other sturgeon species (Karpinsky et al., 1992) therefore heavy load of metals in sediment and benthic suspension feeders which accumulate metals in their body may significantly influence metals concentration in this species. Fish age and related feeding activities also determines the extent of metal accumulation in fish. Marmulla et al., 1990 reported a negative correlation between Cu and age suggesting that lesser feeding activities corresponded with higher age resulting in lower levels of

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metal accumulation. Heavy loads of pollution into the Caspian Sea resulting from oil extraction activities, shift in prey selection or developmental stages of fish may be responsible for these findings. As for fish size we obtained similar results to Honda et al., 1983 which reported negative correlation between P*agothenia borchgrevinkiv* fish size and accumulation of Cu. Al- Yousef et al., (2000) also reported similar results in their studies on *Lethrinus lentjan*. However this trend is not true for Zn in liver tissue where metal concentration increases with fish age.

Another issue which controls metal accumulation is an organism is species uptake, detoxification and elimination mechanisms which depend significantly on the size- specific metabolic rate of organism (Farks, et al., 2003), Thus the negative correlations between concentration of metals and fish size do not necessarily mean that there is a particular metal concentration at the beginning of the growth period and no new metal is further absorbed. The negative relationship between heavy metal concentrations and condition factor suggests relative dilution effects of lipid content of target tissue (Farks et al., 2003). Mac Donald et al. (1997) studied concentrations of Ag, Cr, Cu, Zn and Hg in muscles of *Acipenser transmontanous*. Table 3 compares our findings with other authors. The differences could be related to pollution load of the study area, biological characteristics, migratory and feeding behavior of the organisms even within the same study area (Canli et al., 2003). Bemis et al. (1997) believe that migration either for reproduction or feeding influences metal accumulation in fish. Agusa et al., 2004 studied concentration of heavy metals in sturgeons of the Caspian Sea and their results are presented in table 3. In the present study we collected fish only from Iranian waters therefore we did not have access to fish from northern parts of the Caspian Sea. Copper and Zinc concentration in sediments from Iranian parts is higher than other riparian countries of the Caspian Sea however their concentrations were not considerable (de Mora et al. 2004 ) .Concentration of mercury in sediments from Iranian part is lower than other countries around the Caspian Sea. Although concentration of Hg in sediments of Iranian basins is lower that other countries, however concentration of this metal in muscles is high. This could be due to the rather long half life of Hg in the form of Methyl mercury in body (700-1000 days) in comparison to other metals. On the other hand concentrations of Cu and Zn in sediments of Iranian basins are much higher but their concentrations in muscles are not significant which is similar to de Mora et al. 2004. Probably reduced feeding prior to reproduction migration or feeding migration elsewhere in the Caspian Sea are responsible for this result. As shown in table 4 concentrations of heavy metals Cu, Zn and Hg were lower than the standard and permitted levels set up by EEC but concentrations of Hg were higher than the standard and permitted levels set up by MAFF at the time of this study.

According to FAO (2009) reports, the per capita fish consumption in Iran is 6400g or 18g/day and 126 g/week. The

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estimated daily and weekly intake (EDI and EWI) for an adult person (mean weight= 70 kg) presented in table 5 shows that the EDI and EWI indexes are below the standards (Türkmen et al., 2009; FAO/WHO.2006) therefore stellate sturgeon consumption does not pose threat for human health at the time of this study.

**Table 5: Estimated permissible daily and weekly intake of metals for a mature man based on Stellate sturgeon consumption**

| Species | Cu | Zn | Hg | References |
|---|---|---|---|---|
| **Caspian Sea** | | | | |
| Stellate sturgeon | 0.47 | 5.8 | 2 | this study |
| Stellate sturgeon* | 0.1 | 1.3 | 0.4 | this study |
| Stellate sturgeon | 1.5 | 20.3 | 0.06 | Agusa *et al*(2004) |
| Beluga | 1.42 | 20.2 | 1.4 | Agusa *et al* (2004) |
| Persian sturgeon | 1.74 | 21.7 | 0.33 | Agusa *et al* (2004) |
| Russian sturgeon | 1.73 | 22 | 0.32 | Agusa *et al* (2004) |
| Caspian roach | 0.26 | 18.8 | 0.2 | Anan *et al*(2005) |
| Caspian spart | 1.94 | 57.5 | 0.05 | Anan *et al*.(2005) |
| **Fraser River, Canada** | | | | |
| White sturgeon | 1.36 | 14.2 | 3.37 | MacDonald *et al* (1997) |
| **Gulf and Gulf of Oman** | | | | |
| Orange spotted | 2.18 | 21 | 0.99 | de Mora *et al.(*2004) |
| Spangled emperor | 0.66 | 8.28 | 0.05 | de Mora *et al*.(2004) |
| **Taipei Market, Taiwan** | | | | |
| Sea perch | 1.3 | 38 | 1.045 | Han *et al.(1998)* |
| Milkfish | 3.06 | 60.5 | 1.96 | Agusa *et al* (2004) |
| Tilapia | 1.56 | 64 | 2.54 | Agusa *et al* (2004) |
| Carp | 4.85 | 121 | 2.41 | Agusa *et al* (2004) |
| **Nouakhott, Mauritania** | | | | |
| Peseudupeneus | 0.66 | 13 | 0.14 | Romeo *et al* (1999) |

a: Provisional Permissible Tolerable weekly intake in µg/week/kg body weight

b: PTWI for 60 kg adult person (µg/week/70 kg body weight)

c: PTDI, provisional permissible tolerable weekly intake (µg/week/70 kg body weight)

d: (Türkmen *et al*., 2009)

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# تجمع زیستی فلزات سنگین Cu, Zn, Hg در عضله و کبد ازون برون (*Acipenser stellatus*) دریای خزر و ارتباط آن با پارامتر های رشد

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**چکیده**

تجمع فلزات سنگین Cu, Zn و Hg در عضله و کبد ازون برون (***Acipenser stellatus***) قسمت جنوبی دریای خزراندازه گیری شد. تاثیر فاکتور وضعیت (CF)، سن و شاخص کبد (GSI) بر روی تجمع این فلزات بررسی شد. تعداد 40 عدد ماهی با استفاده از دام گوشگیر در طول زمستان و بهار 1386 و 1387 از سواحل جنوبی دریای خزر تهیه شدند. طول کل (TL) و طول چنگالی (FL) تمامی ماهیان و وزن آنها تعیین شد. برش عرضی از اولین شعاع سخت باله سینه ای تهیه و برای تعیین سن بکار گرفته شد. نمونه های عضله و کبد تهیه شده و پس از انتقال به آزمابشگاه جهت برای بررسی ها ی بعدی در دمای 20- سانتیگراد نگهداری شدند. در آزمایشگاه نمونه ها در داخل آون خشک و با استفاده از $HClO_3$ و $HNO_3$ هضم شد. سپس حجم نمونه ها با استفاده از آب دیونیزه به $50^{cc}$ رسانده شده و غلظت فلزات Cu, Zn و Hg با استفاده از فلیم اسپکترومتر تعیین شد. ترتیب فلزات در بافت عضله ازون برون بصورت Zn>Hg> Cu و در بافت کبد بصورت Zn> Cu> Hg بود. غلظت Zn همبستگی جزئی با سن نمونه ها نشان داد (R= 0/3). همبستگی منفی بین غلظت Zn فاکتور وضعیت (R= -0/3) مشاهده شد. نتایج بدست آمده با استاندارد ها ی بین المللی MAAF و NHMRC مقایسه شدند. بررسی حاضر نشان داد که تجمع فلزات سنگین در ازون برون تحت تاثیر غلظت این فلزات در رسوبات و نیز شرایط فیزیولوژیک ماهی می باشد.

**واژگان کلیدی:** ازون برون، فلزات سنگین، فاکتور وضعیت، شاخص کبدی، کبد، عضله

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