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TRACE ELEMENTS ACCUMULATION IN EDIBLE TISSUES OF FIVE STURGEON SPECIES FROM THE CASPIAN SEA

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Abstract. This study is focused on twenty trace elements (Ag, Ba, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Mn, Mo, Pb, Rb, Sb, Sn, Sr, Ti, V, Zn) accumulation in muscles of five sturgeon species *(Acipenser guldenstaedti, A. persicus, A. nudiventris, A. stellatus* and *Huso huso*) from the southern part of the Caspian Sea. Moreover the relationships between some biological characteristics and the levels of the selected elements as well as inter-elemental relationships were assessed. The samples (10 specimens for each the five species) were collected from two important sturgeon fishery zones located in the Iranian part of the Sea in 2002. Concentrations of the elements were determined using ICP-MS. Only in the case of Cs could significant differences between two selected sampling areas be detected. There were significant differences in levels of Co, Ga, Rb, Sn, Ti, Pb and Bi in muscles of the species. Only in the case of Cd weight related differences among the species were found. The significant length dependent relationships were observed for Ga and Ba. Patterns of elements accumulation were assessed by statistical methods and compared to the other researches. In all the cases, the amounts of toxic trace elements (Cd, Cu, Pb and Zn) were markedly below the international guidelines for human consumption.

Keywords: Caspian Sea, sturgeon, trace elements

1. Introduction

During the past forty years, especially in the last decade, the levels of pollutants (including heavy metals, pesticides, petroleum hydrocarbons and ...) in the Caspian Sea have increased, subsequently the anthropogenic pressures on the coastal and marine ecosystems have grown progressively. Since the Caspian Sea is a closed basin, residence time for contaminants is relatively long and this may be one of the reasons for increasing amounts of pollutants. On the other hand as the Caspian Sea is a shared water body, activities in one riparian country have potential to affect the environment in the other countries. Pollution of the Caspian Sea is a serious issue and one that was raised during the Soviet regime. Untreated wastes and pollution from industrial and oil field activities have been reported in a number of journals and reports (Rich, 1972; Anon., 1988; Glantz and Zonn, 1997; Anon., 1998c).

Caspian Sea supplies food, water, industrial opportunities, and oil and gas to its surrounding nations. Considering the vital role of the Caspian in the lives of millions of people, its increasing pollution is now one of the major concerns of environmentalists worldwide and in particular in the Caspian basin countries (Shaw *et al*., 1998).

The main sources of pollution of the Caspian Sea can be divided into two major sources including land-based and offshore sources. The major land-based sources of pollution to the Caspian Sea enter the sea via industrial and domestic wastewater. Wastewater entering the sea includes warm water from power stations, water from desalinating facilities, treated and untreated water from domestic and industrial factories, contaminated sludge, and runoff from industrial and agricultural activities. Leakage of oil from offshore oil production is a major source of Caspian Sea pollution (Anon., 1998b).

Heavy metals are important for the ecology of the Caspian because they do not decompose, only change chemical bonds. Metals thus gradually accumulate in the sea, in sediment, and in living marine organisms (DHI, 2001).

The major part of the Caspian's southern section, with an area of 155,512 square kilometers belongs to Iran, which is 36% of the whole area of the Caspian Sea (Hamshahri, 1995).

Traditionally the Caspian Sea is regarded as the sea of sturgeon species. The Caspian Sea is the only one over the world where considerable shoal of sturgeons is preserved. The Caspian lake output of sturgeon and caviar is more than 90 percent of their world output. During the last twenty years, however, the sturgeon catch has declined by 88 percent. Six species of sturgeon exist in the Caspian, belonging to the genera of *Huso* and *Acipenser*. Twenty-five sturgeon species and sub-species have been identified so far, from which only three species produce caviar, all living in the Caspian Sea (*Huso huso, Acipenser guldenstaedti, A. stellatus*). Currently, the main source of caviar has been from sturgeon caught in the southern part of the Caspian off the Iranian coast (Anon., 1994; Dumont *et al*., 1997; Anon., 1998a; Anon., 1998c; Clark, 2001; Anon., 2003). Whereas elevated levels of heavy metals have been observed in the blood and tissue of Caspian Sea sturgeons, very few studies have yet been conducted on the status of trace element contamination in these fishes (Karpinsky, 1992).

The primary objective of this study was to measure the levels of different trace elements in the edible tissue (muscle) of the five sturgeon species and to compare them with the guidelines for human consumption. The other main purpose of this investigation was to provide reliable data, which can be compared with previous or future results from other parts of the world. Before conducting the present study, no relatively comprehensive research on trace elements accumulation in sturgeons from southern part of the Caspian Sea has been done. Hence, the results of this project can be very important from health of seafood consumers point of view. The importance of these kinds of studies will be realized if it be taken into consideration that several species of sturgeon are considered threatened with extinction. As a

Figure 1. Map of the Caspian Sea showing area of sample collection. ∗ Golestan catch area ⊙ Mazandaran catch area.

consequence, sturgeon species have been included in the CITES (the convention on international trade in endangered species of wild fauna and flora). This study was conducted in the framework of The Ecotoxicology Project (ECOTOX).

2. Material and methods

2.1. Study area

The areas of sample collection were located in southeastern part of the Caspian Sea (Figure 1). Fisheries Company of Iran has divided the whole area of sturgeon fishery into five zones. The samples were collected from two sturgeon fishery zones in Mazandaran and Golestan Provinces during January and February 2002. These two zones were selected because all the five sturgeon species could be collected at the same time. Moreover, the annual catch from these two zones was markedly higher compared to the other zones.

2.2. Sampling, dissection and transportation

The total number of collected samples was 50. From each of the five sturgeon species (*A. guldenstaedti, A. persicus, A. nudivemtris, A. stellatus and H. huso*) 10 specimens were collected (5 specimens from each sampling area). The specimens were caught using gill net with standardized mesh and dimensions set by Iran Fisheries Research Organization. The biological characteristics of the specimens including total length, weight and age sample were recorded. Individuals were dissected using high quality stainless steel instruments on a clean glass working surface to separate some parts of their muscles. Muscles were separated according to the methods recommended by UNEP (1984). Muscle samples were put in clean dry polyethylene bags and frozen at approximately –20 °C as soon as possible and then shipped to the laboratory (Ehime University, Japan).

2.3. Analytical methods

The general procedure used for measurement of the trace elements levels has been described previously (Yasunaga *et al.*, 2000, Anan *et al.*, 2002). Double distilled deionized water was used throughout the study. All glassware was carefully cleaned with nitric acid followed by rinsing with distilled water. Muscle samples were dried at 80 °C for 12 hr. Pulverization and homogenization were achieved by grinding in a Teflon mortar. About 0.1 g of powdered sample was digested in microwave using suprapure nitric acid (1.5 ml) in a Teflon PTFE tube. Concentrations of 20 trace elements (Ag, Ba, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Mn, Mo, Pb, Rb, Sb, Sn, Sr, Ti, V, Zn) were measured by inductively coupled plasma-mass spectrometry (ICP-MS) (Hewlett-Packard, HP-4500). Accuracy of analyses was examined using standard reference materials, SRM 1577b (National Institute of Standards and Technology, USA) and DORM2 (National Research Council Canada). In the present study, concentrations are expressed on wet weight basis.

2.4. Data analyses

Goodness of fit test was employed to test the null hypothesis that the samples had come from a normal population (Daniel, 1977; Rees, 1991; Zar, 1999). Since the null hypothesis was acceptable, parametric statistical methods could be applied. To detect homogeneity (homoscedasticity) of variances Bartlett's test (Sokal and Rohlf, 1981; Zar, 1999) was used and because the variances were heterogeneous, Tylor's power law (Green, 1979) applied to determine appropriate data transformation. As a result all data were ln (n + 1) transformed.

Eighteen 2-way analysis of covariance (ANCOVA) were used to test for significant differences in each trace element bioaccumulation (5 species $\times$ 2 sampling sites) and to assess the influence of total length, weight and age (as covariates). Seven Duncan's new multiple range tests (Steel *et al.*, 1997; Zar, 1999) were used to determine which group means did not differ from one another. The concentrations of the elements in muscles were compared statistically by means of one-way ANOVA and Duncan's new multiple range test. Three one-way ANOVAs were also used to test the null hypothesis that there were no significant differences among the species, from the biological characteristics (length, weight and age) point of view, followed by Duncan's multiple range tests. Pearson's correlation coefficients were used to examine relationships between the elements as well as between elements and the biological characteristics. Two hierarchical cluster analyses (using

Euclidean measures) were used. One of them to group the species based on accumulation of all the elements in muscle and the other to classify the elements on the basis of the levels in muscles.

3. Results

3.1. Effects of different variables on elements bioaccumulation

Values of In and Sb in all the cases except for concentration of Indium in one of the *H. huso* samples from Golestan catch area (0.002 μg g^{-1} wet weight) were less than the detection limits (< 0.001 μg g^{-1} wet weight). Thus, these data could not be considered in the statistical analyses. Based on the results presented in Table I, it can be concluded that:

(a) No age related differences were found for each species in bioaccumulation of the elements.
(b) Only in the case of Cd weight related differences (negative) could be observed (P = 0.038).
(c) Length dependent relationships were observed for Ga (P = 0.040) and Ba (P = 0.042). In both cases, relatively weak and negative correlations (Pearson) could be observed, namely the levels of the elements in the muscle slightly decreased with increasing fish size.
(d) Only in the case of Cs significant differences (P $\leq$ 0.001) between the selected sites could be detected. In the case of all species, mean Cs concentration in samples from Golestan region was higher compared to Mazandaran area (Figure 2).
(e) There were significant differences among the species for the accumulation of Co, Ga, Rb, Sn, Ti, Pb and Bi in muscle.

In order to determine which species can be categorized in one group (with regards to contents of each metal in muscle), several Duncan's new multiple range tests were conducted (Table II).

Generally, based on Tables II and III patterns of the elements occurrence in the muscle can be summarized as follows:

Co: *A. guldenstaedti, A. nudiventris, H. huso* > *A. stellatus, A. persicus*
Ga: *A. nudiventris, A. stellatus* > *H. huso, A. guldenstaedti, A. persicus*
Rb: *A. guldenstadti, H. huso, A. stellatus* > *A. persicus, A. nudiventris*
Sn: *A. guldenstaedt* > *H. huso* > *A. stellatus, A. persicus* > *A. nudiventris*
Ti: *H. huso, A. persicus* > *A. guldenstaedti, A. nudiventris, A. stellatus*
Pb: *A. stellatus* > *A. persicus, H. huso, A. guldenstaedti, A. nudiventris*
Bi: *A. guldenstaedti* > *A. stellatus, A. nudiventris, H. huso, A. persicus*

Figure 3 shows the dendrogram derived by average linkage clustering of the five species. Arbitrary dashed lines have been used to differentiate the clusters. Comparison among species with respect to all the trace elements indicates at a

TABLE I

Results of 18 two-way ANCOVAs testing species and sites as well as their interactions on accummulation of each element in muscle of five sturgeon species. Weight, length and age used as covariates

Source of variation	DF	V	Cr	Mn	Co	Cu	Zn	Ga	Rb	Sr	Mo	Ag	Cd	Sn	Cs	Ba	Ti	Pb	Bi
		F-ratios																	
Weight	1	0.336	0.054	0.029	0.612	0.744	0.854	0.411 *	0.091	1.595	0.007	0.156	4.618 *	0.132	3.462	0.137 *	0.001	1.450	1.592
Length	1	0.129	0.410	0.342	0.007	0.236	0.444	4.545	0.049	2.807	0.003	0.038	1.207	0.241	2.141	0.850	0.163	2.567	1.294
Age	1	1.146	0.520	0.502	0.154	3.969	0.396	2.857	0.082	1.426	0.280	0.407	2.210	0.293	1.252 ***	2.521	0.594	1.313	0.152
Site	1	0.313	0.046	0.428	3.522 ***	0.967	0.421	0.496 *	3.364 *	0.004	0.085	0.618	1.677	1.546 ***	21.422	0.643	0.025 *	1.174 ***	0.258 *
Species	4	0.675	0.392	2.152 *	6.390 **	1.373 *	0.132	4.462	3.247	1.164	2.254	0.879	0.889	7.225	2.129 ***	1.145	3.092	14.448	4.921
Site × Species	4	0.550	1.459	2.865	4.715	3.171	1.603	0.381	1.040	0.627	0.367	2.115	0.387	0.932	6.015	0.365	0.237	0.994	0.279

Sigificant variance ratios (F) are indicated by: * at $P \leq 0.05$, ** at $P \leq 0.01$ and *** at $P \leq 0.01$.

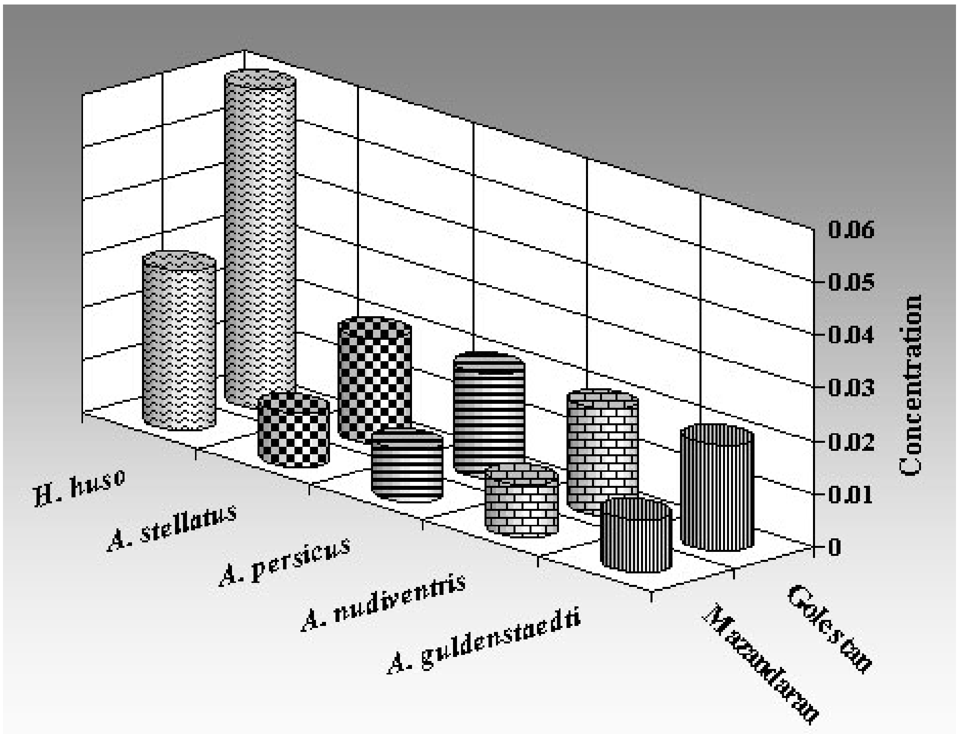

Figure 2. Comparison of mean Cs levels (μg g^{-1} wet weight) in muscle of the sturgeon species collected from two catch areas (Golestan and Mazandaran).

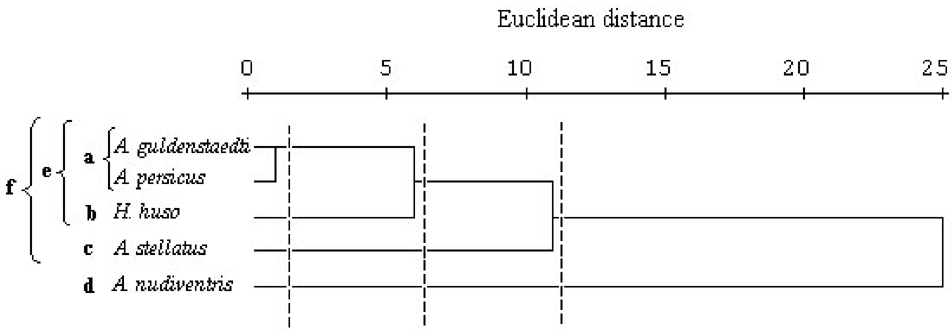

Figure 3. The dendrogram for hierarchical cluster analysis of the five sturgeon species based on concentrations of 18 trace elements in muscle. The vertical dashed lines show the arbitrary division lines for defining clusters.

TABLE II

Results of seven Duncan's new multiple range tests to determine the species can be classified in one group. Minimum, maximum and standard deviation (in parentheses) of the elements concentrations also presented (in μg g^{-1} wet weight). The elements, which have a common letter are not significantly different from one another

Elements	*Acipenser guldenstaedti*	*Acipenser nudiventris*	*Acipenser Persicus*	*Acipenser Stellatus*	*Huso huso*
Co	a	a	b	b	a
	0.005–0.019	0.001–0.027	0.001–0.005	0.001–0.006	0.004–0.014
	(0.004)	(0.008)	(0.001)	(0.002)	(0.003)
Ga	b	a	b	ab	b
	0.003–0.013	0.007–0.074	0.001–0.020	0.001–0.067	0.002–0.021
	(0.003)	(0.025)	(0.006)	(0.021)	(0.007)
Rb	a	c	b	ab	ab
	2.110–4.180	1.370–2.950	2.000–3.130	2.420–3.150	2.250–3.480
	(0.614)	(0.543)	(0.341)	(0.256)	(0.445)
Sn	a	d	cd	c	b
	0.028–0.127	0.001–0.033	0.001–0.046	0.001–0.039	0.027–0.063
	(0.029)	(0.010)	(0.016)	(0.012)	(0.011)
Ti	b	b	a	b	a
	0.001–0.002	0.001–0.002	0.001–0.019	0.001–0.001	0.001–0.010
	(0.0005)	(0.0003)	(0.006)	(0.0000)	(0.003)
Pb	b	b	b	a	b
	0.004–0.015	0.001–0.017	0.001–0.022	0.004–0.067	0.001–0.019
	(0.003)	(0.005)	(0.006)	(0.020)	(0.007)
B	a	b	b	b	b
	0.018–0.143	0.008–0.056	0.001–0.015	0.001–0.137	0.007–0.034
	(0.041)	(0.015)	(0.006)	(0.038)	(0.009)

distance about 1, four distinct clusters: **a** *(A. guldenstaedti* and *A. persicus)*, **b** (*H. huso*), **c** (*A. stellatus*) and **d** (*A. nudiventris*). At a higher distance (about 6) clusters **a** and **b** fuse, forming a single cluster (**e**). The same case can be observed at a distance about 11 (linkage of clusters **a**, **b** and **c** and forming cluster **f**). Generally, the major differences (maximum distance) are in clustering of *A. nudiventris* and the other species. Whereas, the major similarities (the minimum distance) can be observed between *A. guldenstaedti* and *A. persicus*.

Table IV summarizes the results of several statistical analyses (one-way ANOVA and Duncan's new multiple range test) concerning biological characteristics of the specimens.

TABLE III

Mean (± SD) twenty trace element concentrations (μg g^{-1} wet weight) in muscles of five sturgeon species from the Caspian Sea

	Ag	Ba	Bi	Cd	Co	Cr	Cs	Cu	Ga	In
Acipenser guldenstaedti	0.002 (0.001)	0.061 (0.033)	0.068 (0.041)	0.005 (0.006)	0.009 (0.004)	0.325 (0.113)	0.019 (0.007)	1.912 (0.534)	0.007 (0.003)	ND
Acipenser nudiventris	0.002 (0.002)	0.255 (0.224)	0.024 (0.015)	0.001 (0.001)	0.008 (0.007)	0.369 (0.130)	0.016 (0.005)	1.655 (0.759)	0.027 (0.025)	ND
Acipenser persicus	0.001 (0.001)	0.057 (0.052)	0.008 (0.006)	0.006 (0.004)	0.002 (0.001)	0.314 (0.099)	0.015 (0.007)	1.721 (0.367)	0.005 (0.006)	ND
Acipenser stellatus	0.002 (0.002)	0.183 (0.191)	0.032 (0.038)	0.002 (0.001)	0.003 (0.002)	0.401 (0.085)	0.013 (0.007)	1.224 (0.458)	0.018 (0.021)	ND
Huso huso	0.002 (0.001)	0.082 (0.069)	0.023 (0.009)	0.002 (0.002)	0.007 (0.003)	0.372 (0.093)	0.041 (0.018)	1.772 (0.305)	0.010 (0.007)	ND

	Mn	Mo	Pb	Rb	Sb	Sn	Sr	Ti	V	Zn
Acipenser guldenstaedti	0.458 (0.128)	0.011 (0.005)	0.008 (0.003)	3.128 (0.614)	ND	0.058 (0.029)	1.065 (0.204)	0.001 (0.0004)	0.017 (0.016)	21.640 (7.418)
Acipenser nudiventris	0.476 (0.223)	0.010 (0.017)	0.004 (0.005)	2.213 (0.543)	ND	0.007 (0.009)	1.283 (0.668)	0.001 (0.0003)	0.012 (0.005)	20.410 (7.038)
Acipenser persicus	0.323 (0.057)	0.002 (0.001)	0.012 (0.006)	2.582 (0.341)	ND	0.013 (0.015)	0.908 (0.219)	0.005 (0.006)	0.011 (0.006)	18.810 (3.873)
Acipenser stellatus	0.566 (0.287)	0.002 (0.002)	0.037 (0.019)	2.704 (0.256)	ND	0.024 (0.012)	1.069 (0.208)	0.001 (0.048)	0.017 (0.011)	17.950 (3.684)
Huso huso	0.475 (0.245)	0.006 (0.001)	0.011 (0.007)	2.891 (0.445)	ND	0.042 (0.011)	1.120 (0.597)	1.772 (0.008)	0.048 (0.008)	24.470 (5.052)

ND: Non-Detectable.

3.2. Order of elements accumulation

Based on Figure 4 if an arbitrary vertical line crosses the horizontal lines at a distance about 5, three distinct clusters can be observed (**a, b** and **c**). In other words, the pattern of the metal occurrence in the muscles of the sturgeon species can be written in descending order as follows:

Zn > Rb, Sr, Cu > Mn, Cr, Ba, Bi, Sn, Cs, Ga, Pb, V, Mo, Co, Cd, Ti, Ag

Following the conduction of a one-way ANOVA (testing the null hypothesis that there were no significant differences among concentration of trace elements in muscles of all the sturgeon species)($F = 2533.361$, $P \leq 0.0001$) and a Duncan's new multiple range test, relatively similar pattern was observed:

Zn > Rb > Cu > Sr > Mn > Cr > Ba > Bi, Sn, Cs, Pb, Ga, V, Mo, Co, Cd, Ti, Ag

TABLE IV

Results of 3 one-way ANOVAs testing the null hypothesis that there were no significant differences among the species, from the biological characteristics (length, weight and age) point of view ($P \leq 0.0001$). Mean ($\pm$ SD) of the values are also presented. The values have a common letter are not significantly different from one another (Duncan's new multiple range test) ($P \leq 0.05$)

	Acipenser guldenstaedti	*Acipenser nudiventris*	*Acipenser Persicus*	*Acipenser Stellatus*	*Huso huso*	DF	F-ratios
	ab	b	ab	a	c		
Length	135.7	152.3	144.8	126.3	183.1	4	11.762
(cm)	(16.8)	(22.1)	(12.1)	(14.8)	(29.3)		
	ab	b	ab	a	c		
Weight	15.2	22.5	16.1	7.8	48.5	4	28.737
(Kg)	(5.1)	(7.9)	(2.5)	(2.3)	(18.1)		
	b	b	b	a	c		
Age	14.7	13.6	14.7	11.5	18.9	4	16.362
(year)	(2.4)	(1.0)	(1.5)	(2.4)	(2.7)		

DF: Degrees of Freedom

3.3. Associations among the elements

An analysis of correlation coefficient between element concentration pairs in muscles of the five species revealed a highly significant ($P \leq 0.001$) coupling: Ga – Ba (r = 0.996), Rb – Sn (r = 0.604), Cu – Zn (r = 0.571), V – Cr (r = 0.549), Rb – Ba (r = - 0.547) and Ga – Rb (r = – 0.533). The results shown positively correlated in most cases.

4. Discussion

When fish are exposed to elevated metal levels in an aquatic environment, they tend to take these metals up from their direct environment. The metals enter the body of the fish via the gills and skin, or through the intake of contaminated food or drinking water (kotze *et al.*, 1999). All the metals taken up are not accumulated because fish can regulate metal concentrations to a certain extent, whereafter bioaccumulation will occur. Therefore, the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal (Heath, 1991; Kotze, 1997). In other words, the amount of a metal bioaccumulated is influenced by various environmental, biological and genetic factors, leading to

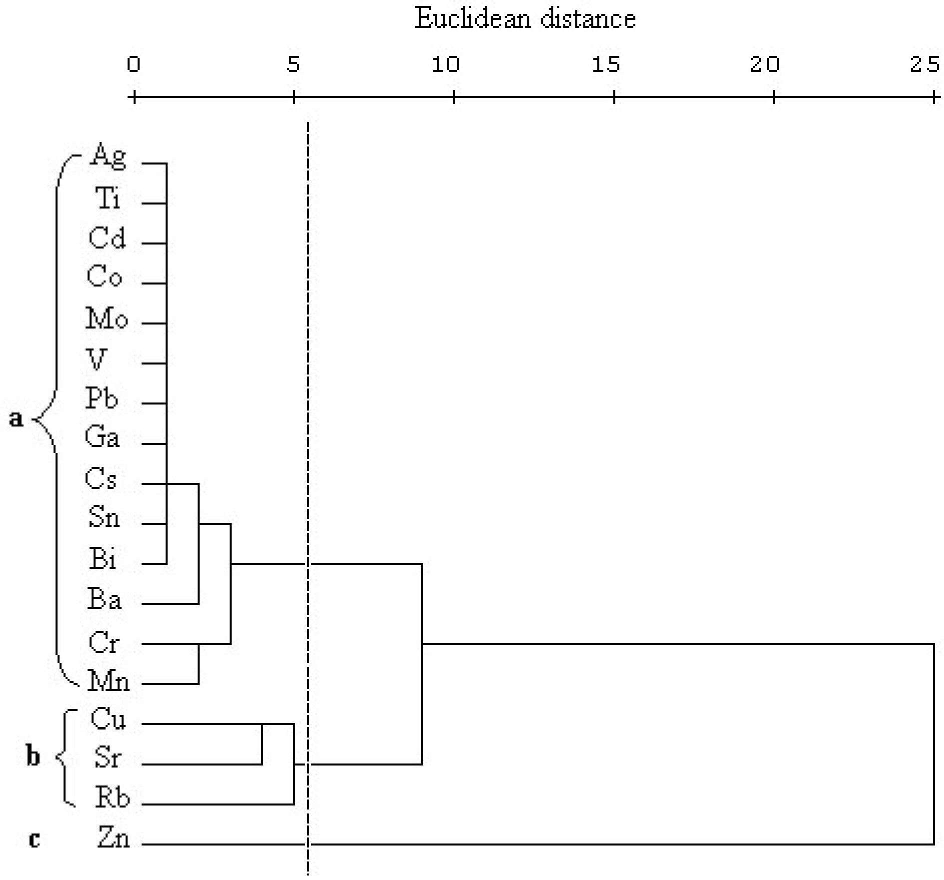

Figure 4. The dendrogram for hierarchical cluster analysis (derived by average linkage clustering) of the trace element levels in muscle of the five sturgeon species. The vertical dashed line shows the arbitrary division lines for defining clusters.

differences in metal bioaccumulation between different individuals, species tissues, seasons and sites (kotze *et al*., 1999).

4.1. Size, weight and age related differences

The relationships between trace element levels in the muscle and the biological characteristics (especially length) have been documented by several investigators. The results varied between the studies. Sometimes even contradictory results have been obtained from different researches. For example, Windom *et al*. (1987) reported that copper in muscle was positively correlated to length of *Coryphaenoides armatus* specimens. But Vas *et al*. (1993) observed a negative correlation between the size and the muscle copper content in several fish species. There were negative correlations between the concentrations of Zn, Cu, Mn, Pb and Al in the muscle of two freshwater fish species (*Clarias gariepinus* and *Labeo umbratus*) from Olifants River (south Africa) and the body length, whereas a significant positive correlation was found in the case of Ni (Coetzee *et al*., 2002). Al-Yousuf *et al*. (2000) reported no clear relationship between the zinc levels in muscle of *Lethrinus lentjan* and fish size. But in the case of copper and cadmium slightly negative and positive relationships were observed, respectively. Nussey *et al*. (2000) found that accumulation of Cr, Mn, Ni and Pb decreased with an increase in the length of *Labeo umbratus* (a cyprinid fish) specimens. As mentioned before, in the present study size dependent relationships (negative) were observed only for Ga and Ba. According to Patrick and Loutit (1978) smaller fish have higher metabolic rates (per gram of body tissue) and therefore are able to take up metals, via food and water, more rapidly than larger fish. The non-existence of a relationship between the other metals concentrations and the length 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 (Paez – Osuna and Ruiz – Fernandez, 1995).

Sophie and Davies (2001) found that in *Nezumia aequalis* (a demersal fish species) the copper content of muscle negatively correlated with weight.

According to Rashed (2001) different trace elements can be classified into three groups from relationship between the concentrations in the muscle of *Tilapia nilotica* and the fish ages: (a) Cu showed increasing tendency with fish age, (b) Cr and Sr were independent of fish ages, (c) Co, Fe, Mn, Ni and Zn increased slightly with fish age. Reduction of element concentration with increasing age was often observed by many other researchers (Medina *et al*., 1986). The new tissues could be formed at a greater rate than metals transported into the tissues to establish a steady state concentration (Vinikour *et al*., 1980).

4.2. Inter-species differences

The interspecies differences in accumulation of the mentioned metals may be arose from species-specific feeding habits as uptake of metals via the food plays an important role (Coetzee *et al*., 2002). Some of sturgeons such as ship *(A. nudiventris*) and Persian sturgeon (*A. persicus*) are mixed feeders. They forage on fishes and bottom invertebrates. The beluga (*H. huso*) feeds on gobies, shads, carps (Ivanov, 1997). Russian sturgeon (*A. guldenstaedti)* is a benthophagous mollusk-eater (Stygar, 1984). *Nereis diversicolor*, along with higher crustaceans represents the main food item of sevruga (*A. stellatus*) (Kashentseva, 2001). On the other side, differences in bioavailability of the elements from surrounding water and sediments can be accounted for the differences among the species in the accumulation of the elements. Bioaccumulation is a function of the bioavailability of contaminants in combination with species-specific uptake and elimination processes (Kennish, 1992). In other words, metal concentrations in a marine organism do not depend on bioavailability alone, but also on the balance between uptake and excretion (Rainbow *et al*., 1990; Weimin *et al*., 1993).

4.3. Inter-site differences

Except the temperature, salinity, dissolved oxygen and pH all exhibit unique patterns of fluctuation, as their amount decrease from the west coast (Port of Astara) toward the east coast (Torkaman Port) (Anon., 1998b). Some researchers (Rowan and Rasmussen, 1993; Wright, 1995; Ke *et al*., 2000) indicated that the bioaccumulation of Cs by fish and other taxa of aquatic organisms was a negative function of the ambient K+ concentration, salinity and suspended sediment concentrations, but a positive function of ambient temperature. With regards to the fluctuation patterns of environmental parameters in the southern part of the Caspian Sea, the difference between the specimens from the two sites in accumulation of Cs can be attributed to the differences in ambient environmental conditions (especially salinity and temperature). Moreover, the possible differences in bioavailability of Cs between the two sites can be cited as another reason for the significant differences in the levels of Cs in the specimens collected from the sites. The partitioning of metals among different phases including the water column, sediment and pore water, affects the bioavailability of metals in an aquatic system. Within each phase the bioavailability determined by several physical, chemical and biological characteristics (Luoma, 1989). In order to gain a better insight into the bioavailability of Cs in the region all the affecting factors should be studied carefully.

4.4. Inter-elemental relationships

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 (Mason and Simkiss, 1983; Paez-Osuna *et al*., 1995). According to Wright (1995) the relationship observed between some of the metal pairs may be a result of a competition or an additional effect on the metal binders and transporters at the cellular level. In the present study, the correlation between Ga and Ba was notably higher than other metals. Hence the relatively similar trends can be expected for them.

4.5. Differences in bioaccumulation patterns between species and elements

With regards to the patterns of elements occurrence in the muscle, no definite trend could be established. Just in the case of Rb and Sn the patterns are relatively similar. This may be due to the relatively similar physicochemical properties. As mentioned before, a highly significant coupling ($P \leq 0.001$, $r = 0.604$) was observed between Sn and Rb.

Although there were significant differences among the species in biological characteristics (length, weight and age) (Table IV), Contrary to our expectation, no relationship could be found between the order (descending or ascending) or grouping of the mean values of the species characteristics (according to Duncan's new multiple range test) and the results of the clustering (Figure 3). This may be attributable to the differences in their diet and lifestyles. The differences in diet (feeding habits) may lead to *A. guldenstaedti* being exposed to larger amounts of trace metals through diet than is the case for other studied species (Sophie & Davies, 2001). Moreover, the possible differences in retention time of the species in polluted waters can be accounted for the differences in the accumulation patterns (Schuhmacher *et al*., 1992).

Table V indicates a general view on the order of trace elements accumulation in muscle of different marine and freshwater fish species. Based on the results, in most cases Zn and Cu show the highest concentration compared to the other studied elements. The reverse case can be observed for Cd and Co. The obtained pattern of the elements accumulation in the present study is in general agreement with those reported by other researchers for other studied fish species (Table V).

4.6. Comparison with guidelines and published data

Table VI presents several guidelines and limits for maximum permissible levels of four heavy metals in seafood. Comparison between the mean concentrations of the four elements in muscle of the species and existing guidelines clearly indicates that the concentrations in all the cases are considerably below the permissible amounts for human consumption.

Comparison between the mean concentrations of the four metals in the muscles of freshwater and marine commercial fishes from the Caspian Sea (Patin, 1982) and the levels obtained from the present study indicates that concentrations of Cu in the sturgeon species are somewhat higher than the marine species, nevertheless considerably below the permissible amounts. As shown in Table V, the mean Zn contents in muscles of the sturgeons are slightly higher than the marine fishes from the sea (especially in the case of *H. huso*).

Pourang (1995) examined the levels of heavy metals (Cu, Zn, Mn and Pb) in seven chosen tissues of two fish species (*Esox lucius* and *Carassius auratus*) from the Anzali wetland (connected to the Caspian Sea). Comparison of the gained data from our study with the mentioned research shows that in case of Cu and Zn the results of the two studies are comparable, while the levels of Pb in muscles of sturgeons are well below the concentrations in the two studied species from the Anzali Wetland.

TABLE V

Patterns of trace elements occurrence in muscle of different species from various parts of the world. The orders are not based on statistical analyses

Species	Order	Sampling region	Reference
Tilapia nilotica	Fe > Zn > Cu > Sr > Cr > Ni > Co > Mn	Nasser Lake, Egypt	Rashed, 2001
Lethrinus lentjan	Zn > Cu > Mn, Cd	Persian Gulf	Al-Yousuf *et al.*, 2000
Leuciscus leuciscus	Hg > Pb > Cd	Eastern England	Barak & Mason, 1990
Esox lucius	Hg > Pb > Cd	Eastern England	Barak & Mason, 1990
Perca fluviatilis	Hg > Pb > Cd	Eastern England	Barak & Mason, 1990
Leuciscus cephalus	Hg > Pb > Cd	Eastern England	Barak & Mason, 1990
Tinca tinca	Hg > Pb, Cd	Eastern England	Barak & Mason, 1990
Barbus grypus	Zn > Pb > Mn > Cu > Cd	Diyala River, Iraq	Khalaf *et al.*, 1985
Gadus morhua	Zn > Pb > Cu > Cr > Cd	Mersey Channel, England	Collings *et al.*, 1996
Merlangius marlangus	Zn > Pb > Cu > Cr > Cd	Mersey Channel, England	Collings *et al.*, 1996
Pleuronectes platessa	Zn > Pb > Cu > Cr > Cd	New Brighton, England	Collings *et al.*, 1996
Platichthys flesus	Zn > Pb > Cu > Cr > Cd	New Brighton, England	Collings *et al.*, 1996
Limanda limanda	Zn > Cu > Pb > Cr > Cd	Mersey Channel, England	Collings *et al.*, 1996
Anguilla anguilla	Zn > Cu > Pb > Cr > Cd	New Brighton, Englang	Collings *et al.*, 1996
Esox lucius	Zn > Mn > Cu > Pb	Anzali Wetland, Iran	Pourang, 1995
Carassius auratus	Zn > Mn > Cu > Pb	Anzali Wetland, Iran	Pourang, 1995
Chelidonichthys kumu	Zn > As > Se > Hg > Cu > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Lepidotrigla argus	Zn > As > Se > Cu > Hg > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Platycephalus marmoratus	Zn > As > Se > Cr > Cu > Hg > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Platycephalus longispinus	Zn > As > Se > Cu > Hg > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Rhabdosargus sarba	Zn > As > Hg > Se > Cu > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Pagrus auratus	As > Zn > Se > Cu > Hg > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Pseudorhombus jenynsii	As > Zn > Se > Cu > Hg > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Meuschenia trachylepis	As > Zn > Se > Hg > Cu > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Nelusetta ayraudi	As > Zn > Se > Cu > Cr > Hg > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Anoplocapros inermis	As > Zn > Se > Cu > Hg > Cr > Pb > Cd	Malabar, Australia	Gibbs & Miskiewicz, 1995
Abramis brama	Zn > Fe > Cu > Mn > Pb > Ni > Cd > Co	Olshanka Rivar, Ukrain	Malyarevskaya & Karasina, 1991
Rutilus rutilus	Zn > Fe > Cu > Mn > Ni > Pb > Co > Cd	Olshanka Rivar, Ukrain	Malyarevskaya & Karasina, 1991
Esox lucius	Zn > Fe > Cu > Mn > Pb > Ni > Cd > Co	Olshanka Rivar, Ukrain	Malyarevskaya & Karasina, 1991
Ctenopharyngodon idella	Zn > Hg > Cu > Pb > Cd > Ni	Lake Balaton, Hungary	Vigh *et al.*, 1996
Mulus barbatus	Fe > Zn > Pb > Cu > Cd	East Mediterranean coast of Turkey	Kargin, 1996
Sparus aurata	Fe > Zn > Pb > Cu > Cd	East Mediterranean coast of Turkey	Kargin, 1996

TABLE VI

Comparison of mean heavy metal concentrations in different fish species (sturgeon and bony fish) from different areas of the world as well as maximum allowable concentrations of several heavy metals in seafood for human consumption. Concentrations are in μg g^{-1} wet weight except the cases are denoted with asterisk, which are in μg g^{-1} dry weight. Assume a wet weight-dry weight conversion factor of 0.2 (Blaylock & Templeton, 1991)

Geographical area/Standards	Species	Cd	Cu	Pb	Zn	References
WHO[1]		0.2	10		1000	Biney & Ameyibor, 1992; Madany *et al*., 1996
NHMRC[2]		0.05	10	1.5	150	Maher, 1986; Darmono & Denton, 1990
Germany		0.5		0.5		Merian, 1991; Radojevic & Bashkin, 1999
New Zealand		1.0	30	2.0	40	Nauen, 1983
Australia		0.2–5.5	10–70	1.5–5.5	40–1000	Nauen, 1983
Hong Kong		2.0		6.0		Nauen, 1983
Switzerland		0.1		1.0		Nauen, 1983
Denmark				2		Huss, 1994
Caspian Sea	*A. guldenstaedti*	0.005	1.91	0.008	21.65	Present study
Caspian Sea	*A. nudiventris*	0.0015	1.66	0.004	20.40	Present study
Caspian Sea	*A. persicus*	0.006	1.72	0.012	18.85	Present study
Caspian Sea	*A. stellatus*	0.0015	1.23	0.037	17.95	Present study
Caspian Sea	*H. huso*	0.0015	1.78	0.011	24.50	Present study
Caspian Sea	*Carassius auratus*		1.3	1.4	55.4	Pourang, 1995
Caspian Sea	*Esox lucius*		2.8	1.2	25.4	Pourang, 1995
Caspian Sea	Freshwater commercial fishes	0.4	0.6	1.7	20.6	Patin, 1982
Caspian Sea	Marine commercial fishes	0.3	1.6	1.1	16.2	Patin, 1982
West of Scotland	*Nezumia aequalis*	0.004	0.21	0.005	3.91	Mormede & Davies, 2001b
West of Scotland	*Lepidion eques*	0.005	0.17	0.002	2.62	Mormede & Davies, 2001b
West of Scotland	*Raja fyllae*	0.012	0.33	0.027	5.53	Mormede & Davies, 2001b
Malabar, Australia	*Platycephalus longispinus*	0.004	0.18	0.007	4.43	Gibbs & Miskiewicz, 1995
Malabar, Australia	*Pagrus auratus*	0.001	0.18	0.02	4.80	Gibbs & Miskiewicz, 1995
Malabar, Australia	*Anoplocapros inermis*	0.003	0.26	0.006	4.57	Gibbs & Miskiewicz, 1995
Diyala Rive, Iraq	*Barbus grypus*	0.157	1.612	3.497	29.307	Khalaf *et al*., 1985
Fish farming, Lake Manzala Egypt	*Oreochromis niloticus*	0.72	4.13	6.35	6.37	Sherief & Mancy, 1995
Fish farming Lake Manzala, Egypt	*Mugil cephalus*	0.93	5.76	7.30	7.20	Sherief & Mancy, 1995
Nile River, Egypt	*Tilapia nilotica*		0.32		3.37	Mohamed *et al*., 1990
Nasser Lake, Egypt	*Tilapia nilotica*		0.26		0.630	Rashed, 2001

[1] World Health Organization; [2] Australian National Health and Medical Research Council; [3] Ministry of Agriculture, Fisheries and Food ND: Non-Detectable.

5. Conclusion

In general, on the basis of the obtained results it can be concluded that the levels of trace elements in all the studied species from southern part of the Caspian Sea are below the international guidelines as well as in most cases lower than other commercial fishes from the sea. Hence, at the present, it seems that consumption of the species do not present any danger for human health.

Patterns of elements accumulation in the studied species were comparable to what has been reported by many other researchers for other fish species.

However, for better interpretation and justification of the obtained results and in order to provide a more comprehensive view of anthropogenic pollutants accumulation in fishes from the Caspian Sea, further and more detailed studies on various trophic levels of the sea need to be conducted.

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