Heavy metal concentrations in the selected tissues of the Persian sturgeon, *Acipenser persicus*, from southern coast of the Caspian Sea

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Abstract

Bioaccumulation of heavy metals, including Cd, Pb, Cu and Zn in muscle, gill, liver, kidney and stomach in *Acipencer persicus* was studied. Fish were collected from the 'Iranian fishery zone 1' located between Astara and Kiyahshahr across five stations in the study area during the autumn catch season in 2001. Samples were analyzed by wet digestion with hydrogen peroxide and nitric acid. Analyses were testified using spike method. A sample of bovine liver (CRM 185R) was also tested to ascertain reliability of analyses. Digested samples were analyzed using Flame Atomic Absorption Spectrophotometer. The bioaccumulation pattern for Zn in different tissues studied was stomach>liver> kidney>gills>muscle tissue. The mean concentration of Zn in the stomach was 136.6±10.70 $\mu g\ g^{-1}$ dry weight. Cu with the maximum concentration of 39.71±8.85$\mu g\ g^{-1}$ dry weight in liver showed a bioaccumulation pattern of liver>kidney>stomach>gills>muscle tissue. The bioaccumulation pattern for Pb was determined as gills>liver>kidney>stomach>muscle tissue. Maximum mean concentrations of Pb 6.87±2.25$\mu g\ g^{-1}$ dry weight belonged to gills. Bioaccumulation pattern for Cd in the different organs studied in *A. persicus* was kidney>liver>gills>stomach>muscle tissue. Maximum mean concentration of 5.1±0.97$\mu g\ g^{-1}$ dry weight belonged to kidneys and the minimum mean concentration of 0.05±0.007$\mu g\ g^{-1}$ dry weight belonged to muscle tissue. Concentration of the metals in the muscles samples were below the most guidelines for human consumption. However, the concentrations for heavy metals were higher than duidelines in some other organs this does not pose health problems as these organs are not used for human consumption.

Keywords: *Acipenser persicus*, Heavy metals, Muscle, Organ, Caspian Sea, Iran

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Introduction

The Caspian Sea is the largest intercontinental water basin and is considered the habitat of several most commercially valuable sturgeon species of the world (Dumont, 1998). Sturgeon catch in the past decade has declined dramatically. Annual sturgeon catch in the Azov and Caspian Sea rose from 24000 tons to 25000 tons during 1970 to 1985, representing 90% of the world catch. However, later the catch figures have dropped down to 2000 tons (Billiard & Lecointre, 2001). This severe decline that poses a threat to sturgeon reproduction is to a great extent associated with over fishing, construction of dams across rivers in which sturgeons migrate and water and sediment pollution (Billiard & Lecointre, 2001).

Among this chemical pollution has been an important factor that affects sturgeon populations in the Caspian Sea. Investigations in the last decade have demonstrated the occurrence of heavy metals in water, sediment and aquatic organisms of the Caspian Sea (ECOTOX Study, 2002; De Mora & Sheikholeslami, 2002; Sadeghi Rad, 2002). Some regions in central and south Caspian are obvious hotspots (in sediments) for copper (Cu) and zinc (Zn).

The Persian sturgeon, *Acipenser persicus* is among the sturgeon species that inhabits the Caspian Sea. Possessing the highest catch numbers and breeding percentage in sturgeon hatcheries, this species is of particular importance in Iran. Survival and sustenance of these fishes is greatly influenced by their productivity, number of healthy breeders, survival of offspring up to maturity and environmental pollution. Therefore, consistent and comprehensive investigations on different aspects of sturgeons have to be conducted to understand the impacts of various pollutants including that of heavy metals in this species and to determine their bioaccumulation in different organs of these fishes. Given the economic significance of meat and caviar, it is important to monitor the accumulation of heavy metals in sturgeons and compare these concentrations with the MAC for these metals based on international standards for human consumption. The present study was conducted to gather information regarding the bio-accumulation of heavy metals Zn, Pb, Cu and Cd in muscle tissue and different organs (gills, liver, kidney and stomach) in *A. persicus* and to determine the health status of muscle tissue for human consumption.

Materials and methods

Five catch stations in the 'Iranian fishery zone 1' located between Astara (48° and 52' east longitude and 38° and 26' north latitude) and Kiyahshahr (49° and 53' east longitude and 37° and 27' north latitude) were selected to collect the samples (Fig. 1). Tissue samples (n=25) were collected from five *A. persicus* specimens caught in the study area during the autumn catch season in 2001. Tissue samples from kidney, liver, gills, stomach and muscles were taken from each fish. A section of pectoral fin ray was removed for age determination. Samples were sealed in plastic bags and were transferred to the laboratory on ice where they were stored at −17°C until they were analyzed.

Analyses of samples were conducted at the chemistry analytical laboratory of the Gilan University. Tissue samples were cut into pieces and dried at 70°C under atmospheric pressure. Samples were analyzed by wet digestion with hydrogen peroxide and nitric acid. About 10-15g of dried sample was transferred to a 250ml volumetric flask and 10ml of deionized water, 2ml of 65% nitric acid and 2ml of 30% hydrogen peroxide was added to it. Samples were refluxed for about an hour. After cooling again 2ml of 65% nitric acid and 2ml of 30% hydrogen peroxide was added and samples were refluxed. This process was repeated three times. Digested samples were filtered and the residue on the filter paper was rinsed with hot water (three times and each time with 15ml water) (Lanza & Budeni, 1975).

The volume of the filtrate was reduced to 40ml and transferred to a 50ml volumetric flask and the volume of the filtrate was made up to 50ml. Digested samples were analyzed using Flame Atomic Absorption Spectrophotometer (Varianspectr AA 220) (detection limit for Pb=0.3-25, Cu=0.1-15 and Cd=0.03-3ppm). Analyses were testified using spike method. A sample of bovine liver (CRM 185R) was also tested to ascertain reliability of analyses. Recovery for Zn, Cu, Cd and Pb were 53, 100.44, 53.3 and 98.8, respectively.

All results obtained were analyzed using Excel and the non-parametric Kruskal-Wallis Test (SPSS for Windows, version 14.0) was used to compare groups of sampled data.

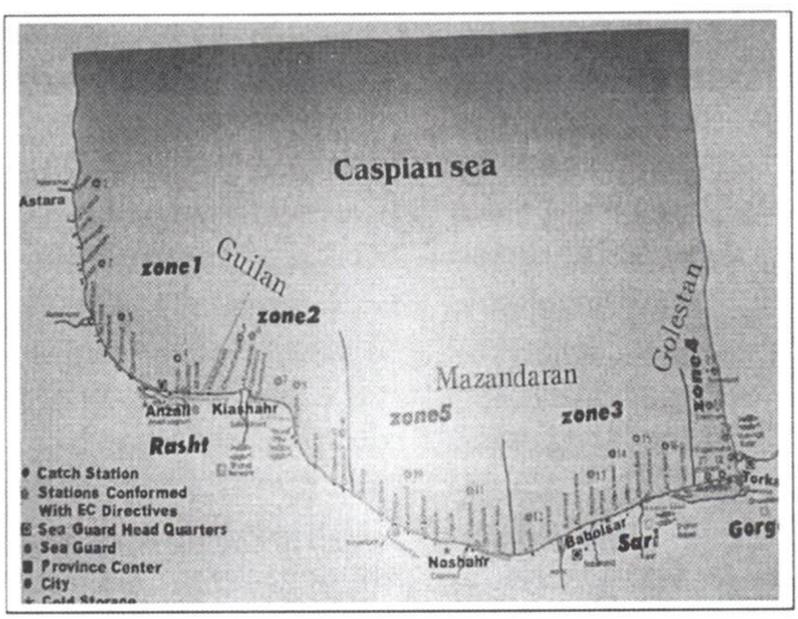

Figure 1: Map of the Iranian coastline of the Caspian Sea and the Iranian fishery zones

Results

Fish specimens were in the 13-18 year classes. The ratio of females to males in the collected samples was 1:1.5.

There was no significant difference in the bioaccumulation of Zn, Cu, Cd and Pb between females and males. Both age groups studied were homogenous. Our results indicated that mean Zn concentrations increases up to the age of 16 years, followed by a decrease at 17 years and reaches to a maximum concentration at 18 years. Mean concentrations for Cu were highest in 17 years old and lowest in 14 years old. The lowest mean values for Pb were found in 17 years old and highest mean values were observed in 16 years old, whereas for Cd, highest concentration was recorded in 13 years old and the highest mean values for this element was observed in 18 years old specimens. No definite pattern was observed for metal accumulation as a function of age. No significant differences were observed among the different catch stations regarding the accumulation of Zn, Cu, Pb and Cd.

Concentrations for Zn ranged from 15.92 to 255.7μg g^{-1} dry weight and mean concentration for Zn was 78.61μg g^{-1} dry weight. Whereas, the maximum mean concentration of Zn observed in stomach tissue (136.6±10.70μg g^{-1} dry weight) and the lowest mean concentration in muscle tissue (20.54±0.57μg g^{-1} dry weight) (Fig. 2). Significant differences were observed for Zn concentrations among various tissues (x^2=74.87, P≤0.0001).

The minimum, maximum and mean concentration of Cu was measured as 0.1, 129 and 11.84μg g^{-1} dry weight, respectively.

The highest mean concentrations of Cu were detected in the liver tissue (39.7± 8.85μg g^{-1} dry weight) and the lowest in muscle tissue (1.66±0.13μg g^{-1} dry weight) (Fig. 3). Significant differences were observed for Cu concentrations among the different tissues (x^2=91.68, P≤0.0001).

Maximum and minimum concentrations of Pb were 37.66 and 0.05μg g^{-1} dry weight. Mean concentration for Pb in the tissues studied was 3.42μg g^{-1} dry weights. The highest mean concentrations were detected in gill (6.87±2.25μg g^{-1} dry weight) and the lowest concentrations belonged to muscle tissue (0.09±0.008μg g^{-1} dry weight) (Fig. 4). Significant differences were observed for Zn (x^2=51.76, P≤0.0001) concentrations among the different tissues examined.

The maximum and minimum concentrations for Cd in the present study were 19.74 and 0.03μg g^{-1} dry weight, respectively. Mean concentration recorded for Cd was 1.88 μg g^{-1} dry weight. Highest mean concentration for Cd (0.05±0.97μg g^{-1} dry weight) was detected in liver tissue (Fig. 5). Significant differences were observed for Cd (x^2=82.31, P≤0.0001) concentrations among the different tissues studied.

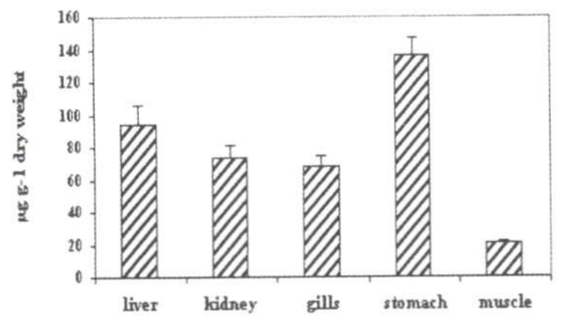

Figure 2: Mean concentration of Zn in *A. persicus*

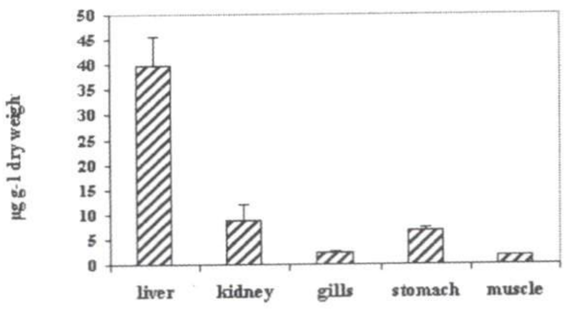

Figure 3: Mean concentration of cu in *A. persicus*

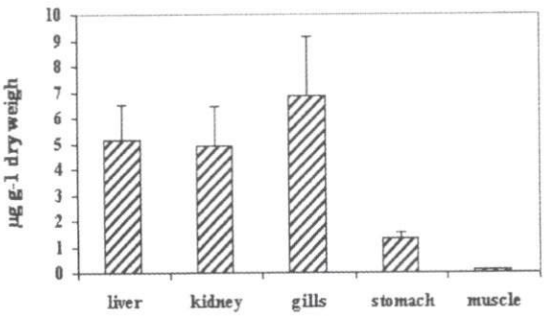

Figure 4: Mean concentration of Pb in *A. persicus*

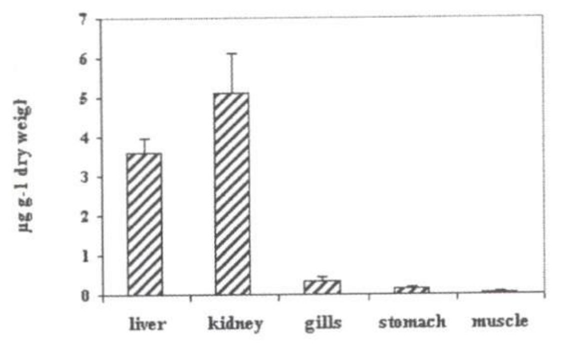

Figure 5: Mean concentration of Cd in *A. persicus*

Discussion

Heavy metals in fish are transferred through the blood to different parts of the body where they bind with proteins. Thus their accumulation in different tissues and organs differs (Romanenko *et al.*, 1986; Heath, 1987). In the present study, the bioaccumulation of Cd, Cu, Zn and Pb in the different organs in *A. persicus* did not follow a similar pattern.

Various accumulation level in different organs/tissues of a fish can primarily be attributed to the differences in the physic-ological role of each organ.

Regulatory ability, behavior and feeding habits are other factors that could influence the accumulation differences in the different organs (Kotz, *et al.*, 1999). The pattern of Zn accumulation in different organs in *A. persicus* in the present study was muscle tissue<gills<kidney<liver< stomach.

The Zn content in gills, muscle tissue and gonads in *A. stellatus* caught in the Volga River during the years 1997-1998 was reported as 188.56±1.94, 57.89±1.49 and 69.6±1.3µg g^{-1} dry weight, respectively (Zaitsev *et al.*, 2000).

In an earlier investigation the concentration of Zn in muscle tissue in *A. persicus* in the south Caspian Sea was determined as 26.9μg g^{-1} dry weight (Sadeghi, 2002) which is comparable to the mean concentration of Zn in the present study (20.54±0.57μg g^{-1} dry weight). In another study conducted by Gapeyova *et al.* (1990) the concentration of Zn in muscle tissue, liver, gills, spleen and blood serum was 4.47, 16.40, 12.80, 15.20 and 2.46mg kg^{-1} wet weight.

The accumulation pattern for Cu in different organs and muscle tissue in *A. persicus* was muscle tissue<gills<stomach< kidney<liver. Liver and kidney are the target organs for Cu accumulation in fishes. The fact that fishes are capable of regulating the intake of heavy metals makes this relationship more complicated (Segner, 1986).

According to Gapeyova *et al.* (1990) the Cu concentrations in liver in *H. huso*, *A. persicus* and *A. stellatus* was 14, 15 and 10 times, respectively, higher than that in muscle tissue. The present study also confirms these findings.

The gills are in direct contact with the contaminated medium (water) and have the thinnest epithelium of all the organs. This is due to its respiratory function which causes it to be optimized for gaseous exchange with the environment (large surface area, short diffusion distances between the body and the water). Metal penetration through the epithelial cell is suggested of particulate fractions or via nutrient carriers (Part, 1987). Furthermore, the mucous layer on the gills almost also most probably plays a role in the loss of metals (Varanasi & Markey, 1978; Segner, 1986; Heath 1987). The accumulation patterns for metal concentrations in different organs in *A. persicus* were as follows:

Muscle tissue=Zn>Cu>Pb>Cd,
Liver=Zn>Cu>Pb>Cd,
Stomach=Zn>Cu>Pb>Cd,
Kidney=Zn>Cu>Cd>Pb,
gills=Zn>Pb>Cu>Cd

The content of essential metals (Cu, Zn) in non polluted areas is always higher than the content of non-essential metals (Pb, Cd) and their presence in tissues is related to the requirement of these trace metals in biochemical cycles (Murphy *et al.*, 1978). The present study also indicates this to some extent.

The variations in metal content in fish tissues are mostly influenced by variations in climatic conditions (rainfall) and also on the rise and fall in pollution load that enters an ecosystem. The accumulation of metals in fishes caught (from different catch stations) in the study region indicate no significant differences in terms of catch region. The metal content detected in muscle tissue could be due to the life long exposure of these fishes to these metals. The bioaccumulation of metals in these fishes is an indicator of fishes being exposed to different levels of metals in the past. The accumulation of metals in fish tissue has received considerable attention for health safety. In the present study the metal contents in muscle tissues are lower than the Maximum Allowable Concentration (MAC) for these metals based on international standards for human's consumption (Table 1). Values in the table for muscle tissue are presented in terms of µg/g dry weight considering that the water in muscle tissue is about 60-70%. So the reported values for heavy metals are much lower than the MAC values (µg/g wet to the Maximum Allowable Concentration or higher than the maximum permissible concentration. These organs are not used for human consumption and thus do not pose any problem. However, they might cause histological and physiological problems in fish that need to be studied further.

Table 1: Comparison of data obtained for muscle tissue with standard Maximum Allowable Concentration (MAC) in µg/g wet weight for human consumption

Source	Cd	Cu	Pb	Zn	Reference
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
MAFF[3]	0.2	20	2	50	Anon, 1993; Collings *et al.*, 1996
Germany	0.50		0.5		Merian, 1991; Raojevic & Bashkin, 1999
Denmark			2		Huss, 1994
HCCR[4]	0.1	10	0.5		Buchtova, 2001
Muscle tissue*	0.049	1.66	0.09	20.43	Present study

1- World Health Organization
2- Australian National Health and Medical Research Council
3- Ministry of Agriculture, Fisheries and Food
4- Health Care of the Czech Republic
*** µg/g dry weight**

Acknowledgment

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References

ANON, 1993. Monitoring on surveillance of non-radioactive contaminant in the aquatic environment and activities regulating the disposal of waste at the Sea, Aquatic environment monitoring report. No.36, Ministry of Agriculture, Fisheries & Food, LOWESTOFT, 78P.

Biney, C.A and Ameyibor, E., 1992. Trace metal concentrations in the pink shrimp "*Penaeus notialis*", from the coastal of Ghana. Water, Air and Soil Pollution. **63:**273-279.

Billard, R. and Lecointre, G., 2001. Biology and conservation of sturgeon and paddlefish. Reviews in Fish Biology and Fisheries. **10:**355-392.

Buchtova, H., 2001. Hygiena a technologie zpracování ryb a ostatnich vodních živočichu, alimentární onemocnění z ryb mrazírenství, Veterinární a farmaceutická Univerzita Brno, 164P.

Collings, S.E., Johnson, M.S. and leach, R.T., 1996. Metal contamination of angler-caught fish from the Mersey Estuary.

Marine Environmental Research. 41**(3):** 281-297.

Darmono, D. and Denton, G.R.W., 1990. Heavy metal concentrations in the banana prawn, *Penaeus merguiensis* and leader prawn, *P. monodon*, in Townsville region of Australia, Bulletin of Environmental Contamination and Toxicology, **44:**479-484.

De Mora, S. and Sheikholeslami, M.R., 2002. ASTP: Contaminant Screening Programme. Final Report: Interpretation of Caspian Sea Sediment Data. 26P.

Dumont, H.J., 1998. The Caspian Lake: History, biota, structure, and function. Limnology and Oceanography, 43**(1):** 44–52.

ECOTOX Study, 2002. Investigation into toxic contaminant accumulation and related pathology in the Caspian sturgeon, seal and bony fish. 17P.

Gapeyova, M.V., Teselmovich, A.L. and Shirokova, A.M., 1990. Heavy metals in sturgeons in North Volga. Ribinisk, Russia. 15P.

Heath, A.G., 1987. Water pollution and fish physiology. CRC Press Inc., Florida, USA. 245P.

Huss, H.H., 1994. Assurance of seafood quality, FAO Fisheries Technical Paper. Rome, Italy. 169P.

Kotz, P., Du Preez, H.H. and Van Vuren, J.H.J., 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarius gariepinus* from the Olifants River, Mpumalanga, South Africa. Department of Zoology, Rand Afrikans University. South Africa. 12P.

Lanza, P. and Budeni, P.L., 1975. Analytical Chemistry Acta. Vol. 75, 149P.

Madany, C.M., Wahab, Z. and Alawi, A.L., 1996. Trace metals concentrations in marine organisms from the coastal areas of Bahrain, Persian Gulf. Water, Air and Soil Pollution. **91:**233-248.

Maher, W.A., 1986. Trace metal concentrations in marine organisms from St. Vincent Gulf, South Australia, Water, Air and Soil Pollution. **29:**77-84.

Merian, E., 1991. Metal and their compounds in the environmental occurrence analysis and biological relevance. VCH, Weinheim. 704P.

Murphy, B.R, Atcchison, G.J. and McIntosh, A.W., 1978. Cd and Zn muscle of Bluegill *(Lepomis macrochirus)* and Largemouth bass *(Micropterus salmoides)* from an industrially contaminated lake. Environmental Pollution. **17:**253-257.

Part, P., 1987. Testing and bioavailabilty of metals in natural waters. *In*: Lectures Notes in Earth Sciences. Springer-Verlag, Germany. 16P.

Radojevic, M. and Bashkin, V.N., 1999. Practical environmental analysis. The Royal Society of Chemistry, UR. 466P.

Romanenko V.D., Malyzheva, T.D. and Yevtushenko N., 1986. The role of various organs in regulating zinc metabolism in fish. Hydrobiology Journal, 21**(3):**7-12.

Sadeghi Rad M., 2002. [Heavy metal determination (Zn, Cu, Cd, Pb and Hg) in muscle tissue and caviar in two sturgeon species *A. persicus* and *A stellatus* in the southern shores of the Caspian Sea]. Final Report. Iranian Fisheries Research Organization. 81P. (in Persian)

Segner H., 1986. Response of fed and starved roach, *Rutilus rutilus* to sublethal Cu contamination. Journal of Fish Biology, **30**:425-437.

Varanasi N. and Markey Y.D., 1978. Uptake and release of lead and cadmium in skin and mucus of coho salmon (*Oncorhynchus kisutch*). Comparative Biochemistry and Physiology, **60C**:187-191.

Zaitsev, V.F., Gordon, Y.V., Shipulin, S.V. and Sokirko, G.I., 2000. Chemical elements in organs and tissues of *Acipenser stellatus* during its riverine life. Book of Abstracts. 9th National Russian Conference on Physiology, Ecology and Biochemistry of Fishes. Yaroslavl, **1**:102-103.