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Trace metals (Cu, Cd, Zn, and Pb) in the liver and the kidney of *Acipenser persicus* with relation to their concentrations in the surficial sediments of the west coast of the southern Caspian Sea

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Abstract: This study was carried out to find any relative concentrations of the trace metals (Cu, Cd, Zn and Pb) in the liver and the kidney of *Acipenser persicus* and in the surficial sediments of the west coast of The Southern Caspian Sea. Twenty five samples of the *Acipenser persicus* (Persian sturgeon) and ten samples of the sediments were taken from five fishing stations in autumn 2001. The tissues were digested by wet method and the sediments were digested by dry method. Every digested sample was separately analysed by AAS (Atomic Absorption Spectroscopy). Recovery, mean, ANOVA, correlation coefficient and hierarchical cluster analysis were calculated. It was concluded that the concentrations of the studied trace metals (Cu, Cd, Zn and Pb) were at the standard level and there was a positive correlation coefficient between the kidney and the liver in the concentrations of Cd and Pb.

Keywords: *Acipenser persicus*, Persian sturgeon, trace metals, Southern Caspian Sea, Iran

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Introduction

The Caspian Sea, a closed aquatic ecosystem, which is divided into north, center and south is scientifically, environmentally and economically important in the world. The deepest region of The Caspian Sea is located in the south (DOE., 2001). One of the main economic fish family of The Caspian Sea is Acipenseridae with five famous species; *A. guldenstadti, A. persicus, A. nudiventris, A. ştellatus and Huso huso* (Williot, 1997 ; UNDP, 1998 ; DOE , 2001). The famous Iranian species which basically lives in the Iran's coasts is *Acipenser persicus*. This species belongs to Acipenseriform order, Acipenseridae family and Acipenser genus. It lives close to the surface and migrates through The Caspian Sea and the fresh water rivers to spawn (Birstein *et al.*, 1997 ; CEP, 2000). Todays, *A. persicus* is in serious danger due to dam, bridge and industry construction, destruction of migration areas and habitat, irregular and overall fishing, discharge of waste and sewage into the sea, which contain various pollutants like trace metals. Trace metals, the most important pollution indicators, are so toxic and stable in the environment. They are usually deposited in the sediments and the tissues and transferred through the food chains. They make complex compound or are compounded (Freedman, 1995). Since there wasn`t enough information on the concentrations of the trace metals in the liver and the kidney of the *Acipenser persicand* and in the sediments of the south regions of the Caspian Sea, this study was carried out.

Material and Methods

Sampling Stations

The study was carried out in the west coast of the southern Caspian Sea (48° , 51'-50° , 39' N and 38° , 36'-36°, 57' E) in the fisheries zone No.1 (under supervision of HACCP), at the 10-15m depth . Due to the lack of enough facilities and the short distance of the stations only five sampling stations located almost every other two stations were selected (Fig.1).

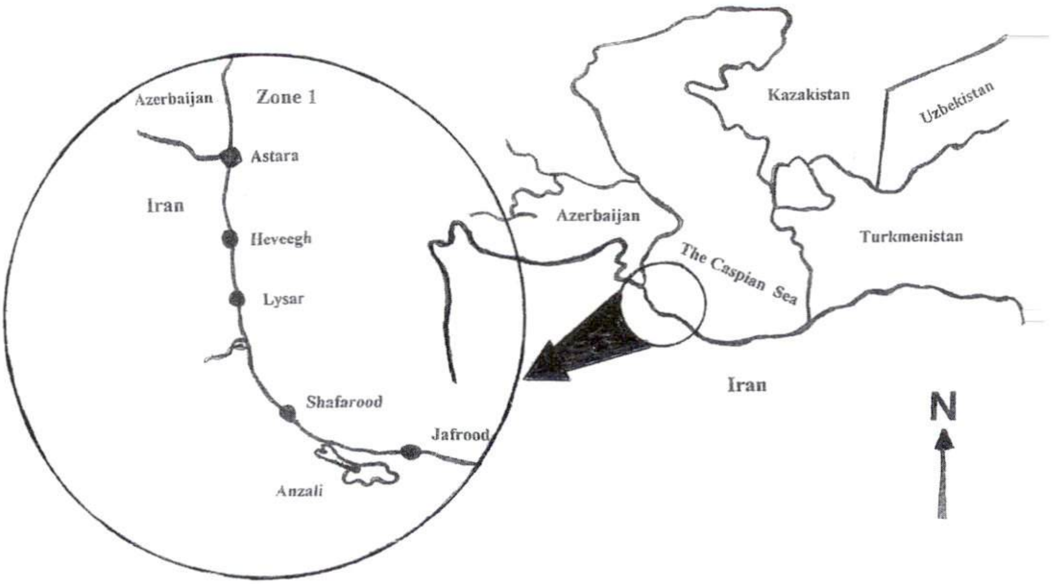

Figure 1: The Caspian Sea and the sampling stations

Sampling

Laboratory apparatus was washed by detergent and tap water, then was soaked into nitric acid 15% for 24hr and rinsed acid out with deionized water (ROPME, 1999). Ten samples of the sediments were taken by Van Veen grab (10 samples = 5 sampling stations × 2 depths). Twenty five samples of the kidneys and twenty five samples of the livers were cut up from twenty five individual fish with a sterile stainless steel scalpel (25 tissues = 5 sampling stations × 5 fish). The samples were individually packed into the sterile labeled polyethylene bags and carried in a ice-box at 4 ° C to the laboratory.

Analytical preparation

The materials were Analytical Grade.To prevent any interference of the trace metals,blank samples were prepared and digested by the wet digestion method (three times). The sediments were digested by the dry method (Dolzal, 1968 ; Langmyhr & Sveen, 1965) and the tissues were digested by the wet method (Lanza & Budeni, 1975) three times. To confirm the reliability of the methods, two reference materials (CRM 185 R – Bovine's kidney and Soil –7 *Community

Bureau Of Reference –BCR) were also analysed by the same methods. The recovery percentages of the trace metals were calculated for the sediments (97% , 10m depth ; 98%, 15m depth), the kidneys (Cu 96% ; Zn 98% ; Pb 92% ; Cd 95%) and the livers (Cu 94% ; Zn 96% ; Pb 94% ; Cd 96%).

Analytical Methods

Tissue digestion

Twenty five samples of the kidneys and twenty five samples of the livers were separately ground in a mortar and homogenised. Fifteen g of the homogenised sample of the kidney and fifteen g of the liver were placed in two clean petridishes. They were thoroughly dried in a vacuum condition at 400 torr pressure and 70 °C for an hour. They were left into decicator to be cooled, then weighted. This procedure was repeated till the weights became stable, then ten g dried sample of the kidney and ten g dried sample of the liver were individually transferred into two 250cc conical flasks and nitric acid 65% (2ml), deionized water (10ml) and hydrogen peroxide 30% (2ml) were added .

Sediment digestion

Ten sediments were homogenised. Fifteen g of the homogenised sample was dried at 760mmHg and 105 °C for one and half hour. The dried sample was left into decicator to be cooled and weighted. This procedure was repeated till the weight became stable. Ten g dried sediment was transferred into a 250 cc conical flask where fluridric acid 20% (25ml) and choloridric acid 15% (25ml) were added. The flask was placed on a water-bath oscillator at 40 °C for six hours and fifteen swings per minute.

Final Procedure

The solutions extracted from the tissues and sediments were separately filtered with a Whatman filter paper (No.1). Each residue was washed with deionized water (15ml) and filtered again. The filtered solutions were separately poured into a beaker and heated untill their volumes reached to 40ml. Then they were separately made up to 50ml with the distilled water.The 50ml volumetric solutions were separately aspirated into Varian spectr AA-220 set to detect the concentrations of the four trace metals.

Statistical Analysis

SPSS 10.0.05, ver.2000 software was used for statistical analyses. The null hypothesis (there is no relation among the concentrations of the trace metals in the kidney and liver of *A. persicus* and in the sediments of the west coast of the southern Caspian Sea) were tested by one-way ANOVA and the relative concentration of the same trace metals in the same tissues and sediments were tested by Pearson's correlation coefficient. The hierarchical cluster analyses (Euclidean distance) was used to classify the sampling stations on the basis of the concentrations of the trace metals in the tissues and the sediments. Means were also calculated to compare the concentrations of the studied trace metals with the standard concentrations and the other studies.

Results

Mean concentrations of the trace metals (Cu, Cd, Zn and Pb) in the sediments and tissues were summarized in table (1).

Table 1: Mean concentrations of the trace metals (Cu, Cd, Zn and Pb) in the sediment of The Southern Caspian Sea(ppm / dry weight) and in the kidney and liver of *Acipenser persicus* (ppm / wet weight)

Sample	Trace Metals			
	Pb	Zn	Cd	Cu
Sediment	7.78+0.54	44.89+1.39	0.62+0.01	9.91+0.7
Liver	1.99+0.35	31.58+2.22	1.31+0.26	13.98+1.42
Kidney	1.16+0.24	20.51+2.1	1.47+0.33	2.65+0.63

It was concluded that the concentrations of Cu, Cd, Zn and Pb were lower than the standard concentrations. The concentrations of Cu, Pb and Zn were lower but the concentration of Cd was higher than the concentrations which had been reported by the other studies (table 2).

Table 2: Mean concentrations of the trace metals in the sediment in comparison with the standard concentrations and the other studies (ppm/ dry weight)

Standards and Studies	Cu	Cd	Zn	Pb	References
Element composition of the earth's crust	50	0.3	75	14	Bowen, 1979
ASTP	13.2-50.9	0.098-0.244	55.9-146	11.3-24.6	Mora & Sheikholeslami, 2002
ISQG	18.7	0.7	124	30.2	Grimwood & Dixon, 1997 Anon, 1999
NOAA (PEL)	108	4.2	271	112	
NOAA (LV)	36		480	530	
NOAA (ERL)	34	1.2	150	46.7	NOAA, 1999
NOAA (ERM)	270	9.6	410	218	
the southern Caspian Sea	9.91	0.62	44.89	7.78	present study

ISQG : Canadian Interim Sediment Quality Guideline
NOAA : National Oceanic and Atmospheric Administration
PEL : Probable Effects Level
LV : Limit Values
ERL : Effects Range Low
ERM : Effects Range Medium
ASTP : At Sea Training Programme (Contaminant Screening Programme)

Mean concentrations of the trace metals in the liver and the kidney of *A. persicus* were compared with the standard concentrations in the muscle(table.3)

Cu : WHO, NHMRC, UK-MAFF (Muscle) > Present Study (Kidney)
WHO, NHMRC (Muscle) < Present Study (Liver)
UK-MAFF (Muscle) > Present Study (Liver)

Cd : WHO, NHMRC, UK-MAFF, Germany (Muscle) < Present Study (Kidney)
WHO, NHMRC, UK- MAFF, Germany (Muscle) < Present Study (Liver)
Zn : WHO, NHMRC, UK- MAFF, (Muscle) > Present study (kidney)
WHO, NHMRC, UK- MAFF, (Muscle) > Present study (Liver)
Pb : NHMRC, UK- MAFF, Denmark (Muscle) > Present study (Kidney)
Germany, (Muscle) < Present study (Kidney)
NHMRC, Germany, (Muscle) < Present study (Liver)
UK-MAFF, Denmark (Muscle) > Present study (Liver)

Table 3: Mean concentrations of the trace metals in the muscle, liver andkidney (ppm / wet weight)

Standards and studies		Muscle				References
		Cu	Cd	Zn	Pb	
WHO		10	0.2	1000	--	Biney & Ameyibor, 1992 Madany *et al.*, 1996
NHMRC		10	0.05	150	1.5	Maher, 1986 Darmono & Denton, 1990
UK (MAFF)		20	0.2	50	2.0	Anon,1993 Collings *et al.*, 1996 Mormede & Davies, 2001
Germany		--	0.5	--	0.5	Merian, 1991 Radojevic & Basknin, 1999
Denmark		--	--	--	2	Huss, 1994
Present study (*A. persicus*)	Liver	13.98	1.31	31.58	1.99	present study
	Kidney	2.65	1.47	20.51	1.16	

NHMRC : Australian National Health and Medical Research Council
WHO: World Health Organization
UK(MAFF): Ministry of Agriculture, Fisheries and Food.

Mean concentrations of the trace metals in the muscle, the liver and the kidney of different fish and *A. persicus* were summarized in table (4).The results indicated that the concentrations of the trace metals in the liver and the kidney of all mentioned species were more than their concentrations in the muscle. It was also concluded that the P values of Cu ($P \leq 0.000$) and Cd ($P \leq 0.003$) were less but the P values of Zn ($P \leq 0.058$) and Pb ($P \leq 0.509$) were more than the significant P value ($P \leq 0.05$). In other words, There were just significant differences in the concentrations of Cu and Cd in the sediments, the livers and the kidneys of *A. persicus* in the west coast of the southern Caspian Sea, but no significant differences were observed in Zn and Pb.

The correlation coefficient (r) showed that there was a relationship between the liver and the kidney in the concentrations of Cd ($r = 0.9457$, $P \leq 0.000$) and Pb ($r = 0.9349$, $P \leq 0.0300$) ,and between the kidney and the sediment in the concentration of Pb ($r = 0.7263$, $P \leq 0.0174$). It meant that any fluctuations in the concentrations of Cd and Pb in liver and kidney and Pb in kidney and sediment were correlated (table.5). No more correlation was observed in the rest.

Fig.2 shows the hierarchical cluster analysis dendrogram of the sampling stations in the west coast of The Southern Caspian Sea on the basis of the concentrations of the trace metals (Cu, Cd, Zn,and Pb) in the sediments , the livers and the kidneys of *A. persicus*.

Arbitrary dashed lines which separate the clusters showed as follows;

1) Three stations (a) at distance four and four stations (b) at distance 12 formed a single cluster ,where the major similarities were observed between these stations duo to the concentrations of the trace metals in the sediments of the west coast of The Southern Caspian Sea.
2) Two stations (b) at distance three and three stations (a) and two stations (c) at distance five formed the single clusters where the major similarities were observed between these stations duo to the concentrations of the trace metals in the liver of *A. persicus*
3) Three stations (a) at distance four and four stations (b) at distance fifteen formed the single cluster where the major similarities were observed between these stations duo to the concentrations of the trace metals in the kidney of *A. persicus*.

Table 4: Mean concentrations of the trace metals in the muscle, liver and kidney of different fish species (ppm/ wet weight)

Species	Muscle				Kidney				Liver				References
	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	
Nezumia aequalis	0.37	0.010	5.79	0.024	--	--	--	--	7.49	0.841	35.08	0.128	Mormedeand Davies 2000-2001
Lophius piscatorius	0.12	0.417	--	0.0026	0.92	0.0051	--	0.0077	7.39	0.0896	--	0.02	
Carassius auratus	0.7-2.9	--	36.8-205.4	0.4-2.3	3.4-28.4	--	119.7-286.2	1.4-8.3	13.6-61.8	--	94.0-83.8	0.6-4.9	Pourang, 1995
Esox lucius	1.3-8.3	--	14.6-45	0.3-2.1	12.1-20.5	--	643.6-1923.3	0.7-3.8	46.9-89.3	--	59.1-187.6	0.6-4.8	
Acipenser stellatus	0.28	0.35	54.86	1.7	16.11	0.43	310.02	356	32.46	0.85	159.93	7.9	Zaitsev, 2002
	1.224	0.002	17.95	0.037	--	--	--	--	--	--	--	--	Pourang et al, 2003
Acipenser persicus	1.721	0.006	18.810	0.012	--	--	--	--	--	--	--	--	
	--	--	--	--	2.65	1.47	20.51	1.16	13.98	1.31	31.58	1.99	Present study

Table 5: One–way ANOVA for the concentrations of the trace metals in the sediment, kidney and liver of *Acipenser persicus* in the west coast of the Southern Caspian Sea.

Trace Metals	df	F ratio	* P value
Cu	2	10.682	0.000
Cd	2	6.617	0.003
Zn	2	2.987	0.058
Pb	2	0.683	0.509

* The mean difference is significant at $P \leq 0.05$

Table 6: Pearson`s correlation coefficient in the sediment, liver and kidney of *A. persicus* for the concentrations of the trace metals in the west coast of the Southern Caspian Sea

Samples		Cu	Cd	Zn	Pb
Liver-Kidney	r	0.1308	0.9457	-0.2200	0.9344
	p	0.5330	* 0.000	0.2905	* 0.0300
Liver – Sediment	r	-0.3355	0.3998	-0.2243	0.6055
	p	0.3432	0.2523	0.5332	0.0636
Kidney – Sediment	r	0.3616	0.5142	0.2999	0.7263
	p	0.3045	0.1283	0.3997	* 0.0174

* The mean difference is significant at $P \leq 0.05$

Mean concentrations of (1) Cu (2) Cd (3) Zn and (4) Pb with 95% significance were summarized as follows:

(1) Cu : Liver > Kidney > Sediment

(2) Cd: Kidney > Liver > Sediment

(3) Zn : Liver > Kidney > Sediment

(4) Pb: Sediment > Liver > Kidney

It was concluded that Cu and Zn in the liver, Cd in the kidney and Pb in the sediment were highly concentrated (fig. 3).

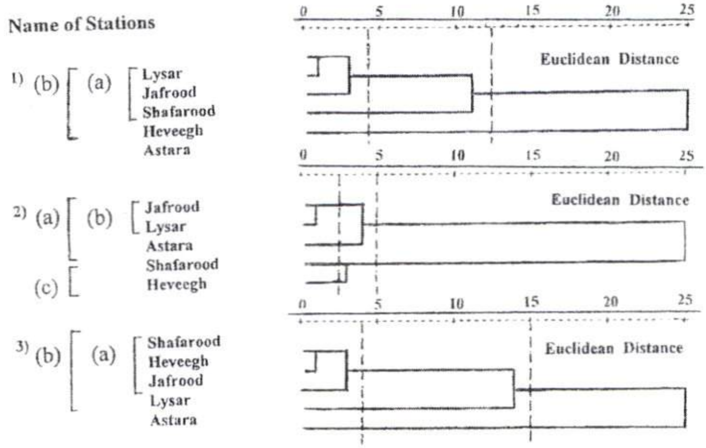

Figure 2: Hierarchical cluster analysis dendrogram of the sampling stations duo to the concentrations of the trace metals (1) in the sediment (2) Liver and (3) Kidney

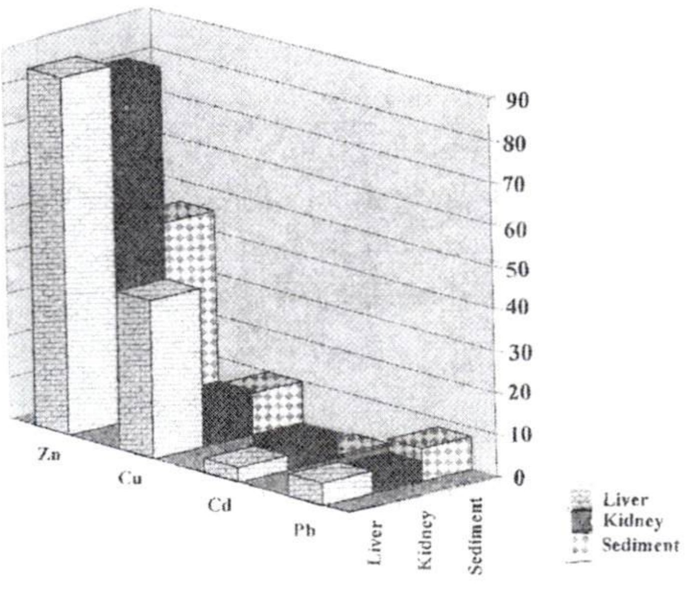

Figure 3: Mean concentrations of the trace metals in the sediment and the tissues (ppm/dry weight)

Discussion

Sediment

The study proved that the concentrations of the four trace metals in the sediments of the west coast of The Southern Caspian Sea were lower than the standard concentrations (table 2). It was revealed that the measured concentration of Cd in this study was higher than the concentration which was reported by Mora & Sheikholeslami (2002), and Bowen (1979). However, the concentrations of the trace metals are different due to time, sampling stations and metal itself. Freedman (1995) states that the concentrations of the trace metals in soil and rock are higher than their concentrations in water but the availability of them for uptaking is high in water, because most of the trace metals are largely insoluble in soil and rock but soluble in water. Among the aquatic environment, sediments contain the high concentrations of the trace metals (Gerhardt, 1990 ; Clements, 1991) because the anthropogenic discharges and effluents into the environment highly increase the accumulation of the trace metals in the sediments (Amini Ranjbar, 1997). It is a good reason to explain how the trace metals have been accumulated in the sediments of the southern Caspian Sea. It should also be considered that most trace metals are naturally ubiquitous in the environment even in a trace concentration. It means that there is also naturally universal metal contamination in the soil and the water without any interference of anthropogenic activities (Freedman, 1995). For instance; different types of soils like superficial soil (3-8mg/l), rocks, crude oil, ore, earth's crust (0.10mg/l), sea water (0.15μg/l) and sedimentary rocks, found in the basin of the Caspian Sea, naturally contain Cd (Malakuti, 2002). So, the concentration of Cd probably have a natural origin in the study area. However any firm statement depends on studying and measuring the concentration of Cd or other trace metals in the soil of the study area in the future.

Density of human activities and their waste discharges as a non-natural origin may affect the concentrations of the trace metals too. Majorities of industries, populated cities, rural areas, farms and agricultural lands, mines and the other activities are located in the southern Caspian Sea due to geographical characteristics and bounderies of the area (waterbodies, cultivated plains, coastal areas, condensed forests, foot hills and mountains). These facilities not only consume regional freshwater but also discharge their polluted and metal contaminated sludges, sewages, effluents, refuses and agricultural wastes into The Caspian Sea by means of irrigation system and local rivers (DOE, 2001; Manghavidel, 1996). Sludges and sewages, discharged into the aquatic environment, contain considerable quantities of the toxic trace metals (Cd, Zn,and Cu) which are accumulated in the sediment (Freedman, 1995). Inorganic pesticides (lead arsenate and copper sulfate) and fertilizers (phosphate) have contaminated agricultural lands by the toxic trace metals for a long time(Chaisemartin, 1983; Rashed, 2001). By-products, waste materials and molten wastes ,produced by mining and refinery activities, contain the trace metals (Cu and Cd). They are carried by irrigation system or precipitation into the rivers and low lands (Freedman, 1995). All these materials and activities probably are the other trace metal contamination sources in the study area. But for any precise estimate, It is recommended that the concentrations of the trace metals in the industrial, civil or agricultural effluents in the study area be measured .

Malakuti (2002) states that the high utilization of water, phosphate fertilizers, solubility of Cd and the drainage-basin of the area have increased the concentration of Cd in the study area. In other words, phosphate fertilizers contain high concentration of Cd ,because they are made from the phosphate ores with high concentration of Cd (10-980mg/kg). Since the high quantities of the fertilizers specially phosphate are used in The Southern Caspian Sea where 71% of Iran's agricultural lands are located (Malakuti, 2002), it is concluded that these fertilizers have probably contributed Cd in the study area.

Temperature, salinity, dissolved oxygen and pH also affect the accumulation of the trace metals in the sediments (Anon, 1998b), so it can be another reason for accumulation of the trace metals in the study area.

The final reaction which causes accumulation of the trace metals in the sediments occures when fresh and brakish water are mixed in the estuary (Duinker *et al.*, 1982 ; Sholkovitz, 1976). This reaction causes the suspended solids ,containing the trace metals, be deposited in the sediments due to flocculation. The same reaction happens in the basin of The Caspian Sea where fresh water rivers flow into the brakish sea (DOE, 2001).

With considering above reasons and comparing the concentrations of the four trace metals with the standard concentrations, It is concluded that the sediments of the west coast of the southern Caspian Sea are not contaminated.

Tissue

Trace metals are so considerable for their toxicity and the potential bioaccumulation in the aquatic species (Blevins, 1985 ; Gupta & Mathur, 1983). Accumulation patterns are different due to behaviour and diet (Mormede & Davies, 2001). However, the bioaccumulation of the trace metals in the fish is influenced by feeding behaviour, rate of growth, temperature, hardness, age, sex, salinity and metal interaction (MacCarty & Henry, 1978 ; Bendell–Young & Harvey, 1989 ; Mance, 1990). There are three ways for the race metals to enter the fish's body, (body surface, gills and digestive tract). Since the body surface doesn't play a dominant role in uptaking trace metals, the role of gills and digestive tract to uptake the trace metals probably became more considerable (Pourang, 1995). Metals are taken from the food (Mormede & Devies, 2001) aquatic plants, lake water, sediment and benthic organisms (Rashed, 2001). However, different feed habits affect the metal accumulation (Coetzee *et al.*, 2002). *A. persicus* is a benthic feeder (CEP, 2003), so it probably takes the trace metals from the benthic organisms which are able to notonly accumulate metals butalso contact with the sediments with the high concentrations of the trace metals. (Gerhardt. 1990 ; Clements, 1991). However, it is essential to measure these four trace metals in the benthic organisms to find how much metal is taken from

With regard to table (3), the concentrations of Cu in the liver, Cd and Pb in both liver and kidney are higher than their concentrations in the muscle in comparison with some standards, but the concentration of Zn is lower. However there aren't any standard references to show the acceptable concentrations of the trace metals in the non-edible or edible tissues of *A. persicus*. Perhaps physiological differences and the position of ȩach tissue influence the biaccumulation of the particular metals (Heath, 1991 ; Kotze, 1997). Pourang's study (1995) on *Esox lucius* and *Carassius auratus* in Anzali wetland revealed that Cu in the liver and Zn in the kidney were accumulated more than the muscle. Malyarevskaya & Karasina (1991) and Kureishy (1993) Obtained the same results. Jaffar and Pervaisshahid (1989), Al-yousuf *et al.* (2000) found that the concentrations of Cu, Zn, and Cd in the liver were higher than their concentrations in the muscle. Results taken from Mormede's & Davies studies (2000) on *Nezumia aequalisand* and *Lophius piscatorius* (table 4) revealed that the concentrations of Cu, Cd, Zn and Pb were higher in the liver and the kidney rather than muscle. Zaitsev (2002) obatined the same results for *Acipenser stellatus* (table 4). According to his study, the concentations of Cu, Cd, Zn and Pb in liver and kidney were several times higher than their concentrations in muscle. With considering these results, the high concentrations of Cu, Cd, Zn and Pb in the liver and the kidney of *A. persicus* are acceptable. By the way, the fish's liver is more recommended environment indicator for water pollution and has high tendency to accumulate pollutants from environment (Al-yousuf *et al.*, 2000 ; Galindo *et al.*, 1986). However the role of the kidney is as important as the role of the liver in the fish. Kidney as a important urinary system collects and excretes waste liquids from the body. It plays a vital role in the conservation of salt and water and in the filtration and the absorption (Benton, 1967). In other words, fish is able to reject or obtain large amount of the trace metals (Dallinger *et al.*, 1987 ; Gerhardt, 1990)

With regard to the mentioned reasons, it is concluded that the high concentrations of the trace metals in the liver and kindly of *A. persicus* are normal, therefore; the fish isnot contaminated.

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