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Full Length Research Paper

Levels of some heavy metal concentration in fishes tissue of southern Caspian Sea

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The level of four heavy metals cadmium (Cd), copper (Cu), lead (Pb) and mercury (Hg) in flesh of *Tinca tinca* and *Pelecus cultratus* as well as water samples (90 each) collected from six different sites of southern Caspian Sea were evaluated. The results of this study revealed that the mean Cd, Cu, Pb and Hg levels in the water samples was significantly lower ($P< 0.05$) than the mean residuals levels in flesh of *T. tinca* and *P. cultratus* samples. The mean residuals levels of Cd were 0.60, 0.77 and 0.54 ppm/wet weight for *T. tinca* , *P. cultratus* and water, respectively. 11.1% of *T. tinca*, 18.6% of *P. cultratus* and 10.4% of water samples had Cd residuals level exceeding the permissible limit. The mean residual levels of Cu were 1.76, 1.94 and 1.28 ppm/wet weight for *T. tinca*, *P. cultratus* and water, respectively. Cu residuals levels in all *T. tinca* and *P. cultratus* sample were within the action limit, while 6.4% of water samples exceeded the action limit. Meanwhile, the mean residuals level of Pb was 1.51, 1.74 and 1.12 ppm/wet weight for *T. tinca*, *P. cultratus* and water, respectively. 20.5% of *T. tinca*, 36% of *P. cultratus* and 28.9% of water samples had Pb residuals level exceeded the permissible limit. The mean residuals levels of Hg were 0.34, 0.17 and 0.08 ppm/wet weight for *T. tinca*, *P. cultratus* and water, respectively. 5.1% of *T. tinca*, 13% of *P. cultratus* and 9.2% of water samples had Hg residuals levels exceeding the permissible limit. These findings recommend that improved water quality control and periodically environmental monitoring are necessary in Caspian Sea. In addition, recommendations regarding the acceptable number of fish meals to be consumed weekly from the health authorities should be taken to fish consumers, especially children and pregnant woman, to control the accumulative effect of heavy metals.

Key words: Heavy metals, fish tissue, Caspian Sea, pollution, water.

INTRODUCTION

Fish, as human food, are considered as a good source of protein, polyunsaturated fatty acids (particularly omega-3 fatty acids), calcium, zinc, and iron (Chan et al., 1999). In the future, seafood will even be a more important source of food protein than they are today and the safety for human consumption of products from aquaculture is of public health interest (WHO, 1999). Metal residues problems in the fish flesh are serious, as reflected by the high metal concentrations recorded in the water and sediments (Wong et al., 2001). Recently, assessment of heavy metals residues in fish flesh has been the purpose of many researches in different countries: Norway (Amundsen et al., 1997), Malaysia (Lau et al., 1998), Greece (Catsiki and Strogyloud, 1999), Mexico (Avila_Perez et al., 1999), Turkey (Zyadah and Chouikhi, 1999); USA (Cohen et al., 2001) and Egypt (Abou-Arab et al., 1996; Rashed, 2001). The Caspian Sea is the largest lake, both by its area and volume. The watershed of this large water body is approximately 3.5 mkm^2 that makes more than 10% of all drain less areas on the earth. The Caspian Sea is not a freshwater lake. Its waters are rather brackish; three times less concentrated

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Abbreviations: Cd, Cadmium; **Cu**, copper; **Pb**, lead; **Hg**, mercury; **FAO**, food and agriculture organization; **FDA**, food and drug administration.

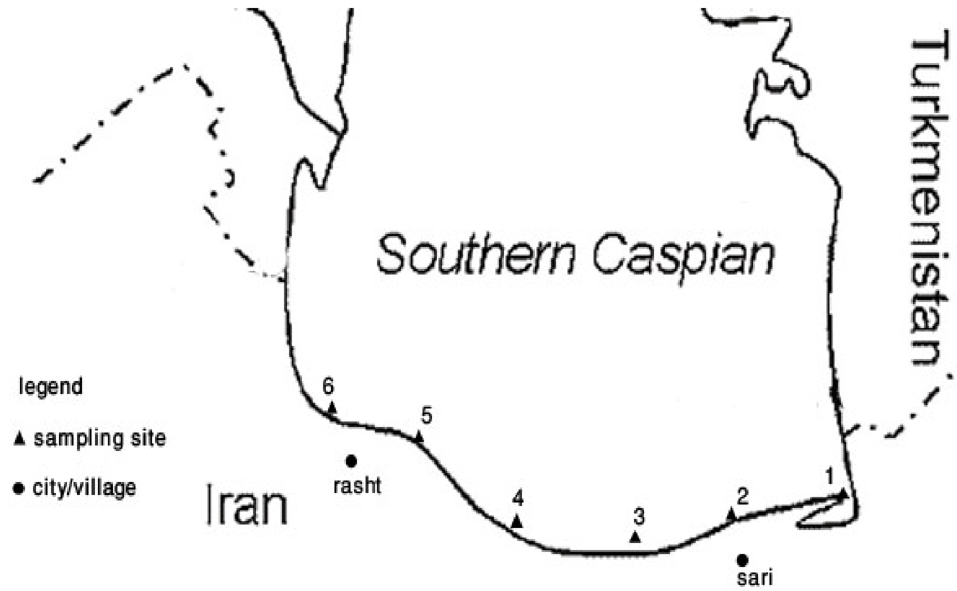

Figure 1. Southern Caspian Sea.

than in the World Ocean. The water salinity is almost stable here fluctuating within 12-13 gr/l. Volga River provides about 80% of the total river runoff which carries heavy metals rich sewage from Russia and water circulation carries that around the Caspian Sea. This lake is considered as one of the major site of commercial and recreational fisheries in Iran. Heavy metal residues in fish flesh and its hazard effects on the health of people are a matter of great concern to food hygienists. The most non-essential heavy metals of particular concern to fish and surface water are cadmium (Cd), Copper (Cu), lead (Pb) and mercury (Hg) which have the way to fish flesh mainly via gills (Tao et al., 1999, 2000), subsequently to safe guard the fish consumers, periodical evaluation of heavy metals residual level in the fish flesh and water from expected polluted area are of major importance (Kock and Hofer, 1998; Medani and Ahmed, 1999). The objective of this study was to evaluate Cd, Cu, Pb and Hg contents of *Tinca tinca*, *Pelecus cultratus* fleshes and water from southern Caspian Sea, Iran.

MATERIAL AND METHODS

Samples collection

T. tinca (n=90); *P. cultratus* (n=90) and water (n=90) samples were collected at quarterly intervals during a period of January, 2009 to January, 2010 from six different sites and average depth 1.5 m (15 samples from each site) at Caspian Sea, Iran (Figure 1). The average weight of fish samples were 150 g ± 12, while water sample was 150 mL. The samples were sent to the laboratory for preparation and analysis. All equipment surfaces and utensils which used in this study were cleaned thoroughly with detergent, rinsed with water, dried and then rinsed with 2-propanol solution. The samples were stored at -2℃ prior to analysis.

Mineral analysis

Cadmium, copper and lead analysis

Cd, Cu and Pb were determined according to AOAC (1994). Fish flesh was minced and well mixed, then approximately 1.0 g was placed in a 150 mL beaker and 10 mL concentrated nitric acid was added. After a short soaking period, 5 mL of 60% perchloric acid was added and the mixture was slowly heated on a hot plate until the conclusion of growth (approximately 2 h). The mixture was then heated until the appearance of dense white fumes that indicate the nitric acid had evaporated and perchloric acid had reached its boiling point. The mixture was cooled, 10 mL of 25% hydrochloric acid was added and the solution was transferred to a 100 mL volumetric flask that was subsequently brought to volume with deionized water. Assessment of metals level in prepared samples were carried out using an atomic absorption spectrophotometer (Model 3100, Perkin-Elmer, Norwalk, Conn, USA) operated under conditions given by EPA (1996). Instruments parameters were: flow rate, 1.5 mL/min; sample pump tube, 0.48 mL/min; pump speed, 90-100 rpm; sample uptake time, 17-21 s; flush time, 30 s; integration time, 10 s; wash time, 30 s; background correction, on; and automatic blank subtraction, on. The lower limits of detection were: Cd, 0.10 ppm; Cu, 0.10 ppm and Pb, 0.50 ppm.

Mercury analysis

Hg was determined according to APHA (1985a). Analysis of Hg was performed by placing 1.0 fish flesh in a 150 mL beaker and adding 10 mL concentrated nitric acid. After a short soaking interval, 5 mL concentrated sulfuric acid was added and the mixture was heated in a microwave digester (CEM, Matthews, N.C. USA) using preset instrument setting. The digested mixture was then cooled and transferred to a 100 mL volumetric flask and brought to volume with deionized water. The elemental analysis for Hg was performed using a PSA atomic fluorescence spectrophotometer (Model, Merlin, P.S. Analytical LTC, and Princeton, N.J., USA) equipped with a PSA random access auto sampler. Instrument setting were: argon carrier (500 mL/min; 99.9999% purity grade); argon sheath

Table 1. Cadmium residues (ppm/wet weight) in flesh of *T. tinca* and *P. cultratus* and water samples from Caspian Sea

Item	LOD*	Mean ± S.D.	Maximum	P.L.**
T. tinca	0.10	$0.60^{a} \pm 0.04$	1.04	0.50
P. cultratus	0.10	$0.77^{b} \pm 0.46$	1.93	0.50
Water	0.0001	$0.54^{c} \pm 0.67$	2.02	0.70

LOD*, Lower limit of detection; P.L.**, permissible limit (WHO, 1990, 1993).
Means in the same column bearing different superscript are significantly different (P < 0.05) n = 90.

Table 2. Copper residues (ppm/wet weight) in flesh of T. tinca and P. cultratus and water samples from Caspian Sea.

Item	LOD*	Mean ± S.D.	Maximum	P.L.**
T. tinca	0.10	$1.76^{a} \pm 0.81$	6.29	20.00
P. cultratus	0.10	$1.94^{a} \pm 1.11$	7.48	20.00
Water	0.10	$1.28^{b} \pm 0.92$	3.61	2.00

LOD*, Lower limit of detection; P.L.**, permissible limit (WHO, 1990, 1993).
Means in the same column bearing different superscript are significantly different (P < 0.05), n = 90.

Table 3. Lead residues (ppm/wet weight) in flesh of *T. tinca* and *P. cultratus* and water samples from Caspian Sea.

Item	LOD*	Mean ± S.D.	Maximum	P.L.**
T. tinca	0.50	$1.51^{a} \pm 0.64$	2.34	2.00
P. cultratus	0.50	$1.74^{b} \pm 1.01$	4.55	2.00
Water	0.001	$1.12^{c} \pm 1.06$	3.66	0.50

LOD*, Lower limit of detection; P.L.**, permissible limit (WHO, 1990, 1993).
Means in the same column bearing different superscript are significantly different (P < 0.05) n = 90.

(300 mL/min); argon dryer gas (3 L/min); solution #1=2.5% stannous chloride (Reagent grade, Fisher Scientific); solution #2=2% nitric acid (Reagent grade, Fisher Scientific). The lower limit of detection for Hg using this method was 0.001 ppm.

Water analysis

Each sample was put in a screw-capped tube and boiled for 1-3 h at 130°C till complete dryness. 10 mL of concentrated nitric acid was added to the sample and boiled close to dryness then diluted to 20 mL with deionzied water. The solution was filtrated, transferred to 100 mL volumetric flask, marked and stored refrigerated till analysis. Cd, Cu and Pb were determined according to APHA (1985b) method using an Electothermal atomic absorption spectrophotometer (Model 3100, Perkin-Elmer, Norwalk, Conn, USA). The lower limits of detection were: Cd, 0.0001 ppm; Cu, 0.10 ppm and Pb, 0.001 ppm. Hg was determined according to APHA (1985b) method using Cold vapor atomic absorption spectrophotometer (Model 3100, Perkin-Elmer, Norwalk, Conn, USA). The lower limit of detection was 0.002 ppm.

Statistical analysis

Data were subjected to a one-way analysis of variance using SPSS program to perform the analysis.

RESULTS

The results of this study revealed that the mean Cd, Cu, Pb and Hg residuals level in water samples were significantly (P < 0.05) lower than the mean residuals level in flesh of *T. tinca* and *P. cultratus* samples (Tables 1, 2, 3 and 4). It is observed that flesh of *P. cultratus* samples had significantly (P < 0.05) higher levels of Cd, Pb and Hg than the flesh of *T. tinca* samples (Tables 1, 3 and 4).

Apparently Cd level in the water samples (Table 1) was significantly (P < 0.05) lower than that of *T. tinca* and *P. cultratus*. On the other hand, *P. cultratus* had significantly (P < 0.05) higher Cd content than *T. tinca*. Cd has effects on fish and their consumers. It acts as a fish stressor, leading to metabolic alterations in *T. tinca*, which increased glucose concentration in white muscle and phosphofructo kinase in red muscle.

Cu was found in all samples 100% of *T. tinca* and *P.*

Table 4. Mercury residues (ppm/wet weight) in flesh of *T. tinca* and *P. cultratus* and water samples from Caspian Sea.

Item	LOD*	Mean ± S.D.	Maximum	P.L.**
T. tinca	0.001	$0.34^a \pm 0.02$	0.41	0.50
P. cultratus	0.001	$0.17^b \pm 0.11$	0.57	0.50
Water	0.002	$0.08^c \pm 0.02$	0.90	0.005

LOD*, Lower limit of detection; P.L.**, permissible limit (WHO, 1990, 1993).
Means in the same column bearing different superscript are significantly different (P < 0.05)
n = 90.

Table 5. Occurrence of cadmium; copper; lead and mercury in the flesh of *T. tinca* and *P. cultratus* and water from Caspian Sea.

Metal	***Tinca tinca* (n = 90)**				***Pelecus cultratus* (n = 90)**				**Water (n = 90)**			
	N.D. (%).	*P. No. (%)*	*< P.L. (%)*	*> P.L. (%)*	*N.D. (%).*	*P. No. (%)*	*< P.L. (%)*	*> P.L. (%)*	*N.D. (%).*	*P. No. (%)*	*< P.L. (%)*	*> P.L. (%)*
Cd	9 (10)	81 (90)	72 (88.9)	9 (11.1)	4 (4.4)	86 (95.6)	70 (81.4)	16 (18.6)	13 (14.4)	77 (85.6)	69 (89.6)	8 (10.4)
Cu	0 (0)	90 (100)	90 (100)	0 (0)	0 (0)	90 (100)	90 (100)	0 (0)	12 (13.3)	78 (86.7)	73 (93.6)	5 (6.4)
Pb	2 (2.2)	88 (97.8)	70 (79.5)	18 (20.5)	1 (1.1)	89 (98.9)	57 (64)	32 (36)	0 (0)	90 (100)	64 (71.1)	26 (28.9)
Hg	12 (13.3)	78 (86.7)	74 (94.9)	4 (5.1)	5 (5.6)	85 (94.4)	74 (87)	11 (13)	14 (15.6)	76 (84.4)	69 (90.8)	7 (9.2)

N.D., Non detected; P.No., positive No.; <P.L., lower than permissible limit; >P.L., higher than permissible limit.

cultratus samples and in 86.7% of water samples (Table 5). Mean Cu levels were 1.76, 1.94 and 1.28 ppm for *T. tinca*, *P. cultratus* and water samples, respectively (Table 2). It is clear from the obtained results that mean Cu level of water was significantly (P > 0.05) lower than the mean Cu level in flesh of *T. tinca* and *P. cultratus* (Table 2). On the other hand, 5 (6.4%) of water samples exceeded the permissible limit of WHO (Table 5).and Pb was detected in 97.8, 98.9 and 100% of *T. tinca*, *P. cultratus* and water samples, respectively (Table 5). The mean Pb levels were 1.51, 1.74 and 1.12 ppm for *T. tinca*, *P. cultratus* and water samples, respectively (Table 3). According to this action limit, 20.5% of *T. tinca*, 36% of *P. cultratus* and 28.9% of water samples had Pb residual levels that exceed the permissible limit (Table 5). It is observed that the mean Pb level in water was lower (P < 0.05) than the mean Pb residual level in fish flesh samples (Table 3). Mean Hg level were 0.34, 0.17 and 0.08 ppm for *T. tinca*, *P. cultratus* and water samples, respectively (Table 4). Such values were far less than those reported by Galab (1997) for the flesh of *Claris lazera* and water samples from Manzala Lake. Subsequently, 5.1% of *T. tinca*, 13% of *P. cultratus* and 9.2% of water samples had Hg content that exceed the permissible limit (Table 5).

In addition, seafood having concentration level that exceed the limit incriminated in Hg were poisoning (Minamata disease) in Japan (WHO, 1999). People eating contaminated fish with Hg are subjected to neurological disorders and Minamata disease (Lodenius and Malm, 1998). It is confirmed that the major health impacts caused by Hg level affect people who are not working directly in Hg related industry but who have a regular fish diet that has Hg residues above the permissible limit (Quiroga et al., 2000).

Advisory actions should be issued to warn at-risk populations like children and pregnant or lactating women to avoid over consumption of fish, which hunted from Caspian Sea to prevent the accumulative effects of the heavy metals. Governmental efforts needed to control environmental pollution and improve the water quality of Caspian Sea.

DISCUSSION

There are direct relationships between heavy metals residuals level in fish flesh and metals pollution level in domestic or agricultural wastes in Iranian northern rivers, which represent sources of Caspian Sea (El-Kader et al., 1993). Opinions differ regard the residuals level of heavy metals in the water and their relation to the residuals level in fish flesh. Kock and Hofer (1998) reported that even low concentrations of heavy metal in the water may result in high concentrations in fish flesh. However, others (Wong et al., 2001) reported that despite high metal levels in the seawater and sediments, concentrations of Cd and Pb in fish flesh did not exceed permissible levels. Cd was detected in 90, 95.6 and 85.6% of *T. tinca*, *P. cultratus* and water samples, respectively (Table 5). Mean Cd level was 0.60, 0.77 and 0.54 ppm for *T. tinca*, *P. cultratus* and water samples, respectively (Table 1). Abou-Arab et al. (1996) reported that Cd residues in sardines and mackerel were 0.086 and 0.077 ppm, respectively. It is clear that the Cd level was higher in fish flesh than those obtained by other (Abou-Arab et al., 1996; Medani; Ahmed, 1999), which could be interpreted as the reported metals pollution in the sources of Caspian Sea (El-Kader et al., 1993). Gutenmann et al. (1988) indicated that a frequently used food safety limit for Cd in food is 2 ppm. In 1993, Food and Agriculture Organization (FAO) limit for Cd is 0.5 ppm. WHO (1990, 1994) indicated that Cd permissible limit is 2.0 ppm for seafood and 0.70 ppm for water. 11.1% of *T. tinca*, 18.6% of *P. cultratus* and 10.4% of water samples (Table 1) had Cd residuals level that exceed the permissible limit set by WHO (1990, 1994). Cd significantly decreased total protein concentrations in liver and white muscle regardless of tissue glycogen levels (Almeida et al., 2001).

On human, ingestion of Cd can be associated with salivation, choking attacks, persistent vomiting, abdominal pains, and spasms of the anal sphincter, vertigo and loss of consciousness (Elinder, 1986). It is seen that the Cu contents of Caspian Sea was lower than the past few years. UNDP (1998) reported that mean Cu level in water of Manzala Lake was 4.6-27.9 ppm and 82% of the examined water samples exceeded the permissible limit of WHO. It is may be due to governmental effort for control of the Cu based industrial wastes from the way to the sources of Caspian Sea before treatment. WHO (1990, 1994) indicated that Cu permissible limit is 20 ppm for fish and 2.00 ppm for water. According to WHO action limit, Cu residual level in all *T. tinca* and *P. cultratus* samples was within the action limit. Cu occurs in foods in many chemical forms and has an important role in the physiological activities of living bodies. Abou-Arab et al. (1996) reported Cu residues in sardines and mackerel of 0.086 and 0.077 ppm, respectively. Cu is considered as public health hazard if an abnormal high level of Cu is ingested. Cu may cause Mediterranean anemia, hemochromatosis, liver cirrhosis and Wilson’s disease (Underwood, 1977). UNDP (1998) detected the level of Pb in the fish flesh and water of Caspian Sea by 0.10 and 4.2 ppm, respectively. Abou-Arab et al. (1996) reported mean Pb residue in whole sardines and mackerel of 11.1 and 12.6 ppm, respectively. Hodson et al. (1984) indicated that the Canadian Pb limit of 10 ppm was discontinued, but that the British limit remains at 2 ppm for fish. Abou-Arab et al. (1996) indicated that the FAO limit (1983) is 2.0 ppm. WHO (1990, 1994) indicated that Pb permissible limit is 2.0 ppm for seafood, and 0.50 ppm for water. Industrial and agricultural discharges are the sources of Pb pollution in Iran. Pb is identified as a serious public health problem particularly for children. The adverse toxic effect caused by Pb on human was recognized (Subramanian, 1988). Neurological defects, renal tubular dysfunction, anemia are the most characterized of Pb poisoning (Forstner and Wittmann, 1983). It could be interpreted as the different in the feeding behavior of *C. lazera* as other fish as well as the governmental effort for control the sources of the Caspian Sea. Food and drug administration (FDA) set an action limit for Hg in seafood of 0.5 ppm in 1969, but this was increased to 1 ppm in 1978 (Yess, 1993). However, WHO (1990, 1993) indicated that Hg permissible limit is 0.50 ppm for seafood and 0.005 ppm for water. Hg is readily absorbed and predominantly (90-100%) found in fish tissue bound to protein in the organic form as methylmercury (EPA, 1994). Methylmercury is a public health hazard, which causes neurological impairment and kidney damage (Bechtel, 1986).

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