Baseline Study of Mercury in Fish of Shatt Al-Arab river by Cold Vapor Atomic Absorption Spectrometry

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Abstract

Cold vapor atomic absorption spectrometric determination of mercury in six fish species from Shatt Al-Arab river, provide values that are lower than the most literature data. We found significant species differences, with *Cyprins Carpio* having the highest levels (0.160 ± 0.045 µg/gm) and *Barbus sharpeyi* the lowest levels (0.053 ± 0.026 µg/gm). Statistically significant differences of total mercury concentration were also found among the individually tested tissues(muscle , kidney, liver, skin and gills), since the muscle of the tested fish accumulate the highest amount of mercury (0.067- 0.160 µg/gm). The data illustrates that Shatt Al-Arab river is still a non mercury– polluted area at the time of analysis.

الخلاصة

اهتم البحث الحالي بتقدير عنصر الزئبق في نماذج من الأسماك بإستخدام تقنية الإمتصاص الذري-التذرية الباردة ،شملت الدراسة ست أصناف من الأسماك جمعت من مواقع مختلفة في نهر شط العرب، فضلا عن شراء بعض النماذج من مراكز البيع المباشر، تضمنت الدراسة الأصناف , *Barbus sharpeyi* , *Tenaulosa ilisha*، والتي تمثل 50 % من غذاء سكان مدينة البصرة من الأسماك. *Cyprins Carpio* , *Barbus lutaus* , *Liza abu* , *Tilapia zillii*, بينت النتائج إحتواء الصنف *CyprinsCarpio* أعلى تركيز من الزئبق (0.160±0.045) مايكروغرام/غرام مقارنة بالأصناف الأخرى كذلك إحتواء عضلات السمكة أعلى تركيز من عنصر الزئبق (0.137±0.053) مايكرو غرام/غرام، بينما احتوت خياشيم السمكة أوطأ تركيز من العنصر (0.056±0.014)مايكرو غرام/غرام. أستنتج من الدراسة أن النتائج المحصلة تدل على إن تركيز الزئبق في أسماك نهر شط العرب قليل مقارنة مع دراسات عالمية أخرى، و أنه أدنى من الحدود المسموح بها للإستهلاك البشري.

Introduction

It is well know that fish can be very nutritious and are packed with great nutrients such as omega-3's, the B vitamins and lean protein that are essential for growth and development [Daviglus et al 2002, Patterson 2002]. But unfortunately, fish can also have some unhealthy contaminants.

Mercury and its compounds are included among the most toxic substances found in the environment [Renner, 1997; Wheatley and Wyzga, 1997]and it is exists in a large number of different physical and chemical forms with a wide range of physical, chemical, and ecotoxicological properties that are of fundamental importance to its environmental behavior [Lambertsson et al., 2001; Queuvallier et al.2000] . Mercury in fish can affect brain development and damaging the central nervous system. The three most important inorganic chemical forms of mercury (elemental mercury – Hg^0, mercurous – Hg_2^{2+} and mercuric – Hg^{2+} ions) are far more soluble and have a strong affinity for many inorganic and organic legends, especially those containing sulfur. Those forms are strongly accumulated in aquatic organisms and also concentrated in aquatic food chains i.e., fish, birds etc. [polyi et al 2004; Ebinghauser et al., 2000]Fishes in the surface water serve as bioindicators of pollutant loads [Teh et al., 1997],it is therefore a product for which suitable measures should be taken to provide chemical monitoring of the risks deriving from its consumption.

The major health impacts caused by mercury affect mostly individuals who have a regular fish diet. Human exposure to mercury intoxication through the ingestion of mercury contaminated fish has been well review[Harada 1995].

The FDA has released guidelines for children, women who are pregnant and women who are trying to become pregnant. These guidelines state that no more than 12 oz of low mercury fish should be consumed weekly. "Highest" mercury fish should be avoided and "high" mercury fish should be kept to only three 6-oz servings per month. Most concern has centred on the presence of mercury in fish since seafood is a major source of

mercury in non-occupationally exposed populations [Faro and Seidler 1960].

Fish and fish products account for most of the organic mercury in food. The average daily intake of mercury from food is in the range 2–20 μg/day, but may be much higher in regions where ambient waters have become contaminated with mercury and where fish constitute a high proportion of the diet [Gorchev 1991].Mercury concentration in fish are influenced by fish age , mercury concentration in water ecosystem , food contamination , chemical , biological and physical process in aquatic environment and seasonal variations [Svobodova et al 2004, Sarica et al 2005].

The people are faced with conflicting information about the risks and benefits of consuming fish.The aim of this study is to determine the mercury concentration levels in the six fish species from Shatt Al-arab river to providing data on the most commonly eaten fish that will help people make informed decisions about risks from fish consumption.

Experimental

Apparatus:

The determination of mercury in the fish samples achieved by means of cold mercury vapor atomic absorption spectrometry (CVAAS) using shimadzu atomic absorption spectrophotometer model (AA-630-12). The cold vapor system used has been described in detail elsewhere [Ali 2006].

Reagents:

Chemicals of analytical – reagent grade and distilled – deionized water were used for the preparation of all solutions:

1- 1000 ppm Hg^{2+} solution, 1.3535 gm of $HgCl_2$ was dissolved in 50 ml of concentrated HCl and diluted to 1 Litre with deionized water .
2- 25% (w/v) SnCl2 ,25 gm of $SnCl_2$ was dissolved in 100 ml of 20% (v/v) HCl .
3- 0.05 % (w/v) $K_2Cr_2O_7$ .0.05 gm of $K_2Cr_2O_7$ was dissolved in 2% (v/v) HNO_3 solution .

4- Crystal potassium persulphate

Sample preparation:

The freshwater Shatt Al-Arab is the longer river in Basrah (180 km). Fish from this river serve as the main source of protein for the population of Basrah city Six species of fish commonly consumed in Basrah were collected in September 2006 at various sites on the shore of Shatt Al-Arab as shown in Fig.1. Some fish samples were bought directly from local fish markets. Total length (TL) of fish was ranged among 22.5 to 44.6 cm. Ages of the fish were between 3–7 years. .Muscle flesh from the mid-dorsal region of the five species was removed. The samples were first sun dried on the beach sand until nearly dry (about 10% of the water remained) .Dried sample (0.5 gm) and 15 ml HNO_3 were placed in a digestion tube fitted with a 40 cm air-cooled condenser .A temperature and time controlled heated digestion unit was used as follows: 25ºC (4 hrs.) ;70ºC (3hrs.) and 140ºC(6 hrs.). The condenser was removed to reduce the volume to 2 ml .After cooling ,5 ml $HClO_4$ was added, the air condenser replaced and the following temperature-time schedule implemented : 140ºC (10 min.), 160ºC (10 min.), 180ºC(10 min.)and finally 210ºC for 1 hr . This gradual increase in temperature is necessary to prevent charring of the material**.** The digestion block was cooled to 170 ºC and the air condenser removed to reduce the volume to 1 ml . After cooling to room temperature , the solution was quantitatively transferred to a standard flask and diluted to 20 ml with deionized water.

Procedure:

Two ml of digested fish samples was introduced in the reduction vessel, 2 ml of $SnCl_2$ solution was added and mixed using magnetic stirrer for 2 min. then the mercury vapor forced by nitrogen gas with rate of 0.25 L/min in range (1.0 – 25 ng/ml) (Fig.2). A calibration graph prepared from aqueous standard mercury solution to which $SnCl_2$ solution was added and measured by the above procedure. The optimum parameters of atomic absorption spectrometer for the determination of mercury were listed in

Table (1). Detection limit calculated following the recommendations of IUPAC was 0.73 ng Hg /gm dry weight.

Statistical analysis:

The statistical analysis were carried out using two-way analysis of variance with unbalanced repeated measurements .Statistical between individual time points was made by using Revised Least Significant Difference (RLSD) test. The probability level for significance was 5% or less .

Result and disscution

Six fish species were selected for monitoring bioaccumulation of total mercury in river ecosystems. There are four possible routes for substances such as mercury to enter fish body. One way is by means of the food ingested [Baudin 1987] and another is by means of absorption of the mercury in its ionic form by the fish through the gills [Heath 1987]. This can occur probably by simple diffusion and possibly through the pores in the gills [Bryan 1979] . Indications are that metal uptake in the gill tissues may be correlated with mass specific rates, with small fish accumulating metals more rapidly than larger ones [Anderson and Spear 1980]. A third possible route is through drinking water [Eddy 1981] while a fourth is by absorption through the skin.

The bioaccumulation of mercury was evaluated in selected fish tissues of *Tenaulosa ilisha* ,the mercury concentrations in the tested tissues are presented in Table (2) .The results showed that the highest concentrations of the total mercury were determined in muscle tissues (0.137 ± 0.053µg/gm) and the lowest concentrations of total mercury were found in gills (0.056 ± 0.014µg/gm) and skin (0.042 ± 0.017µg/gm) of tested fish .Statistically significant differences of mercury concentrations were found among the individually tested tissues with the following exceptions: the skin tissues have statistically insignificant differences compared to the gill ($P< 0.05$) also statistically insignificant differences of the mercury concentrations were found between liver and kidney($P< 0.01$) The mercury content in tested tissues of *Tenaulosa ilisha* decreased in order: muscle >>

kidney ≈ liver > skin ≈ gills .; this can attributed to the influences of the living environment on the total mercury content in muscle tissues of fish where mercury is bound to cysteine rich proteins [Boening 2000,Anonymous 2002] . Also the other authors [Anonymous 1999,2002 ,Dusek et al 2005] observed high concentrations of the total mercury in muscle tissues of various fish species .Vigh et al 1996 found the highest mercury concentrations in the kidney of grass carp .Only the flesh of freshwater fish in England revealed higher values than those in the other tisses [Barak and Mason 1990).

Mercury concentrations between fish species *Cyprins Carpio* , *Barbus lutaus*, *Liza abu, Tilapia zillii,Barbus sharpeyi and Tenaulosa ilisha* were significantly different ($p < 0.0001$). Table (3) showed that *Cyprins Carpio* had the highest mercury concentrations (0.160 ± 0.045µg/gm), followed by *Barbus lutaus* (0.149±0.075µg/gm)The lowest mercury concentrations were found in the two species *Liza abu* and *Barbus sharpeyi* with mean concentrations of (0.067 ± 0.027µg/gm) and (0.053 ± 0.026µg/gm) respectively. The high mean mercury concentrations observed in *Cyprins Carpio* can be attributed to the widest food web (algae, aquatic plants and terrestrial seeds ,larvae and molluse animals). Because of the low range fish age (3-7 years) their age did not influence the content of total mercury in the tested tissues.

In Table(4) literature data for levels of total mercury in fish are presented. Even for the same type of water interspecies comparison has limited value. Consequently our data can only be compared with the scarce values for the same or related *Tilapia* species as shown in Table (5). The total mercury content in the muscle of *Tilapia zillii* from Egypt (Maryut Lake) [El Nabawi et al 1987 was 4-times higher compared to the muscle samples from Egypt (Niger Delta)[Kakulu and Osibanjo 1986] and 65-times lower compared to the muscle samples from Egypt(Wadi El-Raiyan Lake) [Saleh et al 1988]. Levels of mercury for the Egypt (Nile River) fish samples were identical for those found in the fish samples from Egypt (Maryut Lake) [El Nabawi et al 1987] . In the fish samples from Kenya

(Lake Nakuru) the total mercury levels obtained by Koeman et al [1972] were significantly lower than those found by Greichus et al [1978b] .

The FAO limit for total mercury in fish products , which is also adopted by various countries , is 0.5µg/gm ,(FAO 1983). The WHO (1987) recommendeds a permitted value of 0.31 µg/gm .In all fish samples analyzed a concentration level of at least thirty times lower was observed

Mercury in fish has been featured in the media frequently ,and people are faced with conflicting information about the risks and benefits of consuming fish.We suggest that the state agencies responsible for protecting the health of their citizens should obtain information on fish availability in markets and fish preferences of diverse groups of citizens and use this information to select fish for analysis of contaminant levels , providing data on the most commonly eaten fish that will help people make informed decisions about risks from fish consumption.

Conclusions

The levels of mercury determined are higher in *Cyprins Carpio* than in the other fish species, all of which are consumed by humans The muscle tissue had the highest mercury concentrations in comparison with other organism. The levels of mercury in the tested fish species are , as far as could be compared , below published values for other fish and well below the acceptable limits for mercury in foods and thus unlikely to cause adverse effects to aquatic organisms. Our results help us to understand better the accumulation of the individual mercury in the selected tissues of the fish.

Table (1) :Instromental Parameters

Wave length (nm)	253.65
Band width (nm)	0.3
Lamp current (mA)	6.0
Back ground correction	None
Time of mixing (sec.)	60
Flow rate of N_2 gas (L/min)	0.25
Conc. Of $SnCl_2$(%)	25
Volume of $SnCl_2$ (ml)	2.0
Temprature (ºC)	80
Volume of sample (ml)	2.0

Table (2):Concentrations of mercury (µg/gm ,wet weight) in different organs in *Tenaulosa ilisha* **fish from Shatt Al-Arab river.**

Organs	No. of sample	Hg Conc.(Mean±S.D) For 5 replicates
Kidney	8	0.076±0.030
Liver	8	0.079±0.025
Muscle	10	0.137±0.053
Skin	9	0.042±0.017
Gills	8	0.056±0.014

Table (3): Concentrations of mercury (µg/gm ,wet weight) in the muscle of different species of fish from Shatt Al-Arab river.

Species	No. of sample	Hg Conc For 5 replicates. (Mean±S.D)
Liza abu	9	0.067±0.027
Barbus lutaus	11	0.149±0.075
Barbus sharpeyi	10	0.053±0.026
Tenaulosa ilisha	10	0.137±0.053
Cyprins Carpio	10	0.160±0.045
Tilapia zillii	8	0.077±0.036

Table(4): Concentrations of mercury in freshwater fish .

species	Location	No. of samples	Hg contents (µg/gm)	References
Gadus morrhua	Norway (fjords)	-	0.1-0.4	Stenner and Nickless 1974
Commercial species	SW Spain/Portugal (Atlantic coast)	-	0.79	Stenner and Nickless 1975
Composite	South Africa (Transvaal)	-	0.52	Greichus et al 1977
Composite	Zimbabwe (McIlwaine Lake)	3	0.23	Greichus et al 1978a
Twelve species	Finland (Lake Paijanne)	1774	0.05-4. 68	Hattula et al 1978
Lates niloticus	Nigeria (Niger River)	-	0.67	Ndiokwere 1983
All fish	Nigeria (Niger Delta)	-	0.10	Kakulu and Osibanjo 1986
Salmon sole	Pakistan (coastal waters)	16	0.05±0.02	Jaffar and Ashraf 1988
Forimio niger	Pakistan (coastal waters)	16	0.07±0.02	Jaffar and Ashraf 1988
Paralichthys species (sole)	Argentina (Bahia Blanca estuary)	188	0.85±0.18	Marcovecchio et al 1988
Finfish	USA (Louisiana)	52	0.4	Ramelow et al 1989
Four species	Spain (Castellan Coast)	-	0.16-0.84	Hernandes et al 1990
Rutilus rutilus	Eastern England(Rivers)	338	0.01-0.4	Barak and Mason 1990a
Nine fish species	USA (Savannah River)	-	<0.5	Winger et al 1990
Serranus species	Italy (Coastal waters)	-	0.09-0.63	Giadano et al 1991
Commercial species	Czechoslovakia	30	0.01-0.12	Palusova et al 1991
Common fish	Spain (Granada Coast)	32	0.03-1.21	Navarro et al 1992
Two species	Burundi (Lake Tanganyika)	100	<0.05	Benemariya et al. 1991
Six species	Iraq (Shatt Al-Arab River)	58	<0.3	Present study

Table(5): Concentrations of mercury in some *Tilapia* species.

Species	Location	No. of samples	Hg contents (µg/gm)	References
apia grahami	Kenya (Lake Nakuru)	10	0.016	Koeman et al 1972
Tilapia	Kenya (Lake Nakuru)	10	0,22	Greichus et al 1978b
Tilapia melanopleura	Egypt (Niger Delta)	-	1.33	Ndiokwere 1983
Tilapia zillii	Egypt (Niger Delta)	-	0.013	Kakulu and Osibanjo 1986
Tilapia niloticus	Egypt (Nile River)	5	0.05	El Nabawi et al 1987
Tilapia zillii	Egypt (Maryut Lake)	4	0.06	
Tilapia niloticus	Egypt (Maryut Lake)	5	0.04	
Tilapia zillii	Egypt (Wadi El-Raiyan Lake)	-	4.00	Saleh et al 1988
Tilapia zillii	Iraq (Shatt Al-ArabRiver)	10	0.077	Present study

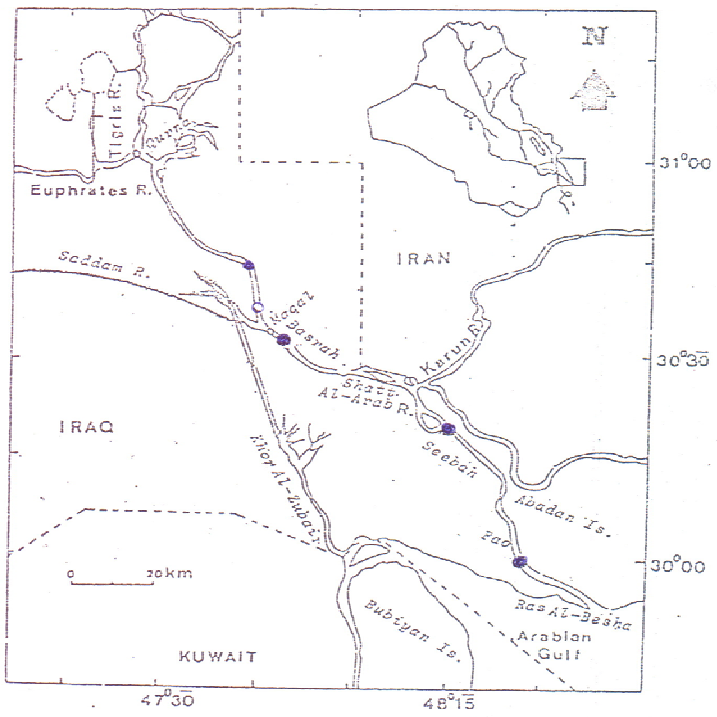

Fig. 1. Shatt Al-Arab River map showing sampling stations

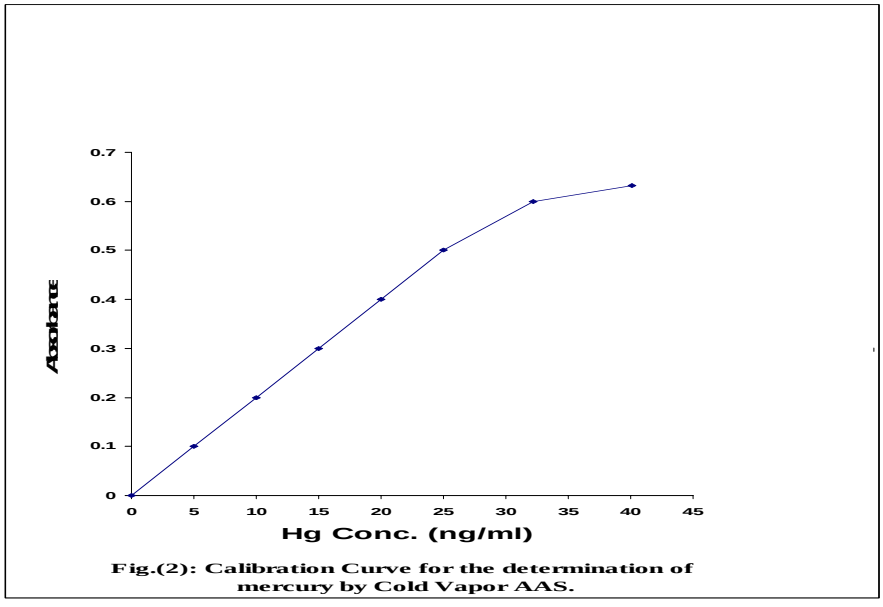

Fig.(2): Calibration Curve for the determination of mercury by Cold Vapor AAS.

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