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DETERMINATION OF SELENIUM IN FISH BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

Nadhum A.N.Awad Hassan T. Abdulsahib and *Amal A. Mahdi

Chemistry Dep., Collage of science, University of Basrah, Basrah,Iraq
**Marine sciences centre, University of Basrah, Basrah,Iraq*

Abstract

Hydride generation atomic absorption spectrometric method was used for the determination of selenium in fish. Different parts of the most important six species of fish *Cyprins carpio*, *Barbus luteus*, *Liza abu, Tilapia zillii, Barbus sharpeyi and Tenaulosa ilisha* is the diet of the local population , have been analyzed after wet destruction with a mixture of concentrated acids ($HClO_4/HNO_3$).Tissue analysis of *Barbus sharpeyi* reveals that liver accumulates the highest amount of selenium .No correlations were observed between selenium levels in the fish and season of collection ,length ,sex and age of fish. . The values obtained are compared with literature data and selenium contents in fish muscle.

Introduction

Fishes are an important source of protein for many people throughout the world, and their importance in the diet has increased among human health .Not only are fishes an important source of nutrients, but fishing is a popular pastime[Burger 2002, Knuth et al 2003],in urban as well as in rural areas[Burger et al 2001, Ramos and Crain 2001]. Fish provide omega-3 (n-3) fatty acids that reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery [Daviglus et al 2002, Patterson 2002] There has been ever –increasing interest in the role of selenium in human nutrition ,since more and more investingators realize the essentiality of this trace element for human health [Levander 1982]. The interest in daily

intake of selenium has resulted not only because of the pathological conditions which induce from deficiency or toxicological reasons, but especially also for the beneficial effect of this element in preventation of various types of cancer [Milac-Devic et al 1992, Comstock et al 1992] and cardiovascular diseases [Bluhm 1990, Singh et al 1992] Selenium is an essential trace element for vertebrates but selenium contamination in drainwater and surface water is a serious problem to fishes and wildlife resources and it has been identified as an environmental contaminant in fishes collected from rivers in industrialized areas [Mierzykowski et al 1997]. National dietary intake of selenium by rainbow trout is approximately 0.07g/g [Hilton et al 1980]. While selenium may cause death in deficient amounts [Eisler 1985], elevated intake of selenium also be harmful. Fishes consuming diets with 10 to 33 g Se/g have experienced toxic effects [Besser et al 1993] .Excessive amounts may be lethal, cause reproductive abnormalities or failure, result in tissue damage, retard growth, or eliminate entire fish communities [Lemly 1996].

In this study the analyzed selenium in different types of fishes in Shatt Al-Arab river has been done to establish natural background levels of this element in a non polluted basin in Basrah; hence, no analytical work seems to have been undertaken on the selenium content of Shatt Al-Arab fish.

Experimental

Apparatus

A shimadzu flame atomic absorption spectrophotometer model (AA-630-12). A pye unicam hallow cathode lamp of selenium was used as a source; The silica tube (18×0.8 cm) is placed in an air – acetylene flame, about 4 mm above the slot of a 10-cm single slot burner head. Nitrogen gas was used as carrier gas for transfer the selenium hydride from the reduction vessel to the absorption cell.

Reagents

All chemicals used were of analytical – reagent grade. The hydrochloric and nitric acids were of “suprapur” quality (Merck) and (60%w/v) perchloric acid “analar” (Merck) was used. 1000-ppm a stock solution of

selenium standard was prepared by dissolving 1 gm of high purity selenium metal (Fluka) in (3:1) hydrochloric acid - nitric acid mixture at 120 ±10 Cº for 15 minutes, the solution was allowed to cool and diluting to 1 liter with demineralised water.

Sodium borohydride (Fisher Scientific Company 98% pure) solution,1%w/v in distilled water was freshly prepared. Hydrochloric acid (1.5 M) was prepared by diluting 6.52 ml of conc. (36%) HCl by deionized water to 50 ml .

Sample preparation

Six species of fishes commonly consumed in Basrah were collected at various sites on the shore of Shatt Al-Arab as shown in Fig.1. Some fishes samples were bought directly from local fish markets. Muscle flesh from the mid-dorsal region of the five species was removed, while for the *Barbus sharpeyi* different parts of organs samples were taken for analysis. 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.)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,1 ml HCl was added, the mixture heated at 100 ºC for 15 min to convert all selenium compounds to selenites and then the solution was quantitatively transferred to a standard flask and diluted to 20 ml with 1.5M HCl

Procedure

A 0.2 ml volume of the sample solution in 1.5 M HCl medium was placed in the reaction vessel and 2 ml of 1% w/v $NaBH_4$ solution were added. The solution was mixed for 20 sec. and the formed selenium hydride

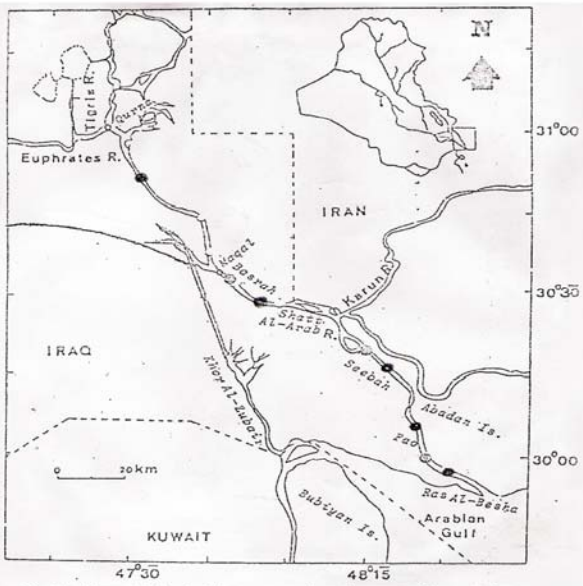
swept by nitrogen gas at a flow rate of 1.2 L/min in to the heated atomization cell, and the absorption signal is recorded. The optimum parameters of atomic absorption spectrometer for the determination of selenium were listed in Table (1). The hydride generation system used has been described in detail elsewhere [Hussain 2006].The calibration curve used in the analysis of selenium was shown in figure (2). Detection limit calculated following the recommendations of IUPAC was 1.6 ng Se /gm dry weight. The linearity of calibration graph ranged between 0.05 and 3.0 µg Se/gm .

Table (1) :Instrumental Parameters

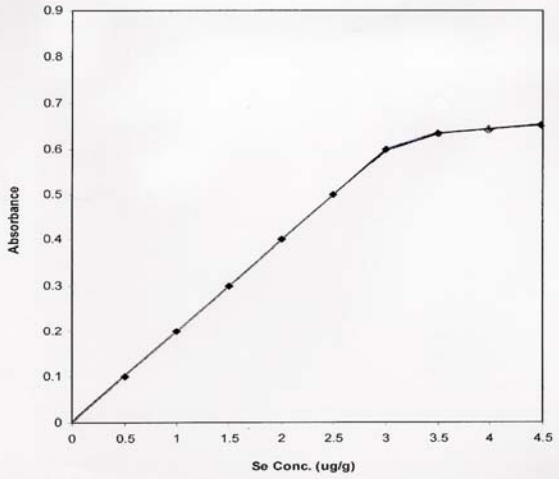
Wave length (nm)	**196.1**
Band width (nm)	**1.9**
Lamp current (m A)	**8.0**
Back ground correction	**None**
Flow rate (L/min)	**Air : 11.5**
	Acetylene:2.5
Time of mixing (sec.)	**20**
Flow rate of carrier gas (L/min)	**1.2**
Conc. Of $NaBH_4$(%)	**1.0**
Conc. Of HCl (M)	**1.5**
Temperature (ºC)	**25(room temp.)**
Volume of sample (ml)	**0.2**

Table (2): Concentrations of selenium (µg/gm, Mean ± S.D) in different organs in *Barbus sharpeyi* fish from Shatt Al-Arab river.

Organs	No. of sample	Se Conc.	
		Dry weight	Wet weight
Skin	7	1.37±0.22	0.35±0.02
Liver	9	1.63±0.31	0.23±0.04
Kidney	9	1.29±0.34	0.39±0.03
Muscle	10	0.94±0.26	0.24±0.05
Gill	7	1.21±0.29	0.26±0.04



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



Fig(2):Calibration curve for the determination of selenium by hydride generation AAS.

Statistical analysis

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

Results and Discussion

The freshwater Shatt Al-Arab is the longer river in Basrah (180 km). Fishes from this river serve as the main source of protein for the population of Basrah city. Fish can accumulate significant amounts of selenium from both water and food. There are four possible routes for substances such as selenium to enter fish body. One way is by means of the food ingested and another is by means of absorption of the selenium in its ionic form by the fish through the gills. This can occur probably by simple diffusion and possibly through the pores in the gills. 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. A third possible route is through drinking water while a fourth is by absorption through the skin. We therefore analyzed the selenium levels in various organs of *Barbus sharpeyi* as shown in Table 2. The data shows that the liver contained the highest concentration (1.63±0.31µg/gm) and the lowest levels was found in muscle(0.94±0.26 µg/gm). Table (3) shows that there are significant differences in selenium levels ($P<0.01$) among *Cyprins carpio*, *Barbus luteus*, *Lizaabu, Tilapia zillii, Barbus sharpeyi and Tenaulosa ilisha*, with *Cyprins carpio* having the highest levels (1.77 ± 0.42µg/gm) and *Barbus luteus* the lowest levels (0.73 ± 0.35 µg/gm). Differences found between species may be explained by the levels of selenium in water and the type and amount of food consumed by the fish. No correlation were found between selenium content and length of the fish, also there is no any correlation between selenium levels and season of collection or age of fish. No sex differentiation was made, since no influence of this factor on selenium content of fish was found [Glover 1979].

The people have now restricted the literature search to economically important species that are consumed by humans. It is difficult to compare selenium contents of different fish because: (a)species sampled are frequently not identified, or sometimes the common name is used instead of the taxonomic indication, (b) various concentration expressions are used (µg/g fresh weight, dry weight or wet weight) or even not mentioned, (c) different organs or tissues of total fish are sampled, (d) there are different feeding habits and (e) differences can be attributed to various forms of

water pollution (and therefore the selenium level of water normally must be taken into account). In Table (4) literature data for levels of selenium in fish are presented. Even for the same type of water interspecies comparison has limited value. Consequently the data can only be compared with the scarce values for the same or related *Tilapia* species. In Table (5) concentrations of selenium in the flesh of *Tilapia* were much higher for the Egyptian fish [Saleh et al 1988], probably due to agricultural waste dumping, containing fertilizers with sodium selenite (Na_2SeO_3). The low levels of selenium found in Burundi [Benemariya et al 1991] fish show that Lake Tanganyika is still a non- polluted area. If all these conditions are fulfilled, sampling the same species at the same point can still result in different figures as a function of time, not only because of the technique of analysis used but because of decreased or increased industrial waste discharges or recently started water treatment, the regulation of this process have been reduced the direct inputs of selenium to the environment. In general, the concentrations of selenium that accumulate in fish are lower now than at any time for which accurate data exist.

Once a personal choice has been made to eat fish, the consumer must decide what types to eat. This decision may be based on several preventive and healthy factors besides price and availability, including selenium concentrations. The feed should not contain more than 5 mg/kg [WHO 1987] Many of the fish species examined in this study had levels of selenium < 2.0 ppm and would pose little risk to a developing fetus. The data suggest that consumers have choice of fish with low selenium levels, and such information should be provided to the public. Information on selenium levels in commercial fish will also be useful to the public in balancing the risks from self-caught and commercial fish .That is , with information on selenium (or other contaminants) in fish from their local lakes or streams, anglers or the family cook can determine whether to eat commercial or self-caught fish and how much of each species to eat. There are a long way from having sufficient information on selenium for people to make these decisions, but the agencies should go in this direction. From a public health standpoint, commercial fish is the main point of intervention to reduce selenium exposure in the public.

Table (3): Concentrations of selenium (µg/gm, Mean ± S.D) in the muscle of different species of fish from Shatt Al-Arab river.

Species	No. of sample	Se Conc.	
		Dry weight	Wet weight
Cyprins carpio	9	1.77±0.42	0.63±0.06
Barbus luteus	11	0.73±0.35	0.33±0.05
Lizaabu	10	1.09±0.34	0.81±0.08
Tilapia zillii	10	1.26±0.38	0.22±0.02
Barbus sharpeyi	10	0.94±0.26	0.26±0.06
Tenaulosa ilisha	8	1.02±0.47	0.42±0.02

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

Species	**No. of samples**	**Se contents (µg/gm)**	**Country**	**References**
Esox lucius (pike)	14	0.13	Netherlands	Luten et al 1980
Perca flaviatilis (perch)	15	0.24		
Shizostedion lucioperca (pike-perch)	11	0.26		
Salmo trutta (brown trout)	18	0.25±0.04	Norway	Froslie et al.1985
Lota lota (burbot)	25	0.27±0.05		
Esox lucius (pike)	40	0.21±0.03		
Perca flaviatilis (perch)	145	0.41±0.16		
Esox lucius (pike)	-	0.35-0.44	USA	Cappon &smith 1981
	-	(<0.20-0.62)	Canada	Speyer 1980
Freshwater fish	-	0.56	USA	May & McKinney 1981
	-	0.46	USA	Lowe et al .1985
Anguilla anguilla		0.86	Belgium	Vandelanoote et al. 1988
Rutilus rutilus	-	1.25		
Perca flaviatilis		0.75		

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

Species	No. of samples	Se contents (µg/gm)	Sample area	References
Tilapia zillii	-	1.4-5.0	Wadi El-Raijan (Egypt)	Saleh et al. 1988
Tilapia niloticus	11	1.5-1.9	Lake Tanganyika (Burundi)	Benemariya et al. 1991
Tilapia zillii	10	1.26±	Shatt Al-Arab river (Iraq)	This work

Conclusion

The level of selenium is higher in *C. carpio* than the other species. Liver contained the highest concentration of selenium than the other organs of *Barbus sharpeyi*. Fish from Shatt Al-Arab river in Basrah are good source of selenium, this food contributes 50 % of the daily intake of selenium. Taking into account the scarce data on fish consumption, the mean daily intake of selenium which provide by fish was 35 µg/day for ten families residing around Shatt Al-Arab river. Selenium concentration in shatt Al-Arab fish appears to reflect natural background levels rather than pollution.

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تقدير السلينيوم في الأسماك بوساطة تقنية الأمتصاص الذري- توليد الهيدريد

دكتور ناظم عبد النبي عواد حسن ثامر عبدالصاحب أمل عبدالجليل مهدي*

قسم الكيمياء – كلية العلوم – جامعة البصرة –العراق

**** مركز علوم البحار – جامعة البصرة - العراق***

المستخلص

تم في البحث الحالي تقدير عنصر السلينيوم في نماذج من الأسماك بإستخدام تقنية الإمتصاص الذري-توليد الهيدريد ، تضمنت الدراسة ستة أصناف من الأسماك المتوفرة والتي جمعت من مواقع مختلفة في نهر شط العرب، كذلك تم شراء بعض النماذج من مراكز البيع المباشر، أجريت الدراسة على الأصناف *Cyprins carpio, Liza abu, Tilapia zillii, Barbus sharpeyi, Tenaulosa ilisha, Barbus lateu* والتي تمثل 50% من غذاء سكان مدينة البصرة. بينت الدراسة إحتواء الصنف *Cyprins carpio* أعلى تركيز من لسلينيوم (0.42 ± 1.77) مايكروغرام /غرام مقارنة بالأصناف الأخرى كذلك بينت الدراسة احتواء كبد السمكة *Barbus sharpeyi* أعلى تركيز من عنصر السلينيوم (0.31 ± 1.63) مايكروغرام/غرام، بينما احتوت عضلات السمكة أوطأ تركيز من العنصر (0.26±0.94) مايكروغرام/غرام. أستنتج من الدراسة عدم وجود علاقة بين كل من موسم جمع النماذج، الطول، الجنس، عمر السمكة وبين محتوى السلينيوم في الأسماك. تم مقارنة النتائج المحصلة مع دراسات عالمية أخرى.