Mercury Determination in Muscle Tissue of Fish Samples Using Microwave Digestion and CVAAS Analysis

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ABSTRACT

In this study, the mercury (Hg) levels in muscle tissue of 19 different fish species were determined. The fish samples were collected from the Sakarya River, Sakarya Cark Stream, Sapanca Lake and the Western Black Sea, solubilized in a microwave digestion system and analyzed by cold vapor atomic absorption spectrometry (CVAAS). The accuracy of the method was validated with certified reference material DORM-3 Fish Protein. Various analytical parameters were optimized. The relative standard deviation was found to be below 10%. The Hg levels ranged from 0.046 ± 0.07 μg g^{-1} to 0.755 ± 0.02 μg g^{-1}. The Hg recoveries for the external calibration standards and the known amounts of mercury added to the fish samples indicated minimal or no loss of Hg during microwave digestion.

INTRODUCTION

Fish meat is widely consumed and considered a main source of nutrition in many coastal communities (1). It contributes to a healthy diet by providing high-value amino acids and nutrients (vitamins and minerals) and is an excellent source of essential omega-3 fatty acids associated with many health benefits (2). Although highly nutritious, high consumption of some fish meat can have significant adverse effects on human health due to the bioaccumulation of heavy metals in fish muscles from the surrounding aquatic environment (3).

In recent years, the marine environment has been extensively contaminated as a result of human activities. The danger of heavy metals contamination is further increased because they are neither chemically nor biologically degradable and can remain for hundreds of years (4). Bioaccumulation of metals in fish has been widely investigated and several comprehensive studies focus on metal toxicity in fish (5 - 7). Mercury (Hg) is one of the most toxic elements in our environment including in the lithosphere, hydrosphere, atmosphere, and biosphere (8). Humans get mercury from food, environmental and industrial endeavors, and via amalgam compounds. There are two major types of Hg classified as inorganic and organic mercury. Some microorganisms transform mercury into methyl mercury, which is then consumed by fish (9). Mercury can cause serious problems in a variety of ways. Breathing in of mercury vapor damages the developing nervous system of the fetus. Mercury values can also rise to dangerous levels in people who work or live near mercury-related industries such as in mercury waste-containing areas and thermal power plants (10 - 14). The discharge of Hg has resulted in elevated levels in the air and various types of water bodies including rivers, lakes, and coastal waters, as well as in soils and sediments. Of particular concern to public health is the Hg in fish which is considered the most important vector linking environmental Hg to humans (15, 16).

There are a number of analytical methods available for the determination of low concentrations of mercury. The most commonly used are cold vapor atomic absorption spectrometry (CVAAS) and cold vapor atomic fluorescence spectrometry (CVAFS) (11, 17). Because of simplicity, higher sensitivity, less time, and relatively free from interference, CVAAS is the preferred method (10).

In this study, the levels of Hg were measured by CVAAS in 16 different fish species caught in the waters of the Sakarya River, Cark Stream, Sapanca Lake, and Western Black Sea during September and October 2012. Several microwave (MW) digestion techniques were studied for the preparation of the fish samples. The accuracy of these methods was checked by the analysis of the certified reference material DORM-3 Fish Protein for Trace Metals (National Research Council Canada). The results were compared with studies reported in the literature and using the standard values published by the World Health Organization (WHO) and the U.S. Food and Agriculture Organization (FAO) .

EXPERIMENTAL

Instrumentation

A GBC Avanta Σ cold vapor atomic absorption spectrometer (GBC Scientific Equipment Pty. Ltd., England) was used for this study (Figure 1). All measurements were carried out using high purity argon. A Hg hollow cathode lamp,

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operated at 3.0 mA, was utilized as the radiation source. The wavelength and slit width for Hg were 257.3 nm and 0.5 nm, respectively. The measurements were carried out in integrated absorbance (peak height) mode at 253.7 nm and using a spectral bandwidth of 0.5 nm. Argon 99.99% was the carrier gas.

Reagents and Standard Solutions

All reagents were of analytical grade. Double deionized water obtained with a Milli-Q™ system (Millipore Corporation, USA) was used for all dilutions (18.2 M cm^{-1} resistivity). Hydrochloric acid, nitric acid, sodium chloride, sodium hydroxide, and sodium tetrahydroborate were from Merck (Darmstadt, Germany). Stock mercury standard solution was prepared by diluting (1000 mg/L) with 3.0 M HCl solution for the determination of the mercury by CVAAS and some experimental parameters were optimized. The Hg(II) stock standard solution (1000 mg L^{-1}) was prepared for the calibration. $NaBH_4$ solution was used to form elemental Hg as the reducing agent with (0.8% w/v) NaOH.

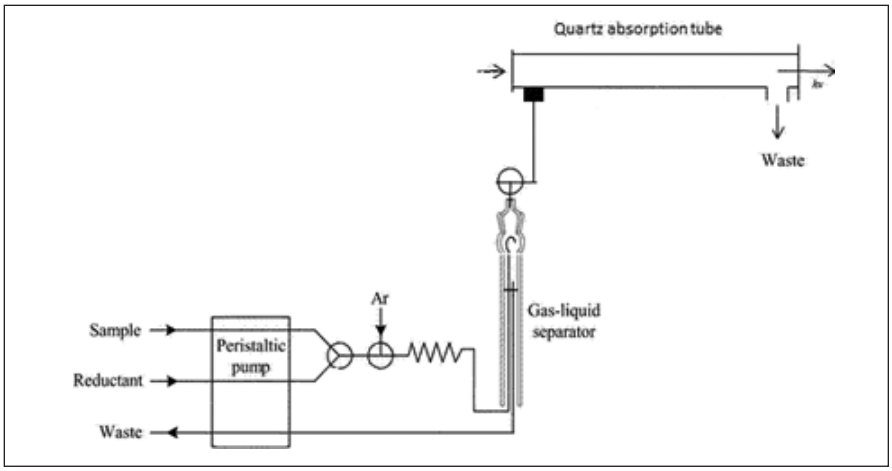

Fig. 1. Schematic of CVAAS system for mercury determination.

TABLE I
Fish Species and Area in Turkey Where Found

Sample Place	Location	Fish Species
Sapanca Lake	Sapanca	*Esox lucius* *Cyprinus carpio* *Scardinius erythrophtholmus* *Tinca tinca*
Sakarya River	Sakarya	*Silurus glanis* *Capoeta pestai*
	Pamukova	*Cyprinus carpio* *Mugil cephalus* *Barbus capito*
Cark Stream	Sakarya	*Blicca bjoerkna* *Mugil cepholus* *Scardinius erythrophtholmus*
West Black Sea	Karasu	*Trachurus mediterraneus* *Sadra sarda* *Mullus barbatu* *Engraulis encrasicolus* *Merlangius merlangus* *Belone belone* *Pamatomus saltarix*

Sample Preparation

19 samples were collected from fish species randomly caught in the Sakarya River, Cark Stream, Sapanca Lake, and the Western Black Sea (see Table I). The fish species anlyzed were: *Silurus glanis, Blicca bjoerkna, Capoeta pestai, Cyprinus carpio, Scardinius erythrophtholmus, Mugil cepholus, Barbus capito, Esox lucius, Tinca tinca, Trachurus mediterraneus, Sadra sarda, Mullus barbatus, Engraulis encrasicolus, Merlangius merlangus, Belone belone, Pamatomus saltarix*. The scales and skin of the fish samples were separated, the viscera removed, the bones cleaned, and the flesh parts homogenized. These were then placed into polyethylene containers, kept in 1:10 nitric acid solution in order to prevent metals contamination, and stored at –20 °C. The homogenized muscle tissues were dried at 50 °C for 48 hours, then placed into a desiccator until they reached ambient temperature.

Microwave Digestion Procedure for Fish Samples

The dried samples were prepared for the microwave incineration process using a Milestone Ethos D microwave system (Sorisole-Bg Italy) with maximum pressure of 1450 psi and maximum temperature of 300 °C. A 0.5 g sample was digested with 6 mL concentrated Suprapur® HNO_3 (65%) (Merck, Darmstadt, Germany) and 2 mL

concentrated Suprapur® H_2O_2 (30%) (Merck) and diluted to 10 mL with double deionized water. A blank digest was carried out in the same way. The digestion conditions for the microwave system for the samples were applied (10-11) (see Table II). The microwave sample preparation method is preferred because it accelerates the incineration process and decreases the contamination level (18). After the incineration process, the samples were filtered and brought to 25-mL volume with pure double distilled Water.

The accuracy of the method was validated with certified reference material (CRM) DORM-3 Fish Protein. Analysis of the CRM was carried out by dilution to linear range of the calibration curve. Table III shows that the results are in accordance with the certified values.

TAB;E II
Microwave Digestion Program

Step	Power (W)	Ramp (min)	Ventilation (min)
1	0	2	8
2	250	2	8
3	250	6	8
4	400	5	8
5	550	8	8

Optimization of Experimental Conditions

The variables were optimized for the simultaneous determination of Hg in the standards, the CRM, and different fish species. $NaBH_4$ solution was used as the reducing material in order to form elemental Hg with 0.8% (w/v) NaOH. The analysis of the CRM was performed by diluting the linear range of the calibration curve. The results were in accordance with the certified values (see Table III). The influence of the argon flow rate (Figure 2), concentration of HNO_3 (Figure 3), and sodium borohyride (Figure 4) on the signal of Hg were investigated.

TABLE III
CRM DORM-3 Fish Protein Analysis Rresults (μg g^{-1}), (N=4)

Hg Certified Value	0.382±0.060
Hg Found Value	0.355±0.056
Relative Error	–7.06%

Analytical Figures of Merit

The linear range of the calibration curve reached the quantification limit up to 20 μg L^{-1} (see Figure 5). The detection and quantification limits for Hg were calculated by LOD=3σ and LOQ=10σ, respectively. The RSD values for the measurements were found to be less than 5.6% (N=5).

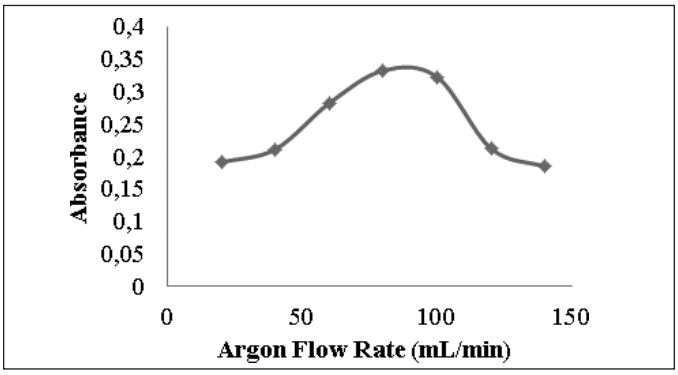

Fig. 2. Optimization of carrier gas flow rate.

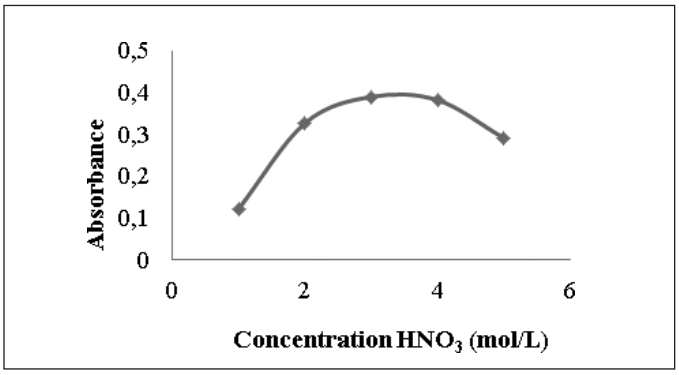

Fig 3. Optimization of acid concentration.

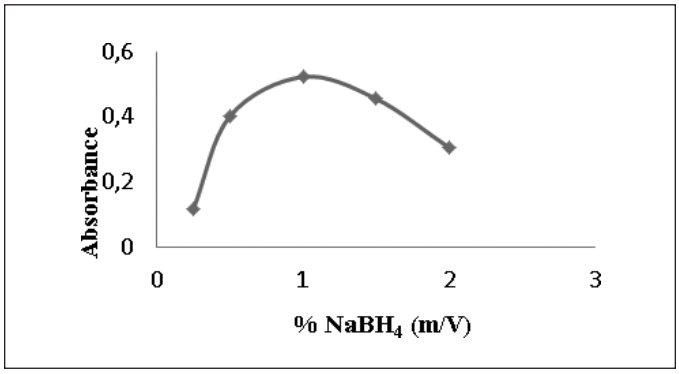

Fig. 4. Optimization of reducing agent concentration

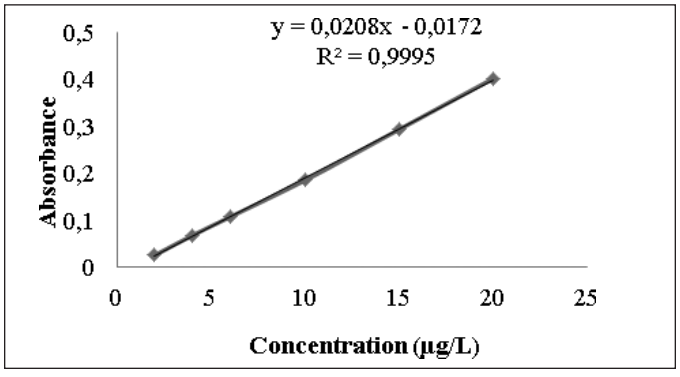

Fig. 5. Calibration curve of determination of Hg by CVAAS.

RESULTS AND DISCUSSION

Several countries have established limitations for allowable mercury levels in fish and generally vary between 0.5–1.0 μg g^{-1}. While determining these limitations, fish consumption and trade were considered. The Turkish National Food Codex, WHO, and FAO advise the Hg levels not to be in excess of 0.5 μg g^{-1} (8–14).

The total Hg level in the fish samples and the % RSD found in this study are listed in Table IV. According to these results, the Hg levels of *S. glanis* were at 0.755 μg g^{-1}, while in all other fish samples the level was 0.5 μg g^{-1} which is acceptable in accordance with the FAO/WHO and the National Food Codex of Turkey regulations (18).

Many studies have been published regarding Hg levels in fish. However, to our knowledge the present study is the first study for fish found in Turkey. In 2015, Doker and Basgelmez (9) developed a simple and sensitive procedure in order to specify and extract Hg. Species separation was accomplished with reverse phase-high performance liquid chromatography (HPLC) and hyphenated to inductively coupled plasma mass spectrometry (ICP-MS). They analyzed six fish species from the Black Sea in Turkey and found Hg levels at 0.2 and 0.1 ng g^{-1}. The differences in Hg content in fish may arise from various factors such as species, gender, diet, habitat, and season (9). In 2009, Tüzen (10) performed a CVAAS method and reported mercury levels of 25 μg kg^{-1} for *S. sarda*, and the highest levels were found at 84 μg kg^{-1} for *M. merlangus*. Thus, these Hg levels are below the 0.5 mg kg^{-1} level as mandated by the Turkish Food Codex (11, 19).

TABLE IV
Mercury Levels in Fish Species (μg g^{-1}), N=4

Fish Species	Total Hg	RSD (%)
Esox lucius	0.082±0.01	0.9
Cyprinus carpio	0.166±0.07	0.4
Scardinius erythrophtholmus	0.221±0.02	0.5
Tinca tinca	0.097±0.01	0.5
Silurus glanis	0.755±0.05	2.3
Capoeta pestai	BDL[a]	BDL
Cyprinus carpio	0.104±0.01	0.6
Mugil cephalus	0.152±0.02	1.0
Barbus capito	0.195±0.01	0.3
Blicca bjoerkna	0.329±0.03	1.4
Mugil cepholus	0.110±0.01	0.9
Scardinius erythrophtholmus	0.474±0.03	0.4
Trachurus mediterraneus	0.046±0.01	0.3
Sadra sarda	0.058±0.02	0.7
Mullus barbatus	BDL[a]	BDL
Engraulis encrasicolus	0.089±0.01	0.5
Merlangius merlangus	0.076±0.01	0.5
Belone belone	BDL[a]	BDL
Pamatomus saltarix	0.098±0.02	0.5

[a] BDL = Below detection limit.

In 2015, Fernandez et al. (4) analyzed fish samples for the determination of cadmium and lead by ICP-AES and mercury by CVAAS (4).

Eperesi et al. (20) specified Hg levels in tuna fish using microwave digestion and cold vapor AAS. The Hg levels found were 0.19±0.07 μg g^{-1} and 3.60±0.17 μg g^{-1}, also below the legal limits.

Pan et al. (16) reported that in fish in China Hg was present in 82% of the 54 different fish (within a total of 571 fish species) with levels at 30 ng g^{-1}. They found that lower Hg concentration levels are connected with feeding habits and trophic levels (16).

Voegborla and Akagi (21) identified Hg levels between 0.004–0.122 μg g^{-1} in 13 of 56 fish samples from the Atlantic coast region in Ghana. These values were lower than the limit of 0.5 μg g^{-1} as per WHO (22).

Squadrone et al. (23) examined mercury and selenium accumulation in *S. glanis* found in the lakes and rivers of Italy. The Hg levels in this fish were 1.4 mg kg^{-1}. *S. glanis* is the largest freshwater fish in Europe and is a favorite food owing to the boneless white flesh, also low in fat and highly palatable (22, 23).

This specie is also widely found in the rivers and lakes of Turkey and sold in supermarkets and other similar stores. In our study, the level of Hg in *S. glanis* from Turkey was found at 0.755 μg g^{-1}, while the Hg levels in all other fish species were below 0.5 mg kg^{-1}.

The variation of Hg concentration in fish of the same species is mostly associated with the size (weight) of the fish. Larger fish usually have higher concentrations than their smaller counterparts (24). We consider that as *S. glanis* is bigger than the other fish and since it feeds on other small fish, the Hg level may be higher.

CONCLUSION

In this study, the mercury level was determined in various fish samples from the Sakarya River, Sakarya Cark Stream, Sapanca Lake, and the Black Sea. Microwave digestion was used to prepare the samples for analysis by cold vapor atomic absorption spectrometry (CVAAS). Quality control of the CVAAS method was performed with CRM DORM 3 Fish Protein, and good agreement was found between the certified values and the experimental results. Several countries have set limitations for the allowable mercury levels in fish and vary between 0.5–1.0 mg kg^{-1}. WHO advises the mercury levels not to exceed 0.5 mg kg^{-1}. In this study, the mercury levels of *S. glanis* harvested in Turkey were found to be below 0.5 mg kg^{-1} which is a positive result for the population in Turkey. Reducing the pollutant load in the region appears to be the best approach to decreasing the presence of heavy metals. According to the data obtained and considering the high fish consumption, the fish of this area of Turkey is not seen as a threat to public health.

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