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# Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry

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**Abstract**

The concentrations of heavy metals (Pb, Cd, Fe, Cu, Mn and Zn) in fish samples were determined using graphite furnace atomic absorption spectrometry after dry ashing and wet ashing methods. Different matrix modifiers were used for the stabilization of the analyte. Good accuracy was assured by the analysis of biological reference materials. Recoveries were quantitative for all elements studied ($\geqslant$95%). The relative standard deviations were less than 7% for all elements.


*Keywords:* Heavy metals; Digestion; Fish; Graphite furnace atomic absorption spectrometry

## 1. Introduction

The heavy metal pollution of the marine environment has long been recognized as a serious environmental concern (Balkas, Tugrel, & Salhogln, 1982; Tarıq, Jaffar, & Moazzam, 1991). In the sea, pollutants are potentially accumulated in marine organisms and sediments, and subsequently transferred to man through the food chain (Giordano et al., 1991). For these reasons, it is important to determine the chemical quality of the marine organisms, particularly the contents of heavy metals, in order to evaluate the possible risk, to human health, of fish consumption (Cid, Boia, Pombo, & Rebelo, 2001). Metals such as iron, copper, zinc and manganese, are essential metals since they play an important role in biological systems, whereas mercury, lead and cadmium are non-essential metals, as they are toxic, even in traces (Schroeder, 1973; Somer, 1974). The essential metals can also produce toxic effects when the metal intake is excessively elevated. Levels of heavy metals in fish have been widely reported (Pujin, Djukic, Maletin, Obradovic, & Kostic, 1990; Sharif, Mustafa, Mirza, & Safiullah, 1991; Tarıq, Jaffar, & Ashraf, 1991; Ubillus, Alegria, Barbera, Farre, & Lagerda, 2000; Voegborlo, El-Methnani, & Abedin, 1999).

Decomposition of solid samples is an important step in combined analytical methods. In most cases, when using highly sensitive measuring methods, such as flame AAS, graphite furnace AAS, ICP–OES, ICP–MS or inverse voltammetry, the sample is measured in an aqueous solution (Knapp, 1991). Combined analytical methods are favoured for multi element analysis of environmental and biological samples at very high speed. Sequential and simultaneous determinations of the elements can be made using the above analytical techniques. Determination of heavy metals (Pb and Cd) in fish has been performed with electrothermal AAS (detection limits of Pb: 6.4 ng/ml and Cd: 0.14 ng/ml) (Sures, Taraschewski, & Haug, 1995). The concentrations of Zn, Cd, Cu and Pb in fish have been determined by using ICP–AES and FAAS (Zhuang, Wang, Yang, Zhu, & Yang, 1995). Heavy metal concentrations have been determined in fish and biological samples using flame AAS with detection limits (as μg/l) Cd: 0.6, Cu: 3, Fe: 5, Mn: 3, Pb: 20 and Zn: 2 (Karadede & Ünlü, 2000). Levels of heavy metals in biological samples have been determined by flame AAS with detection limits (as μg/g) Cd: 0.03, Cu: 0.03, Zn: 0.07, Fe: 0.04 and Mn: 0.01 (Kress, Hornung, & Herut, 1998). Trace metals have been determined in fish samples by electrothermal AAS with detection limits (as μg/l) Cd: 0.0788, Cu: 0.344, Ni: 1.825, Pb: 0.968 and Zn: 0.0127 (μg/ml by FAAS) (Cid et al., 2001). Detection limits of molybdenum have been

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reported in geological samples and seawater, using combined analytical methods, as 0.75 μg/l for ICP–AES, 0.08 μg/g for ICP–MS, 0.18 μg/ml for FAAS and 3 ng/l for GFAAS, respectively (Santos, Korn, & Ferreira, 2001).

Graphite furnace atomic absorption spectrometry (GFAAS) is one of the suitable methods for the determination of trace metals in food and biological samples because of its speed, minimum need for sample preparation, the possibility of automation, good sensitivity and low detection limit (Acar, 2001; Acar, Kılıç, & Türker, 2000; Doner & Akman, 2000; Blust, Vander der Linden, Verheyen, & Decleir, 1988; Huang et al., 2000; Lynch & Littlejohn, 1989). However, the determination of heavy metals in fish samples by GFAAS is difficult because the influence of a complicated matrix greatly affects the analytical results. Therefore, different chemical modifiers are used for the stabilization of the analyte.

In this study, an analytical method was developed for the determination of Pb, Cd, Fe, Cu, Mn and Zn, in fish samples, using graphite furnace atomic absorption spectrometry.

## 2. Materials and methods

### 2.1. Apparatus

A Varian Model Spectr AA 220 atomic absorption spectrometer, equipped with a Varian GTA-110 graphite furnace, was used. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak areas. The instrument settings and furnace programmes for analysis of trace elements are described in Table 1.

### 2.2. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solutions. All the plastic and glassware were soaked in nitric acid for 15 min and rinsed with deionized water before use. The stock solutions of metals (1000 mg/l) were obtained by dissolving appropriate salts of the corresponding metals (E. Merck) and further diluted prior to use. High purity Argon was used as inert gas.

### 2.3. Sampling

The fish species were collected from random commercial catches in the Middle Black Sea coasts in Samsun (Turkey) between September 2000 and May 2001. The samples were washed with distilled water, dried in filter paper, homogenized, packed in polyethylene bags and stored below −20 °C until analysis. Five species of fish, namely *Alosa caspia*, *Engraulis encrasicholus*, *Trachurus trachurus*, *Sarda sarda*, and *Clupea sprattus* were included in the study.

### 2.4. Digestion procedures

Two types of digestion procedures were applied. Optimum digestion conditions are given below. The samples were dried to constant weight at 110 °C.

#### 2.4.1. Dry-ashing

A sample (1 g) was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 °C in 1 h. The samples were ashed for about 4 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of $HNO_3$ (25% v/v) and the mixture, where necessary, was heated slowly to dissolve the residue. The solution was transferred to a 25 ml volumetric flask and made up to volume (Vaidya & Rantala, 1996). A blank digest was carried out in the same way. All metals were determined against aqueous standards.

#### 2.4.2. Wet-ashing

The samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion

Table 1
Instrument settings and furnace programmes for analysis of trace elements by AAS

| Working conditions | Fe | Cu | Mn | Zn | Pb | Cd |
|---|---|---|---|---|---|---|
| Wavelength (nm) | 248.3 | 324.8 | 279.5 | 307.6 | 283.3 | 228.8 |
| Slit width (nm) | 0.2 | 0.5 | 0.2 | 0.7 | 0.5 | 0.7 |
| Lamp current (mA) | 7 | 4 | 5 | 5 | 5 | 4 |
| Ar flow (ml/min) | 250 | 250 | 250 | 250 | 250 | 250 |
| | | | (flow interrupted at atomization stage) | | | |
| Injection volume (μl) | 20 | 20 | 20 | 20 | 15 | 15 |
| Heating programme temperature °C [ramp time (s), hold time (s)] | | | | | | |
| Drying 1 | 110(1, 20) | 110(1, 20) | 110(1, 20) | 110(1, 20) | 110(1, 20) | 110(1, 20) |
| Drying 2 | 130(5, 30) | 130(5, 30) | 130(5, 30) | 130(5,30) | 130(5,30) | 130(5,30) |
| Pyrolysis | 1100(15,10) | 1000(15,10) | 1300(15,10) | 1000(15,10) | 900(15,10) | 800(15,10) |
| Atomization | 2200(0,5) | 2100(0,5) | 1900(0,5) | 1800(0,5) | 2000(0,5) | 1900(0,5) |
| Cleaning | 2600(1,2) | 2600(1,2) | 2400(1,2) | 2400(1,2) | 2500(1,2) | 2400(1,2) |

bomb. A sample (1 g) was placed into Teflon container and 5 ml of concentrated $HNO_3$ was added. The system was heated to 130 °C for 90 min and finally diluted to 25 ml with deionized water. The sample solution was clear. A blank digest was carried out in the same way. All metals were determined against aqueous standards.

### 2.5. Analytical procedure

Determinations of all metal concentrations were carried out by graphite furnace AAS. During analyses, internal argon flow rate through the graphite tube was 250 ml/min; gas flow was interrupted during atomization. Sample volume, ramp and hold times for the drying, pyrolysis, atomization and cleaning temperatures were optimized before analysis to obtain maximum absorbance and minimum background. The details are given in Table 1.

Matrix modifiers added were 50 μg $(NH_4)_2HPO_4 + 3$ μg $Mg(NO_3)_2$ for Cd and Pb, 5 μg Pd + 3 μg $Mg(NO_3)_2$ for Mn, Fe, Cu, and 5 μg $Al_2SO_4$ for Zn.

In order to validate the method for accuracy and precision, certified reference materials (Oyster tissue and Mussel tissue) were analysed for each element. The results are shown in Table 2.

The detection limit is defined as the concentration corresponding to 3 times the standard deviation of 10 blanks.

## 3. Results and discussion

The concentrations of the heavy metals in fish species are given in Table 3. When the dry ashing method is compared with wet-ashing method for testing metal concentrations, the *t*-test indicates that the differences are not significant at the 95% confidence level, but the standard deviation of the dry-ashing method is higher than that of the wet-ashing method. In addition, the recovery of heavy metals in the dry-ashing method is lower than that of the wet-ashing method. Dry-ashing is slow and time-consuming. Wet-ashing favours organic matter destruction, shortens the time needed for the analysis and offers the advantage of simple, fast organic matter destruction, minimum reagent volume, reduction of possible analyte losses by volatilization or retention and elimination of the environmental contamination risks (Silvestre, Lagarda, Farre, Martinez-Costa, & Brines, 2000). An open beaker method for dry-ashing fish samples was found to be susceptible to contamination from external sources which could be prevented by carrying out the digestion in the closed system used in this study.

Detection limits, precision, and accuracy of analyses were determined by repeated analyses of two biological reference material standards. The results from the analysis of SRM were all within the 95% confidence limit of the SRMs. Excellent recoveries for all metals were obtained compared to the certified values. The detection limits for the methods were found to be (μg/l), Cu:0.36, Mn:0.23, Zn:0.25, Fe:0.42, Pb:0.98 and Cd:0.065 (n:10, 3s). The detection limits of the elements determined in this study were found to be 10 times and 100 times lower than those reported in other studies using flame AAS (Karadede & Ünlü, 2000; Kress et al., 1998).

It is desirable to use a higher pyrolysis temperature in order to remove the matrix efficiently and a temperature of at least 1000 °C should be aimed at for many analytes in food, biological and environmental samples (Bin & Zhe-Ming, 1996; Lynch & Littlejohn, 1989; Zong, Parsons, & Slavin, 1996). The pyrolysis and atomization temperatures of heavy metals were increased using different chemical modifiers. The maximum pyrolysis and atomization temperatures obtained were 800 and 1900 °C for Cd; 900 and 2000 °C for Pb; 1000 and 1800 °C for Zn; 1300 and 1900 °C for Mn; 1000 and 2100 °C for Cu; 1100 and 2200 °C for Fe. Most of the matrix was removed before the atomization step and less interference occurred during atomization. The method of standard additions was used to avoid the matrix effect.

The fish species analysed are used for human consumption. All metal concentrations were determined on a dry weight basis. According to the results (Table 3),

Table 2
Observed and certified values of elemental concentrations, as μg/g dry weight, in standard reference materials (SRM)

| Element | NIES-6 | | Recovery (%) | NBS-1566 | | Recovery (%) |
|---|---|---|---|---|---|---|
| | Certified value | Observed value | | Certified value | Observed value | |
| Cu | 4.9±0.3 | 4.8±0.8 | 98 | 63.0±3.5 | 64.4±2.8 | 102 |
| Fe | 158±8 | 160±12 | 101 | 195±34 | 200±10 | 103 |
| Mn | 16.3±1.2 | 15.8±1.9 | 97 | 17.5±1.2 | 17.9±1.8 | 102 |
| Zn | 106±6 | 105±8 | 99 | 852±14 | 861±10 | 101 |
| Cd | 0.82±0.03 | 0.78±0.05 | 95 | 3.50±0.40 | 3.55±0.65 | 101 |
| Pb | 0.91±0.04 | 0.88±0.11 | 97 | 0.48±0.04 | 0.50±0.10 | 104 |

Each value is the average of five determinations. NIES-6: National Institute of Environmental Science, Japan; mussel tissue homogenate. NBS-1566: National Bureau of Standards and Technology; oyster tissue.

Table 3
Metal concentrations, as μg/g dry weight, in fish species (the number of determinations on each sample is 10)

| Sample No.[a] | Method[b] | Cd | Pb | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|
| 1 | A | 0.35±0.05 | 0.52±0.16 | 2.93±0.18 | 16.08±1.15 | 1.57±0.24 | 20.41±1.75 |
| | B | 0.34±0.08 | 0.51±0.21 | 2.90±0.31 | 15.50±2.10 | 1.56±0.14 | 22.94±1.60 |
| 2 | A | 0.20±0.03 | 0.38±0.02 | 1.94±0.10 | 10.45±1.63 | 1.96±0.12 | 17.38±2.01 |
| | B | 0.18±0.02 | 0.39±0.07 | 1.96±0.17 | 10.32±1.05 | 1.98±0.32 | 18.85±1.72 |
| 3 | A | 0.47±0.10 | 0.85±0.16 | 1.52±0.35 | 32.40±2.70 | 3.76±0.45 | 12.05±2.30 |
| | B | 0.48±0.08 | 0.83±0.36 | 1.55±0.26 | 31.26±1.73 | 3.50±0.58 | 11.41±1.15 |
| 4 | A | 0.09±0.02 | 0.22±0.04 | 1.28±0.14 | 9.52±0.81 | 1.06±0.27 | 11.20±1.44 |
| | B | 0.10±0.01 | 0.26±0.07 | 1.29±0.32 | 10.14±1.11 | 1.33±0.42 | 13.72±1.32 |
| 5 | A | 0.30±0.15 | 0.74±0.11 | 1.79±0.62 | 25.48±3.18 | 2.82±0.24 | 9.50±0.60 |
| | B | 0.30±0.28 | 0.68±0.17 | 1.83±0.44 | 24.12±2.06 | 2.74±0.44 | 10.36±1.29 |

[a] 1, *Alosa caspia*; 2, *Engraulis encrasicholus*; 3, *Trachurus trachurus*; 4, *Sarda sarda*; 5, *Clupea sprattus*.
[b] a, Dry ash; b, wet ash.

the metal contents in the samples studied depend on the analyzed species. The concentration of cadmium varied from 0.09 to 0.48 μg/g; for lead it ranged from 0.22 to 0.85 μg/g. The lowest and highest values of these elements were found in *Sarda sarda* and *Trachurus trachurus* species, respectively. Levels of the essential metals in the fish samples were higher than those of the non-essential metals. Among the six metals under study, zinc showed the highest level of accumulation. A similar situation was observed in studies (Cid et al., 2001). The concentration of zinc varied from 9.50 to 22.94 μg/g. The lowest and highest values of this element were observed in *C. sprattus* and *A. caspia* species, respectively. The concentrations of copper, iron and manganese in the *S. sarda* species were lower than those found in other analysed fish species. The highest iron and manganese values were found in *T. trachurus* species.

The fact that toxic metals are present in high concentrations in marine organisms is of particular importance in relation to the FAO/WHO (1976) standards for Pb and Cd as toxic metals. The maximum permissible doses for an adult are 3 mg Pb and 0.5 mg Cd per week, but the recommended doses are only one-fifth of those quantities.

Results achieved for heavy metals in fish samples collected from the middle Black Sea, Turkey were in good agreement with other reported data from the literature.

## 4. Conclusions

The proposed method was efficient for simple, rapid and reliable determination of some heavy metals in the fish species. The accuracy of the method was checked and confirmed by standard reference materials. The recoveries of the heavy metals in standard reference materials were in the range of 95–104%. The relative standard deviations were less than 7%. The wet-digestion in closed vessels was the best. It was suitable for routine analysis. It was found that the concentrations of heavy metals in the fish samples were below those of Public Health Regulation in Turkey (Anonymous, 1995).

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