

The Determination of Trace Metals in Marine Organisms by Atomic Absorption Spectrometry

G. RAMELOW, S. TUĞRUL, M. A. ÖZKAN, G. TUNCEL,
C. SAYDAM and T. I. BALKAS

*Department of Marine Sciences, Middle East Technical University,
Ankara, Turkey*

(Received July 26, 1977)

The application of atomic absorption spectrometry to the analysis of trace metals in marine organisms is described. The samples are digested with nitric acid in teflon decomposition vessels. Mercury is determined by the cold-vapor method while the other elements are determined by flame atomization or flameless graphite-furnace atomization. Several marine species of economic importance are analyzed for seven trace elements.

KEY WORDS: Trace metals, marine organisms, atomic absorption.

INTRODUCTION

Recently heavy-metal contamination of the environment has been recognized as a serious pollution problem. In particular, mercury has received much attention due to the well-known toxic effects of this metal.^{1,2} Another element, cadmium, whose toxic effects have been well documented,³⁻⁵ has received less attention.

In addition to mercury and cadmium, it is of interest and importance to investigate the levels of other metals as pollution indicators and to establish base-line levels of these elements in the environment.⁶

The effects of pollution on marine organisms are especially important to countries which derive considerable economic benefit from the sea. Previous studies^{7,8} have shown the level of mercury in fish and mussels caught in the Aegean and Marmara Seas to be generally lower than the limits set in the U.S.A.

Atomic absorption spectrometry is proving very useful for the determination of trace metals in marine specimens.⁹⁻¹² Particularly useful are the cold-vapor flameless method for mercury¹³ and the graphite-furnace

methods¹⁴⁻¹⁶ which offer the great sensitivity essential for the detection of the levels of metals commonly found in marine samples.

In this paper procedures are described for the determination of selected trace elements in marine organisms by atomic absorption spectrometry. The results of analyses of seven metals in several species of economic importance are given.

MATERIALS AND METHODS

Apparatus

The basic instrumentation consisted of a Varian-Techtron Model AA-6 atomic absorption spectrophotometer equipped with a Model A-25 recorder. The hollow cathode lamps used were Varian mercury, cadmium, lead and zinc single-element and a copper-iron-chromium-manganese-nickel-cobalt multi-element lamp, all operated at the manufacturer's recommended currents.

Cold-Vapor Mercury Determination For the analysis of mercury an absorption cell constructed of glass with quartz windows cemented in place was fastened to the standard air-acetylene burner head. Two types of reduction vessel were used, a continuous-flow vessel which has been previously described,¹⁷ and a small gas-washing bottle of about 30 ml capacity. Air was supplied to the vessel from the compressor of the AA instrument. An adjustable flow meter was used to control the air flow to the reduction vessel.

Flame Atomization Flame atomization was used for the analysis of zinc, iron, and in some cases, copper. The standard air-acetylene flame was used.

Graphite-Furnace Atomization For the analysis of cadmium, lead, nickel, manganese and chromium a Perkin-Elmer Model HGA-74 graphite furnace was mounted in place of the normal nebulizer-burner. The sample aliquots were introduced into the furnace with an adjustable micropipet (Finnpipet, 5-50 μ l).

Reagents

Stock solutions of the elements (1 gram/liter) were prepared from the appropriate pure element or suitable compound of that element. Working standards were prepared fresh daily.

The nitric acid used in the digestions was obtained from Merck. It was found to be low in trace-metal content and therefore suitable for use with flameless techniques.

Distilled, deionized water was used throughout.

Sample preparation

Fresh-weight samples were prepared by cutting from 0.5 to 1.0 gram amounts from the dorsal area when possible and digesting in 2 to 3 ml concentrated nitric acid in a digestion bomb (Parr Instruments or Uniseal Decomposition Vessels, Ltd.) at 150°C for 1 to 1.5 hours. Dried samples of 0.1 to 0.4 gram were digested in the same manner. At the end of the digestion period the liquid samples were transferred to 25, 50 or 100 ml volumetric flasks as required and diluted to volume with water.

Analytical procedures

Cold-Vapor Mercury Determination Two different analytical procedures for mercury were used. With the continuous-flow reduction vessel a standard addition method was employed as follows: 0.5 ml of 5% SnCl_2 is added to the vessel while air is passing through and the instrument zeroed. One ml of sample digest and the proper amount of aqueous standard are then added simultaneously and the absorption peak recorded.

An alternative procedure was to use a small gas-washing bottle as reduction vessel with an interrupted-flow technique as follows. The reducing agent is added to the vessel while air is flowing and the instrument zeroed. The air is then turned off and the sample added. After a suitable period of time, usually 90 seconds, during which the sample is vigorously shaken, the air is turned on and the absorption peak observed.

Flame Atomization For those elements whose concentration in the samples was within the range of detection of conventional flame atomization, the standard air-acetylene flame was used by directly aspirating the diluted digests. The standard addition method, used in all cases, was as follows. Selected amounts of aqueous standards are added in increasing amounts to three 10-ml volumetric flasks and then topped up with the digested sample.

Graphite-Furnace Atomization Operating conditions for all elements using the HGA-74 graphite furnace are given in Table I. Two different standard addition techniques were used with this furnace. In one method the standard additions were made in 10-ml volumetric flasks and then aliquots of these solutions introduced into the furnace. In the other method aqueous standards were added to the furnace, dried and then the digested sample introduced and carried through the entire sequence of programmed heating steps. No difference was observed between the results of the two methods.

TABLE I
HGA-74 operating conditions

	Cd	Cu	Cr	Pb	Mn	Ni
Wavelength (nm)	228.8	324.7	(357.9) [†]	217.0	279.5	232.0
Slit width (nm)	0.5	0.2	0.2	1.0	0.2	0.2
Sample aliquot (μ l)	10-30	10-30	30-50	10-30	10-30	30-50
Dry (sec, digits)	30-60, 138	30-60, 138	90, 138	30-60, 138	30-60, 138	90, 138
Ash (sec, digits)	15, 245	15, 413	15, 480	15, 375	15, 434	15, 480
Atomize (sec, digits)	6-8, 689	6-8, 905	8-10, 999	8-10, 745	6-8, 967	10-12, 999
Clean (sec, digits)	4, 999	4, 999	—	4, 999	4, 999	—
Background Correction [‡]	Yes	Yes	No	Yes	No	Yes

[†]Spectral interferences caused by the other elements used in the Varian multi-element lamp make it inadvisable to use the normal 357.9 nm line for chromium determinations. The less sensitive 425.4 nm line is recommended for this lamp.

[‡]Hydrogen continuum lamp cannot be used to measure and correct for non-atomic absorption above 350 nm.

RESULTS AND DISCUSSION

Determination of mercury

Effect of Reduction-Vessel Design As mentioned above two different types of reduction vessels were evaluated for the analysis of mercury. Most work in the literature has involved the use of vessels in which the carrier-gas flow is interrupted for a period of time while the sample is vigorously agitated to effect the reduction process and then allowed to pass through the sample.

However, recently a continuous-flow type of reduction cell has been described¹⁸ which has several advantages over the other types. We have constructed a similar type of vessel in our laboratory and used it for the analysis of mercury in simple aqueous solutions.¹⁷ However, when this vessel is used for digested fish solutions, the peak heights obtained are significantly less than the interrupted-flow system. This is due to the slowness of the reduction process in the digested fish samples compared with simple aqueous solutions. In addition, the precision is also less because the mixing is effected by the air bubbles passing through the solution. With this vessel the standard addition method is mandatory while with the interrupted-flow type it has been found that calibration graphs prepared with aqueous standards can be used.

It can be concluded that either of the two methods is satisfactory for the analysis of mercury in marine specimens. The choice of which method to use will be made after evaluating the advantages and disadvantages of each.

Analysis of Mercury in Marine Organisms The results of the analysis of mercury are given in Table II. In general the mercury levels are less than the maximum levels of consumption established in Europe and the U.S.A. However, the two samples of white bream which were caught in the Izmir harbor, a heavily polluted area, contained dangerously high levels of mercury.

Other trace metals

Several different marine species were analyzed for six trace metals by the techniques described. The concentrations of zinc were high enough in all cases to allow the use of flame atomization. The other elements, copper, cadmium, manganese, lead and chromium, were analyzed using graphite-furnace atomization. The results of all analyses are given in Table III.

Trace-metal concentration of mussel as a function of size

Four arbitrarily chosen sizes of Mediterranean Mussel, collected near Gemlik on the Sea of Marmara in Western Turkey, were analyzed for mercury, cadmium, copper and lead. The analytical results together with the weights and shell length are given in Table IV.

TABLE II
Analytical results of mercury analysis of various marine organisms

Species	$\mu\text{g/g}$ (fresh weight)
Turbot	0.20
Blue fish (sample 1)	0.24
Blue fish (sample 2)	0.11
White bream (sample 1)	1.12
White bream (sample 2)	1.70
Sardine (sample 1)	0.03
Sardine (sample 2)	0.04
Gilt-head bream (sample 1)	0.30
Gilt-head bream (sample 2)	0.29
Grey mullet	0.02
Swordfish	0.25
Mackerel	0.09
Mussel	0.03
Oyster	0.16†

†Dry weight

TABLE III
Analytical results of analysis of trace metals in various marine organisms ($\mu\text{g/g}$ fresh weight)

Species	Cd	Pb	Cu	Mn	Zn	Cr
White bream	0.04	0.61	1.11	0.51	10.6	0.58
Sardine	0.02	0.57	2.18	1.63	6.3	0.28
Gilt-head bream	0.03	0.68	1.20	—	9.5	0.49
Grey mullet	0.09	1.36	1.70	0.33	12.2	0.10
Horse mackerel	0.17	1.05	0.99	0.63	4.3	0.65
Striped mullet	0.02	0.12	0.68	0.22	6.4	0.14
Shrimp	0.02	0.34	1.77	0.26	9.3	0.33
Crab (leg)	0.03	0.27	10.34	0.29	39.3	0.36
Dried oyster†	0.27	0.26	43.56	—	460.0	3.68

†Dry weight

The ability of shellfish to concentrate many elements is well known.^{19,20} It is thus expected that the mussel which gets its nourishment by effectively filtering the surrounding water, might show an increase in the concentration of some elements with size (and thus age). Such a trend is indicated in Table IV for cadmium and copper although more data is needed to be conclusive.

TABLE IV
Trace metal concentration of Mediterranean mussel versus size^a

Size	Total weight (g)	Fresh weight (g)	Shell length (cm)	$\mu\text{g/g}$ (fresh weight)				
				Hg	Cd	Cu	Pb	
Very small (I)	6.2	1.0	4.0	0.03	—	0.75	0.48	
Very small (II)	7.2	1.7	4.4	—	—	—	—	
Small (I)	11.8	2.3	5.5	0.05	0.07	0.89	0.54	
Small (II)	17.9	5.8	5.8	—	0.24	1.27	0.61	
Medium	27.7	5.9	7.3	0.02	0.40	2.65	0.57	
Large	67.8	7.4	8.7	0.05	—	—	—	

^aArbitrarily determined on basis of total weight and shell length.

Calibration check of instruments and methods

In order to check the accuracy of the instruments and methods used in this study, an analysis of a dried oyster sample provided by the International Laboratory of Marine Radioactivity was made for Hg, Cd, Cu, Pb, Zn, Mn, Cr, Fe and Ni. This oyster homogenate is being used in an interlaboratory calibration program for the trace metal analysis of marine environmental samples.²¹ Our results agreed well with the average values calculated from the reports of 60 laboratories using a variety of analytical techniques. The largest variations were observed for chromium and nickel which were obtained with a faulty hydrogen lamp background corrector.

Acknowledgment

This work was supported by the United Nations Environmental Program (UNEP) as part of the Joint FAO-UNEP Coordinated Project on Pollution in the Mediterranean - UNEP project number FP 0503-75-07.

References

1. "Mercury and the Environment", Organization for Economic Co-operation and Development, Paris, 1974.
2. L. Friberg and J. Vostal, "Mercury in the Environment", CRC Press, Cleveland, 1972.
3. W. Friberg, M. Piscator and M. Nordberg, "Cadmium in the Environment", Environment Protection Agency Report PB 199, 795 (1971).
4. "Baseline Studies of Pollutants in the Marine Environment and Research Recommendations", The IDOE Baseline Conference, New York, 1972.
5. W. Eulerson and H. Coeller, "Cadmium, the Dissipated Element", Oak Ridge National Laboratory, NSF Environmental Program, 1973.
6. M. Bernhard, "Manual of Methods in Aquatic Environment Research, Part 3 Sampling and Analysis of Biological Material", FAO Technical Paper No. 158, Rome, 1976.
7. I. Ölmez and N. K. Aras, *Radiochem. Radioanal. Letters* **22**, (1), 19 (1975).
8. J. Garden and H. Özbil, Proceedings, 2nd National Spectroscopy Congress, Ankara, 1976.
9. R. A. Grieg, *Anal. Chem.* **47**, 1682 (1975).
10. G. Topping, *Aquaculture* **1**, 373 (1973).
11. G. Topping, *Aquaculture* **1**, 379 (1973).
12. K. Julshamn and O. R. Braekkan, *At. Abs. Newsletter* **14**, 49 (1975).
13. A. M. Ure, *Anal. Chim. Acta* **76**, 1 (1975).
14. G. K. Pagenkopf, D. R. Neuman and R. Woodruff, *Anal. Chem.* **44**, 2248 (1972).
15. D. A. Segar, *Intern. J. Environ. Anal. Chem.* **3**, 107 (1973).
16. D. A. Lord, J. W. McLaren and R. C. Wheeler, *Anal. Chem.* **49** (2), 257 (1977).
17. G. Ramelow and T. I. Balkas, *Atomic Absorption Newsletter* **15**, 55 (1976).
18. J. E. Hawley and J. D. Ingle, Jr., *Anal. Chem.* **47**, 719 (1975).
19. R. R. Brooks and M. G. Rumsby, *Limnol. Oceanogr.* **10**, 521 (1965).
20. D. W. Hood, "Impingement of Man on the Oceans", (Wiley-Interscience, 1971), pp. 471-472.
21. "Intercalibration of Analytical Methods on Marine Environmental Samples", Progress Report No. 13, International Laboratory of Marine Radioactivity, Monaco, June, 1976.