Reprinted from Atomic Absorption Newsletter, Vol. 17, No. 3, 1978

AN INVESTIGATION INTO POSSIBLE MERCURY LOSSES DURING LYOPHILIZATION OF MARINE BIOLOGICAL SAMPLES

Gerald Ramelow
Department of Marine Sciences
Middle East Technical University
Ankara, Turkey
and
Hava Hornung
Israel Oceanographic & Limnological Research Ltd.
Tel-Shikmona
P.O.B. 1793
Haifa, Israel

INTRODUCTION

The preservation of fresh tissue of fish and other marine biological organisms is important in environmental analysis. Often large numbers of samples must be stored for long periods of time before analysis. Three methods are commonly used to preserve samples: 1) freezing the wet sample; 2) removing the water by oven drying or 3) lyophilization. The latter two methods are probably more convenient than freezing since the storage of dried samples is simpler. It is also preferable to report analytical results on a dry-weight basis.

Oven-dried material can be used for the determination of many elements but not for mercury. It has been demonstrated (1) that considerable losses of mercury can occur at normal drying temperatures.

Lyophilization of samples of marine organisms, on the other hand, preserves the samples by rapid freezing and drying at low temperatures. The samples are converted into a form suitable for indefinite storage and are also effectively concentrated, an important consideration when dealing with trace elements. Mercury is an extremely volatile element; therefore, it is important to know whether any losses of this element occur during the lyophilization step.

The possible loss of mercury from biological materials upon lyophilization has been studied by several workers. Hsu *et al.* (1) and Litman, *et al.* (2) report losses of mercury from fish samples ranging up to 71%. In contrast, Lever (3), studying the organs of laboratory animals, and Perkins, *et al.* (4) have not detected any mercury losses. The analytical method used by all these workers to measure mercury was activation analysis.

In view of the conflicting nature of the experimental results reported to date and the potential importance that lyophilization may have to programs where mercury levels in fish and other marine biological samples are constantly monitored, the necessity of additional experimental work is obvious. In this paper, we present the results of an investigation into possible mercury losses during the lyophilization of marine biological samples. Several different species of Mediterranean fish and a mussel were tested.

EXPERIMENTAL

Equipment and Reagents

A Coleman Perkin-Elmer MAS 50 Mercury Analyzer was used to analyze the samples. The standard BOD bottle reduction vessel was replaced by a ground glass-stoppered, 100-ml Erlenmeyer flask fitted with a fritted-glass bubbler. Ice traps were placed between the outlet of the flask and the inlet of the absorption cell was used to remove water vapor from the air stream.

Since a different reduction vessel was used, it was necessary to calibrate the instrument with aqueous mercury standards. In practice, the slope of the calibration plot was slightly greater than one.

A Leybold Heraeus GT2 freeze-drying apparatus was used to lyophilize the samples. System pressure was 10^{-1} torr.

A 1000-ppm stock solution of mercury was used to prepare fresh intermediate standards daily. The reducing agent was a 20% solution of stannous chloride in conc HCl. Two ml of this solution was used to reduce the mercury in 25 ml of a digested sample.

Preparation and Storage of Samples

Fish and mussel samples were collected and handled according to UNEP suggestions (5). This involved preparing the fish samples by homogenizing fresh filets of single or several individual specimens in a blender. The homogenized samples were then frozen until needed in plastic bags.

The homogenized samples were spread around the inside of small glass bottles and frozen overnight in a freezer. Before placing the samples in the lyophilizer, they were frozen at −70°C in a slurry of ethanol and dry ice and left in the lyophilizer for ~ 24 hr.

The samples of mussel were prepared by grinding the soft parts of several individual specimens in an agate mortar until visibly homogeneous. They were then treated in the same manner as the fish samples.

Digestion of Samples

The fresh fish and mussel samples were prepared for analysis by digesting amounts of wet tissue ranging from 0.3-1.0 g in Teflon-lined, high pressure decomposition vessels (Uniseal Decomposition Vessels, Ltd.). Two different sizes of vessel were available for use: a large vessel with a capacity of 70 ml and a smaller vessel of 23-ml capacity. In the large vessel, sample weights up to 1 g were digested with 5 ml of conc nitric acid; in the small vessel, weights up to 0.5 g were digested with 3 ml of acid. All samples were heated at 130-140°C for 1.5 hr.

Lyophilized samples were digested in the same manner as fresh samples; sample weights were ~ 0.1-0.5 g. It was found that lyophilized sample weight should not exceed 0.3 g for the small bomb and 0.5 g for the large bomb. If larger samples were used, dissolution was not completed under the conditions described.

After digestion, all samples were transferred to 50-ml volumetric flasks and diluted to volume with mercury-

TABLE I

A Comparison of Mercury Concentration in Biological Samples Using Wet and Lyophilized Samples

Organisms	Hg Concentration (μg/g wet weight)					
	Wet Sample			Lyophilized Sample		
	No. of Samples	Range	Average ± s.d.	No. of Samples	Range	Average ± s.d.
FISH						
SYNODONTIDAE						
Saurida sp.	9	0.05-0.65	0.24±0.19	9	0.07-0.70	0.27±0.20
CARANGIDAE						
Trachurus sp.	3	0.06-0.19	0.12±0.07	3	0.07-0.19	0.12±0.06
MULLIDAE						
Mullus sp. 1	25	0.02-0.35	0.13±0.06	25	0.03-0.23	0.15±0.05
Mullus sp. 2	1	0.09	—	1	0.13	—
SPARIDAE						
Boops sp.	2	0.07-0.08	0.08±0.007	2	0.08-0.08	0.08±0
Pagellus sp.	6	0.07-0.28	0.17±0.07	6	0.08-0.28	0.18±0.08
SPHYRAENIDAE						
Sphyraena sp.	2	0.08-0.16	0.12±0.06	2	0.08-0.17	0.12±0.06
TRIGLIDAE						
Chelidonichthys sp.	1	0.09	—	1	0.13	—
MOLLUSCA						
DONACIDAE						
Donax sp.	8	0.07-0.80	0.38±0.29	8	0.08-0.93	0.37±0.26

ter water. To obtain duplicate readings of each sample, aliquots of 25 ml were analyzed. Each sample was done in triplicate.

RESULTS AND DISCUSSION

Eight different species of fish were examined in this study. Care was taken to use exactly the same procedures throughout for both fresh and lyophilized samples. The analytical results of wet and lyophilized samples are given in Table I. The concentration of mercury in the lyophilized samples has been converted to wet weight, based on the water loss during lyophilization. The water loss of lyophilized samples averaged 75.9% for fish and 74.3% for the mussel.

The results given in Table I indicate no significant losses of mercury from the fish samples upon lyophilization. Even though the mercury concentrations in the lyophilized portions varied somewhat from the wet, both negatively and positively, the overall averages of 0.152 μg/g for the wet and 0.168 μg/g for the lyophilized fish samples are in good agreement.

The 49 fish samples were divided into two groups: 1) samples with values from 0.10-0.65 μg/g Hg and 2) from 0.02-0.09 μg/g. The t-test for the first group showed no significant difference between the wet and lyophilized samples. In the second group the t-test was significant. The low values (0.02-0.09 μg/g) in the wet samples give higher results after lyophilization. This could be explained by the difference in the size of the sample. The lyophilized sample is more concentrated, and more material can be used for analysis, while the readings on the small wet samples are often close to the limit of detection.

The results obtained with the mussel *Donax* sp. are also given in Table I. Again the averages of the wet and lyophilized samples are in good agreement (0.38 μg/g wet, 0.37 μg/g lyophilized), and the difference was insignificant using the t-test. It should be noted, however, that difficulties were encountered in the lyophilization of mussel samples. When the mussels were homogenized while wet in a mortar and pestle, and the homogenized material was then lyophilized, only one sample gave a dry powder. The other samples appeared oily and progressively assumed an unattractive black color upon storage in closed containers. However, when the mussels were lyophilized whole and homogenized after lyophilization, dry white powders were obtained.

ACKNOWLEDGMENTS

This work was carried out as part of an FAO/UNEP joint project on the determination of baseline levels of heavy metals, particularly, mercury and cadmium, in the Mediterranean Sea. Gerald Ramelow gratefully acknowledges his fellowship from FAO/UNEP.

The authors wish to thank Mrs. L. Zismann for identifying the fish.

Received March 27, 1978

REFERENCES

1. K. K. Pillay, C. C. Thomas, Jr., J. A. Sondel and C. M. Hyche, Anal. Chem. **43**, 1419 (1971).
2. R. Litman, H. L. Finston and E. T. Williams, Anal. Chem. **47**, 2364 (1975).
3. P. D. LaFleur, Anal. Chem. **45**, 1534 (1973).
4. M. Friedman, E. Miller and J. Tanner, Anal. Chem. **46**, 236 (1974).
5. M. Bernhard, FAO Fish. Tech. Pap. No. 158 (1976).