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Effect of Different Preservation Methods on the Trace Element Concentrations of Fish and Mussel Tissues

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An important consideration in the determination of the concentrations of trace elements in biological samples is the prevention of contamination and elemental losses during sample collection and storage. The choice of container material and the methods used in cleaning them have been recognized, both as sources of contamination and as factors in adsorption losses (MOODY & LINDSTROM 1977; STRUEMPLER 1973). This study investigates the effect of different methods used to preserve biological samples for storage on the trace element content of fish muscle and liver, and the soft tissues of marine mussels.

The tissues from the commercially important marine fish, whiting, Merluccius bilearis, were preserved by freezing or by formaldehyde immersion and were analyzed for 12 trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sn, Te, V and Zn). In addition, edible marine mussels, Mytilus edulis, were preserved in their shells by freezing, formaldehyde immersion or freeze drying. These samples were analyzed for the same 12 trace elements.

MATERIALS AND METHODS

Samples. The whiting were caught off the Rockaway Inlet in the New York Bight by a commercial trawler. To minimize variation due to body size, all 30 fish selected for analysis were of similar size and weight, averaging 330±60 g wet weight and 36±2 cm in length. Approximately 3 kg of live mussels, Mytilus edulis, from Maine were purchased from a commercial fishing establishment. The soft tissues ranged in wet weight from 2.7-20.4 g.

Preservation procedure. (a) Freezing. Ten whole fish and 40 mussels were stored at 0° C in acid-cleaned polyethylene bags.

(b) Formaldehyde. Ten whole fish and 40 mussels were preserved in 10% formaldehyde solution prepared from reagent grade formaldehyde. In addition, 10 whole fish were preserved in the same 10% formaldehyde solution which was buffered by the addition of 3 g $Na_2B_4O_7 \cdot H_2O$ per L 10% formaldehyde solution. The formaldehyde

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solutions were injected into the body cavity with acid-cleaned plastic syringes with stainless steel needles. Enough solution (~ 50 cm^3) was injected to fill the body cavity. Care was taken not to puncture any organs. The fish were then placed in acid-cleaned plastic bags with enough formaldehyde solution to keep them moist. A similar procedure was used for the mussels. Unbuffered 10% reagent grade formaldehyde solution (~ 20 m^3) was injected between the shells into the soft tissues. Samples of fish and mussels preserved with formaldehyde were then stored at room temperature.

(c) Freeze-drying. Twenty mussels were frozen at 0° C then freeze-dried for 5 days at 8-10° C. The dried tissue was then removed from the shell using a Teflon-coated scalpel, placed in acid-cleaned polyethylene bottles, and frozen at 0° C until analyzed.

Sampling procedure. The preserved whole fish and mussels were stored for a period of about 1 year before the entire soft tissue of the mussels and 3-13 g sections of the dorsal muscle and the entire liver (1.7 - 15 g) of the whiting were removed for trace metal analysis. All incisions and contact with the tissue samples were made with titanium blades and Teflon-coated utensils to minimize contamination (HEIT & KLUSEK 1980). As a precaution against airborne contamination, the fish were dissected in a laminar flow clean air station. The excised samples were placed in acid cleaned polyethylene bottles and frozen at 0° C until analyzed.

Analyses. All of the samples were analyzed by a contractor laboratory, Dr. H. L. Windom, Skidaway Institute of Oceanography, Savannah, GA. Prior to digestion the wet samples were homogenized using a Teflon spatula and then dried at 60° C. The percent moisture was determined.

Samples to be analyzed for As, Cd, Cr, Cu, Ni, Pb, Se, Sn, Te, V and Zn were wet ashed using ~ 20 mL of concentrated HNO_3 in acid-precleaned Teflon beakers on a hot plate. After complete digestion (~ 6-8 h) the samples were taken to near dryness and then dissolved in 10% nitric acid and brought to a volume of 10 mL. Vials were stored under refrigeration until analyzed.

For Hg analysis, the samples were wet digested overnight in Pyrex beakers in a water bath, at ~ 60° C, using 4 mL of concentrated H_2SO_4 and 3 mL of concentrated HNO_3. Just prior to analysis the samples were further oxidized by the addition of potassium permanganate and potassium persulfate. All of the samples were analyzed within 24 h of the initiation of digestion.

The samples were analyzed by atomic absorption spectrophotometry (AAS). Mercury analysis was by cold vapor flameless AAS

following reduction of the Hg with stannous sulfate. Cd, Cr, Cu, Ni, Pb, Sn, Te and V were analyzed by flameless AAS using a heated graphite furnace and deuterium background corrector. Zn was analyzed using flame AAS methods. As and Se were also analyzed by graphite furnace AAS, however, these elements were first converted to hydrides using oxalic acid and sodium borohydride. The metal hydrides were purged into the graphite furnace with helium.

Quality control. Replicate determinations were made of the 10% formaldehyde solutions used in the preservation process. Liver and dorsal muscle samples were split and submitted as blind duplicates.

All comparisons of the concentrations of trace elements found in the samples preserved by different methods were made using the nonparametric Kruskal-Wallis test at the 0.01 level of significance (HOLLANDER & WOLFE 1973).

RESULTS AND DISCUSSION

The results of the analysis of the 10% formaldehyde solution for As, Cd, Cr, Hg, Ni, Pb, Se and Tl are shown in Table 1. The trace element levels in the 10% formaldehyde solution were found to be so low (ng/mL) as not to be a factor in tissue contamination. The concentration of the elements discussed in this report occur at levels of at least an order of magnitude above that found in the formaldehyde preservative.

Table 1. Trace Element Concentration in Formaldehyde Solutions (ng/mL)*

	As	Cd	Cr	Hg	Ni	Pb	Se	Tl
10% formaldehyde, Stored in acid washed polyethylene bags	1.3	<1.0	<1.0	<0.1	15	2.0	<1.0	<1.0
10% formaldehyde, Stored in deionized H_2O rinsed polyethylene bags	0.9	<1.0	2.0	<0.1	15	3.6	<1.0	<1.0

*Mean of two replicate determinations.

In addition, there were no apparent differences in the levels of the trace elements in the 10% formaldehyde stored for a period of

3 weeks in polyethylene bags cleaned with HNO_3 compared to the solution stored for the same period in polyethylene bags rinsed with deionized water. It was assumed that acid cleaning removes all available metals from the walls of the bags. Therefore, the concentrations of the trace elements in the 10% formaldehyde solution were not affected by leaching during storage in the polyethylene bags. Thus, polyethylene bags can be used for storage of samples without fear of contamination.

Concentrations in fish muscle. The average concentrations of the trace elements in the fish muscle samples are shown in Table 2. Although Cd, Cu, Ni and Pb show significant differences in concentration between samples preserved by the 3 methods, these differences are not systematic and may be due to other factors. For example, analytical variability overshadowed the generally lower range of Cd, Pb and Ni concentrations found in the formaldehyde preserved muscle samples as compared to the frozen samples. Table 3 shows the results of the quality control samples. Less than 10% variability in the analysis was considered excellent; variability > 30% was unacceptable. Results of the analysis of splits of fish muscle samples indicated variable precision (22±19%) for Cd and poor precision for Ni (35±43%) and Pb (67±45%). It should be noted that the distribution of trace metals in the dorsal muscle of a striped bass, Morone saxatilis, has been shown to be nonhomogeneous (HEIT 1979). Thus, it is possible that for these elements some of the variability may be due to nonhomogeneous distribution.

Concentrations in fish liver. Although statistically significant differences in the concentration of Cd, Cu, Pb and Zn were seen in the samples preserved by the different methods, these differences again may be due to poor analytical precision rather than the method of preservation. The precision for the analysis of the split liver samples (Table 3) were found to be quite variable. Again, the possibility that nonhomogeneity (HEIT 1979) may contribute to this general lack of precision should not be overlooked.

Concentrations in mussels. No significant differences were seen in the concentrations of the trace elements in the mussels with the exception of As. However, the range of As concentrations overlap for the frozen and formaldehyde preserved samples and for the formaldehyde and freeze dried samples. Since no differences in the As concentrations were seen in the fish tissues preserved by the same methods, we believe that the differences suggested by the statistical test may be due to factors other than the method of preservation.

Table 2. Average Concentration of Trace Metals in Fish Muscle and Liver and in Marine Mussels ($\mu g/g$ dry weight)

		No. of samples*	Dry wt. (g)	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Sn	Te	V	Zn
Fish muscle	Frozen	10	1.3±0.5	3±1	0.04±0.03	0.3±0.2	1.7±0.4	0.4±0.1	0.8±0.5	0.2±0.1	0.2±0.1	<0.5	3±2(9)	<0.1	20±5
	10% formaldehyde buffered	10	1.2±0.2	2±1	0.04±0.02	0.2±0.1	3.0±0.7	0.4±0.2	0.8±0.2	0.08±0.05	0.3±0.2	<0.5	2±1	<0.1	16±8
	10% formaldehyde	10	1.1±0.3	3±1	0.02±0.01	0.3±0.2	1.7±0.3	0.3±0.1	0.4±0.2	0.04±0.03	0.2±0.2	<0.5	4±2	<0.1	17±9
Fish liver	Frozen	10	1.2±0.5	3±1	0.4±0.6	0.1±0.1	8±3	0.2±0.2	0.7±0.6	0.12±0.08	0.5±0.2	<0.5	1.0±0.6	<0.1	60±20
	10% formaldehyde buffered	10	1.9±0.8	3±1	0.04±0.03	0.2±0.2(9)	2±1	0.13±0.05	0.7±0.6	0.17±0.04	0.3±0.2	<0.5	1.7±0.7	0.2±0.1(7)	12±4
	10% formaldehyde	10	1.9±0.9	3±1	0.24±0.07	0.1±0.1	2±1	0.1±0.1	1.4±0.8	0.4±0.2	0.6±0.3	<0.5	0.9±0.7(5)	<0.1	50±20
Mussel	Frozen	12	2.6±2.	5±1	0.8±0.2	1±1	6±2	0.11±0.06	1.3±0.6	1±1	0.9±0.5	1.1±0.3	0.7±0.5	<0.1	80±40
	10% formaldehyde	12	2.4±1.	3±1	0.6±0.1	1±2	6±2	0.09±0.04	4±2	0.7±0.6	0.8±0.3	<0.5	0.9±0.5(9)	1.1±0.4(11)	70±50
	Freeze dried	8	2.3±1.	2±1	1.1±0.6	2±2	6±2	0.09±0.04	3±2	0.6±0.3	1.2±0.2	<0.5	1.3±0.7	1.0±0.4	70±20

*No. of samples except where indicated in ( ).

Table 3. Average Precision of Analysis for Trace Elements in Fish Muscle and Liver*

Element	Tissue	
	Muscle	Liver
As	35 ± 30	28 ± 19
Cd	22 ± 19	52 ± 23
Cr	37 ± 39	19 ± 12
Cu	16 ± 12	12 ± 12
Hg	3 ± 2	11 ± 9
Ni	35 ± 43	41 ± 27
Pb	67 ± 45	27 ± 23
Se	54 ± 23	36 ± 29
Te	20 ± 16	43 ± 29
Zn	26 ± 14	19 ± 14

*Average % coefficient of variation for 6 sets of duplicate samples; no averages are given for Sn and V as the concentrations were reported as less than values.

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