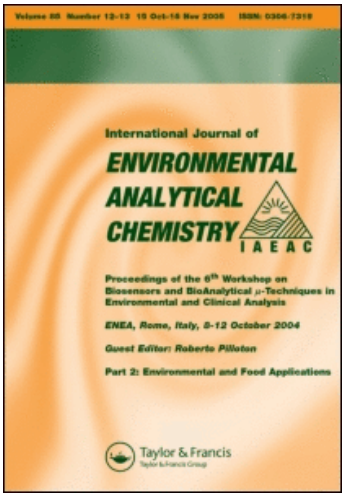

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A COMPARATIVE STUDY OF ANALYTICAL METHODS: DETERMINATION OF HEAVY METALS IN MUSSELS (*MYTILUS EDULIS*) FROM EASTERN CANADA

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Dry ashing and microwave digestion procedures used for dissolution of biological tissue samples were compared as well as determining the heavy metals Cd, Cu, Pb, and Zn by ICP-AES and FAAS/GFAAS methods. Marine reference samples from an inter-laboratory exercise and natural mussel samples (*Mytilus edulis*) collected in the Atlantic Region were used. The results indicate that microwave digestion in a closed system accompanied by the low detection limit graphite furnace AAS method is the preferred combination for obtaining good quality results especially for those metals which are present at very low concentrations.

KEY WORDS: Mussels, metal analysis, microwave digestion, dry ashing.

INTRODUCTION

A pilot scale monitoring of blue mussels (*Mytilus edulis*) in shellfish growing areas of the Atlantic Region required analysis of heavy metals in mussel samples. For the mineralization of metals in organic matrices wet and dry ashing techniques are commonly used. Wet digestion involving perchloric acid is well known for its dangerous characteristics; at high temperatures perchloric acid forms explosive mixtures with organic materials. The digestion requires constant operator attention and adherence to established protocols to avoid explosions. Also, it must be carried out in specially designed fumehoods that are safe for perchloric acid fumes. Dry ashing involves combustion of the sample and has the potential of losing elements which are present in volatile forms. There is also a risk of external contamination.

Both the wet and dry ashing procedures are slow and time consuming. The mineralization of biological matrices by microwave digestion has been gaining wider acceptance in recent years. Several workers[1,2,3] have reported the usefulness of microwave system for acid decomposition of biological samples. It is reported that the procedure is simple, rapid and reliable for the digestion of a variety of sample matrices. In this work, the performance of microwave digestion was compared to the dry ashing

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method for mineralization of metals in natural blue mussel (*Mytilus edulis*) samples. For comparison of instrumental methods the metals were determined by atomic absorption spectrometric and simultaneous multi-element ICP-AES methods respectively.

EXPERIMENTAL

Materials and methods

The mussel samples were collected from indigenous populations at the different sites. The complete sampling procedure is described by Machell *et al.*[4]. The composite sample from each site comprised 10 individuals. The samples were stored in jars washed with nitric acid and rinsed with deionized distilled water and then shipped immediately to the laboratory for analysis.

After the shells had been removed, the extracts were homogenized using a Polytron homogenizer (Brinkmann Instruments) with stainless steel blades. The blended sample was compressed between filter papers to remove extraneous moisture. The homogenized samples were subsampled (four replicates) for dry ashing and microwave digestion.

Chemicals were reagent grade except where noted and deionized distilled water was always used.

Dry Ashing – 10 mL nitric acid (Baker Ultrex II ultrapure reagent) was added to a subsample (four replicates of about 1 g) of wet mussel tissue or dry reference material (0.5 g) in an acid washed 150 mL tall form beaker. The beaker was covered with a small glass petri dish and left to react overnight. Twenty-four hours later the sample was heated at 80°C until dry. The dried sample was moved to a muffle furnace and the temperature was slowly increased from room temperature to 450°C in one hour. The combustion was not observed during this process. The sample was heated for about 4 hrs 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 25 mL volumetric flask and made up to volume.

Microwave Digestion – wet mussel homogenate (about 0.5 g) or dry reference material (0.1 g) was digested in replicate with 3 mL of Seastar ultrapure HNO_3 in 40 mL Lorran PTFE bombs (Lorran International, Porters Lake, N.S. Canada, BOJ 2S0). Four bombs containing the mixtures were placed in a microwave pressure cooker as a precautionary measure to prevent the leakage of acid fumes into the microwave oven. The pressure cooker and a beaker containing 50 mL water were placed in an unmodified domestic type microwave oven (Panasonic NE-7970C) equipped with a turntable. The samples were microwaved for 90 seconds on full power (700 W) followed by 5 min on medium/low power. These conditions were chosen after a series of tests that resulted in complete digestion without leakage. The bombs were cooled in water and opened slowly until brown vapours were observed to escape through the pressure relief hole incorporated into the cap of the bomb. When the vapours stopped the cap was opened slightly and this opening was continued gradually until no further vapour was observed. The contents were transferred to a 25 mL volumetric flask and brought to volume. The microwave digestion method used here may be adopted to any microwave oven after calibration of power output. The calibration is carried out by measuring the temperature rise of one liter of water from room temperature. In our microwave oven for one liter water in the teflon FEP beaker the temperature rise in ninety seconds at full power was 16°C. A combination of ninety seconds of high power and 5 minutes at medium/low power resulted in rise of 43°C.

Inductively coupled plasma atomic emission spectrometric (ICP-AES) measurements were made on a Thermo Jarrell Ash 61E simultaneous emission spectrometer. The system was controlled by an IBM PS/2 model 50 computer. A standard torch and fixed position cross flow nebulizer controlled by a peristaltic pump working at a flow rate of 1 mL min^{-1} were used. The instrument operating parameters were RF Power incident 1.1 K.W., torch height 12 mm above the coil (adjusted for maximum ratio for peak height/background for Cd 1 ppm solution), exposure time 5 sec., and spectrum shifter 4 cycles for background correction.

Calibration standard solutions with 1 milligram per litre metal were prepared by diluting 1000 milligram per litre SPEX Plasma Standards (SPEX Industries Inc. 3880 Park Ave. N.J. U.S.A.). Instrument calibration was verified with certified wastewater quality control samples acquired from Environmental Resource Associates, Arvada, Colorado, U.S.A. This sample was also used as a control to monitor performance of the instrument during analysis.

Flame atomic absorption spectrophotometric (FAAS) measurements for Cu and Zn and graphite furnace atomic absorption spectrophotometric (GFAAS) for Cd and Pb were made on Perkin Elmer 4000 Atomic Absorption Spectrophotometer, and on model 306, equipped with Perkin Elmer HGA 500 graphite furnace respectively. Uncoated platforms made in the laboratory and gas interrupt during atomization were employed. The details of the method are described by Loring & Rantala[5] (1992). Single element standards in 12% HNO_3 (v/v) were used for direct calibration for analysis of microwave digested samples, while standards prepared for ICP-AES were used with dry ashed samples.

National Research Council of Canada interlaboratory comparison samples[6] dry homogenized dogfish muscle (NRC-D), scallop tissue (NRC-E), and swordfish muscle (NRC-F), were used as reference materials (four replicates) to assess the relative accuracy and precision of the methods.

RESULTS AND DISCUSSION

The NRC Canada inter-comparison study and natural mussel (*Mytilus edulis*) tissue samples were mineralized by dry ash and microwave digestion techniques. The efficiency of the mineralization was examined by comparing the Cd, Cu, Pb and Zn results obtained for the dry ashed and microwave digested NRCC samples by FAAS/GFAAS methods. The metal concentrations found in the samples were compared with the NRCC recommended values and are presented in Table 1. The metal concentrations in the dry ashed NRCC samples determined by ICP-AES and FAAS/GFAAS methods are compared with the NRCC recommended values in Table 2. In natural mussel samples the concentrations determined by ICP-AES method in dry ashed samples were compared with the concentrations measured by AAS method in the microwave digested samples and the results are shown in Table 3. The statistical test using a range of results[7] was applied for estimating similarities or difference in the experimental results. The results for each element are discussed below.

Cadmium

The concentrations of cadmium found in NRCC samples prepared by dry ashing (0.057, 0.44, and 0.27 μg g^{-1} Cd) and microwave digestion (0.059, 0.43, and 0.24, μg g^{-1} Cd respectively) agreed closely when the concentration of the metal was measured by the GFAAS method (Table 1). These values are also in good agreement with the NRCC

Table 1 Concentration of metals (μg g^{-1} dry weight; n = 4) in NRCC reference tissue samples measured by AAS; Comparison of dry ash and microwave digestion techniques.

Sample	*Method*	*Cd*		*Cu*		*Pb*		*Zn*	
		mean	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*
Dogfish									
NRC-D	dry ash	0.057	5.4	5.5	8.7	7.5	16	27	12
	microwave	0.059	32	4.3	20	9.1	57	25	3.8
	NRCC Rec. Val.	0.08	26	6.1	17	9.0	24	28	8.0
Scallop									
NRC-E	dry ash	0.44	3.0	2.0	68	N.M.		41	2.0
	microwave	0.43	6.0	1.1	15	0.07	14	46	1.1
	NRCC Rec. Val.	0.48	13	1.2	20	0.1*		46	7.0
Swordfish									
NRC-F	dry ash	0.27	3.6	3.2	39	N.M.		30	1.7
	microwave	0.24	4.0	2.1	6.2	0.16	15	34	1.5
	NRCC Rec. Val.	0.31	19	2.6	13	0.24	26	34	7.0

RSD Relative Standard Deviation
Rec. Val. Recommended Value
N.M. Not Measured (high blank reading)
* Estimated True Value (Berman & Boyko 1985)

Table 2 Concentration of metals (μg g^{-1} dry weight; n = 4) in dry ashed NRCC Reference Tissue Samples; Comparison of ICP-AES and AAS techniques.

Sample	*Method*	*Cd*		*Cu*		*Pb*		*Zn*	
		mean	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*
Dogfish									
NRC-D	ICP-AES	0.34	6.2	4.8	3.5	8.0*	14	28	8.6
	AAS	0.057	5.4	5.5	8.7	7.5	16	27	12
	NRCC Rec. Val.	0.08	26	6.1	17	9.0	24	28	8
Scallop									
NRC-E	ICP-AES	0.85	3.2	1.1	100	2.4*	12	42	2.2
	AAS	0.44	3.0	2.0	68	N.M.		41	2.0
	NRCC Rec. Val.	0.48	13	1.2	20	0.1**		46	7
Swordfish									
NRC-F	ICP-AES	0.50	3.2	2.3	39	2.1*	14	31	0.8
	AAS	0.27	3.6	3.2	39	N.M.		30	1.7
	NRCC Rec. Val.	0.31	19	2.6	13	0.24	26	34	7

RSD Relative Standard Deviation
Rec. Val. Recommended Value
N.M. Not Measured (high blank reading)
* Blank Correction not Applied
** Estimated True Value (Berman & Boyko 1985)

recommended values (0.08 ± 0.02, 0.48 ± 0.06, and 0.31 ± 0.06 μg g^{-1} Cd). The determination of Cd in the dry ashed samples by ICP-AES method showed relatively higher concentrations of Cd (0.34, 0.85, 0.54 μg g^{-1} Cd respectively) in the samples (Table 2). These values are significantly higher than the values determined by the GFAAS method and also higher than the NRCC suggested values. The high values observed for Cd in the NRCC samples by ICP-AES method may likely be due to Cd being measured at close to detection limit of the method and high variance can occur.

Table 3 Concentration of metals (μg g^{-1} wet weight; n = 4) in natural mussel samples.

Location	*Method*		*Cd*		*Cu*		*Pb*		*Zn*	
			mean	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*	*mean*	*RSD %*
Cardigan River	dry ash	ICP-AES	0.47	7.0	2.3	6.7	0.56*	55	22.9	4.4
	microwave	AAS	0.24	14	3.2	5.6	0.04	9.3	25.6	4.5
Sydney Harbor	dry ash	ICP-AES	0.46	5.7	5.5	38.0	1.5*	55	19.1	29
	microwave	AAS	0.28	4.6	10.0	6.6	0.61	13	28.4	6.0
Seal Island-1	dry ash	ICP-AES	1.6	18	25.5	8.2	3.5*	11	86	5.4
	microwave	AAS	1.8	8.3	31.7	2.6	0.54	8.0	104	6.3
Seal Island-2	dry ash	ICP-AES	1.3	38	16.9	5.6	0.87*	41	93	5.4
	microwave	AAS	2.1	7.1	21.1	5.3	0.51	4.9	107	5.9

RSD = Relative Standard Deviation
* Blank Correction not Applied

The results were similar for the measurement of low concentration Cd in the natural samples (Table 3).

The concentrations of Cd found by the dry ash/ICP-AES method in Cardigan River (0.47 μg g^{-1} Cd) and Sydney Harbour (0.46 μg g^{-1} Cd) samples were relatively higher than those found by the microwave/GFAAS method (0.24, 0.28 μg g^{-1} Cd respectively). For Seal Island sample 1 (1.6 μg g^{-1} Cd) and sample 2 (1.3 μg g^{-1} Cd) the dry ash/ICP-AES method results were lower than those obtained by the microwave/GFAAS method (1.8, 2.1 μg g^{-1} Cd respectively). Since the non-homogeneity of the natural samples cannot be ruled out this data was not used to compare the performance of the method. However a statistical test using a range of results indicated that the two methods produced significantly different results. The differences were more visible in samples that contained low concentrations of cadmium such as Cardigan River and Sydney Harbour.

Copper

The average concentrations of copper found in NRCC samples by dry ash/ICP-AES (4.8, 1.1, 2.3 μg g^{-1} Cu), dry ash/FAAS (5.5, 2.0, 3.2 μg g^{-1} Cu), and microwave/FAAS (4.3, 1.1, and 2.1 μg g^{-1} Cu) methods showed a reasonably good agreement with the NRCC's statistically accepted values (6.1 ± 1.0, 1.2 ± 0.2, 2.6 ± 0.3 μg g^{-1} Cu).

In natural samples the concentrations found by dry ash/ICP-AES method for the four samples (2.3, 5.5, 25.5, 16.9 μg g^{-1} Cu) were consistently lower than those found by the microwave/FAAS (3.2, 10.0, 31.7, and 21.1 μg g^{-1} Cu) method. The bias observed in natural samples was not apparent in the NRCC reference samples. The statistical test using a range of results at the 95% confidence level indicated that the two methods produced significantly different results.

Lead

The samples were found to be contaminated with lead during dry ashing. The lead contamination of dry ashed blank sample solutions was found by the GFAAS method.

The concentration of Pb in blank solutions varied between 0.006 and 0.016 milligrams per liter. In one blank sample solution the reading was even higher than that observed for one of the actual samples. The microwave digested blank sample solutions were clean, so the contamination was attributed to external sources during the sample preparation steps. However, ICP-AES method did not show the presence of lead in the blank solutions possibly because the concentration was lower than the detection limit of this method, therefore, ICP-AES results were not corrected for the blanks.

The average concentrations of lead in the NRCC dogfish sample found by dry ash/ICP-AES (8.0 $\mu g\ g^{-1}$ Pb), dry ash/GFAAS (7.5 $\mu g\ g^{-1}$ Pb) and the microwave/GFAAS (9.1 $\mu g\ g^{-1}$ Pb) showed a reasonably good agreement with the suggested value (9.0 ± 2.1 $\mu g\ g^{-1}$ Pb). But for scallop and swordfish samples the dry ash/ICP-AES method gave much higher results (2.4, 2.1 $\mu g\ g^{-1}$ Pb) than those obtained by the microwave/GFAAS method (0.07, 0.16 $\mu g\ g^{-1}$ Pb). Because of contamination, the dry ash/GFAAS values could not be determined for these samples. The microwave/GFAAS method results obtained for these samples were in agreement with the recommended values (0.1 and 0.24 ± 0.06 $\mu g\ g^{-1}$ Pb). The high concentration values obtained by dry ash/ICP-AES method for the scallop and swordfish samples may have resulted from contamination of the samples as reported above. The high bias for Pb may also be due to measurements being made close to detection limit of the ICP-AES, method as noted earlier for the determination of Cd. A similar trend was observed for natural samples.

The concentrations of Pb in all four natural samples determined by the dry ash/ICP-AES method (0.56, 1.5, 3.5, 0.87 $\mu g\ g^{-1}$ Pb) were found to be high compared to the values obtained by the microwave/GFAAS method (0.04, 0.61, 0.54, 0.51 $\mu g\ g^{-1}$ Pb). As expected the high bias was more pronounced on samples with low concentrations of Pb as compared to those samples like dogfish containing the metal in high concentration.

Zinc

Concentrations of Zn in the reference samples obtained by dry ash/ICP-AES (28, 42, 31 $\mu g\ g^{-1}$ Zn), dry ash/FAAS, (27, 41, 30 $\mu g\ g^{-1}$ Zn) and microwave/FAAS methods (25, 46, 34 $\mu g\ g^{-1}$ Zn) compared well and within the experimental errors were in good agreement with the recommended values for the respective samples (27.9 ± 2.3, 46.0 ± 3.0, 34 ± 2.4 $\mu g\ g^{-1}$ Zn).

For natural samples, however the zinc concentrations found by dry ash/ICP-AES method (22.9, 19.1, 86, 93 $\mu g\ g^{-1}$ Zn) were consistently lower than the values obtained by the microwave/FAAS method (25.6, 28.4, 104, 107 $\mu g\ g^{-1}$ Zn). The bias showed a pattern similar to that observed in the determination of copper noted above.

CONCLUSIONS

An open beaker method for dry ashing biological tissue samples was found to be susceptible to contamination from external sources. The contamination was found to influence significantly the analytical results for lead in samples with low concentrations. The contamination from external sources can be prevented by carrying out the digestion in a closed system such as the microwave digestion and PTFE bomb used in our study.

Tests based on the range of results at 95% confidence level indicated that dry ashing and microwave digestion methods produced statistically different results. The difference was more pronounced for determination of metals in natural samples than in reference

samples. This could be attributed to non-homogeneity of the samples. It was observed that sample preparation by microwave digestion (a closed system) accompanied by the GFAAS determination with a lower detection capability compared to ICP-AES is a better combination for the determination of Cd and Pb when they are present in low concentrations.

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