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Microwave digestion and analysis of foliage for total mercury by cold vapor atomic fluorescence spectroscopy

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Abstract. A microwave technique for digesting foliage samples was developed and evaluated for quantifying low levels of Hg by cold vapor atomic fluorescence spectroscopy, CVAFS. The method meets three criteria: (1) to digest all sample material completely and consistently, (2) to reduce sample digestion time to less than one hour, and (3) to maintain a low analytical blank. Mean recovery of NIST standards was 90±6%. Samples that were analyzed by this technique and by Instrumental Neutron Activation Analysis compared within 15%. This method also compared within 15% of hot acid digestion methods on samples prepared and analyzed by CVAFS at different laboratories in the First International Mercury in Foliage Intercomparison of Methods $(FIM)^2$. The largest source of variability in all of the interlaboratory comparisons was sample inhomogeneity rather than analytical error.

Introduction

Throughout the world, vegetation, fungi, and mosses grown near areas of elevated levels of mercury (Hg) (i.e. volcanoes, mines, chlor-alkali plants, and incinerators) have extremely high levels of Hg in their tissues (Lindberg et al. 1979; Siegel et al. 1984; Shaw & Panigrahi 1986; Barghigiani & Bauleo 1992; Carpi et al. 1994; Fischer et al. 1995). As a result, plants have been used as Hg biomonitors for decades. At these high levels (less than 1 to over 10,000 μg/g) sample preparation for samples of vegetation, fungi, mosses, and lichen by hot acid digestion and analysis by cold vapor atomic absorption spectrometry (CVAAS) or cold vapor atomic fluorescence spectroscopy (CVAFS) has proved sufficient (Stokes et al. 1983; Shaw & Panigrahi 1986; Kojo & Lodenius 1989; Barghigiani et al. 1990; Bargagli et al. 1991; Carpi et al. 1994; Moore et al. 1995). Using extreme care to avoid contamination and analytical error, hot acid digestion and analysis by CVAAS or CVAFS has been successfully carried out on vegetation samples with much lower total Hg concentrations (Rasmussen et al. 1991; Iverfeldt 1991; Munthe et al. 1995; Lindberg 1996).

Studies have shown that there are many sources of variability in the analysis of plant tissues for total Hg. Sources of variation include not only differences between plants of the same species (Rasmussen et al. 1991) but

also within different species at the same site (Kovalevskii 1986; Barghigiani et al. 1990). Additionally, tissue age, type, and calcium content have been found to influence the variability of Hg within plants (Rasmussen 1994). Sources of analytical variability include incomplete digestion and inadequate mixing of the sample (Rasmussen 1994). Also, the concentrated nitric (14M) and/or sulfuric acids (18M) frequently used to digest samples can cause elevated reagent blanks. Despite efforts to homogenize vegetation samples without causing contamination, variability within the sample matrix remains a problem, especially at low Hg levels. Generally, within sample replication can be kept to within 10% (Siegel et al. 1984; Rasmussen et al. 1991; Rasmussen 1994; Carpi et al. 1994).

Microwave digestion has been used successfully to extract organic samples for other types of analyses, such as atomic absorption, inductively coupled plasma mass spectroscopy, and chromatography. Microwave digestion and analysis of *Rosmarinus officinalis* L. (rosemary) for total Hg by CVAAS has been reported, but the method was not described (Barghigiani & Ristori 1995). Our laboratory has successfully used microwave digestion of glass-fiber filters for trace level Hg (pg) analysis (Keeler et al. 1995). A similar technique was investigated for digesting foliage samples that would (1) digest all of the sample material completely and consistently, (2) require little effort and rapid digestion (less than 1 h), and (3) require less concentrated acid thereby reducing reagent blanks.

Methods

Foliage collection and processing

Foliage samples were collected from a site located at the Proctor Maple Research Center in Underhill Center, Vermont (44°31′ N, 72°52′ W, 400 m elevation) on 30 August 94. The species sampled were *Acer saccharum* Marsh. (sugar maple), *Betula alleghaniensis* Britt. (yellow birch), *Fagus grandifolia* Ehrh. (American beech), and *Ostrya virginiana* (Mill.) K. Koch (hop-hornbeam). These samples were collected as part of a larger Hg in throughfall and litterfall study (Rea et al. 1996). To determine if there were any significant differences between individual trees of the same species or between tree species, three samples were collected from three trees of each species. Foliage samples were collected using a pole pruner with four to six extensions to clip branches from trees at a height of 12 m. Branches were caught before hitting the ground and the leaves were carefully picked from the branches and placed into plastic bags. Approximately 20 leaves from each branch were collected into plastic bags for Hg analysis. These samples were

triple bagged and frozen until dried at the University of Michigan Air Quality Laboratory (UMAQL). Particle-free gloves were worn at all times during sample collection and handling.

Clean techniques were incorporated into all phases of the drying and digestion procedures in order to minimize contamination or volatilization of Hg from the leaves. Oven-drying plant samples for Hg analysis has been shown to cause losses of Hg (Rasmussen et al. 1991; Carpi et al. 1994). Therefore, to minimize Hg losses due to volatilization, samples were kept triple bagged and frozen until dried in a Class 100 clean bench at room temperature. The foliage samples were dried inside acid-cleaned polyethylene buckets with HEPA filtered air (class 100) drawn through the buckets by vacuum pumps for three to four days. Particle-free gloves were worn during all stages of sample handling and were changed between samples to prevent cross-contamination. Petioles were removed to prevent dilution of the sample (Rasmussen et al. 1991). Petioles were removed by breaking them from the leaf base. Each sample was placed in an acid-cleaned Teflon bottle and ground to approximately 0.5–1 mm in diameter using an acid-cleaned polyethylene spatula; a clean spatula was used for each sample. The bottles were sealed with Teflon-tape, triple bagged, and stored at room temperature until digested and analyzed for Hg.

Rasmussen et al. (1991) found that air dried samples retained approximately 5% of their moisture content. Therefore, a second set of 20 leaves from each branch was collected to determine differences in the dry weight of leaves dried at room temperature versus conventional drying techniques. The petioles were also removed from these leaves before they were oven-dried. The dry weight ratio of leaves dried in an oven to leaves dried at room temperature was 0.94–0.96. Therefore, it was determined that samples for Hg analysis were sufficiently dried at room temperature.

Microwave digestion and analytical procedures

Foliage samples to be analyzed for total Hg were handled while wearing particle-free gloves in the Class 100 clean bench and analyzed in a Class 100 clean room. The microwave sample preparation accessories described below are from CEM Corporation for use with the MDS-2000. Lined digestion vessels with vent stems were used to microwave the samples. After thorough mixing of the sample, a 0.25 g aliquot was weighed into the acid-cleaned Teflon microwave vessel using an acid-cleaned polyethylene spatula. Next, 20 mL of 1.6 M HNO_3 (Suprapur, EM Science) were added to the vessel. The Teflon vessels and lids were sealed inside vessel shells. The MDS-2000 has an internal pressure control system which passively monitors the pressure inside one vessel without contaminating the sample. The microwave oven

was programmed in three stages. The pressure was first raised and held at 40 PSI for three minutes, then it was raised to 85 PSI and held for three minutes, and, finally, the pressure was raised to 150 PSI and held for five minutes (CEM 1991). A transparent, deep yellow solution, with no visible solid or particulate material, remained in the vessel after digestion. The microwave digestion procedure took approximately 35 minutes to complete.

After the vessels had cooled, the Hg forms in the extract were oxidized to Hg^{+2} with 0.2N BrCl to a 0.005N BrCl solution and allowed to react for at least 12 h before analysis. A 1 mL aliquot of the extract was taken for analysis, the excess BrCl was reduced with NH_2OH and the Hg^{+2} in solution was reduced to Hg^0 with $SnCl_2$. The solution was purged with high purity N_2 and the Hg^0 liberated was collected onto a gold-coated bead trap. The gold traps were thermally desorbed in a high purity He stream and analyzed by CVAFS using the dual amalgamation technique (Fitzgerald & Gill 1979). Calibration curves were run on each day of analysis. Control standards were checked throughout the course of analysis. If a control standard was not within 10% of the expected value, the instrument was recalibrated. Reagent blanks were run on each day of analysis and contributed to less than 3% of the sample concentration. The detection limit, calculated using three times the mean of the reagent blank for a 1 mL aliquot, was 2.8 ng/g. All foliage samples were analyzed in duplicate and reported concentrations have been blank corrected.

Results and discussion

NIST recoveries

To test this digestion technique, 20 aliquots of National Institute of Standards & Technology (NIST) Standard Reference Material (SRM) #1515 (*Pyrus* spp) were digested and analyzed in duplicate. Mean recovery of NIST SRM #1515 after microwave digestion was $90 \pm 6\%$ of the certified value. On each day of analysis, two aliquots of the NIST standard were digested and analyzed in duplicate; variation between analytical duplicates ranged from 0.01–9.0%. The NIST suggests that digestion in nitric acid alone is incomplete and hydrofluoric (HF) acid should also be used to provide full recovery. Since samples are purged in acid-cleaned glassware, our analytical system cannot tolerate HF and it was not used. Although recovery usually fell within the certified range, the lack of HF in the sample digestion could account for recovery being less than 100%.

Table 1. Mean ± standard deviation of replicate foliage samples analyzed for total Hg by Instrumental Neutron Activation Analysis (INAA) and microwave digestion and analysis by cold vapor atomic fluorescence spectroscopy (CVAFS)(ng Hg/g dry weight).

Species	Replicate	MIT[1] (INAA)	UMAQL[2] (mic. dig. & CVAFS)
Acer saccharum	1	39 ± 5	38.8 ± 3
	2	36 ± 5	35.3 ± 2
Betula alleghaniensis	1	36 ± 4	32.0 ± 5
	2	33 ± 3	33.1 ± 9
Ostrya virginiana	1	41 ± 6	38.0 ± 5
	2	24 ± 4	39.4 ± 2

[1] MIT: Massachusetts Institute of Technology
[2] UMAQL: University of Michigan Air Quality Laboratory

Interlaboratory comparisons

As a test of this microwave digestion procedure, two 0.1 g aliquots of dried sample from *Acer saccharum*, *Betula alleghaniensis*, and *Ostrya virginiana* were analyzed for Hg by Instrumental Neutron Activation Analysis (INAA) at the Nuclear Reactor Laboratory at the Massachusetts Institute of Technology (Olmez 1995). Since the entire sample is analyzed, any problems associated with incomplete digestion are avoided with INAA. Two aliquots of these same samples were digested and analyzed for Hg by CVAFS at the UMAQL. The results of these analyses are presented in Table 1. Agreement between the two analytical methods was better than 15% except for one replicate of *Ostrya virginiana*. Both sample inhomogeneity and differences in analytical procedures probably account for the differences observed between laboratories. Despite our efforts to thoroughly grind and mix the samples, the largest source of variability appears to be inhomogeneity within the sample. The differences between INAA and CVAFS may be due to variability between the individual leaves in the sample or, more likely, to more vein tissue and less leaf tissue being present in one aliquot versus another. It was observed upon grinding the leaves, that the vein tissue was thicker and more difficult to break apart than rest of the leaf tissue. Rasmussen et al. (1991) observed that the addition of the petiole to foliar samples caused dilution of the sample for total Hg. The same influence may be seen in vein tissue if it was not thoroughly ground and mixed within the sample.

The microwave digestion procedure was also tested against other sample preparation procedures in the First International Mercury in Foliage Intercomparison of Methods (FIM)[2] (Lindberg 1997). Foliage samples were dried at

Table 2. Mean ± coefficient of variation expressed in ng/g of a live foliage and a litterfall sample prepared and analyzed for total Hg by cold vapor atomic fluorescence spectroscopy in separate laboratories involved in the First International Mercury in Foliage Intercomparison of Methods $(FIM)^2$ (ng Hg/g dry weight).

	ORNL[1]	FGS[2]	IVL[3]	UMAQL[4]
Preparation method	Heated H_2SO_4/HNO_3	Heated H_2SO_4/HNO_3	Heated H_2SO_4/HNO_3	Micowave digestion
Quercus prinus	52.5 ± 2.5	54.4 ± 2.2	n/a	47.5 ± 4.5
Mixed-hardwood litterfall	115 ± 4.9	110.4 ± 1.5	120	103.3 ± 7.1

[1] ORNL: Oak Ridge National Laboratory
[2] FGS: Frontier GeoSciences
[3] IVL: Swedish Environmental Research Laboratory
[4] UMAQL: University of Michigan Air Quality Laboratory

room temperature, ground, and homogenized at Oak Ridge National Laboratory (ORNL) and were prepared and analyzed by CVAFS at the different laboratories involved in the $(FIM)^2$. The laboratories involved were ORNL, Frontier GeoSciences (FGS), the Swedish Environmental Research Institute (IVL), and the UMAQL. ORNL digested samples using EPA Hg digestion method 245.5 CLP-M which heats samples at 95 °C in a sulfuric/nitric acid mixture. FGS and IVL also digested the samples in a nitric/sulfuric acid mixture followed by reflux heating in a glass vessel. The UMAQL used the microwave digestion procedure described in this paper.

Results of two of the foliage samples analyzed by the laboratories are shown in Table 2. Data shown include effects of differences in sample digestion procedures. Agreement between the laboratories ranged from 4 to 14% on the *Quercus prinus* L. (chestnut oak) sample and between 4 and 15% on the litterfall sample. The similarity between the digestion procedures used by ORNL, FGS, and IVL most likely accounts for the closer agreement between those methods than the microwave digestion method used by the UMAQL. The lower recovery (6–14%) on the samples in the $(FIM)^2$ by the UMAQL may be due to the digestion procedure, as a similarly low recovery was found for the NIST reference material. The results obtained by each laboratory, however, fell within the error associated with each sample value. The differences between laboratories may also be attributed to inhomogeneity between the sample aliquots. This would account for the good internal laboratory precision and the larger variation between laboratory analyses of these samples.

Foliar analysis

The results of the foliar analysis for samples collected in the Lake Champlain Watershed are summarized in Table 3. All of the samples were analyzed in

Table 3. Mean ± standard deviation, minimum, and maximum total Hg concentrations (ng/g dry weight) of four species of foliage collected from Underhill Center, Vermont on 30 Aug 94.

	Acer saccharum	*Betula alleghaniensis*	*Fagus grandifolia*	*Ostrya virginiana*
Mean ± std. dev.	39.1 ± 6.0	30.4 ± 4.1	34.9 ± 5.2	36.7 ± 7.3
Minimum	33.0	21.5	27.7	24.2
Maximum	49.1	35.5	40.7	45.9
n	9	9	8	9

duplicate with less than 10% variation between duplicate analyses. Total Hg concentrations ranged from 21.5 to 49.1 ng Hg/g (dry weight) for the species sampled in this study. The source of the variation in these samples is not incomplete digestion, as there were no traces of leaf material in the sample digests. More likely, the source of the variation was the inhomogeneity of the ground foliar samples.

Due to the large variation between samples of the same species, no significant differences were found ($p < 0.05$) between trees of the same species or between the four species sampled at this site. Rasmussen et al. (1991) also observed similarity in total Hg concentrations between leaves/needles of *Acer saccharum*, *Abies balsamea* (L.) Mill (balsam fir), and *Picea glauca* (Moench) Voss (white spruce). The total Hg concentrations measured in this study fall within the ranges reported for other forest species: *Picea mariana* (Mill.) BSP. (black spruce), *Pinus banksiana* Lamb. (jack pine), *Betula papyrifera* Marsh. (American white birch), and *Larix laricina* (Du Roi) K. Koch (Tamarack) (Moore et al. 1995) and *Acer saccharum* and *Abies balsamea* (Rasmussen 1994).

Conclusions

We have developed a microwave digestion procedure for deciduous leaves that is suitable for low level Hg determination by CVAFS. This method has accomplished all of our objectives; it (1) completely digests all sample material with no solids remaining, (2) requires little effort while reducing sample digestion time to approximately 35 minutes, and (3) requires only 1.6 m nitric acid instead of concentrated acids, which reduces analytical blanks. Mean recovery of NIST standards was 90±6% and agreement with INAA was generally less than 15%. The microwave digestion procedure compared within 15% of other digestion procedures in the $(FIM)^2$. The largest source of

variability between laboratories appears to be sample inhomogeneity rather than analytical error.

The problem of sample inhomogeneity which was brought out in this study has led to the development and use of acid-cleaned ground glass mortars and pestles. Not only do foliar samples appear to be much more homogeneous (sample variability is typically less than 5%), but also vein tissue is more easily broken down and the process of grinding samples is much faster and easier.

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