Determination of Trace Metals in Fresh Water Mussels by Atomic Absorption Spectrometry with Direct Solid Sample Injection

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Freeze-dried residues of fresh water mussels (exclusive of shell) from the Kingston Basin of Lake Ontario are analyzed for Al, Cr, Cu, Pb, and Zn by direct firing of solid samples in a graphite furnace atomizer. Naphthalene is used as a diluent. Calibration using aqueous standards appears sufficiently accurate for slowly volatilized elements (Al, Cr, Cu). Calibration tests with NBS Standard Material 1571 (orchard leaves) indicate that aqueous standards cannot be used with accuracy for volatile elements (Pb, Zn).

Considerable interest has developed in trace metal determinations at extremely low levels in various marine samples (*1–3*). Recent advances in atomic absorption spectrometry have improved analytical sensitivities by one or two orders of magnitude, particularly for the ubiquitous first row transition metals (*4, 5*). Much of the development is centered on the use of flameless atomizing devices of various kinds with lower ultimate detection limits. This implies that the sample digestion procedures must be scrupulously pure, or a fortiori, that handling is minimal. A common experience has been to introduce an appreciable contamination with Zn, Cd, Cu, or Pb, using analytical grades of acids, water, or even clean glassware.

Many of the biological samples collected are awkward in the sense that the dissolution of the tissue is often difficult and time consuming. There has been some concern expressed that better methods be developed for trace metal determinations in such samples (*6*). The direct insertion of solid biological samples in a Massmann type furnace is appealing as handling is limited. The instrument operates in a discontinuous batch mode, however, and requires the correct weight of sample, such that 10^{-10} to 10^{-12} g of analyte atoms is vaporized. If the metal content is extremely low, near detection limit levels, then samples of several mg can be weighed and transferred with some precision. But if moderate amounts of the element (μg per g levels) are involved, there remains the near impossible task of inserting microgram weights of the solid.

To be useful, a direct insertion method must cover the wide range of trace metal levels found in natural biological samples particularly marine fauna (*1, 7*). Some means of mixing inert diluents with freeze-dried residues is required. Forming a slurry with a liquid and micropipetting the suspension has been suggested (*3, 8*), but this requires a stable emulsification. Some success has been obtained with inorganic rock specimens by grinding samples with powdered graphite (*9, 10*), thus gaining an extended furnace wall surface and an enhanced reduction of the sample. But with organic matrices of varying origin, it might be more advantageous to use solid diluents which have a similar reducing nature and yet a greater volatility.

Flameless atomization often involves greater background absorptions and more complicated matrix effects. For some analytes, the amplifying and recording electronics of many conventional spectrometers contribute in part to the matrix effects. In a batch mode, control of the interferences is of paramount importance if a suitable calibration is to be made. For some directly inserted solids, aqueous standards have proved adequate (*10, 11*), but this is not generally the case. It is perhaps the inherent difficulty in accurately synthesizing the sample matrix in the laboratory and the consequent uncertainty in calibration that has kept the method of direct solid insertion from widespread use. The work described below shows how some of the difficulties of method might be removed.

EXPERIMENTAL

Apparatus. Figure 1 is a block diagram of the spectrometer and recording circuits used. Samples were vaporized in a graphite Massmann furnace, a Perkin-Elmer HGA-2000 with a conventional electronic control. The monochromator was a Jarrell-Ash 0.5-m Ebert type with curved bilateral slits and a grating blazed for maximum energy at 250 nm. The output of the lock-in amplifier was recorded directly (slow response), or routed through the Biomation transient waveform recorder (fast response).

The chart recorder limited the bandwidth of the slow response system with a time constant of 0.25–0.40 s (full scale). This range was determined from a Bode plot (*12*), using test waveforms of varying frequency, from pure sine to symmetric-triangular. The recorder showed a nonlinear decrease in the time constant with the fraction of full scale deflection used.

The lock-in amplifier determined the time constant in the fast response system and was fixed at 0.03 s or less. With such wide bandwidths, this system was not optimized at the maximum signal/noise response (*13*). However, metal concentrations in the samples were generally large and measurements of detection-limit levels were never a concern here.

Measurements of absorption peak heights were made from the ultimate chart record in either response system. Some determinations of peak areas were also made. In this case, the output of the lock-in amplifier was routed directly to a linear integrator with a time constant of 0.45 s (slow response), or stored in the transient recorder for later integration (fast response).

Samples. Fresh water mussels were collected from various points in the Kingston Basin of Lake Ontario (*7*) for the purpose of monitoring the water quality of the Great Lakes drainage system. The whole animal except for the shell was freeze-dried and the residue used as the analytical sample.

Direct vaporization of extremely small quantities of the pure animal residues indicated that the trace metal contents were very high so that less sensitive resonance lines were often employed. Dilution of the freeze-dried residue was also made with pure solid naphthalene where necessary. Dried animal residues were ground in an agate mortar with 10 times (w/w) the diluent.

Pellets of the homogenate were formed under pressure between nylon pistons in a uniform bore, thick walled, glass tube. Pellet weights (usually in the range 1–5 mg) were determined by the difference in weight of tantalum transfer boats. Transfer was made via a Perkin-Elmer sampling spoon. The tantalum boat containing the pellet was inserted end-on, the boat inverted and then withdrawn. Care was exercised in this operation to ensure a reproducible location of the pellet in the center of the furnace.

Some mussel residues were dissolved for test analyses using standard additions. Samples, 0.1–0.15 g, were treated in a Teflon pressure vessel (1½-inch dia. × 1½-inch) with 1 ml of concd HNO_3 for 2 h at

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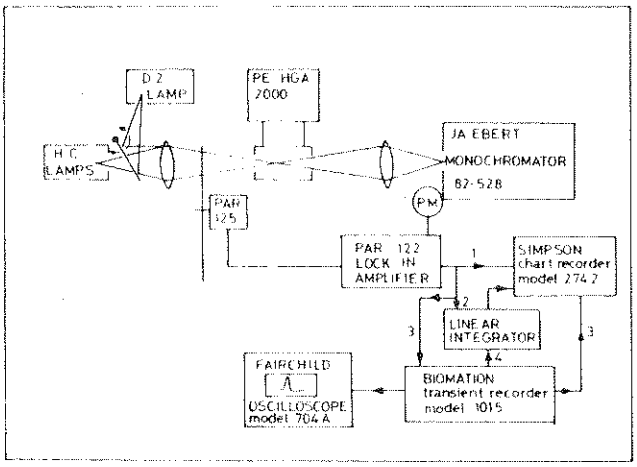

Figure 1. Signal collection circuits

(1) Peak height, slow response; (2) peak area, slow response; (3) peak height, fast response; (4) peak area, fast response

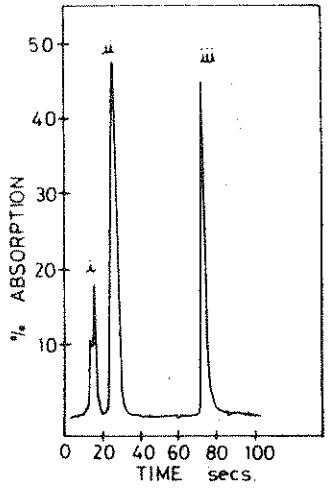

Figure 2. Analytical trace of Cu analysis in pellet sample C19L; dry 20 s, 150 °C; char 50 s, 1000 °C; atomize 25 s, 2200 °C

(i) naphthalene smoke, (ii) organic matrix smoke, (iii) Cu absorption

105 °C. On cooling, 0.75 ml of 1:1 $HClO_4/H_2O$ mixture was added and the sample reheated for 2 h at 105 °C. This treatment dissolved all but a trace of siliceous material. These dissolved samples were diluted quantitatively with doubly distilled water and micropipetted into the furnace.

Reagents and Standards. Naphthalene (Fisher N-134), used as the diluent was tested for trace metal contamination prior to use and none was found for 7 elements (Al, Cd, Co, Cr, Cu, Pb, Zn). Mineral acids (Aristar), used for acidification, cleaning of glassware, and dissolution were also tested. If detectable levels of any of the 7 elements were found, the stock was discarded.

For the preparation of liquid standards, Fisher Certified AA Standard Solutions (1000 μg per ml) were used. Dilute solutions were prepared from doubly distilled water and the pH of these solutions was adjusted to 1 or lower.

NBS Standard Reference Material No. 1571 (orchard leaves) was also dissolved in Teflon decomposition vessels for subsequent Pb, Zn, and Cu calibrations. Several samples of weight 0.05–0.15 g were heated for 6 h at 115 °C in 2 ml concd HNO_3 and 1 ml 30% H_2O_2 and when cooled, diluted to 50 ml quantitatively. This treatment was sufficient to take the whole of the sample into solution.

Procedure. To gain stability in single beam measurements, the source and photomultiplier were switched on at operating current values for 2 h before use. The optical volume of the furnace tube was kept small to avoid light blockage by pellet samples or liquid droplets.

Each solid sample was cycled through the steps of drying (130 °C), charring, and atomization. Temperatures for optimum charring and atomization were determined from the results of serial experiments with increasing values. These temperatures were not measured directly; relative values indicated by the electric current flow through the tube walls were used.

The analysis of each solid sample was a four-step procedure: (i) H.C. line absorption of the sample, (ii) H.C. line absorption of the standard, (iii) H.C. line absorption of a naphthalene blank of appropriate weight, (iv) D_2 arc background absorption. From 4 to 9 analyses for each element were performed; however, it was not found necessary to repeat steps (iii) and (iv) each time. Absorption measurements were made with a N_2 purge of 14 ml s^{-1}. The instrument settings for each element are indicated in Table I.

Usually 25 μl (Eppendorf) aliquots of the dissolved samples and standards were subjected to the same regimen as above. From 4 to 8 replicates were run to establish the precision in each case. Where standard additions were made, these were added directly to the stock solution.

RESULTS AND DISCUSSION

Analytical Trace. Figure 2 is an analytical trace typical of pellet volatilization/sublimation. The peaks in chronological sequence are for the scattering or absorption of, (i) naphthalene, (ii) organic mussel residue, (iii) elemental atomic vapor.

Naphthalene was found to sublime or vaporize completely if the pellet was heated sufficiently long at 150 °C. Naphthalene can be obtained at high purity with extremely low levels of trace metals. It constitutes an excellent diluent for biological samples for this and other reasons. As a pure hydrocarbon, it will speed the reduction of the organic matrix at low temperatures. Langmyhr and associates (*9, 10*) have employed powdered graphite as a diluent in petrographic samples which were analyzed by direct insertion of the solids. Evidence was obtained of sharpened peaks for some metals, notably Ag and Zn. This was attributed to an enhanced volatility, at least kinetically, owing to an expected acceleration in the rate of metal oxide reduction. Mixtures of graphite with mussel residue have not been tried here but it is believed that it is more advantageous to use a hydrocarbon. Solid phase diffusion rates of H or H_2, even at very low charring temperatures are likely to be much larger than carbon species. H-reduced intermediates would gain volatility and further reduction of oxides would be sponsored.

Table I. Pellet Analysis Atomization Heating Rates

Element	λ nm	Char temp, °C	Heating rate Temp, °C	Heating rate Time, s
Al	394.4	1000	2500	25
Cr	360.5	900	2500	25
Cu	324.7, 327.4	1000	2200	25
Pb	283.3	500	2000	15
Zn	307.6	500	2000	15

Charring Temperature. Optimal temperatures for charring were decided by a procedure similar to that used for Pb analyses in milk (*14*). A plot of peak absorbance per mg of sample vs. charring temperature for Cr is shown in Figure 3. Low temperatures, below 500 °C in this case, show abnormal background absorptions, whereas high temperatures, above 1400 °C, show a loss of Cr. All elements investigated behaved in this way. Charring temperatures were selected from the plateau region. Cu, Al, and Cr show a fairly broad plateau (600 to 1200 °C), while Zn and Pb have much lower and narrower ranges for safe charring (500 °C <Zn < 900 °C; 400 °C < Pb < 500 °C).

Final Temperature Using a Slow Response System. Power is supplied to the graphite furnace in a HGA 2000 at constant wattage, so that any temperature setting will refer not only to the appropriate final temperature of the tube walls,

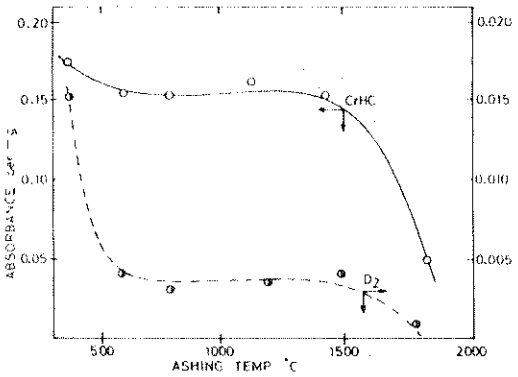

Figure 3. Atomizing absorbance variations with charring (ashing) temperature; Cr in mussel–naphthalene pellet

but also to the initial heating rate. These heating rates are only constant during the beginning phases of the atomization cycle; a nonlinear decay to zero occurs as the final temperature is approached. In practice, initial heating rates of 400 to 575 °C s^{-1} were achieved corresponding to final atomizing temperatures of 1500 to 2500 °C.

Wall temperatures measured (*15*) during the atomizing cycle show a bidirectional gradient downwards from a central maximum. If a purge gas interrupt mode is used, similar gradients will develop in the vapor, leading to a complicated thermal and mass diffusion of the analyte atoms. The resulting concentration gradients on the optical axis would mean that the absorbance is not a simple linear function of concentration (Beer's law), but must be of the form,

$$A = \int_{\text{path}} \epsilon c(t,l)\mathrm{d}\, l$$

where the concentration c is a function of spatial and temporal coordinates. If a constant flow of purge gas is used, the convection tends to reduce the vapor gradients in c and diminish the spatial dependency. Instantaneous values of A then become approximations to the linear Beer's law form,

$$A_t = \epsilon c_t l$$

A second feature which tends to linearize A is its logarithmic nature. Absorption peaks whose heights or areas are near linear with the weight of sample injected usually produce linear absorbance vs. concentration plots.

Figures 4 and 5 show absorption peak height and area measurements as a function of the final atomization temperature for five elements vaporized directly from mussel residue–naphthalene pellets. These data are for a constant weight of mussel residue (0.5 mg) and were obtained using the slow response system. Areas and heights are adjusted to arbitrary scales for comparison purposes. Hwang and Thomas (*16*) and Posma and associates (*13, 17*) have shown that peak heights are limited by the bandwidths (time constants) of the amplifying and recording systems used. With the slow response obtained on direct input of the lock-in amplifier to the chart recorder, there is a considerable distortion of the signal. Moreover, Posma, Smit, and Rooze (*13*) have demonstrated that a severe peak height limitation then occurs and that this attenuation is most pronounced at higher atomizing temperatures. This effect is apparent in Figures 4 and 5, particularly with the more volatile elements, Pb and Zn.

On the other hand, Al and Cr are more slowly volatilized, either as elements or refractory oxides and evidently the bandwidth of this slow system is sufficient, capturing signals with little distortion or peak cut-off.

The diminution in peak areas at higher temperatures for the volatile elements and the fact that there is a temperature

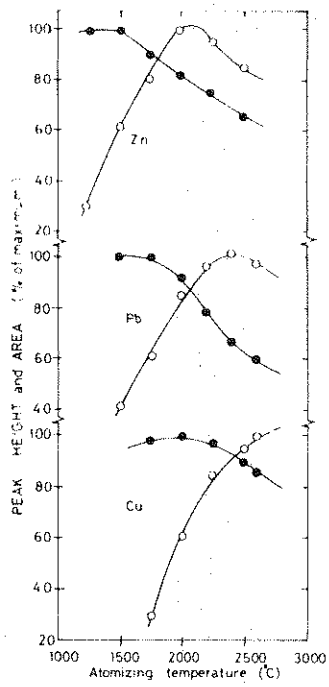

Figure 4. Peak height and area variations for Zn, Pb, and Cu in mussel–naphthalene pellets with atomizing temperature; slow response; (O) height; (●) area

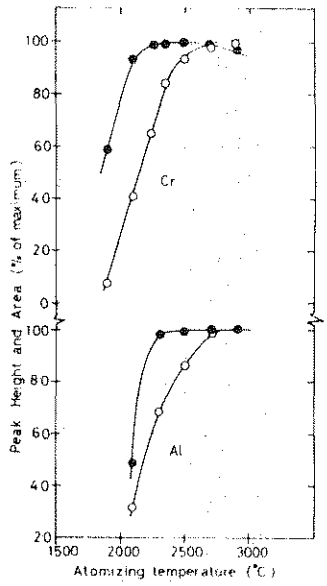

Figure 5. Peak height and area variations for Cr and Al in mussel–naphthalene pellets with atomizing temperature; slow response; (O) height; (●) area

separation of the individual height and area maxima confirms that there is a progressive truncation of the peaks of the higher atomization heating rates. Ideally, peak areas will only be a function of atom residence times and independent of the generation or vaporization rates (*5, 18*). At extreme temperatures, the residence time will be reduced by accelerated mass and thermal diffusion through the tube ends. With the high N_2 purge flow rates used here, this effect will only be noticeable above the convective loss at very high temperatures. Increased diffusion may in part be the reason for the coincident inflection in both heights and areas at the upper temperatures for Zn and Pb.

Early models of flame atomic absorption spectrometers

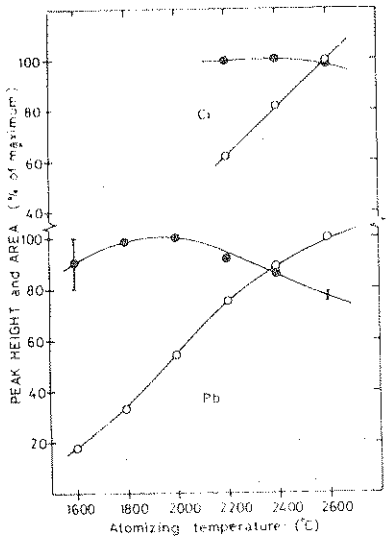

Figure 6. Peak height and area variations for Cr and Pb in aqueous standards with atomizing temperature; fast response; (O) height; (●) area

converted to flameless operation generally fall into the category of slow response. Providing a calibration can be made, absorption measurements obtained with those instruments of directly vaporized solids may be quite useable, if one can accept peak cut-off and in consequence a reduced sensitivity. The degree of signal distortion by reduced bandwidths (overlong time constants) is strikingly illustrated in the Cu results of Posma, Smit, and Rooze.

It should be recognized that in flameless atomization with a slow response record, there may be a considerable electronic contribution to the general mixture of interferences assigned to matrix effects. Optimization of the system may be difficult and day-to-day precision poor if electronic stabilization or constant instrumental procedures are not achieved.

The results in Figures 4 and 5 suggest that peak areas are less a function of temperature than are heights, particularly at lower atomizing temperatures. In some analyses, it may be preferable to optimize the system using areas even though the data retrieval is more difficult or slower. Schramel's results (*19*) acquired in a P-E HGA 72 with a slow response system also confirm this. Analytical curves for 7 elements show a marked improvement in linearity when areas as opposed to heights are used. This is to be expected with sharp peaks as truncation affects the heights much more severely than the areas.

Final Temperature Using a Fast Response System. In Figure 6 are the area and height data for Pb and Cr standards, using the fast response system. The points are the means of 4–9 individual determinations at each temperature. Precision in the area measurements is least at the lower temperatures, ±10% relative standard deviation (RSD), owing to the large fraction of the area in the shoulders in broader peaks. Upper temperature areas are obtained from sharper peaks with better precision, ±4% (RSD). Both sets of area measurements indicate a lesser temperature dependence than the corresponding data at slow response. Both area curves show diffusion losses at the highest temperature but this effect is more noticeable with Pb.

The peak height curves of both elements maintain a high sensitivity with no limiting plateau at the upper temperatures, confirming a negligible distortion in the signals (*13*). The analytical sensitivity is considerably improved for Pb but only moderately for Cr. The determination of volatile analytes such as Pb, Zn, and Cd in any sample, solid or liquid, would benefit from the use of a fast response system. An increased sensitivity

Table II. Trace Metal Analytical Results[a,b]

	Mussel (B2L) samples		NBS 1571 (orchard leaves)	
Element	A[c]	B[c]	C[c]	D[c]
Al	127 ± 20	132 ± 17		
Cr	15 ± 2	14 ± 2		
Cu	35 ± 4	35 ± 3	12 ± 2	12 ± 1
Pb	3.5 ± 2	6.5 ± 3	(18 ± 8)[e], (38 ± 3)[d]	45 ± 3
Zn	342 ± 40	28.2 ± 35	28 ± 5	25 ± 3

[a] μg per g of freeze-dried mussel residue or dried tissue. [b] Data are the means and standard deviations of 4–9 samples. [c] From peak height measurements. [d] From peak area measurements. [e] A, directly inserted solid samples, calibration by standard additions; B, C, dissolved samples, calibration by aqueous standards; and D, certified analysis.

combined with an undistorted signal would simplify the optimization procedures. These elements have short residence times, a considerable fraction of which is involved with the reduction and generation processes in the condensed residue, prior to vapor formation and convective elimination.

Calibration. Direct atomization of solids often leads to large background absorptions and considerable matrix effects, including signal distortion. The first requirement of the method is the development of a secure calibration.

The initial work on the mussel residue–naphthalene homogenates using the slow response system showed that Al, Cr, and Cu were volatilized rather slowly with insignificant background absorptions. In consequence, clear sharp elemental peaks were obtained. Calibration by spike addition of aqueous standards during the freeze-drying stage and the use of peak heights proved feasible. For Zn and Pb the accuracy of this technique was considerably less.

First, homogeneity tests were performed on selected aggregate samples. Twelve animals of the same species (*Lampsilis radiata*) from the same collection area were combined, freeze-dried, ground with naphthalene diluent, and the Cu content in a standard weight of pellet was determined by standard addition. Thirty individual measurements were made for each aggregate and 4 such samples from differing source areas were homogenized and tested. The relative Cu contents, as μg per g dry weight of freeze-dried mussel residue with the corresponding standard deviations ($\bar{x} \pm s$) were found to be, 21.5 ± 2, 22.0 ± 2, 37.0 ± 4, and 34.5 ± 4. The accuracy in obtaining a representative sample was then limited to 15–20% (RSD).

One aggregate was analyzed for five trace elements by two procedures, (a) aqueous standards were added during freeze-drying, (b) unspiked mussel residues were dissolved and 25 μl of the diluted solutions used against subsequent firings of aqueous standards. Table II shows the results, obtained from peak height measurements with the slow response system. Morgenthaler (*8*) recommends that standard additions be made between the charring and atomization stages as this is expected to remove interferences from the organic sample matrix. The homogeneity of the samples analyzed here was limited to ±15% (RSD) and little difference in the result was noted between test additions at this time and earlier.

The Cu, Al, and Cr results display satisfactory agreement between the methods. For these slowly volatilized elements, it appears that any standard addition is reduced to similar, if not identical, inorganic residues as those from the biological sample. The analyte atoms, whether from the standard or the sample, are finally atomized at coincident times and the absorption peak is a single-valued sum from both sources. This is not always the case as is seen later in the Pb results for the NBS standard. Nevertheless, for elements which are sublimated slowly for reasons of low volatility or refractory nature,

a method of standard additions, based on aqueous standards is sufficiently accurate to calibrate directly fired solids. The general lack of background and matrix effects with these analytes is also reassuring and a slow response system with a consequent loss of sensitivity is still usable with value.

The Zn and Pb results in Table II show a satisfactory precision in each case based on the known sample homogeneity, but there is a marked lack of agreement between the methods. The slow response system may disguise aberrations in the peak heights which are specific to the post-charring residues for such rapidly atomized elements. In addition, the Zn content of these animals is particularly high and that in itself may lead to inaccuracies, not only in the determination of [Zn] but also other volatiles. Inter-element interferences are suspect at these levels as has been found by Smeyers-Verbeke et al. (*20*).

The solution of the NBS Reference Material was used to investigate the problems with volatile analytes. Analyses were made for Zn, Pb, and for reference, Cu, in 25-μl aliquots using the fast response system. The results are also shown in Table II. As expected the Cu result against aqueous standards is in excelent agreement with the certified value. Zn calibrations with aqueous standards are probably acceptable for such low Zn bearing samples, but it is doubtful whether mussel residues can be calibrated in this way. Moreover, some difficulties with Zn were evident. The peak appearance time after the initiation of the atomization cycle was slightly different for the NBS sample compared to the aqueous standard. This suggests that the charring residues were not identical.

Even with undistorted signal recording, the Pb analyses of the orchard leaves against aqueous standards were quite disappointing and totally unsatisfactory. The post-charring residues from the sample and aqueous standard differed so greatly that the appearance times of the peaks differed by as much as 0.65 s, a distinctive feature which was quite reproducible. The apparent Pb concentration was found to be a function of the size of the sample dissolved. Peak area measurements showed an improved accuracy, but not enough to be useful. A closer match between the synthetic and sample matrices is obviously required for organics. Figure 7 illustrates the decay in the observed Pb content with sample weight, measured against aqueous standards. Points on the abscissa indicate the sample masses which were digested and diluted to 50 ml in each case. Calibrations of the orchard leaves solutions were also attempted by the method of standard additions with no greater success.

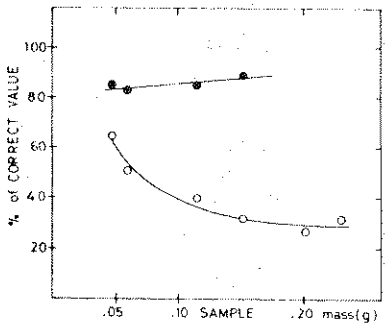

Figure 7. Pb results as a function of the increasing mass of NBS 1571 (orchard leaves) dissolved for analysis; (O) peak height; (●) peak area

This particular feature of two quite dissimilar reduction products for Pb (and possibly for other rapidly atomized elements) is currently under study.

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