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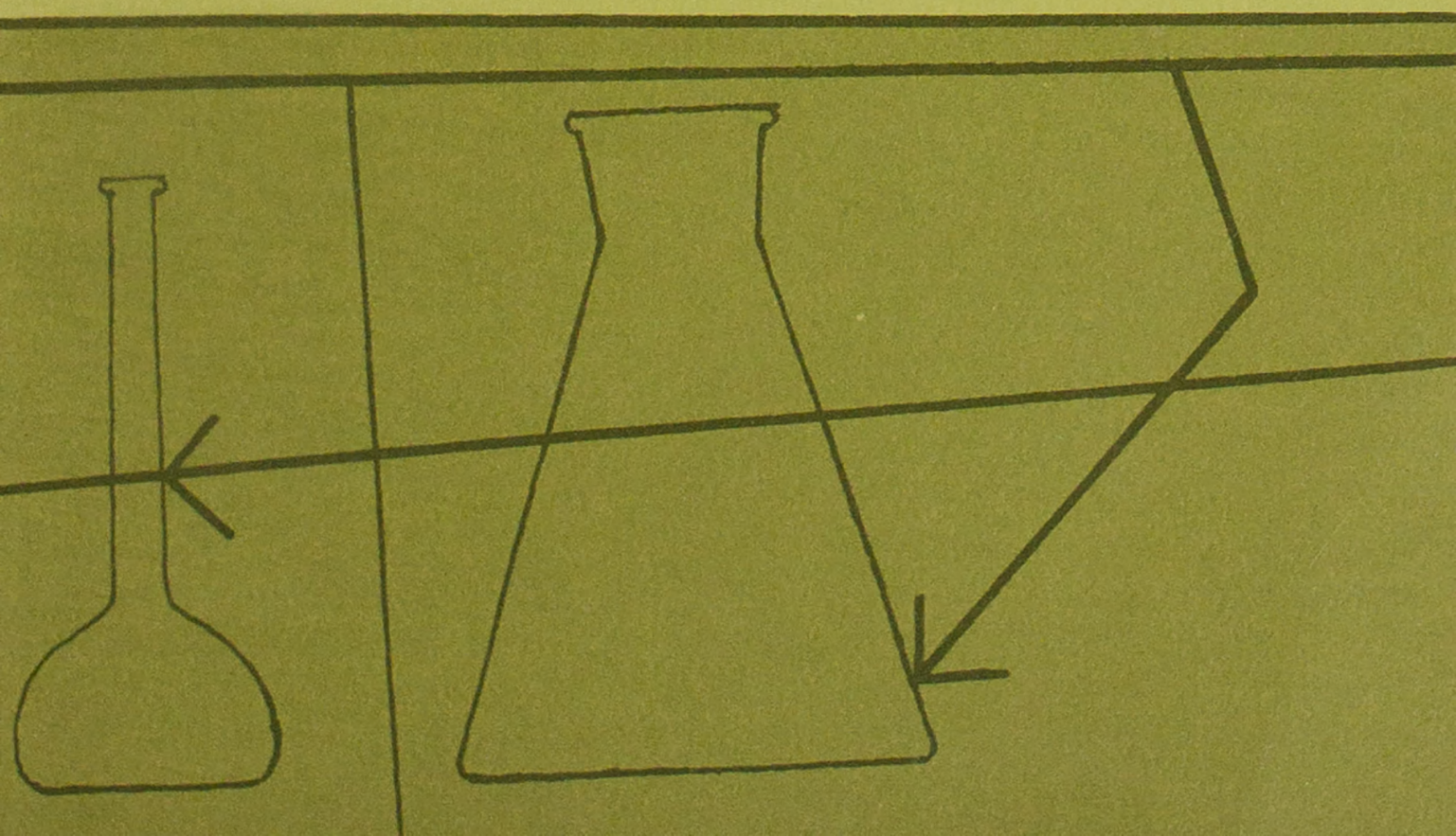

Rapid Wet Ash Digestion of Coniferous Foliage for Analysis of Potassium, Phosphorus, Calcium and Magnesium[1]

By Mark J. Behan[2] and Thomas Kinraide[3]

Abstract

The authors have developed a rapid, safe method of ashing coniferous foliage using nitric and perchloric acids. By this method foliage can be ashed in about 20 minutes. Compatibility of the digestion procedure with routine methods of analysis for potassium, phosphorus, calcium, and magnesium was examined and found to be satisfactory. Although the determination of calcium plus magnesium by EDTA titration was satisfactory, the determination of calcium alone by EDTA was not reliable.

Introduction

Foliar analysis is an important technique in the diagnosis of mineral deficiencies and in the study of mineral cycling in conifers (4). This analysis generally requires that the organic matter be destroyed, usually by ashing in a muffle furnace or by wet ash digestion using strong acids. Although perchloric acid is the most powerful of the acids ordinarily used in wet ash digestion, its use has not been common for three main reasons: 1) Under certain conditions the acid can create an explosion hazard; 2) most existing perchloric acid digestion methods are quite time-consuming, requiring both a separate predigestion with nitric acid

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and a boiling away of the perchloric acid not used in the digestion; and 3) special equipment and facilities may be required to dispose of the excess perchloric acid fumes when the digests are evaporated to near dryness (1,2). This bulletin describes a safe method of wet ash digestion by which conifer foliage can be ashed in about 20 minutes. The cost of the apparatus is relatively low, and subsequent analyses, even those which require a known hydrogen ion concentration, can easily be standardized.

Theory and Safety Precautions

Hot, concentrated perchloric acid is one of the most powerful oxidizing agents known, and if easily oxidizable organic matter (e.g., conifer foliage and filter paper) are mixed with it a violent, uncontrolled reaction is likely to result. However, dilute or cool perchloric acid acts like, and is as safe to handle and store as, any other common, concentrated laboratory acid. For example, if cellulose is placed in cold, concentrated perchloric acid, it merely dissolves. However, if cellulose is added to hot, concentrated perchloric acid a reaction of explosive intensity is likely to occur. All methods of wet ash digestion using perchloric acid require that the easily oxidizable organic components be eliminated before the sample is subjected to the full oxidation potential of hot, concentrated perchloric acid, and our technique does not vary from that important requirement. In most other methods the predigestion is generally accomplished by an initial digestion of the sample with nitric acid which is then evaporated. A mixture of nitric and perchloric acids is then added for the final digestion. Smith (3) could find no advantage or additional safety in this separation, and it is not a part of the new method.

With this procedure controlled oxidation of organic matter is achieved by placing the ground sample in a mixture of nitric and perchloric acids at room temperature. The digest is then placed on a preheated hot plate. As the mixture is heated to about 120°C the nitric acid acts as the principal oxidant, destroying the easily oxidizable material in a smooth and controlled manner with the concomitant evolution of copious amounts of reddish brown nitrogen oxide fumes. The nitric acid completes its reactivity as it is heated from 120°C to 130°C and distills from the reaction mixture. At 130°C the perchloric acid first exhibits low oxidation potentials, but continued heating concentrates the perchloric acid from less than 40% to 72.5% at the azeotropic boiling

point of 203°C. The oxidation potential of perchloric acid is a function of its concentration and temperature, and, because the boiling point is determined by concentration, a smooth, controlled increase in oxidation potential can be achieved as water is evaporated by continued heating. When the azeotrope is reached the full oxidizing power of perchloric acid is finally brought to bear on the last remnants of organic matter. This reaction has been thoroughly studied and documented by Smith (3) who experimented with a variety of organic materials. He determined the oxidation potential of perchloric acid at various strengths and temperatures and thoroughly discussed the theory and safety of the reaction.

The principal safety precaution required is to insure that hot, concentrated perchloric acid does not come into contact with organic matter that has not been subjected to prior oxidation by nitric acid. In our laboratory the nitric and perchloric acids are premixed and then dispensed with an automatic acid burette. Bottles of perchloric acid are never fitted with acid burettes, nor stored in the vicinity where samples are prepared. Further, if straight perchloric acid were to be combined with organic matter, the potentially hazardous error would become immediately apparent when no nitrogen oxide fumes evolved. Even at room temperature these fumes are generated on contact of the perchloric-nitric acid mixture with organic material. The digestion may be carried out in a fume hood, but no organic materials should be permitted in the hood during the procedure. Certain organic materials, such as alcohols, glycerols, ketones, and aldehydes, form particularly explosive mixtures with perchloric acid. Fume hoods made with glycerin-litharge joint cements must not be used since spilled perchloric acid may be soaked up by these cements forming a violent, shock sensitive, explosive, perchloric ester of glycerin. Several fume hood manufacturers have attached the statement "not for use with perchloric acid" to some of their hood models. These warnings should be heeded. Additional information concerning the use of hoods with perchloric acid may be obtained from hood manufacturers or from the G. Frederick Smith Co. (3).

Very little perchloric acid is actually released into the hood by this method. Titrations of the final digests indicate that over 90% of the perchloric acid added remains at the end of the digestion period. The hazard of perchloric acid fumes condensing on

the walls and ducts of the hood is thus much less in this process than in methods in which samples are evaporated to near dryness. Even so, we recommend that the hood and ductwork used in this process be washed down with water at least after each 1,000 digestions. This procedure reduces the buildup of potentially hazardous perchloric acid and its salts.

Inexpensive and quite satisfactory glass fume eradicators have also been described (1,3) for use in lieu of a hood. The use of protective aprons, eye glasses, and perhaps shielding should be routine in any laboratory.

Methods

A. Sample Collection and Preparation

Needles were collected in mid-August from a branch in the upper portion of the crown of a single tree of each of the following species: Engelmann spruce (*Picea engelmannii* Parry), western larch (*Larix occidentalis* Nutt.), lodgepole pine (*Pinus contorta* Dougl. var. *latifolia*) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco). The needles were dried in a forced-draft oven at 70°C for 48 hours, milled to pass a 40-mesh screen, and then stored in airtight bottles.

B. Determination of Time Required for Complete Digestion

A 1.000 g sample of the dried, milled tissue was placed in a 250 ml conical flask, and 20 ml of a 1:1 mixture of concentrated HNO_3 and concentrated (60%) $HClO_4$ were added. Trials with smaller volumes of the acid mixture caused the samples to char. The flask was placed in the fume hood directly on a hot plate preheated to 210°C. In order to determine the time required for complete digestion, 5 mg of potassium dichromate ($K_2Cr_2O_7$) were added to a series of samples of each species. The Cr^{6+} ion was reduced to the green Cr^{3+} ion by the organic matter still present at the conclusion of the nitric acid oxidation phase (cessation of nitrogen oxide emission), which was completed in about 3 minutes. Approximately 70% boiling $HClO_4$ oxidizes the green Cr^{3+} ion to the orange chromic oxide (CrO_3) instantly after complete removal of organic matter (3). Thus, the appearance of the orange CrO_3 was used as an indicator of completeness of oxidation, which generally occurred in from 9 to 13 minutes. The variation was associated with position on the hot plate.

The possible catalytic effect of $K_2Cr_2O_7$ was examined by adding 5, 10, 20, and 40 mg of $K_2Cr_2O_7$ to foliage digests of each species and timing both the period required for cessation of the nitrogen oxide fume emission and the appearance of the orange CrO_3. No catalytic effect could be detected. Vanadium has been found to be a catalyst in some perchloric acid oxidations (3), but additions of up to 20 mg of ammonium vanadate (NH_4VO_3) did not reduce the digestion period for larch.

C. Perchloric Acid Digestion Procedure

As a result of the above determinations, the following procedure was adopted: A 1.000 g sample of dried, milled foliage was placed in a 250 ml conical flask with 20 ml of the 1:1 nitric-perchloric acid mixture. The flask was then placed on a hot plate preheated to 210°C. The samples were removed from the hot plate after 20 minutes. This was nearly twice as long as the longest digestion interval observed in over 50 trials using the $K_2Cr_2O_7$ indicator. After the 20-minute digestion period the flask was removed from the hot plate and cooled under the hood, and then 25 ml of water were added. The silica was brought into suspension with a rubber policeman, and the solution was filtered through Whatman #40 filter paper into a 50 ml volumetric flask. The flask and filter paper were rinsed several times with hot 0.01 N HCl. The flask was cooled and filled to volume with distilled water. An alternate procedure would be to centrifuge the silica and remove the supernatant for analysis.

A precipitate of potassium perchlorate may form upon cooling of the undiluted digest. This may easily be removed by dilution and subsequent heating.

D. Determination and Recovery of Added Phosphorus, Potassium, Calcium, and Magnesium

In an effort to determine the compatibility of this method of digestion and routine methods of analysis, we analyzed samples for N, P, K, Ca, and Mg with and without added salts (see Table 1).

Six 1.000 g samples each of larch, pine, spruce, and Douglas-fir were placed in separate conical flasks. One milliliter of water was added to three samples of each species, and 1 ml of a composite solution containing 1.50 mg of P, 3.00 mg of K, 5.00 mg of Ca, and 1.00 mg of Mg was added to each of the remaining samples. All the samples were then digested as described above.

TABLE 1.—Determination of Phosphorus, Potassium, Calcium, and Magnesium in Perchloric Acid Digests of Conifer Foliage

(Average of three determinations)

Species	% P	% Added P Recovered	% K	% Added K Recovered	% Ca	% Added Ca Recovered	% Mg	% Added Mg Recovered
Composite Solution	0.147	98	0.303	101	0.500	100	0.100	100
Larch	0.127	101	0.648	98	0.202	100	0.092	96
Spruce	0.098	100	0.367	99	0.588	109	0.055	95
Pine	0.090	98	0.395	100	0.366	98	0.110	99
Douglas-fir	0.121	99	0.583	97	0.836	105	0.146	100
Mean*		99.1		98.0		102.9		97.5
Standard Error*		0.73		0.91		1.46		1.19

*Does not include Composite Solution.

The amount of P in the resulting digest was determined by the vanadomolybdophosphoric yellow color method (1). K was determined with the Beckman DU-2 flame spectrophotometer using a 1:50 or 1:100 dilution. Ca + Mg was determined by adding 1 ml of 10% hydroxylamine HCl, 2 ml of 50% triethanolamine, and 3 ml of buffer solution (67.5 g NH_4Cl, 507 ml concd NH_4OH, and 10 g KCN per liter) to a 5 or 10 ml aliquot of the digest. This mixture was titrated against 0.02 N EDTA using a 5 ml semi-micro burette and Calmagite indicator. Mg was determined by Unicam SP 90 Atomic Absorption using a 1:10 dilution.

The determination of Ca alone by the EDTA method proved unreliable in the case of both the tissue digests and the artificial solutions with P, Ca, and Mg compositions similar to those of the tissue digests. When the pH was raised to from 12 to 12.5, as is required for the determination of Ca in the presence of Mg, calcium phosphates were apparently formed which were only slowly, and perhaps never completely, dissolved by the EDTA. This caused continually fading endpoints and low titers. When an excess of EDTA was added before raising the pH and a back titration with standard Ca attempted, the results were invariably too high, indicating the formation of an Mg-EDTA complex which was not totally dissociated by the addition of standard Ca. No difficulty was encountered at the pH of 10 to 10.5 required for the determination of Ca + Mg by EDTA.

We tried to determine nitrogen content by the Kjeldahl distillation of the digests, but, unfortunately, our recovery of N was

quite low, usually about 30%. This was probably because some of the ammonium nitrogen in the samples was oxidized by chlorine liberated during the cooling of the digests and was lost as nitrogen gas. Nonetheless, many other minerals have been successfully recovered from a variety of organic materials subjected to perchloric acid digestion (3).

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