PLANT ANALYSIS REFERENCE PROCEDURES FOR THE SOUTHERN REGION OF THE UNITED STATES

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Editor

C.O. Plank

ABSTRACT

This bulletin is the instrument used by the Southern Extension Research Activity-Information Exchange Group-6 (SERA-IEG-6) to document in summary form procedures used by state university plant analysis programs. This document, records detailed analytical methodologies that are used by the various laboratories throughout the Southern Region. Other procedures are available for many of the analyses presented in this publication. For information related to similar procedures, each state university maintains laboratory manuals that may be of further assistance to the reader. The intent of this document is to provide a reference for current and most widely used plant analysis methods.

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Plant Analysis Reference Procedures for the Southern Region of the United States

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Preface

Plant analysis has evolved into one of the important tools in crop production. It is a process in which plant samples are collected from a plant at a specific time during the growing season and analyzed in a laboratory for various essential nutrients. Nutrients of primary interest are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), molybdenum (Mo), iron (Fe), boron (B), copper (Cu) and zinc (Zn). The plant analysis process also includes an evaluation of the analytical data to determine whether an element is low, sufficient, or high and finally the formulation of a recommendation.

Each step in the plant analysis process is equally important. In the laboratory phase many different procedures are involved which include decontamination, drying, grinding, weighing, ashing, and analysis for 11 to 12 essential nutrients. Consequently, performing a plant analysis involves the use of a variety of laboratory instruments.

Several laboratories in the Southern Region of the United States offer plant analysis services to researchers and growers. This bulletin contains reference procedures commonly used by laboratories in the region. Procedures were selected on the basis of their accuracy and precision as well as their popularity and acceptance by workers in the area of plant analysis. These procedures also provide a reference for laboratories to exchange samples to evaluate current plant analysis procedures or to implement new ones.

C. Owen Plank
Editor

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Sample Preparation

C. R. Campbell and C. O. Plank[*]

Sample preparation is critical in obtaining accurate analytical data and reliable interpretation of plant analysis results. Proven procedures must be followed during decontamination, drying, particle-size reduction, storage, and organic matter destruction. Each of these preparatory procedures provide opportunities to enhance the accuracy and reliability of the analytical results.

A. Decontamination

1. Principle

1.1 Plant materials must be clean and free of extraneous substances including soil and dust particles, and foliar spray residues that may influence analytical results. Generally, the elements most affected by soil and dust particles are Fe, Al, Si, and Mn. Foliar nutrient spray and fungicide residues can affect several elements and should be taken into account in the decontamination process and when evaluating the analytical results. The decontamination process must be thorough while still preserving sample integrity. ***Therefore, decontamination procedures involving washing and rinsing should only be used for fresh, fully-turgid plant samples.***

2. Reagents and Apparatus

2.1 Deionized water.

2.2 0.1 to 0.3% detergent solution (non-phosphate).

2.3 Medium-stiff nylon bristle brush.

2.4 Plastic containers suitable for washing and rinsing tissue samples.

3. Procedure

3.1 Examine fresh plant tissue samples to determine physical condition and extent of contamination. Unless leaf tissue is visibly coated with foreign substances, decontamination is usually not required except when Fe (Wallace et al., 1982) Al, Si, or Mn are to be determined (Jones and Case, 1990).

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3.2 When Al, Si, Mn, and Fe are not of primary interest, plant leaves should be brushed briskly to remove visible soil and dust particles.

3.3 When plant samples show visible residues from spray applications and when Al, Si, Fe (Wallace et al., 1982), and Mn are elements of interest, leaves should be washed in a 0.1 to 0.3% detergent solution (Ashby, 1969 and Wallace et al., 1980) followed by rinsing in deionized water. The wash and rinse periods should be as short as possible (Sonneveld and Van Dijk, 1982) to avoid danger of N, B, K, and Cl leaching from the tissue (Bhan et al., 1959).

3.4 After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

4. Remarks

4.1 When proper sampling techniques have been utilized, decontamination should be minimized.

4.2 Decontamination is generally not necessary where tissue has been exposed to frequent rainfall and/or not exposed to nutrient or fungicidal sprays (Jones et al., 1991). Small plants that have been splattered with soil are the exception to this rule.

4.3 Excessive washing is likely worse than no decontamination since soluble elements, including B, K, and N, are likely to leach from the tissue.

4.4 Samples should be dipped quickly in the wash and rinse solutions. Sonneveld and van Dijk (1982) recommended a time of 15 seconds.

4.5 Relatively high concentrations of Al (>100 mg kg^{-1}), Fe (>100 mg kg^{-1}), and Si (>1.0%) are strong indicators of contamination (Jones et al., 1991). Titanium (Ti) has also been suggested as an indicator of soil or dust contamination (Cherney and Robinson, 1982).

B. Drying

1. Principle

1.1 Water is removed from plant tisssue to stop enzymatic reactions and to stabilize the sample. Removal of combined water also facilitates particle size reduction, homogenization, and weighing.

2. Apparatus

2.1 Forced-air oven equivalent to Blue M Model POM-166E.

3. Procedure

3.1 Separate or loosen tissue samples and place in paper containers.

3.2 Place containers in forced-air oven and dry at 80°C for 12 to 24 hours. NOTE: The original condition and sample size will affect drying time.

4. Remarks

4.1 Drying times longer than 24 hours may be required depending on the type and number of plant samples in the dryer.

4.2 Drying at temperatures under 80°C may not remove all combined water (Jones et al., 1991) and may result in poor homogenization and incorrect analytical results.

4.3 Drying temperatures above 80°C may result in thermal decomposition and reduction in dry weight (Jones et al., 1991).

4.4 Enzymes present in plant tissue are rendered inactive at temperatures above 60°C (Tauber, 1949). As a result, air drying may not stabilize samples and prevent enzymatic decomposition. Samples should, therefore, be properly dried as soon after taking the sample as possible.

4.5 Quick drying of a limited number of samples can be accomplished using a microwave oven provided the samples are turned often and the drying process is closely monitored (Carlier and van Hee, 1971; Shuman and Rauzi, 1981; and Jones et al., 1991).

4.6 If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis. Drying time required will vary. Dry to constant weight by making periodic weighings.

C. Particle-Size Reduction

1. Principle

1.1 Plant tissue samples are reduced to 0.5- to 1.0-mm particle size to ensure homogeneity and to facilitate organic matter destruction.

2. Apparatus

2.1 Standard and intermediate Wiley-type mills equipped with 20-, 40-, and 60-mesh screens and stainless steel contact points.

2.2 Cyclotec or equivalent high-speed grinder.

2.3 Medium bristle brush.

2.4 Vacuum system.

3. Procedure

3.1 After drying, samples should be ground to pass a 1.0-mm screen (20 mesh) using the appropriate Wiley Mill. A 20-mesh sieve is adequate if the sample aliquot to be assayed is >0.5 g. However, if the sample aliquot to be assayed is <0.5 g, a 40-mesh screen should be utilized (Jones and Case, 1990).

3.2 After grinding, the sample should be thoroughly mixed and a 5- to 8-g aliquot withdrawn for analyses and storage.

3.3 Using a brush or vacuum system, clean the grinding apparatus after grinding each sample.

4. Remarks

4.1 Uniform grinding and mixing are critical in obtaining accurate analytical results.

4.2 Exercise care when grinding very small samples or plant material that is pubescent, deliquescent, or that has a fibrous texture. These samples are difficult to grind in Wiley mills and the operator should allow sufficient time for the sample to pass through the screen to ensure homogeneity. In these instances, experience has shown that Cyclotec or equivalent high-speed grinders are preferable.

4.3 Most mechanical mills contribute some contamination of the sample with one or more elements (Hood et al., 1944). The extent of contamination depends on condition of the mill and exposure time (Jones and Case, 1990). Grier (1966) recommended use of stainless steel for cutting and sieving surfaces to minimize contamination.

4.4 Routine maintenance should be performed on mills to ensure optimum operating conditions. Cutting knives or blades should be maintained in sharp condition and in adjustment.

D. Storage

1. Principle

1.1 After particle size reduction and homogenization, samples should be stored in conditions that will minimize deterioration and maintain sample integrity for weighing and follow-up analytical work.

2. Apparatus

2.1 Airtight plastic storage containers.

2.2 Storage cabinet located in cool, dark, moisture-free environment.

2.3 Refrigerator.

3. Procedure

3.1 After grinding and homogenization, a representative subsample is taken from the ground plant material for analyses and storage. The sample should be placed in a container that can be securely sealed.

3.2 Containers should then be placed in a cool, dry place for storage.

3.3 For long term storage, ground samples should be thoroughly dried, sealed, and placed under refrigerated conditions (4^{o}C) until the required analysis can be completed.

4. Remarks

4.1 If samples are placed in a cool (4^{o}C), dark, dry environment, storage life is indefinite (Jones et al., 1991).

4.2 Coin envelopes can also be used for sample storage, however, somewhat greater care must be exercised in handling to prevent absorption of moisture. Collecting the ground sample in the envelope and immediately placing into a desiccator cabinet or desiccator will minimize moisture absorption.

E. Organic Matter Destruction - Dry Ashing

Plant tissue samples previously dried, ground, and weighed are prepared for elemental analysis through decomposition or destruction of organic matter. Extensive work has been done to evaluate published methods and to develop new and improved procedures. The best overviews on organic matter destruction are found in books by Gorsuch (1970) and Bock (1978) and in the review articles by Tolg (1974) and Gorsuch (1976). Two commonly used methods of organic matter destruction include dry ashing (high temperature combustion) and wet ashing (acid digestion) (Jones et al., 1991). Both methods are based on the oxidation of organic matter through the use of heat and/or acids.

1. Principle

1.1 Dry ashing is conducted in a muffle furnace at temperatures of 500 to 550^{o}C for 4 to 8 hours. For tissue high in carbohydrates and oils, ashing aids (Horwitz, 1980) may be required to achieve complete decomposition of organic matter. After ashing, the vessel is removed, cooled, and the ash is dissolved in nitric (HNO_3) and/or hydrochloric (HCl) acid. The vessel is filled to volume and diluted as needed to meet range requirements of the analytical instrument.

2. Reagents and Apparatus

2.1 Muffle furnace with dual time and temperature control.

2.2 Fume hood.

2.3 Hot plate.

2.4 Porcelain or quartz crucibles, 30 mL.

2.5 Pyrex beakers, 50 mL.

2.6 Deionized water.

2.7 Hydrochloric acid (HCl), concentrated.

2.8 Nitric acid (HNO_3), concentrated.

2.9 Sulfuric acid (H_2SO_4), 10%.

2.10 Magnesium nitrate [$Mg(NO_3)_2 \cdot 6H_2O$], 7%.

2.11 Dilute aqua regia (300 mL HCl and 100 mL HNO_3 in 1 L deionized H_2O).

3. Procedure

3.1 Weigh 0.5 to 1.0 g of dried (80°C) plant material which has been ground (0.5 to 1.0 mm) and thoroughly homogenized, into a high-form 30-mL porcelain or quartz crucible or 100-mL pyrex beaker.

3.2 Place samples into a cool muffle furnace.

3.3 Set temperature control of the furnace to allow a gradual increase (2 hours) to the ashing temperature (500 to 550°C) and maintain for 4 to 8 hours.

3.4 Turn furnace off, open door, and allow samples to cool.

3.5 Check the ash to determine extent of destruction. If a clean white ash is obtained, proceed with step 3.9. If a clean white ash is not obtained, repeat step 3.1.

3.6 Moisten the tissue with concentrated HNO_3.

3.7 Place the container on a hot plate and evaporate the HNO_3 from the sample. Make sure the residue is completely free of moisture before placing it into the muffle furnace.

3.8 Remove the container from the hot plate and repeat steps 3.2, 3.3, and 3.4 above.

3.9 Depending upon subsequent analytical procedures, the ash can be solubilized using the appropriate acid and/or mixture of acids.

4. Remarks

4.1 Critical factors in dry ashing procedures include selection of ashing vessel, sample number, placement in furnace, ashing temperature, time, selection of acid to solubilize the ash, and final volume (Jones and Case, 1990). The analyst has less latitude in choosing an ashing temperature (Baker et al., 1964; Gorsuch, 1959, 1970, 1976; Isaac and Jones, 1972) than in selecting the other parameters of the procedure. Placement of vessels and ashing time are dependent on the type and number of samples. Selection of an ashing vessel, the solubilizing acid, and final volume are dependent on the elements of interest and subsequent analytical procedures. A number of combinations of these factors have been used successfully.

4.2 If a clean white ash remains after muffling, oxidation is complete and ashing aids are not required.

4.3 Plant materials with high sugar or oil content (highly carbonaceous) may require an ashing aid. Aids commonly used are 10% H_2SO_4, concentrated HNO_3, and 7% $Mg(NO_3)_2 \cdot 6H_2O$ solutions. The latter is recommended when the tissue is to be assayed for S as SO_4^{2-}. Gorsuch (1970), Horwitz (1980), and Jones et al. (1991) provide details on the use of these oxidizing aids.

4.4 Dry ashing is not recommended for plant materials that are high in Si. Dry ashing these materials results in low micronutrient values, especially Zn.

4.5 Dry ashing results in lower Fe and Al values than wet ashing (Jones and Case, 1990).

F. Organic Matter Destruction-Wet Ashing

1. Principle

1.1 Wet digestion involves the destruction of organic matter through the use of both heat and acids. Acids that have been used in these procedures include H_2SO_4, HNO_3, and $HClO_4$, either alone or in combination. Hydrogen peroxide (H_2O_2) is also used to enhance reaction speed and complete digestion. Most laboratories have eliminated the use of $HClO_4$ due to risk of explosion. Safety regulations require specially designed hoods where $HClO_4$ is utilized. Hot plates or digestion blocks are utilized to maintain temperatures of 80 to 125°C. After digestion is complete and the sample is cooled, the vessel is filled to volume and dilutions are made to meet analytical requirements.

2. Reagents and Apparatus

2.1 Hot plate.

2.2 Block digester.

2.3 Fume hood.

2.4 Nitric acid (HNO_3), concentrated.

2.5 Sulfuric acid (H_2SO_4), concentrated.

2.6 Hydrogen peroxide (H_2O_2), 30%.

2.7 200-mL tall-form beakers or digestion tubes.

2.8 Deionized water.

3. Procedure

3.1 Weigh 0.5 to 1.0 g of dried (80°C) plant material that has been ground (0.5 to 1.0 mm) and thoroughly homogenized and place in a tall-form beaker or digestion tube.

3.2 Add 5.0 mL concentrated HNO_3 and cover beaker with watch glass or place a funnel in the mouth of digestion tube and allow to stand overnight or until frothing subsides.

3.3 Place covered beaker on hot plate or digestion tube into block digester and heat at 125°C for 1 hour. (Where elemental analysis is by ICP, the digestion time can be extended to 4 hours and steps 3.5 and 3.6 omitted).

3.4 Remove beaker or digestion tube and allow to cool.

3.5 Add 1 to 2 mL 30% H_2O_2 and digest at the same temperature. Repeat heating and 30% H_2O_2 additions until digest is clear. Add additional HNO_3 as needed to maintain a wet digest.

3.6 After sample digest is clear, remove watch glass or funnel and lower temperature to 80°C. Continue heating until near dryness. The residue should be clear or white if digestion is complete.

3.7 Add dilute HNO_3, HCl, or a combination of the two acids and deionized water to dissolve digest residue and bring sample to final volume depending upon requirements of subsequent analytical procedures.

4. Remarks

4.1 Critical factors in wet digestion procedures include selection of the digestion vessel, temperature and its control, time, the digestion mixture, and final volume. Selection of a digestion vessel is dependent on the elements of interest and the heat source. Digestion blocks have been developed (Tucker, 1974; Gallaher et al., 1975) and used successfully. They shorten digestion time and allow very uniform temperature control. Time and temperature are interrelated and are dependent on the digestion mixture. A number of ashing mixtures have been utilized and include those reported by: Jones et al. (1991), Jones and Case (1990), Wolf (1982), Parkenson and Allen (1975), Cresser and Parsons (1979), Zasoski and Buran (1977), Adler and Wilcox (1985), Halvin and Soltanpour (1980), Zarcinas et al. (1987), and Huang and Schulte (1985). Wet digestion procedures generally require greater analyst supervision and intervention than dry procedures.

4.2 Nitric acid (HNO_3) is used in most wet oxidation procedures. The addition of H_2SO_4 is used to raise digestion temperature while $HClO_4$ or 30% H_2O_2 are used to increase speed of

reaction and ensure complete digestion (Jones and Case, 1990).

4.3 Most wet digestion procedures can be completed using covered beakers on hot plates but digestion blocks are preferred due to enhanced temperature control.

4.4 Wet ashing is recommended for plant materials that are high in Si.

4.5 Wet ashing results in higher Fe and Al values than dry ashing (Jones and Case, 1990).

G. Organic Matter Destruction-Accelerated Wet Digestion

1. Principle

1.1 Relatively new alternatives for organic matter destruction include wet oxidation procedures which utilize pressure and/or high temperature to shorten digestion time. Closed or open vessels are used either with conventional hot plates or in microwave ovens (White and Douthit, 1985).

A limited number of procedures have been developed which utilize microwave as a source of heat. These procedures are generally classified as closed or open vessel. Closed vessel procedures utilize heat and pressure to increase reaction rate and to decrease digestion time. Element loss is controlled with a reflux valve. Open vessel procedures do not utilize pressure containers and must be monitored closely to avoid excess frothing and sample loss. The following procedure was developed by Campbell and Whitfield (1991) and should be considered as a "tentative procedure" for the Southern Region.

2. Reagents And Apparatus

2.1 CEM Microwave Digestion System. (CEM Corporation, P. O. Box 9, Indian Trail, NC 28079).

2.2 Fume hood and scrubber.

2.3 Nitric acid (HNO_3), concentrated.

2.4 Hydrogen peroxide (H_2O_2), 30%.

2.5 50-mL Erlenmeyer flask. Flasks must be heat-acid washed and relatively free from scratches to avoid boron contamination.

2.6 Deionized water.

3. Procedure

3.1 Weigh 0.5 to 1.0 g of dried (80^oC) plant material that has been ground to <1.0 mm and thoroughly homogenized. Place this material in a 50-mL Erlenmeyer flask.

3.2 Add 10 to 15 mL (10 mL for 0.5-g sample) concentrated HNO_3 to each sample.

3.3 Place in specially designed microwave oven (see 2.1) and digest for 30 minutes at 30% power (210 watts).

3.4 Flush sides of Erlenmeyer flasks with 30% H_2O_2.

3.5 Digest for 5 minutes at 60% power (390 watts).

3.6 While sample is still warm, fill to 50-mL volume with deionized water and shake well.

3.7 Filter digest using #1 Whatman paper and transfer a 10-mL aliquot to a centrifuge tube or other suitable container.

3.8 Digest is ready for analysis with or without further dilution. Procedure is designed for analysis of digest on ICP emission spectrometer.

4. Remarks

4.1 Microwave digestion procedures require the use of specially designed ovens to handle acid fumes. Ideally, the microwave exhaust should be removed to a scrubber before being released to a fume exhaust system. Special safety precautions are required for microwave digestion. See manufacturer's specifications for details.

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Determination of Total Nitrogen In Plant Samples by Kjeldahl

W. H. Baker and T. L. Thompson*

1. Principle of the Method

1.1 The determination of total nitrogen (N) by Kjeldahl involves the transformation of organic N to ammonium (NH_4^+) by digesting the sample with concentrated sulfuric acid (H_2SO_4) and then measuring the amount of NH_4^+ produced. Potassium sulfate (K_2SO_4) or sodium sulfate (Na_2SO_4) salts are commonly used to increase the boiling point, and thus speed up the conversion reaction. The rate of organic matter oxidation is also increased under most conditions by the addition of copper (Cu), selenium (Se), or mercury (Hg) which serve as catalysts. If Hg is the catalyst selected, then sodium sulfide (Na_2S), sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$), or zinc (Zn) dust must be added to the digest to decompose the $Hg\text{-}NH_4^+$ complex.

1.2 The NH_4^+-N created during the digestion procedure is determined by making the digest strongly alkaline, commonly with sodium hydroxide (NaOH), and collecting the volatilized ammonia (NH_3) into a boric acid indicator solution by steam distillation. This reaction is illustrated in equation 1. Standard acid is then used to titrimetrically determine the NH_4^+ trapped as ammonium borate. This reaction is illustrated in equation 2.

$$NH_3 + H_3BO_3 = NH_4^+ + H_2BO_3^- \quad \text{[eq. 1]}$$

$$H^+ + H_2BO_3^- = H_3BO_3 \quad \text{[eq. 2]}$$

1.3 The determination of Kjeldahl N in plant tissue may not be the total N content unless a method for recovery of NO_3-N is also included in the analysis.

2. Apparatus

2.1 Micro-Kjeldahl digestion tubes.

2.2 Digestion block and rack.

2.3 Stand for cooling the digestion tubes.

2.4 Steam distillation apparatus.

3. Reagents and Solutions

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3.1 Concentrated sulfuric acid, 18 *M*.

3.2 Standardized hydrochloric acid, (0.01 *M*) or sulfuric acid, 0.005 *M*.

3.3 Salt/Catalyst mixture - Mix 200 g of K_2SO_4, 20 g of cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) and 2 g of Se. The K_2SO_4 and/or $CuSO_4 \cdot 5H_2O$ may need to be ground using a mortar and pestle if the crystals are too large. It is important that the salt and catalyst be well mixed.

3.4 Sodium hydroxide solution, 10 *M* - Add 1600 g of NaOH to 2 L of carbon dioxide (CO_2)-free deionized water in a 4-L narrow neck polypropylene container. Stopper and cool the solution before bringing it to a volume of 4 L. Protect the solution from contamination by atmospheric CO_2 with an ascarite guard tube at the air inlet.

3.5 Mixed indicator - Dissolve 0.01 g of bromocresol green and 0.07 g of methyl red in 100 mL of ethyl alcohol (90%).

3.6 Boric acid indicator solution, 4% - Dissolve 40 g of H_3BO_3 in 800 mL of boiling deionized water in a 2-L volumetric flask. Cool the solution and dilute to 1900 mL. Add 40 mL of the mixed indicator solution. Carefully adjust the pH of this mixture with 0.1 *M* NaOH and 0.05 *M* H_2SO_4 until the solution turns a reddish purple color (pH 5.0) and then bring the solution to 2 L volume.

4. Procedure

4.1 The Kjeldahl method for determination of total N has been the subject of many modifications according to the type of material to be analyzed. For instance, some plant materials do not require a digestion temperature as high as that recommended in paragraph 4.4 for complete digestion. The user may desire to alter digestion times, temperatures, or reagent proportions for a specific application. Useful references for this purpose are Nelson and Sommers (1973, 1980) and Schuman et al. (1973).

4.2 Place sample (weight depends on N content) or standard in Kjeldahl digestion tube and add 1.1 g of salt/catalyst mixture.

4.3 Digest blanks containing only reagents with each set of samples.

4.4 Add 3 mL of concentrated H_2SO_4. Slowly heat to 200°C. Once the frothing has subsided, bring the temperature up to 350 to 375°C and heat until the digest clears. Digest at 350 to 375°C for an additional 35 minutes to 1 hour past clearing.

4.5 Cool the digest and add 20 mL of deionized water. If solidification has occurred within the digest, it is important to mix the tube contents using a vortex mixer to dissolve the solid.

4.6 Add 5 mL of H_3BO_3 indicator solution to a 50-mL flask and place the flask under the condenser with the condenser tube below the surface of the indicator solution.

4.7 Add 20 mL of 10 *M* NaOH to the digested sample. Immediately transfer the tube to the Kjeldahl distillation apparatus and begin distillation. Collect distillate until the level in the H_3BO_3 flask has reached approximately 35 mL (usually 12 minutes).

4.8 Titrate the NH_3 distilled into the H_3BO_3 solution using standard 0.01 *M* HCl or 0.005 *M* H_2SO_4. The end point is reached when the solution goes to a pink color.

5. Calibration and Standards

5.1 To verify that the digestion procedure is sufficient to digest organic N in the plant sample, primary N standards should be digested with each set of samples. Primary standards should be of a known N content, high purity, and non-hygroscopic for ease of storage. An effective series of standard reference materials (available from Hach Co., Loveland, CO) are some derivatives of para-toluene sulfonate (PTSA); NH_4-PTSA (requires no digestion), glycine-PTSA (relatively easy to digest), and nicotinic acid-PTSA (very difficult to digest). Reference standards are also provided by the National Institute of Standards and Technology, Office of Standard Reference Materials, Gaithersburg, MD.

6. Calculations

6.1 $$\%N = \frac{(T - B) \times N \times 1.401}{\text{g sample}}$$

where: T = mL of sample titrated
B = mL of blank titrated
N = acid normality

7. Remarks

7.1 Samples should contain about 1 mg of N (no more than 5 mg). The sensitivity of the procedure depends upon a number of factors, including weight of the sample, strength of the acid, and accuracy of titration.

7.2 If the ratio of acid to salt is low at the end of the digestion step, a significant amount of NH_3 can be volatilized during the digestion process (Bremner and Mulvaney, 1982). Other situations which may cause N loss during digestion and should be avoided are: localized heating in the digestion flask (temperatures above 410^{o}C), and the use of 30% hydrogen peroxide (H_2O_2) as an oxidant.

7.3 The time required for digestion will be affected by the catalyst, temperature, and type of plant tissue. It is important to allow an equal length of additional time after the sample clears. Jones et. al (1991) reported that as much as 10% of the organic N may not yet be converted to NH_4^+ at clearing.

7.4 Homogeneity of the sample is very important for greatest precision. For best results, dried tissue should pass a 40-mesh sieve. In cases where sample size is less than 0.25 g, special care should be taken to insure sample homogeneity (Jones et al., 1991).

7.5 Digested samples may be stored for several days provided samples are covered and placed in a cool area.

7.6 Samples distilled into the H_3BO_3 solution should be titrated within a short time to avoid absorption of atmospheric CO_2.

8. References

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Determination of Nitrogen in Plant Tissue Using Continuous Flow, Segmented Stream Autoanalyzer

R. A. Isaac and W. C. Johnson, Jr.[*]

1. Principle of the Method

1.1 This is an instrumental method which involves delivering a liquid digest to an analytical cartridge by means of a peristaltic pump. The sample is then combined with reagents and air bubbles in a continuous moving stream, ultimately producing a color which is specific for the analyte in the sample. The stream then flows to a photometer where the color intensity is converted to an electronic signal and displayed on a chart recorder.

1.2 Plant tissue samples may be digested by either AOAC method 976.06G (Helrich, 1990) or the method reported by Isaac and Johnson (1976) for the conversion of nitrogen compounds to ammonia.

1.3 Sample digests are analyzed for ammonia on a continuous flow, segmented stream, autoanalyzer, utilizing the Berthelot reaction.

1.4 Ammonia reacts with sodium phenoxide in the presence of sodium hypochlorite to form a green colored complex.

1.5 Potassium sodium tartrate is added to the sample stream in order to prevent the precipitation of heavy metal hydroxides.

2. Apparatus

2.1 Alpkem RFA300 system equipped with an ammonia cartridge, or a Technicon AutoAnalyzer II system equipped with an ammonia cartridge, or any other equivalent continuous flow, segmented stream autoanalyzer system.

3. Reagents

3.1 Potassium sodium tartrate.

3.2 Sodium hydroxide.

3.3 Disodium ethylenediamine tetraacetate.

3.4 Phenol.

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3.5 Sodium hypochlorite.

3.6 Ammonium sulfate.

4. Autoanalyzer Solutions

4.1 Potassium sodium tartrate solution - Dissolve 50 g potassium sodium tartrate, 28 g sodium hydroxide, and 5 g disodium ethylenediamine tetraacetate in 1 L deionized water.

4.2 Sodium phenoxide solution - Dissolve 200 g sodium hydroxide in 500 mL deionized water, let cool and slowly add 276 g phenol. Dilute to 1 L with deionized water and store in a dark polyethylene bottle.

4.3 Sodium hypochlorite solution - Use a commercial grade bleach such as Chlorox (5.25% NaOCl) without dilution.

5. Standards

5.1 2500 mg N L^{-1} - Weigh 11.79 g $(NH_4)_2SO_4$ into a 1-L volumetric flask, dissolve and dilute to volume with deionized water.

5.2 Working standards - To 50-mL volumetric flasks containing 20 mL deionized water and an equivalent volume of digestion reagent, pipet 1, 2, 3, 4, 5, and 6 mL of 2500 mg L^{-1} N standard. Allow to cool and dilute to volume with deionized water. When using a 250 mg sample and diluted to 50 mL volume, these standards are equivalent to 1, 2, 3, 4, 5, and 6% N, respectively.

6. Procedure

6.1 Turn on all system modules and engage the peristaltic pump.

6.2 Pump reagents through the system for 10 minutes before beginning analysis of samples and standards.

6.3 Before analysis of samples and standards, inspect the system and verify consistent air bubble patterns throughout the system and also verify that the stream is flowing smoothly.

6.4 Begin analysis of samples and standards.

6.5 When analysis is complete, place reagent lines in water and allow the system to run for 10 minutes.

7. Remarks

7.1 Nitrogen concentrations in plant tissue samples may be determined in a range of 0.10 to 6.00 %.

7.2 Isaac and Johnson (1976) reported an average difference of 0.02% N between an AutoAnalyzer nitrogen method and AOAC method 2.049 (Horwitz, 1975) on eight plant tissue samples.

7.3 Analysis time per sample is 60 seconds.

8. References

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Determination of Total Nitrogen in Plant Tissue by Combustion

C. R. Campbell[*]

1. Principle of the Method

1.1 Modern elemental organic analysis (EOA) has evolved from the work of a number of scientists and is based on measurements of one or more physical properties of oxidation gases including pressure, light adsorption, or thermal and electrical conductivity (Pella, 1990). Major obstacles of incompatibility between the combustion process and gas chromatography separation of combustion products were overcome with the dynamic flash combustion principle first introduced in 1973 (Pella and Colombo, 1973).

1.2 Flash combustion is achieved by dropping an organic sample into a quartz reactor (1030^{o}C) in which the helium carrier gas is enriched with oxygen. The quartz reactor consists of a layer of chromic oxide (Cr_2O_3) and cobaltous-cobaltic oxide (Co_3O_4) coated with silver to compliment oxidation and adsorb halogens and sulfur oxides, respectively.

1.3 The combustion gases are reduced in a copper column (650^{o}C). Water is then removed using a trap containing magnesium perchlorate [$Mg(ClO_4)_2$] or molecular sieve (3Å). Carbon dioxide is removed in a second trap containing ascarite, Na_2O, or LiOH.

1.4 For quantification, the nitrogen gas is carried to a chromatographic column (Porapak QS 2/m) and then to a thermal conductivity detector. The resulting signal is passed to an electronic recorder and integrator.

2. Apparatus

2.1 Sartorius 4504MP8 microbalance or equivalent.

2.2 Carlo Erba NA 1500 automatic N analyzer or equivalent.

2.3 Unisys PW 500 or equivalent IBM compatible computer.

2.4 Eager 100 Software for weight collection, instrument control, and integration.

2.5 Sperry 115 printer or equivalent.

2.6 Desiccator.

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3. Reagents

3.1 Chromic oxide (Cr_2O_3).

3.2 Silvered cobaltous-cobaltic oxide (Co_3O_4/Ag layer).

3.3 Quartz wool.

3.4 Quartz turnings (SiO_2).

3.5 Reduction copper (Cu).

3.6 Magnesium perchlorate [$Mg(ClO_4)_2$] or molecular sieve 3Å.

3.7 Tin capsules (Conroy 4001 or equivalent).

3.8 Na_2O, LiOH, or ascarite.

3.9 Helium (99.996% purity).

3.10 Air (breathing quality).

3.11 Oxygen (99.996% purity).

3.12 NBS standard plant material with certified N value.

3.13 Acidanilide.

4. Procedure

4.1 Prepare and install columns for combustion and reduction chambers.

4.2 Prepare and install traps for H_2O and CO_2.

4.3 Check gas flow rates.

4.4 Preheat combustion and reduction chambers and turn on filament heat.

4.5 Check instrument settings for carrier gas flow and oxygen injection.

4.6 Run 3 to 4 conditioning samples.

4.7 Standardize for linear regression model using 4 to 5 acidanilide samples of varying weights to cover the working range of nitrogen concentration in unknowns.

4.8 Weigh internal checks and unknowns.

4.9 Place internal checks and unknowns in autosampler and activate analytical procedure.

4.10 Capture data electronically or record results manually.

5 Remarks

5.1 Total nitrogen concentrations in organic materials can be determined over a range of 0.01 to 100.00%.

5.2 Results are equal or superior to those of traditional Kjeldahl procedures (Sweeney, 1989; Bellomonte et al., 1987; Colombo and Giazzi, 1982). Accuracy varies with N concentration and ranges from "10% deviation at 0.01% to "0.4% deviation at 50.0%. Reproducibility is better than "0.1% of absolute value.

5.3 For best results, samples must be ground to pass at least a 1-mm screen size and thoroughly mixed. Finer grinds improve precision and accuracy. A suitable grind is one which results in a relative standard deviation (RSD) # 2.0% for ten successive determinations (Sweeney, 1989).

5.4 Sample size can range from 3 to 30 mg. Research does not indicate that sample size limits accuracy and precision (Colombo and Giazzi, 1982) as long as sample preparation is sufficient.

5.5 Samples must be weighed to 0.001 g to ensure accuracy and precision. Large sample quantities are not necessary provided the sample has been properly homogenized and blended. (Colombo and Giazzi, 1982).

5.6 Analysis time is one sample per 160 seconds.

5.7 Routine maintenance is approximately one half hour per day. One operator can run 100 to 150 samples per average work day.

6. References

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Determination of Nitrate Nitrogen in Plant Samples by Selective Ion Electrode

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1. Principle of the Method

1.1 The nitrate (NO_3) electrode contains an internal reference solution in contact with a porous plastic organophilic membrane. This membrane acts as a selective nitrate ion (NO_3^-) exchanger. When the membrane is exposed to NO_3^-, a potential develops across the membrane. This potential, E, is measured against a constant reference electrode potential, E^o.

1.2 The magnitude of E is dependent on the NO_3 activity (A_{NO3}) and can be described as the sum of several individual potentials by the empirical equation:

$$E = E^o + [\ S \text{ x } \log (A_{NO3} + \Sigma K a_i)\ ] + E_J \qquad \text{[eq. 1]}$$

where the potential across the liquid junction, E_J, represents the mobility of the cation and anion between the outer sample solution and the reference electrode filling solution, the slope, *S (59.16 mV at 25°C)*, embodies the Faraday and gas constants, the valence of the ion, and the solution temperature, and because the NO_3 electrode is not completely specific, the selectivity ratio, $\Sigma K a_i$, in principle, encompasses the error resulting from ions that are sensed by the electrode falsely as NO_3^-. The components between brackets in equation 1 form the basis of the Nernst equation which describes the potential across the nitrate electrode membrane. Assuming that E_J and temperature are constant between samples and there are no interfering ions, the change in potential will be a direct linear function of A_{NO3}:

$$E = E^o + [\ S \text{ x } \log(A_{NO3})\ ] \qquad \text{[eq. 2]}$$

1.3 The relationship between A_{NO3} and concentration of NO_3 (C_{NO3}) is a composite function of the solution ion concentration and their respective valences. This relationship is expressed as ionic strength, I:

$$I = 1/2 \Sigma C_i Z_i^2 \qquad \text{[eq. 3]}$$

where C_i is the concentration in moles L^{-1} and Z_i is the valence of ion i.

1.4 In pure, dilute solutions A_{NO3} closely approximates C_{NO3}. At higher ionic strengths, A_{NO3} will decrease with respect to C_{NO3} due to the interaction of ions of opposite charge. The ratio of ion activity to its concentration is termed the activity coefficient (g_i):

$$g_{NO3} = A_{NO3} / C_{NO3}. \qquad \text{[eq. 4]}$$

1.5 To avoid problems with standard and sample solutions having different ionic strengths, a

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background salt solution (ionic strength adjuster, or ISA) of high ionic strength relative to C_{NO3} can be added. This results in a reasonably constant g_{NO3} making A_{NO3} directly proportional to C_{NO3} and stable between standard and sample solutions.

2. Apparatus

2.1 Nitrate electrode.

2.2 Double junction reference electrode (fill outer chamber with 0.02 *M* $(NH_4)_2SO_4$ solution).

2.3 Specific ion meter or a pH/millivolt (mV) meter with readability to 0.1 mV.

3. Reagents and Solutions

3.1 Reference electrode filling solution - 0.02 *M* $(NH_4)_2SO_4$. Add 2.64 g of $(NH_4)_2SO_4$ in a 1-L volumetric flask and fill to volume with deionized water. Note, chloride has a high selectivity constant and significant contamination can occur if KCl is used as the internal filling solution.

3.2 Preservative solution - dissolve 0.1 g of phenylmercuric acetate in 20 mL of dioxane in a 100-mL volumetric flask. Fill to volume with deionized water. Note, this solution is very toxic and should not contact the skin.

3.3 500 mg nitrate-N L^{-1} stock solution - add 3.609 g of oven dried KNO_3 into a 1-L volumetric flask. Bring to volume with deionized water.

3.4 2 *M* ammonium sulfate ISA - add 26.4 g of $(NH_4)_2SO_4$ to a 100-mL volumetric flask and fill to volume with deionized water.

3.5 0.025 *M* aluminum sulfate + 10 mg nitrate-N L^{-1} extracting solution - add 32 g of $Al_2(SO_4)_3$, 40 mL of 500 mg NO_3-N L^{-1} stock solution, and 2 mL of preservative solution into a 2-L volumetric flask. Bring to volume with deionized water.

3.6 40 mg nitrate-N L^{-1} standard - add 16 g of $Al_2(SO_4)_3$, 80 mL of 500 mg NO_3-N L^{-1} stock solution, and 1 mL of preservative to a 1-L volumetric flask. Bring to volume with deionized water.

4. Procedure

4.1 Weight out 0.10 g of dried plant tissue that has been ground to pass through a 20-mesh sieve.

4.2 Add 30 mL of $Al_2(SO_4)_3$ extracting solution and shake for 15 minutes. If deionized water is used as the extracting solution, omit $Al_2(SO_4)_3$ in the NO_3 standard described in sections 3.5 and 3.6 and use 2 mL of the $(NH_4)_2SO_4$ ISA solution for every 100 mL of standard and sample solution.

4.3 Record meter reading.

5. Calibration and Standards

5.1 Standards are made up in the 0.025 *M* $Al_2(SO_4)_3$ background solution. It is important that the concentration of the samples be within the range of the standards. The concentration is determined by comparison to the standards. For most potentials between 1 and 40 mg NO_3-N L^{-1}, suitable results can be obtained using the 40 mg NO_3-N L^{-1} solution in section 3.6 as the high standard and using the 0.025 *M* $Al_2(SO_4)_3$ + 10 mg NO_3-N L^{-1} extracting solution in section 3.5 as the low solution. The 0.025 *M* $Al_2(SO_4)_3$ + 10 mg NO_3-N L^{-1} extracting solution should be set to read zero to subtract out the 10 mg NO_3-N L^{-1} background in the sample solutions.

5.2 The purpose of using 0.025 *M* $Al_2(SO_4)_3$ as an extractant is to acidify the sample to a pH near 3 and provide a constant ionic strength background (Baker and Smith, 1969; Carlson and Keeney, 1971). The Al in solution complexes organic acids and the lower solution pH suppresses their ionization.

5.3 The extraction solution described in section 3.5 contains 10 mg NO_3-N L^{-1} to maintain a linear calibration curve, depress the effect of interfering anions, and shorten the electrode equilibration time (Baker and Smith, 1969). Errors will increase rapidly as sample NO_3 becomes small compared with the background NO_3 in the extracting solution.

6. Calculations

6.1 The sample meter concentration reading should be multiplied by 300 to correct for dilution.

7. Remarks

7.1 The NO_3 electrode has a working range of 0.14 to 1400 mg NO_3-N L^{-1}. Deviation from linear calibration curves will begin at NO_3-N concentrations below 1.40 mg L^{-1}. Above 140 mg NO_3-N L^{-1}, effects due to differential cation and anion mobilities at the reference electrode interface can significantly bias A_{NO3} measurement.

7.2 Additionally, some salts may become fixed into the electrode membrane at high salt concentrations resulting in poor performance.

7.3 Serious interference occurs in the presence of perchlorate ($HClO_4^-$), chloride (Cl^-), phosphate ($H_2PO_4^-$, HPO_4^{2-}, HPO_4^{3-}), and nitrite ions (NO_2^-). In plant tissue, Cl^- is the main concern. Orion Research, Inc. (1986) offers a more complete list of common interfering ions and their respective selectivity constants.

7.4 Electrode measurements reproducible to $\pm$2% can be obtained using a well calibrated

instrument for solutions in the range of 14 mg NO_3-N L^{-1} with temperature fluctuations within $\pm 1^{o}C$ (Orion Research, Inc., 1986).

7.5 Plant concentrations of most interfering ions will be too low to adversely affect the determination of NO_3-N. However, where Cl^- is a concern the inclusion of a buffer solution containing 0.01 *M* Ag_2SO_4 in the extracting solution can be used to precipitate Cl^- (Mills, 1980).

7.6 All samples should be analyzed within half a day of extraction to ensure measurement uniformity and avoid any bacterial degradation of NO_3 in the sample.

7.7 The NO_3 electrode should soak in a 0.01 *M* NO_3 solution between each analysis period.

8. References

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Determination of Phosphorus in Plant Tissue by Colorimetry

K. P. Moore [*]

1. Principle of the Method

1.1 An acidified solution of ammonium molybdate containing ascorbic acid and antimony is added to a digested plant tissue sample. The phosphate in the plant tissue sample reacts with the acidified ammonium molybdate to form an ammonium molydiphosphate complex. A blue colored solution is generated from the reduction of the ammonium molydiphosphate complex by ascorbic acid. The intensity of the blue color is proportional to the amount of molybdophosphorus present. Antimony potassium tartrate accelerates the color development and stabilizes the color for several hours.

1.2 The amount of light absorbed by the solution at 660 nm is measured with a spectrophotometer.

1.3 The procedure is described by Murphy and Riley (1962) and Watanabe and Olsen (1965).

2. Apparatus

2.1 Analytical balance.

2.2 150-mL beakers or 50-mL porcelain crucibles.

2.3 Wet oxidation hot plate and hood or furnace.

2.4 1-L and 2-L volumetric flasks.

2.5 100-mL volumetric flasks.

2.6 1:100 dilutor dispenser.

2.7 Test tubes for color development.

2.8 Visible spectrophotometer with 1-cm light path. Spectrophotometers equipped with a flowcell accessory can be used to read samples directly from the color development test tubes.

2.9 Pipettes for making reagents, standards, and any appropriate dilutions.

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3. Reagents

3.1 Nitric acid (HNO_3) - for wet ashing.

3.2 Perchloric acid ($HClO_4$) - for wet ashing.

3.3 0.1 *M* hydrochloric acid (HCl) - for dry ashing.

3.4 Acid molybdate stock solution - In a 2-L volumetric flask, dissolve 125 g ammonium molybdate [$(NH_4)_6Mo_7O_{24}•4H_2O$] in 400 mL distilled water by heating to 60°C. Allow to cool, then dissolve 2.9 g antimony potassium tartrate [$K(SbO)C_4H_4O_6•1/2H_2O$] in the molybdate solution. Place the flask in an ice bath and slowly add 1500 mL concentrated sulfuric acid (H_2SO_4). Cool the mixture in the ice bath and slowly dilute to volume with distilled water. Store in a brown bottle at 4 °C.

3.5 Ascorbic acid stock solution - Dissolve 211.2 g ascorbic acid ($C_6H_8O_6$) in 1500 mL distilled water and dilute to 2 L with distilled water. Store in a brown bottle at 4 °C.

3.6 Working solution (prepare fresh daily) - Add 20 mL of the acid molybdate stock solution and 10 mL of the ascorbic acid stock solution to 800 mL distilled water, then dilute to 1 L with distilled water.

3.7 Standards - To make 1,000 ppm phosphorus stock solution, dissolve 4.3937 g of dried monopotassium phosphate (KH_2PO_4) in distilled water then dilute to 1 L. To make 20, 40, 60, and 80 mg P L^{-1} standards, pipet 2, 4, 6, and 8 mL, respectively, of the 1000 mg L^{-1} stock solution into separate 100-mL volumetric flasks and dilute to volume with distilled water.

4. Procedure

4.1 Digestion - Weigh 1.0000 g $\pm$ 0.0005 g of dried and ground plant tissue into 150-mL beakers or 50-mL porcelain crucibles. Digest samples using the wet oxidation procedure or the dry ashing procedure, respectively. Quantitatively transfer samples into 100-mL volumetric flasks and dilute with distilled water.

4.2 Color Development - Using a dilutor-dispenser, dilute the samples and the 20, 40, 60, and 80 mg P L^{-1} standards 1:100 with the working solution. Allow color to develop for at least 30 minutes before reading. Read the concentration at 660 nm with a visible spectrophotometer.

5. Calibration and Standards

5.1 To calibrate the spectrophotometer for routine analysis, use the working solution (see 3.6) as the blank and the developed 0.80 mg P L^{-1} standard to establish the slope of the line. To check for linearity, read the developed 0.20, 0.40, and 0.60 mg P L^{-1} standards. If the sample concentration lies above the linear working range, dilute the samples appropriately.

6. Calculations

6.1 The instrument reading may be read as percent P in the dried plant tissue.

7. Remarks

7.1 Phosphorus in plant material may commonly range from 0.03% to 1.50%. Following a 1:10,000 dilution, the blue colored complex which is formed using this method obeys Beer's law up to a P concentration of 1.5 mg L^{-1}.

7.2 This method gives excellent reproducibility (Murphy and Riley, 1962).

8. References

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

Nelson, W. L., A. Mehlich, and E. Winters. 1953. The development evaluation and use of soil tests for phosphorus availability. p 153-188. *In* W. H. Pierre and A. G. Norman (ed.). Soil and fertilizer phosphorus, Vol. IV Agronomy, A Series of Monographs, Academic Press, Inc., NY.

Watanabe, F. S., and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Amer. Pro., 29:677-678.

Determination of Potassium, Calcium, and Magnesium in Plants by Atomic Absorption Techniques

E. A. Hanlon*

1. Principle of the Method

1.1 The elements K, Ca, and Mg must be brought into solution by one of the procedures described in the organic matter destruction section. The resulting solution can then be aspirated and subsequently introduced into an energy source. The source is usually an acetylene/air flame producing a temperature of about 2,300°C.

1.2 Atomic absorption. A solution containing the elements in question is atomized and introduced into the acetylene/air mixture. The burner design and adjustment of the fuel/oxidant mixture provides conditions in which the species in question are converted to a nonexcited, nonionized, ground state. In this energy state, atoms are capable of absorbing light energy characteristic of that species. The light source is provided, in most cases, by a hollow cathode lamp in which the element or elements under investigation act as the cathode. When current is provided to the lamp, photons characteristic of the element or element(s) acting as the cathode are emitted. This light is directed into the flame where it is absorbed proportional to the concentration of the element in the flame. Absorption is calculated based upon the measured difference in light intensity passing around the flame and that passing through the flame.

1.3 Flame emission. The process of flame emission is essentially the reverse of atomic absorption. The same instrument is often designed to provide both atomic absorption and flame emission capabilities. Once introduced into the flame, elements are excited beyond the ground state used in atomic absorption. The excited atoms give off characteristic light energy in proportion to their concentration in the flame.

1.4 Measurement of either atomic absorption or flame emission usually includes a movable monochrometer, a method of controlling the light path which is often done by specifically designed slits, and photodetectors which are sensitive to the wavelength(s) in question. An excellent discussion of the above principles and the needed equipment is given by Isaac and Kerber (1971).

2. Reagents and Apparatus

2.1 Concentrated reagent-grade HCl (12 *M*).

2.2 1,000 mg La L^{-1} solution. This solution may be prepared from either La_2O_3 or from

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$LaCl_3 \cdot H_2O$. The former source must be brought into solution using HCl, but is much cheaper than the more readily soluble hydrated chloride source.

2.2.1 For La_2O_3, prepare a slurry by adding a small volume of deionized water to 1.1727 g La_2O_3 in 1-L volumetric flask. Slowly add 8 mL concentrated HCl and stir. Dilute to final volume with additional deionized water.

2.2.2 For $LaCl_3 \cdot H_2O$, dissolve 2.6738 g $LaCl_3 \cdot H_2O$ with deionized water. Slowly add 8 mL of concentrated HCl and bring to volume with additional deionized water. The resulting standard, regardless of the source of La, will contain 0.1 *M* HCl.

2.3 Deionized water.

2.4 1000 mg K L^{-1} standard. Weigh 1.9067 g of oven-dried and desiccated KCl. Quantitatively transfer to a 1-L volumetric flask and bring to volume. Alternately, a commercially prepared standard solution can be purchased.

2.5 1000 mg Ca L^{-1} standard. Weigh 2.4973 g of oven-dried and desiccated $CaCO_3$. Slowly add (dropwise) approximately 8 mL concentrated HCl. Bring to 1 L final volume with deionized water. Alternately, a commercially prepared standard solution can be purchased.

2.6 1000 mg Mg L^{-1} standard. Weigh 1.000 g Mg metal ribbon. Dissolve with 8 mL deionized water and 8 mL of concentrated HCl. Bring to 1 L final volume with additional deionized water.

2.7 Atomic absorption/flame emission spectrophotometer.

3. Procedure

3.1 After performing a dry ashing or wet digestion procedure on a known dry weight of tissue, the resulting product should be wetted with a small amount of deionized water and brought into solution using 2 mL concentrated HCl. Dilute to 100-mL final volume with deionized water.

The final dilution should be based upon the predicted concentrations of the needed elements to insure that the resulting actual concentrations are not at or below detection limits. For the three elements in this section, the 100-mL final volume should provide concentrations sufficiently above the detection limit for a 1-g sample of most plant materials.

3.2 After bringing to final volume, the solution should be mixed by inversion of the volumetric flask several times.

3.3 Potassium may be determined either by atomic absorption or flame emission in this solution. However, flame emission has been reported to be somewhat more sensitive (Anonymous, 1976).

3.4 Serial dilutions should be made until the K concentration reading is within the standardized range of the instrument using 0.1 to 0.3 *M* HCl diluent.

The actual linear range of K is between 0 and 10 mg K L^{-1}. However, commercial

instrumentation can be programmed with three to five serial dilutions of the K standard to extend the upper limit of the working range to between 50 to 100 mg K L^{-1}.

3.5 For determinations of Ca and Mg by atomic absorption, an aliquot of the above solution (3.1) should be diluted with the La standard (2.2) using 1 mL of unknown + 9 mL of 1000 mg La L^{-1}.

3.6 The linear working range for Ca is from 0 to 10 mg Ca L^{-1}, while that of Mg is 0 to 0.5 mg Mg L^{-1}. These ranges can often be extended greatly depending upon the capabilities of the instrumentation, as discussed in 3.4. Typical working ranges are approximately 0 to 50 mg L^{-1}.

4. Remarks

4.1 Jones and Steyn (1973) and Jones (1972) present reviews of the various analytical approaches used to analyze tissue and to interpret the resulting numbers. In summary, K, Ca, and Mg appear to have lower variabilities when atomic absorption methods are used, as compared to flame emission.

4.2 The selection of a digestion procedure does not necessarily mean that the choice of a diluent is clear. For example, several methods of bringing the resulting dry ash into solution have been described (Isaac and Kerber, 1971; Hanlon and DeVore, 1989; and Isaac and Jones, 1972). In general, a small volume of a strong acid, such as HCl or HNO_3, is used regardless of the digestion technique to enhance elemental solubility. The resulting solution is then diluted, most often, with deionized water producing a final acidic solution between 0.1 and 0.3 *M*, depending upon the acid and final dilution. A secondary advantage of such acidification is the biological control resulting from the lowered pH. The procedure described above uses HCl.

4.3 *Interferences*

4.3.1 Potassium. Partial ionization of K occurs in an air/acetylene flame. Addition of 1,000 mg of other alkali salts has been reported to decrease such ionization (Anonymous, 1976).

4.3.2 Calcium. Silicon, P, Al, and sulfate depress Ca absorption. The addition of La in excess greatly reduces these anionic effects, as well as suppressing Ca ionization interferences. The literature reports use of La in concentrations from 0.1% to as high as 5%. However, excellent results have been obtained using the procedure described above for plant material with a substantial savings of La salts.

4.3.3 Magnesium. The elements Si and Al depress Mg absorption. The addition of La in excess greatly reduces these effects.

5. References

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Hanlon, E.A., and J.M. DeVore. 1989. IFAS extension soil testing laboratory chemical procedures and training manual. Circ. No. 812. Fla. Coop. Ext. Ser., IFAS, Univ. of Florida, Gainesville, FL.

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Jones, J. B., Jr., and W. J. A. Steyn. 1973. Sampling, handling, and analyzing plant tissue samples. p. 249-270. *In* L. M. Walsh and J. D. Beaton (ed) Soil testing and plant analysis. Soil Sci. Soc. Am., Madison, WI.

Determination of P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in Plant Tissue by Inductively Coupled Plasma (ICP) Emission Spectroscopy

S. J. Donohue and D. W. Aho [*]

1. Principle of the Method

1.1 Plant tissue digests are efficiently ionized by direct injection into a plasma formed with argon gas ionized in an applied radio frequency field. Each element has characteristic emission spectra when ionized. Resultant ionic emission spectra are monitored at pre-selected wavelengths, allowing effective multi-element determination.

1.2 Light intensity at each specified wavelength is proportional to sample element concentration.

1.3 A linear dynamic range of four to six orders of magnitude is observed for many elements, allowing for two point calibration (Anonymous, 1989).

2. Apparatus

2.1 Inductively-coupled argon plasma spectrometer.

2.2 Analytical balance.

2.3 Porcelain crucibles, 50 mL.

2.4 Whatman No. 42 ashless filter paper (or equivalent), 11 cm.

2.5 Volumetric flasks and pipettes as required for preparation of reagents and standard solutions.

3. Reagents

3.1 To avoid ionic contamination, use only *plasma pure standard reference solutions*, deionized water, and acid washed glassware and storage bottles.

3.2 Concentrated Hydrochloric Acid (HCl)

3.3 Standard #1: 1.2 *M* HCl blank - To approximately 500 mL of deionized water in a 1-L volumetric flask, add 100 mL of concentrated reagent grade HCl. Dilute to volume with deionized water.

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3.4 Standard #2: 400 mg P L^{-1}, 10 mg Zn L^{-1}, and 10 mg B L^{-1} - To approximately 500 mL of deionized water in a 1-L volumetric flask, pipet 100 mL of concentrated reagent grade HCl, 400 mL of 1000 mg P L^{-1} reference standard, 10 mL of 1000 mg Zn L^{-1} reference standard, and 10 mL of 1000 mg B L^{-1} reference standard. Dilute to volume with deionized water.

3.5 Standard #3: 100 mg Al L^{-1} and 100 mg Fe L^{-1} - To approximately 500 mL of deionized water in a 1-L volumetric flask, pipet 100 mL of concentrated reagent grade HCl, 100 mL of 1000 mg Al L^{-1} reference standard, and 100 mL 1000 mg Fe L^{-1} reference standard. Dilute to volume with deionized water.

3.6 Standard #4: 1000 mg Ca L^{-1}, 1000 mg K L^{-1}, 100 mg Mg L^{-1}, 10 mg Mn L^{-1}, and 10 mg Cu L^{-1} - To approximately 500 mL of deionized water in a 1-L volumetric flask, pipet 100 mL of concentrated reagent grade HCl, 100 mL of 10,000 mg Ca L^{-1} reference standard, 100 mL of 10,000 mg K L^{-1} reference standard, 100 mL of 1000 mg Mg L^{-1} reference standard, 10 mL of 1000 mg Mn L^{-1} reference standard, and 10 mL of 1000 mg Cu L^{-1} reference standard. Dilute to volume with deionized water.

3.7 Quality control standard - To approximately 500 mL of deionized water in a 1-L volumetric flask, pipet 100 mL of concentrated reagent grade HCl and the following amounts of each reference standard. Dilute to volume with deionized water.

Reference Solution	Amount added mL	Final Concentration
10,000 mg K L^{-1}	10	100 mg K L^{-1}
10,000 mg Ca L^{-1}	10	100 mg Ca L^{-1}
1000 mg P L^{-1}	50	50 mg P L^{-1}
1000 mg Mg L^{-1}	50	50 mg Mg L^{-1}
1000 mg Fe L^{-1}	50	50 mg Fe L^{-1}
1000 mg Al L^{-1}	50	50 mg Al L^{-1}
1000 mg Zn L^{-1}	10	10 mg Zn L^{-1}
1000 mg B L^{-1}	10	10 mg B L^{-1}
1000 mg Mn L^{-1}	10	10 mg Mn L^{-1}
1000 mg Cu L^{-1}	10	10 mg Cu L^{-1}

4. Procedure

4.1 Digestion - Weigh 1.000 $\pm$ 0.010 g of dried, ground plant tissue into a 50-mL ceramic crucible and dry ash according to standard procedures (See Organic Matter Destruction). Allow to cool, then pipet 5 mL concentrated HCl into the crucible and gently swirl to dissolve the ash. Let stand for 30 minutes, then pipet 10 mL deionized water and allow to stand for 20 minutes. Add 35 mL deionized water to give a final volume of 50 mL and a 1.2 *M* HCl matrix. Filter through Whatman #42 (or equivalent) ashless filter paper. Include 1 blank and 1 test sample (Anonymous, 1989) with each sample set.

4.2 Analysis - Transfer samples to appropriate autosampler vials and analyze on the ICP. Compare results for the test sample (Anonymous, 1989) with established values.

5. Calibrations and Standards

5.1 Standard Reference Plant Material - Prepare a reference plant tissue sample along with each sample set to monitor sample preparation reagents and procedures. Test samples prepared in-house should be checked against NITS standard reference plant material.

5.2 Instrument Calibration - Daily profile optics according to manufacturer's instructions. Standardize instrument using standards in sections 3.3 to 3.6.

5.3 Calibration Check - Check standardization with the quality control sample after performing standardization and after each sample set. Measured element concentration of the quality control sample should be within 5%.

6. Calculations

6.1 Phosphorus, K, Ca, and Mg are expressed as % in tissue. Manganese, Fe, Al, B, Cu, and Zn are expressed as mg kg^{-1} in tissue.

6.2 For elements determined by dry ash:

mg kg^{-1} in tissue = (mg L^{-1} in solution @ 50) / sample weight, g

% in tissue = mg kg^{-1} in tissue @ 0.0001

7. Remarks

7.1 *Range and Sensitivity*

7.1.1 Potential analytical range of plant tissue concentrations for this method are as follows:

0.0003	- 1.25%	P	0.30 -	7500	mg B	L^{-1}
0.0015	- 5.00%	K	0.10 -	7500	mg Cu	L^{-1}
0.0005	- 7.50%	Ca	0.25 -	7500	mg Fe	L^{-1}
0.00005	- 1.75%	Mg	0.05 -	7500	mg Mn	L^{-1}
			0.20 -	7500	mg Zn	L^{-1}
			1.25 -	25,000	mg Al	L^{-1}

7.1.2 Analytical ranges are dependent upon sample extraction method, instrumentation, and selected wavelength.

7.2 *Interferences*

7.2.1 Various spectral interferences can contribute to apparent net signal intensity (Anonymous, 1989). Elements which have overlapping spectral lines with elements of interest must be measured and the appropriate corrections made.

7.2.2 Differences in acid or dissolved solid concentration between sample matrix and calibration standards can cause significant error (Anonymous, 1989). Compensate by using matrix matched standards.

7.3 *Precision and Accuracy*

7.3.1 Please refer to the instrument manufacturer and (Anonymous, 1989) for ICP precision and data bias.

7.4 *Effects of Storage*

7.4.1 Filtrate may be capped and refrigerated overnight. Samples should be allowed to equilibrate to room temperature before ICP analysis.

8. References

Anonymous. 1989. Standard methods for the examination of water and wastewater. Am. Public Health Assoc., Washington, DC, sec. 3120.

Determination of P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in Plant Tissue by Emission Spectroscopy

R. A. Isaac and W. C. Johnson, Jr.[*]

1. Principle of the Method

1.1 This method describes the simultaneous analysis of total P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in plant tissue samples.

1.2 Sample digests are burned in a carbon arc, causing each element to emit unique wavelengths of light. The amount of light emitted by each element is directly proportional to the concentration of the element in the sample. The wavelength chosen for the analysis of each element is selected on the basis of desirable intensity and freedom from spectral interference by other elements.

1.3 Lithium is used as an internal standard.

1.4 A detailed description of the method is given in the Official Methods of Analysis of the Association of Official Analytical Chemists.

2. Apparatus

2.1 Arc-Spark emission spectrograph.

2.2 Analytical balance.

2.3 Furnace.

2.4 Porcelain crucibles, 10 mL.

2.6 Buret, 50 mL.

2.7 Drying oven.

2.8 Wiley mill.

2.9 Volumetric flasks and pipets as required for preparation of reagents and standard solutions.

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3. Reagents

3.1 Buffer solution: Weigh 50 g lithium carbonate (Li_2CO_3) into a 1-L volumetric flask. Slowly add 200 mL concentrated nitric acid and (HNO_3). Let cool and dilute to volume with deionized water.

3.2 Phosphorus stock solution 10% - Pipet 220.4 mL phosphoric acid (H_3PO_4) into a 1-L volumetric flask and dilute to volume with deionized water.

3.3 Potassium stock solution 12.5% - Weigh 238.35 g potassium chloride (KCl) into a 1-L volumetric flask, dissolve and dilute to volume with deionized water.

3.4 Calcium stock solution 4% - Weigh 99.89 g calcium carbonate ($CaCO_3$) into a 1-L volumetric flask. Dissolve slowly in a minimum amount of 1 to 1 HCl and dilute to volume with deionized water.

3.5 Magnesium stock solution 2% - Weigh 33.16 g magnesium oxide (MgO) into a 1-L volumetric flask. Dissolve in a minimum amount of concentrated HCl and dilute to volume with deionized water.

3.6 Manganese, iron, and aluminum stock solution 1000 mg L^{-1} - Weigh 1.5824 g manganese dioxide (MnO_2) into a 150-mL beaker. Add 50 mL of 1 to 1 nitric acid. Place the beaker on a 100°C hot plate and add dropwise 30% hydrogen peroxide solution, swirling the beaker after each addition until the manganese dioxide is completely dissolved. Boil the solution for 5 minutes in order to expel any excess hydrogen peroxide. Weigh 1.0000 g iron wire into a 150-mL beaker. Add 50 mL of 1 to 1 nitric acid and heat on a 100°C hot plate until dissolved. Weigh 1.0000 g aluminum wire into a 150-mL beaker. Add 50 mL of 1 to 1 hydrochloric acid and heat on a 100°C hot plate until dissolved. Combine all three beakers into a 1-L volumetric flask and dilute to volume with deionized water.

3.7 Boron, copper, and zinc stock solution 1000 mg L^{-1} - Weigh 5.7144 g boric acid (H_3BO_3) into a 150-mL beaker. Add 50 mL deionized water and heat on a 100°C hot plate until dissolved. Weigh 1.0000 g copper wire into a 150-mL beaker. Add 50 mL of 1 to 1 nitric acid and heat on a 100°C hot plate until dissolved. Weigh 1.000 g zinc dust into a 150-mL beaker. Add 50 mL of 1 to 1 nitric acid and heat on a 100°C hot plate until dissolved. Combine all three beakers into a 1-L volumetric flask and dilute to volume with deionized water.

4. Procedure

4.1 Digestion - Weigh 1.000 g dried ground plant material into a 10-mL porcelain crucible. Place the crucible in a cool muffle furnace and ash for 4 hours at 500°C. Remove the crucible from the furnace and allow to cool. Add 5 mL of buffer solution (3.1) to the crucible and gently swirl to dissolve the ash.

4.2 Analysis - Transfer the digest to a teflon boat and analyze on the direct reading arc-spark emission spectrograph.

5. Calibration and Standards

5.1 Calibration standards - Make up five mixed element standards covering the range of concentrations given in section 7.1. Each standard solution should contain 200 mL HNO_3 and 50 g Li_2CO_3 L^{-1}

5.2 Instrument calibration - Analyze each calibration standard four times with the instrument in the background corrected mode. Enter the average of the background corrected values into the instrument's computer, thereby constructing a concentration curve for each element. This calibration is very stable, but should be checked on a monthly basis.

5.3 Daily calibration - Follow the steps given in the instrument operation manual to select one of the mid-range calibration standards as a single point calibration source. Calibrate the instrument for sample analysis by analyzing the mid-range standard four times with the instrument in the standardization mode. The computer will automatically adjust the values obtained to fit the curves programmed into the instrument in section 5.2. This single point calibration should be done at least twice each day.

5.4 Standards - Include at least one plant tissue sample having known values with each set of samples analyzed. "In house" check samples are permissible. The values for "in house" check standards should be established vs. a known plant tissue standard such as NIST SRM #1572 Citrus Leaves.

6. Calculations

6.1 Phosphorus, Ca, and Mg concentrations are expressed as %. Values for Mn, Fe, Al, B, Ca, and Zn are expressed as mg kg^{-1}.

7. Remarks

7.1 Phosphorus, K, Ca, and Mg can be determined in a range of plant tissue concentrations from 0.10 to 0.70% P, 0.50 to 5.00% K, 0.20 to 2.00% Ca, and 0.10 to 1.00% Mg. Manganese, Fe, and Al can be determined in the range from 25 to 500 mg kg^{-1}. Boron, Cu, and Zn can be determined in the range from 5 to 50 mg kg^{-1}. The ranges may be extended by dilution, or calibrating the instrument for higher concentrations.

7.2 The sensitivity will vary depending on the instrument used and the wavelength selected for each element.

7.3 This method has very few interferences. However, interferences due to spectral overlap may occur. This type of interference occurs when an element emits a wavelength which is very close to the wavelength of an element being analyzed and the optical system of the instrument is incapable of resolving the two wavelengths.

7.4 In a collaborative study involving 11 different instruments, coefficients of variation ranged from 3 to 7% for Ca, Mg, and Mn; 7 to 15% for P, K, B, Cu, and Zn; and >15% for Fe and Al (Jones, 1975).

7.5 Samples to be analyzed after storage should be placed in an oven at 30°C for four hours

before weighing.

8. References

AOAC method 980.03 in Official methods of analysis of the association of analytical chemists, 15th Edition, p. 41.

Jones, J. B., Jr. 1975. Collaborative study of the elemental analysis of plant tissue by direct reading emission spectroscopy. J. Assoc. Off. Anal. Chem. 58:764-769.

Determination of Sulfur in Plant Tissue by Combustion

R. A. Isaac and W. C. Johnson, Jr.[*]

1. Principle of the Method

1.1 Plant tissue samples are burned at 1370°C in an oxygen atmosphere, converting sulfur compounds to SO_2 gas. After removal of moisture, the SO_2 gas is measured by a solid state infrared detector.

1.2 A detailed description of the method is given by Isaac and Johnson (1984).

2. Apparatus

2.1 LECO SC-132 Sulfur Determinator or equivalent.

2.2 Ceramic boats.

2.3 Drying oven.

3. Reagents

3.1 Magnesium perchlorate ($MgClO_4$) - This is packed in a glass tube and used to remove moisture from the gases produced during combustion of the sample.

3.2 Vanadium pentoxide (V_2O_5).

3.3 Oxygen.

4. Procedure

4.1 Place a ceramic boat on the balance pan of the LECO SC-132. The boat will be automatically tared.

4.2 Weigh 50 to 100 mg of ground plant tissue (20-mesh) into the ceramic boat and enter the weight on the console of the computer. The computer automatically compensates for different weights.

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4.3 Add enough vanadium pentoxide to cover the surface of the plant tissue sample (0.1 to 0.2 g).

4.4 Press the analyze button on the computer console.

4.5 When the load furnace message appears on the computer console, push the sample into the furnace.

4.6 The analysis will begin automatically when the first amount of SO_2 gas reaches the solid state infrared detector. For most samples, analysis time is 1 minute.

5. Calibration and Standards

5.1 Calibration - The instrument is calibrated using a sample with known sulfur content.

5.2 Place the instrument in the calibration mode.

5.3 Enter the known S value when prompted by the computer.

5.4 Analyze the known sample three times. After three analyses, the computer will automatically calibrate the instrument based on the average value of the three analyses.

5.5 Standards - NIST SRM #1572 Citrus Leaves (0.407% S) is recommended. Coal standards may also be used and are available in a wide range of sulfur concentrations. If a coal standard is used for calibration, the accuracy of the calibration should be verified by analyzing a plant tissue sample having a known S content.

6. Calculations

6.1 Sulfur concentrations are expressed as percent.

7. Remarks

7.1 Sulfur can be determined in plant tissue concentrations from 0.05 to 2.00%.

7.2 There are no significant interferences.

7.3 In a study done at the University of Georgia Soil Testing and Plant Tissue Laboratory, consisting of ten replicates of nine different plant materials, the percent relative standard deviation ranged from 0.77 to 2.46%. The range of S values on the samples was 0.150 to l.08%.

7.4 Samples analyzed after storage should be placed in an oven at 30 ^{o}C and dried for 4 hours before weighing.

8. References

Isaac, R. A., and W.C. Johnson. 1984. Methodology for the analysis of soil, plant, feed, water and fertilizer samples. The University of Georgia. pp. 92-93.

Determination of Sulfur in Plant Tissue by Turbidimetry

C. C. Mitchell[*]

1. Principle of the Method

1.1 This procedure is a modification of the Massoumi and Cornfield (1963) and the Chaudry and Cornfield (1966) methods. Sulfate-sulfur is precipitated in aqueous solution by adding barium chloride. The finely divided barium sulfate crystals remain suspended in the solution, diffracting light. The effect on light transmission through the solution is measured with a spectrophotometer.

2. Reagents and Apparatus

2.1 Acetic/phosphoric acid solution: Mix 75 mL concentrated acetic acid with 25 mL concentrated H_3PO_4 and dilute to 1 L.

2.2 Gum acacia solution: Dissolve 5 g gum acacia in 500 mL hot water. Filter hot through a Whatman no. 42 filter paper on a Buchner funnel using suction. Cool and dilute to 1 L with acetic acid.

2.3 Barium sulfate seed suspension: Dissolve 18 g $BaCl_2 \cdot 2H_2O$ in 44 mL hot water. Add 0.5 mL of the 2,000 mg S L^{-1} standard. Bring to a boil, and cool quickly. Add 4 mL of the gum acacia solution and mix well. Prepare fresh daily.

2.4 Barium chloride solution: Add 200 g $BaCl_2 \cdot 2H_2O$ to a 1-L volumetric flask. Add enough hot water to dissolve. Cool and dilute to volume.

2.5 Standard sulfate solution (2,000 mg S L^{-1}): Dissolve 1.0875 g of oven-dried K_2SO_4 in 0.1 *M* HCl and dilute to 100 mL. Prepare working standards containing 10, 20, 30, 40, 50, and 100 mg S L^{-1} by diluting appropriate aliquots of this stock with demineralized water. New working standards should be prepared every 2 to 3 days.

3. Instrumentation

3.1 A spectrophotometer with digital display capable of measuring absorbance to 0.001 is required. A vortex stirrer is desirable for uniform mixing.

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4. Procedure

4.1 Pipette 1-mL aliquots of each standard and digested sample into standard test tubes. Do not run more than 30 samples with a single set of standards.

4.2 Add 22 mL of the acetic/phosphoric acid solution. Mix on a vortex mixer.

4.3 Add exactly 0.5 mL of the barium sulfate seed suspension.

4.4 Add 1 mL of the barium chloride solution and mix each tube exactly the same length of time on a vortex mixer.

4.5 Add 1 mL of the gum acacia solution. Mix again.

4.6 Allow mixtures to set for 30 minutes.

4.7 Mix each sample uniformly just prior to reading absorbance or transmittance on a spectrophotometer set on a wavelength of 440 nm. The wavelength is not critical since only light blockage and not absorbance by the barium sulfate suspension is measured. Plot absorbance or transmittance vs. S concentration. The standard curve is usually linear from 10 to 80 mg S L^{-1}.

5. Remarks

5.1 This turbidimetric method is rapid and sensitive within the range of S found in digested plant samples (20 to 80 mg S L^{-1}) prepared for most other analyses. It is inexpensive and can be used without large investments in specialized instrumentation. Wet oxidation techniques are preferred for sample preparation. However, dry ashing can be used on most plant tissue if high furnace temperatures are avoided and samples are heated slowly.

Uniform precipitating conditions are essential to obtain reproducible results. Acetic and phosphoric acids buffer the pH and reduce variability due to acid concentrations in the sample. A seed suspension of small and uniformly sized barium sulfate crystals acts as nuclei and ensures more rapid precipitation and uniform results at low concentrations. Uniform and carefully stirred test tubes also help to ensure uniform precipitating conditions. Gum acacia added to the solution acts as a stabilizer and provides for greater reproducibility of the suspensions. Reference samples of established concentrations should be digested and analyzed frequently for quality control.

This procedure has worked well for the author on dry ashed plant samples, dry ashed samples digested with magnesium nitrate, and wet ashed samples digested with nitric-perchloric acid. It has compared favorably with total S by combustion techniques and ICP spectrophotometry on plant digestions.

6. References

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Determination of Total Manganese, Iron, Copper and Zinc in Plants by Atomic Absorption Techniques

E. A. Hanlon*

1. Principle of the Method

1.1 The elements Mn, Fe, Cu, and Zn must be brought into solution by one of the procedures described in the organic matter destruction section. The resulting solution can then be aspirated and subsequently introduced into an energy source. The source is usually an acetylene/air flame producing a temperature of about 2,300^{o}C.

1.2 A detailed discussion of atomic absorption and flame emission is provided by Isaac and Kerber (1971).

2. Reagents and Apparatus

2.1 Concentrated reagent-grade HCl (12 *M*).

2.2 Deionized water.

2.3 1000 mg Mn L^{-1} standard. Weigh 1.000 g of Mn metal. Dissolve with a minimum of equal parts deionized water and nitric acid. Add 8 mL of 12 *M* HCl and bring to 1 L final volume with additional deionized water. Alternately, a commercially prepared standard solution can be purchased.

2.4 1000 mg Fe L^{-1} standard. Weigh 1.000 g of Fe wire. Dissolve with approximately 8 mL deionized water and 8 mL of 12 *M* HCl. Bring to 1 L final volume with deionized water. Alternately, a commercially prepared standard solution can be purchased.

2.5 1000 mg Cu L^{-1} standard. Weigh 1.000 g Cu metal. Dissolve with 8 mL deionized water and 8 mL of concentrated HCl. Bring to 1 L final volume with additional deionized water. Alternately, a commercially prepared standard solution can be purchased.

2.6 1000 mg Zn L^{-1} standard. Weigh 1.000 g Zn metal ribbon. Dissolve with 8 mL deionized water and 8 mL of concentrated HCl. Bring to 1 L final volume with additional deionized water. Alternately, a commercially prepared standard solution can be purchased.

2.7 Atomic absorption/flame emission spectrophotometer.

3. Procedure

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3.1 After performing a dry ashing or wet digestion procedure on a known dry weight of tissue, the resulting product should be wetted with a small amount of deionized water and brought into solution using 2 mL concentrated HCl. Dilute to 50 mL final volume with deionized water.

3.2 After bringing to final volume, the solution should be mixed by inversion of the volumetric flask several times.

3.3 The published upper linear ranges are: Zn - 1 mg L^{-1}; Mn - 3 mg L^{-1}; and for Cu and Fe - 5 mg L^{-1} (Anonymous, 1976). With the curve-fitting ability of most atomic absorption/flame emission spectrophotometers, a useful upper working limit of 10 mg L^{-1} can be achieved with good accuracy and precision (Hanlon and DeVore, 1989).

3.4 The preferred method of analysis is by atomic absorption for Fe and Zn. Either atomic absorption or flame emission can be used for Cu and Mn (Isaac and Kerber, 1971). Since these elements are often determined as a group, all four are usually determined by atomic absorption (Jones, 1972).

4. Remarks

4.1 The final dilution should be based upon the predicted concentrations of the needed elements to insure that the resulting actual concentrations are not at or below detection limits. For the four elements in this section, the 50 mL final volume should provide concentrations sufficiently above the detection limit for a 1-g sample of most plant materials.

For example, Isaac and Kerber (1971) present typical elemental ranges for plant tissue, with the range for Cu given as 1 to 25 mg Cu kg^{-1} dry plant tissue. The published detection limit is 0.002 ug Cu mL^{-1} (Anonymous, 1976). However, such a low detection limit should never be assumed, but measured. A daily working detection limit of 0.05 ug Cu mL^{-1} is more realistic for high volume laboratory operations. Using the latter, results reported in the tissue would have a lower limit of 2.5 mg Cu kg^{-1} dry tissue. Should lower levels be expected, the final dilution volume should be reduced. One should keep in mind that the amount of statistical uncertainty surrounding a measurement increases sharply as the reading approaches within ten times of the detection limit (Taylor, 1989).

4.2 *Interferences*

4.2.1 There are no reported interferences for Mn, Cu, and Zn.

4.2.2 The presence of HNO_3 and Ni, or Si may depress the sensitivity of Fe. Use of a very hot, lean-burning, air/acetylene flame appears to overcome these interferences (Isaac and Kerber, 1971; Anonymous, 1976).

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Determination of Boron in Plants by Azomethine-H Method

W. E. Sabbe [*]

1. Principle

1.1 Azomethine-H -- a condensation product of salicylaldehyde and H-Acid (8-amino-1-naphthol-3,6-disulphonic acid) -- was suggested for quantitative determination of boron by Patrovsky (1963). Basson et al., 1969, developed a procedure of utilizing azomethine-H for the determination of B in plant tissue following dry ashing and dissolving the ash in dilute sulfuric acid. This procedure for plant tissue had the great advantage of being free of concentrated acids (Berger and Truog, 1944) and adaptable to automated methodology. Wolf (1971, 1974) expanded the procedure to include soil extracts, irrigation waters, nutrient solutions, composts, manures, and plant tissues.

2. Reagents and Apparatus

The reagents, digestion, and color development are those reported by Wolf (1971, 1974).

2.1 *Reagents*

2.1.1 Water for Reagents. Treat all water used for reagents and dilutions with activated charcoal (Darco G-69) by adding 20 mL (two level tablespoons) charcoal L^{-1} of deionized water, stirring for 5 minutes, and filtering through Whatman #1 paper.

2.1.2 Buffer masking agent. Dissolve 280 g ammonium acetate (NH_4OAc), 20 g of potassium acetate (KOAc), 20 g tetrasodium salt of ethylenedinitrilotetracetic acid (EDTA) and 18 g disodium salt of nitrilotriacetic acid (NTA) in 240 mL of water in a 2000-mL plastic beaker. After partial dissolution in a warm water bath, add 70 mL of acetic acid (HOAc) slowly with constant stirring until dissolved. Let stand overnight and filter through a Whatman #1 paper if a precipitate is present.

2.1.3 Azomethine-H. Dissolve 0.9 g azomethine-H and 2 g ascorbic acid in 10 mL of water in a water bath. After dissolution and cooling, make to a 100 mL volume. If turbid, reheat in warm bath. This reagent should keep for 14 days if refrigerated.

2.1.4 Sulfuric acid (H_2SO_4), concentrated.

2.1.5 Hydrogen peroxide (H_2O_2), 30%.

2.1.6 Boron Standards

2.1.6.1 Primary standard. (100 mg B L^{-1}) Dissolve 0.572 g boric acid (H_3BO_3) in water to a final volume of 1 L.

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2.1.6.2 Secondary standard (4 mg B L^{-1}). Dilute 1 mL of primary standard with wet-ashed blank to a 25 mL volume. Directions for preparation of the wet-ashed blank are given in the digestion procedure.

2.2 *Apparatus*

2.2.1 Glassware for storage of reagents should be low-B glass.

2.2.2 Whatman #40 filter paper.

2.2.3 Spectrophotometer. The spectrophotometer will be set at 420 nm.

3. Procedure

3.1 Digestion. Weigh a 0.30-g sample (0 to 200 mg B kg^{-1}) of dried plant material (passing 40-mesh) into a digestion tube (preferably Vycor). Add 2 mL of concentrated H_2SO_4, rotate the tube so that the plant material is thoroughly moistened, and add 1 mL of 30% H_2O_2. Mix well and place the tube in a cold heating block. Raise the block temperature to 500°C and after white dense fumes have been produced for about 5 minutes remove the tube for cooling. After cooling, add 1 mL of 30% H_2O_2 and replace in block until white dense fumes are produced for 20 minutes. Repeat this procedure until the tube contents are colorless. Note: To insure complete removal of H_2O_2, the final heating time should be 40 minutes after the last H_2O_2 addition. After cooling add water to a 15-mL volume and filter through a Whatman #40 paper. A wet-ash blank using 0.3 g sucrose is prepared in the same manner.

3.2 Color Development. Pipette 2 mL of the wet-ashed digest directly into a photometric tube, add 4 mL of the buffer masking reagent, and 2 mL of the azomethine-H. Immediately mix thoroughly by inverting the tube, then allow 2 hours for color development. Read at 420 nm.

The sample readings are compared to a set of standards (ranging from 0 to 4.0 mg B L^{-1}), prepared at the same time and treated exactly as the digests. These standards are prepared by diluting the 4 mg B L^{-1} standard with the wet-ash blank. The wet-ash blank should be used as the 0 mg B L^{-1} standard.

4. Remarks

4.1 The following were cited by Wolf (1974):

Possible interferences by: 1) NH_4 was overcome by the use of NH_4OAc; 2) K was overcome by use of KOAc; and 3) Al, Cu, and Fe was overcome by use of EDTA and NTA.

The importance of water quality for reagents, etc. was stressed. He indicated that the treatment of deionized water with charcoal would remove organic substances that may interfere with the color development.

The possible interference of residual H_2O_2 with color development was eliminated by a heating time of 40 minutes following the last H_2O_2 addition. The use of photometric tubes for color development does eliminate the use of additional plastic or glassware; however, the

importance of immediate and thorough mixing is stressed.

5. References

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Determination of Total Boron in Plants by the Curcumin Method

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1. Principle of the Method

1.1 The procedure described is Wikner and Uppström's (1980) modification to the traditional curcumin procedure (presented for soils by Wear, 1965). Wikner and Uppström's procedure is faster and requires less specialized equipment than the traditional method. A modified dry ashing procedure to decrease contamination of the samples and to avoid losses of boron is included.

2. Reagents and Apparatus

2.1 *Dry Ashing*

2.1.1 Hydrochloric acid (HCl) solution: Dilute 83 mL of concentrated HCl to 100 mL with deionized water.

2.1.2 Porcelain evaporating dishes.

2.1.3 Muffle furnace.

2.2 *Boron Determinations*

2.2.1 Curcumin reagent: Dissolve 2 g curcumin in 1 L of methyl isobutyl ketone. Use a plastic bottle in the fume hood and filter the reagent before using.

2.2.2 Acid mix: Mix 500 mL concentrated sulfuric acid (H_2SO_4) with 500 mL concentrated acetic acid (HOAc) in a plastic bottle.

2.2.3 100 mg B L^{-1} standard: Dissolve 0.5716 g boric acid in 1 L deionized water. Do not dry the boric acid in a drying oven as this may cause it to polymerize.

2.2.4 0 to 1 mg B L^{-1} working standards: Dilute appropriate amounts of the 100 mg B L^{-1} standard with deionized water.

2.2.5 50-mL plastic Erlenmeyer flasks with plastic screw caps (Teflon is preferred).

2.2.6 Syringe pipettes.

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2.2.7 Visible spectrophotometer with stoppered glass cuvette (disposable plastic cuvette will dissolve in methyl isobutyl ketone).

3. Procedure

3.1 *Dry Ashing*

3.1.1 Weigh 1 g of dry, ground plant material into a porcelain evaporating dish.

3.1.2 Place in muffle furnace and ash at 485°C for 4 hours or overnight.

3.1.3 Cool, moisten with deionized water, and add 1 mL of HCl solution (see 2.1.1).

3.1.4 Transfer the sample to a 100-mL plastic volumetric flask using deionized water and a plastic stirring rod, make to volume, shake, and allow the silica to settle overnight.

3.1.5 Filter part of the sample through Whatman #40 filter paper into a plastic bottle for storage. Do not wash the filter paper or add water in this step.

3.1.6 Always run a blank. The final concentration of HCl in the sample is 0.1 *M.*

3.2 *Boron Determination*

Warning: This procedure must be performed in a good fume hood.

3.2.1 Pipet 1 mL of either sample, blank, or working standard into a 50-mL plastic Erlenmeyer flask.

3.2.2 Add 10 mL of acid mix (see 2.2.2).

3.2.3 Add 10 mL of curcumin reagent (see 2.2.1) and allow the color to develop for 10 minutes.

3.2.4 Add 30 mL of deionized water to stop color formation, cap the samples, and allow to cool for 45 minutes.

3.2.5 Transfer part of the top (organic) phase with a syringe pipet into a stoppered glass cuvette and determine the absorbance at 550 nm.

4. Remarks

4.1 Wet ashing with acids or dry ashing procedures that involve heating an acidic sample must not be used for boron determination. Boron is lost from acidic samples heated to temperatures greater than 55°C.

4.2 Since high temperature glassware contains B, porcelain, metal or plastic sample containers should be used in the ashing and B determination steps until the color is formed.

4.3 Organic removal and multiple stages of deionization may be necessary to secure a water

purity that will give acceptable blank values in this procedure.

4.4 Samples containing high levels of nitrate will require a separation step described by Wikner and Uppström that involves extraction of the B with 2,2-dimethyl-1,3-hexanediol in chloroform.

4.5 The methyl isobutyl ketone used in this procedure will attack many plastics. Erlenmeyer flasks that are not teflon will discolor on repeated use. Spectrometer flow cells that are fed by tubing pumps will not work with this solvent.

4.6 Methyl isobutyl ketone is a highly flammable liquid that may be absorbed into the body by inhalation and attacks the nervous system (Dutch Association of Safety Experts, 1980). This procedure must be carried out in a good fume hood.

4.7 Since curcumin is not soluble in water, it is necessary to rinse all glassware used in the procedure in an alcohol bath in the fume hood before cleaning with soap solution.

4.8 The modified dry ashing procedure may be used with AA or ICAP spectrophotometer for determination of other elements. However, for some samples, P determined by this ashing procedure will be lower than P determined using wet ashing with perchloric acid.

5. References

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Determination of Molybdenum in Plants

J. L. Sims and J. D. Crutchfield*

1. Principle of the Method

1.1 Molybdenum may be determined by measuring its catalytic effect on the reaction rate of the oxidation of KI by H_2O_2 in acid medium according to the equation:

$$2KI + H_2O_2 + 2H^+ \xrightarrow{Mo} 2H_2O + I_2 + 2K^+$$

If the reaction time is held constant and all other conditions are carefully adjusted, then changes in I_2 produced are a function of the concentration of the Mo catalyst. An automated continuous flow system is employed to colormetrically measure the quantity of I_2 produced when samples containing unknown concentrations of Mo are introduced.

1.2 The procedure was first developed and automated for the determination of Mo in geological and biological samples by Fuge (1970) and in plant materials by Bradfield and Strickland (1975) and Quin and Woods (1979). Significant improvements in sensitivity and reproducibility have been made in the method by Eivazi et al. (1982) and in the current procedure. The method has been used successfully for determination of Mo in tobacco *Nicotiana tabacum* L. and soybean *Glycine max* L. Merr. both in Kentucky and Tennessee (personal communication with G. L. Lessman).

2. Apparatus

2.1 Erlenmeyer flasks, 25-mL.

2.2 Muffle furnace.

2.3 Electric or steam hot plate.

2.4 Autoanalyzer System II or equivalent. See Fig. 1 for modules and configuration details.

2.5 Analytical balance.

2.6 Reagent dispensers, watch glasses, volumetric flasks, disposable sample cups, and assorted common laboratory glassware.

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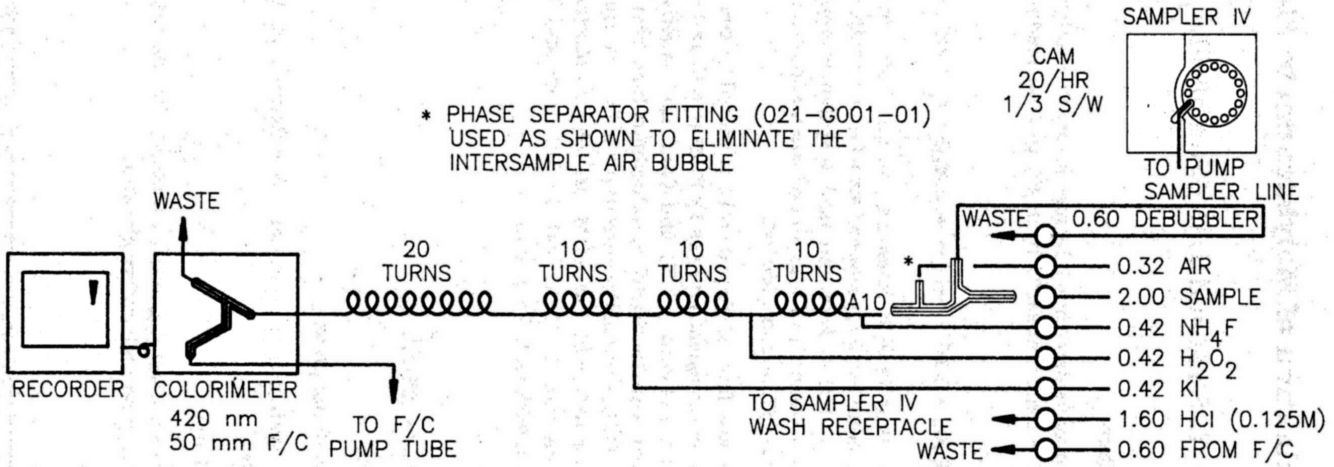

Figure 1. AutoAnalyzer configuration for Molybdenum analysis

3. Reagents and Solutions

3.1 Hydrochloric acid, 2 *M*.

3.2 Hydrogen peroxide, 30%.

3.3 Hydrochloric acid, 0.125 *M*[1]. Add 0.5 mL L^{-1} of Triton X-100.

3.4 Potassium iodide, 0.5% w/v. Add 0.5 mL L^{-1} of Triton X-100.

3.5 Hydrogen peroxide solution. Dilute 0.65 mL of 30% H_2O_2 to 1 L.

3.6 Ammonium fluoride solution, 0.25% w/v.

3.7 Molybdenum stock solution, 1000 mg Mo L^{-1}. Commercially available.

3.8 Working standards. See 5.1 and 5.2.

4. Procedure

4.1 Accurately weigh 0.5 g of dried plant material into carefully cleaned 25-mL Erlenmeyer flasks. Distribute each sample evenly over the bottom of the flask.

4.2 Dry ash the flasks in a muffle furnace for at least 4 hours at 500°C. Samples may be left longer if more convenient.

4.3 After cooling, add 1 mL of 30% H_2O_2, 4 mL of 2 *M* HCl and two glass beads to each flask. Prepare blanks with each set of samples.

4.4 Heat, uncovered, on a hot plate at approximately 80 °C until dry. Allow to cool.

4.5 Add 10 mL of 0.125 *M* HCl (3.3), cover and heat for 20 minutes at 80°C. Swirl flasks to insure dissolution of the sample.

4.6 Allow the samples to settle overnight before determining the Mo content. Carefully decant sufficient liquid into disposable sample cups for analysis on the Autoanalyzer.

4.7 Turn on the Autoanalyzer and pump the reagents until the baseline on the recorder is stable. Run the highest standard and adjust the peak heights to 75 to 80% of full scale, then begin the analysis. Blanks are run with each tray of samples. Keep the trays covered at all times.

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[1]A large quantity of this reagent will be consumed with each batch of samples. Prepare using freshly deionized water and keep tightly sealed at all times (see note 5.2).

4.8 In the current procedure, the timing cam on the Autoanalyzer Sampler IV has been changed from 30/hour, 1:2 (sample:wash) ratio to 20/hour, 1:3 ratio.[1] While daily output was reduced by 50%, baseline resolution between peaks on the recorder was achieved. This enabled much more accurate interpolation of the data. Even at the slower rate, an experienced operator can analyze more than 100 samples per day.

5. Calibration and Standards

5.1 Prepare a 10 mg Mo L^{-1} standard by pipetting 5 mL of the 1000 mg Mo L^{-1} commercial stock solution (3.7) into a 500-mL volumetric flask and diluting to volume with 0.125 *M* HCl solution (3.3).

5.2 Prepare working standards containing 0.04 to 0.20 mg Mo L^{-1} daily from the 10 mg L^{-1} standard, always using the same 0.125 *M* HCl solution. NOTE: It is imperative that the acid concentration of the samples, standards, and intersample wash solution be exactly the same. Any difference will result in a baseline offset that may cause undetectable error. Also, failure to rinse out the sampler wash receptacle with fresh 0.125 *M* HCl solution daily will cause unacceptable baseline drift.

6. Calculations

6.1 A plot of peak height of the standards vs. concentration will yield a sigmoidal curve which is nearly linear in the middle range. Very low samples can be approximated by dissolving the original dry sample in 10 mL of a middle range standard, then subtracting the value of that standard from the sample value read from the standard curve. High samples should be diluted to fall in the optimum range. If 0.5 g of tissue and 10 mL of acid is used, the mg L^{-1} readings from the standard curve should be multiplied by 20.

7. Remarks

7.1 *Range and Sensitivity*

7.1.1 The baseline rate of formation of iodine is a function of the concentration of KI, H_2O_2, and pH. The reagent concentrations used in this procedure are optimized to produce maximum linearity for the range between 0.04 and 0.20 mg L^{-1} and for the given Autoanalyzer configuration (see Fig. 1).

7.1.2 Concentration of Mo in plant materials will range from <0.1 mg kg^{-1} in plants grown on acidic soils heavily fertilized with ammonium-N to 25 mg kg^{-1} in plants grown on neutral or alkaline soils fertilized with Mo. The concentration of Mo may also vary greatly depending on whether the Mo fertilizer was applied broadcast (low), banded with the seed (high), or placed in transplant water near plant roots (high).

7.1.3 Sensitivity of the procedure is <0.1 mg kg^{-1} of Mo in plant material, using a 0.5-g plant sample.

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[1]The 20/hour, 1:3 timing cam was made by altering a 40/hour, 1:1 timing cam.

7.2 *Interference*

7.2.1 Iron (III) and various heavy metals such as Zr, Nb, Ta, Hf, Ti, V, Cr, and W have been shown to catalyze the oxidation of iodide by H_2O_2. However, most of these elements are present in plant materials in low concentrations. The addition of NH_4F to the system and a revised sample preparation protocol greatly reduced Fe interference and also that of Ti, V, Cr, and W (Quin and Woods, 1979).

Perchlorate salts may also interfere with the iodide-H_2O_2 reaction and cannot be quantitatively decomposed by drying. Thus, wet ashing techniques using perchloric acid should be avoided. A dry ashing technique is described (4.1).

7.2.2 High concentrations of Ca, Mg, K, and Na salts may affect the rate of iodide-H_2O_2 reaction. If excessive matrix variation between samples and standards is suspected, the magnitude of the associated error may be estimated by using the method of Single Standard Addition (see 7.3.1).

7.2.3 Excess amounts of phosphate, arsenate, oxalate, and tartrate may cause the catalytic action of molybdenum to disappear completely. Adding the H_2O_2 prior to KI in the automated system removed this effect (Eivazi et al., 1982). Little problem should be expected from phosphate in the concentration range common for plant material.

7.2.4 In the current procedure, the method of sample introduction into the Autoanalyzer has been modified to eliminate the mechanical interference associated with the large inter-sample air bubble (see Fig. 1).

7.3 *Precision and Accuracy*

7.3.1 The accuracy of the method depends on the extent to which the standard and sample matrices are matched. This can be estimated by using the Single Standard Addition Technique described below:

Extract and analyze several test samples using the current procedure. Note the shape of the standard curve and select those samples which have absorbance readings in the middle of the linear portion of the curve. Take two aliquots of each of these samples. To one add a quantity of Mo standard which will give a combined absorbance reading not above the linear portion of the curve. To the other aliquot, add the same volume of blank solution. Analyze the samples and calculate the corrected concentration (C_s) of each according to the equation:

$$\frac{C_s}{C_s + a} = \frac{A_s}{A_a} \quad OR \quad C_s = \frac{a * A_s}{A_a - A_s}$$

where C_s = Sample concentration,
A_s = Abs. of sample aliquot with blank added,
a = Weight of added Mo, and
A_a = Abs. of sample aliquot with Mo added.

A Comparison of the corrected values with those derived from the original standard curve should be a measure of error from the matrix effect.

7.3.2 Repeated analysis of the same plant sample for Mo should yield coefficients of variability of about 5%.

7.4 *Effect of Storage*

7.4.1 Samples may be stored for an indefinite period of any stage until the final 0.125 *M* HCl solution has been added, then they should be analyzed the next day.

7.4.2 Samples and solutions should be covered as much as feasible to avoid contamination from CO_2 in the air.

7.4.3 The KI and NH_4F reagents will keep for two or three days if covered. The peroxide should be prepared fresh daily.

7.5 Molybdenum values by this method have not been calibrated directly to crop response for any crop. However, when using tobacco and soybean tissues with varying levels of Mo, a good correlation ($r = 0.975^{**}$) existed between values by this method and those of the manual thiocyanate spectrophotometric procedure (Eivazi et al., 1982). Earlier, values by this manual procedure were calibrated to dry weight of tobacco after 40 days growth (green, oven-dry tissue) and at harvest (cured leaf tissue) (Sims et al., 1975; Sims and Atkinson, 1976). Maximum growth and cured leaf yields were associated with a Mo concentration of 0.40 :g Mo g^{-1} tissue in those studies. In another study (Hawes et al., 1976), maximum wheat and soybean seed yields were associated with Mo concentrations of 0.41 and 2.50 :g Mo g^{-1} seed, respectively, using the thiocyanate method.

7.6 As contrasted to the manual procedure (Eivazi et al., 1982), the advantages of this automated procedure are increased productivity (20 determinations of digested samples per hour), and lower skill and experience requirements.

8. References

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Determination of Molybdenum in Plant Tissue Using the Graphite Furnace

D. O. Wilson[*]

1. Principle

1.1 Flameless (graphite furnace) atomic absorption spectrophotometry can be used to determine Mo in digests of plant tissue (Henning and Jackson, 1973; Wilson, 1979). An aliquot of the plant digest is dried, charred, and atomized in a small electrically heated graphite sample tube. The thermally dissociated free atoms pass into a light beam from a hollow-cathode discharge lamp and the resulting absorption of light energy is measured and quantified in the same manner as conventional atomic absorption spectrometry. Analytical sensitivity is typically much greater with flameless compared to conventional atomic absorption due primarily to the longer residence times of free atoms in the light beam.

2. Reagent and Apparatus

2.1 *Instrument*

2.1.1 Atomic absorption spectrometer having a deuterium arc lamp for background correction and fitted with a graphite furnace having temperature ramp and purge gas interrupt capabilities.

2.1.2 Rapid response recorder to record the Mo absorption signal.

2.2 *Supplies*

2.2.1 Argon gas for purging.

2.2.2 Pyrolytic-graphite-coated sample tubes.

2.2.3 Micropipet- 25 :L size with disposable tips.

2.3 *Standard Solutions*

2.3.1 Standard solutions of Mo should be prepared in a matrix that is similar in composition to the diluted plant tissue digest. Use glass-distilled water and high purity reagents to prepare the matrix solution.

2.3.2 Dilute a concentrated commercial Mo standard solution with the matrix solution to 0, 2, 5, 10, 25, 50, 75, and 100 :g Mo L^{-1}.

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3. Procedure

3.1 *Plant Tissue Digestion*

3.1.1 Accurately weigh 0.5 g of plant tissue into preweighed borosilicate glass digestion tubes.

3.1.2 Add 3 mL 15 *M* HNO_3 and 2 mL 11 *M* $HClO_4$ and allow to digest overnight at ambient temperature.

3.1.3 Include a few tubes with each set of samples that receive acid additions only to use as digestion blanks.

3.1.4 Place tubes in heated aluminum block and digest until clear and approximately 0.5 mL of liquid remains in the bottom of the tubes. A convenient time-temperature regime is: 1 hour at 170 ^{o}C; 2 hour at 210 ^{o}C; 2 hour at 210 ^{o}C with small funnels in the tubes for refluxing.

3.1.5 Cool the digest and dilute to 10.5 g (10.0 mL) by adding glass-distilled water by weight.

3.1.6 Mix well and transfer to clean polyethylene capped vials.

3.2 *Instrument Setings*

3.2.1 Warm up and balance output of deuterium arc background correction lamp and purge optics as specified by manufacturer.

3.2.2 Set wavelength peak at 313.3 nm.

3.2.3 Set argon purge gas flow at 300 mL min^{-1} and stop flow for atomization.

3.2.4 Furnace time-temperature regime:

Activity	Temperature ^{o}C	Ramp Time Sec	Hold Time Sec
Dry	150	5	20
Char	1800	30	20
Cool	20	2	15
Atomize	2650	2	6
Clean out	2700	2	6

3.3 *Furnace Operation*

3.3.1 Transfer a 25-:L aliquot of solution to the furnace sample tube with a micropipet.

3.3.2 Initiate furnace time-temperature regime.

3.3.3 Record sample designation on peak produced on recorder.

3.3.4 Wait for furnace to cool before transferring the next aliquot to the sample tube.

3.4 *Standard Curves*

3.4.1 Prepare one standard curve by plotting corrected peak height (standard minus 0 Mo blank) for 0, 0.05, 0.125, and 0.25 ng Mo corresponding to 25-:L aliquots of 0, 2, 5, and 10 :g Mo L^{-1} standard solutions, respectively.

3.4.2 Similarly prepare a standard curve for 0, 0.25, 0.625, 1.25, 1.875, and 2.50 ng Mo corresponding to 25-:L aliquots of 0, 10, 25, 50, 75, and 100 :g Mo L^{-1} standard solutions, respectively.

3.5 *Analysis of Plant Tissue Digests*

3.5.1 From the standard curves, determine the ng of Mo in 25-:L aliquots of plant digests and digestion blanks.

3.5.2 If readings are above the highest standard, dilute digests with 0 Mo standard solution (simulated plant digest matrix solution).

3.6 *Calculations*

3.6.1 The equation below may be used to calculate the concentration of Mo in the plant tissue sample, provided that the digests are diluted to 10.0 mL and that 25-:L aliquots are taken for analysis

$$\text{Tissue Mo } (:g\ g^{-1}) = \frac{(\text{ng Mo in digest} - \text{ng Mo in digestion blank}) \times 0.4}{\text{tissue sample weight (g)}}$$

4. Remarks

4.1 *Precision and Sensitivity*

4.1.1 Replicate analyses using the procedure as described for plant material having a Mo concentration > 0.1 :g g^{-1} should give values having a coefficient of variability <10%. Precision decreases substantially with tissue values below 0.05 :g Mo g^{-1}. For the procedure as described, plant tissue Mo can be determined in the range of 0.05 to 2 :g Mo g^{-1}. Digests of tissue with greater than 2 :g Mo g^{-1} can be diluted as described previously.

4.1.2 The Mo concentration of standard orchard leaves (SRM 1571) was found to be 0.28 $\pm$ 0.01 :g Mo g^{-1} (Wilson, 1979) compared to a certified value of 0.3 $\pm$ 0.1 :g Mo g^{-1}. Recovery of Mo added to digests of plant tissue ranged from 95 to 105%.

4.2 *Interferences*

4.2.1 No interferences were reported for a similar procedure using dry-ashed plant samples (Henning and Jackson, 1973).

4.2.2 Perchloric acid digests have been shown to have interferences (Wilson, 1979). Compared to Mo solutions containing a simulated plant digest matrix, observed Mo values were higher when the macroelements, microelements, and/or perchloric acid were omitted. A rather wide range of each of these components can be tolerated, such that if Mo standard solutions contain a matrix that broadly approximates the plant digest matrix, excellent results can be obtained.

4.3 *Sample Combustion Tubes*

4.3.1 Standard (as opposed to platform) pyrolytic-graphite-coated sample tubes are recommended since Mo is best determined using wall atomization.

4.3.2 Perchloric acid or other constituents in plant digests may degrade the interior surface of sample tubes and cause a decrease in apparent Mo sensitivity with time. If this problem is severe, countermeasures are detailed by Wilson (1979).

5. References

Henning, S., and T. L. Jackson. 1973. Determination of molybdenum in plant tissue using flameless atomic absorption. Atom. Abs. Newsletter 12:100-101.

Wilson, D. O. 1979. Determination of molybdenum in wet-ashed digests of plant material using flameless atomic absorption. Commun. Soil Sci. Plant Anal. 10:1319-1330.

CONVERSION TABLE

Conversion Factors for SI and non-SI Units

To convert Column 1 into Column 2, multiple by	Column 1 SI Unit	Column 2 non-SI Unit	To convert Column 2 into Column 1, multiply by
	Length		
1.094	meter, m	yard, yd	0.914
3.28	meter, m	foot, ft	0.304
1.0	micrometer, : m (10^{-6} m)	micron, :	1.0
3.94 X 10^{-2}	millimeter, mm (10^{-3} m)	inch, in	25.4
10	nanometer, nm (10^{-9} m)	Angstrom Å	0.1
	Volume		
2.84 X 10^{-2}	liter, L (10^{-3} m^3)	bushel, bu	35.24
1.057	liter, L (10^{-3} m^3)	quart (liquid), qt	0.946
3.53 X 10^{-2}	liter, L (10^{-3} m^3)	cubic foot, ft^3	28.3
0.265	liter, L (10^{-3} m^3)	gallon	3.78
33.78	liter, L (10^{-3} m^3)	ounce (fluid), oz	2.96 x 10^{-2}
2.11	liter, L (10^{-3} m^3)	pint (fluid), pt	0.473
	Mass		
2.20 X 10^{-3}	gram, g (10^{-3} kg)	pound, lb	454
3.52 X 10^{-2}	gram, g (10^{-3} kg)	ounce (avdp), oz	28.4
2.205	kilogram, kg	pound, lb	0.454
0.01	kilogram, kg	quintal (metric), q	100
1.10 X 10^{-3}	kilgram, kg	ton (2000 lb), ton	907
1.102	megagram, Mg (tonne)	ton (U.S.), ton	0.907
1.102	tonne, t	ton (U.S.), ton	0.907
	Temperature		
1.00 (K - 273)	Kelvin, K	Celsius, ^oC	1.00 (^oC + 273)
(9/5 ^oC) + 32	Celsius, ^oC	Fahrenheit, ^oF	5/9 (^oF - 32)
	Concentrations		
1	centimole per kilogram, cmol kg^{-1}	milliequivalents per 100 grams, meq, 100 g^{-1}	1
0.1	gram per kilogram, g kg^{-1}	percent, %	10
1	milligram per kilogram, mg kg^{-1}	parts per million, ppm	1
	Plant Nutrient Conversion		
	Elemental	**Oxide**	
2.29	P	P_2O_5	0.437
1.20	K	K_2O	0.830
1.39	Ca	CaO	0.715
1.66	Mg	MgO	0.602