

Scope and Application

The method semi-quantitatively determines the concentration of Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Mo, Na, Ni, K, Se, Ag, Na, Th, Ti, V, and Zn in soil materials utilizing a nitric acid extraction/dissolution in conjunction with heating on a hot plate. This method closely follows that outline in EPA method 3050A. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Phosphorus, S, and B analyses require an ICP-AES with a vacuum spectrometer. Potassium, Ca, Mg, Na, Zn, Cu, Mn, and Fe can be analyzed by AAS or ICP-AES. Nitric acid digests may not provide 100% recovery of Al, Si, Fe, and Se. The method has a detection limit of approximately 0.01% for P, K, Ca, and Mg and 0.2 mg kg⁻¹ for B, Zn, Cu, Fe, Mn and Mo (sample dry basis). The method can also be used for the determination of trace elements (Co, Cd, Ni, Pb, etc.) and is generally reproducible within $\pm 7.0\%$ for all analytes.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.1 mg.
2. Hot plate system, capable of 150 °C.
3. Repipette dispensers, calibrated to 0.5 \pm 0.05 mL and 2.0 \pm 0.08 mL
4. Polypropylene or teflon digest beaker, 50 mL volume.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum or purged system.

Reagent

1. Deionized water, ASTM Type II grade.
2. Micro® clean detergent.
3. Concentrated nitric acid, trace metal grade, 12 N.
4. Concentrated Hydrochloric Acid, ACS reagent grade.
5. Standard Calibration solutions of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Th, Ti, V, and Zn. Prepare five multielement standards: of K, Ca, Mg ranging from 5-500 mg L⁻¹; P, S, and Na ranging from 1.0 - 100 mg L⁻¹; and B, Zn, Mn, Fe, Mo and Cu, ranging from 0.10-10.0 mg L⁻¹. Dilute standard calibration solutions with 5 % nitric acid.

Procedure

1. Weigh 1000 \pm 5.0 mg of air-dried soil pulverized to pass 20 mesh sieve (< 0.80 mm) soil material (See Comment #1, #2 and #3) and place in appropriate microwave vessel. For hydrocarbon contaminated soils use no more than 200.0 mg. Include a method blank.
2. Using repipettes add 9.0 \pm 0.1 mL of trace metal grade concentrated nitric acid and 3.0 \pm 0.1 mL of concentrated hydrochloric acid. Ensure that the sample is completely wetted by the reagents.
3. Place digestion beaker on hot plate at 120 °C for 4 hours and allow to digest.

6. Quantitatively transfer the sample to polypropylene labware. Samples containing suspended particulates will require centrifugation or filtering.
7. Quantitatively transfer the contents of the digestion vessel into the centrifuge tube, dilute to 15 mL final volume, cap centrifuge tube, invert three times and store (See Comment #5, #6 and #7).
8. Elemental analysis of soil digests can be made using atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES), or other methodologies. The method chosen will determine specific matrix modifications, calibration standards used, and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) may be facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L⁻¹.

Calculations

Report Elemental constituents to the nearest 3 significant digits as mg kg⁻¹:

$$\text{Analyte Content} = \frac{(\text{mg L}^{-1} - \text{method blank}) \times (30) \times (0.0001)}{\text{Sample Mass (mg)}}$$

Comments

1. Teflon PFA digestion vessel (liners) should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C. Do not brush containers to clean.
2. Sample material must be ground to pass a 20 mesh screen (< 0.40 mm opening), to ensure homogeneity.
3. Check repipette dispensing volume, calibrate using an analytical balance.
4. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.
5. Centrifuging may be necessary to clear the digest.
6. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.
7. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

Kalra, Y. P., D. G. Maynard, and F. G. Radford. 1989. Microwave digestion of tree foliage for multi-element analysis. Can. J. For. Res. 19: 981-985.

Kingston, H. M. and L. B. Jassie. 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Anal Chem. 58: 2534-2541.

Kingston, H. M. and Lois B. Jassie. 1988. Monitoring and predicting parameters in microwave dissolution, p. 97-148. *In*: M. H. Kingston and L. B. Jassie (ed.) Introduction to microwave sample preparation. American Chemical Society, Washington DC.

Meyer, G.A. and P.N. Keller. 1992. An overview of analysis by Inductively Coupled Plasma-Atomic Emission Spectrometry. *In*: Akbar Montaser and D.W. Golightly (ed.) Inductively Coupled Plasma-Atomic Emission Spectrometry. VCH Publishers Inc, New York,

Sah, R.N., and R.O. Miller. 1992. Spontaneous reaction for acid dissolution of biological tissue in closed vessels. *Anal. Chem.* 64: 230-233.

Soltanpour, P.N, G.W. Johnson, S.M. Workman, J.B. Jones and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry, p. 91-139. *In*: J.M. Bartel et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. 3rd. ed. ASA and SSSA, Madison, WI. Book series no. 5.

Stripp, R.A. and D. Bogen. 1989. The rapid decomposition of biological materials by using microwave acid digestion bomb. *J. Anal. Toxic.* 13: 57-59.

Scope and Application

The method semi-quantitatively determines the concentration of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Ti, V, and Zn in soil materials utilizing a nitric acid/hydrochloric acid extraction/dissolution in conjunction with microwave heating in closed teflon vessels. This method closely follows that outlined in EPA method 3051A. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Potassium, Ca, Mg, Na, Zn, Cu, Mn, and Fe can be analyzed by AAS or ICP-AES. Microwave nitric acid/hydrogen peroxide digests may not provide 100% recovery of all metals. The method has a detection limit of approximately 1.0 mg kg⁻¹ mg for Al, Ca, Fe, K, Mg, Na, P, and S; and 0.01 mg kg⁻¹ for Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Pb, Mn, Hg, Mo, Ni, Se, Ag, Na, Sr, Ti, V, and Zn (sample dry basis). The method is generally reproducible within $\pm 7.0\%$ for all analytes.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Microwave digestion system and teflon double wall digestion vessels equipped with a controlled pressure relief mechanism and temperature and/or pressure feedback control (See Microwave Calibration, Comment #1).
3. Repipette dispensers, calibrated to 0.5 ± 0.05 mL and 2.0 ± 0.08 mL
4. Polypropylene centrifuge tube with cap, 15 mL graduated.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum or purged system.

Reagent

1. Deionized water, ASTM Type II grade.
2. Micro® clean detergent.
3. Concentrated nitric acid, trace metal grade, 12 N.
4. Concentrated hydrochloric acid.
5. Standard Calibration solutions of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Ti, V, and Zn. Prepare five multi element standards: Dilute standard calibration solutions with 5% nitric acid.

Procedure

1. Weigh 500 ± 5.0 mg of air-dried soil pulverized to pass 20 mesh sieve (< 0.800 mm) soil material (See Comment #2, and #3) and place in appropriate microwave vessel. For oil contaminated soils use no more than 200.0 mg. Include a method blank.
2. Using repipettes add 9.0 ± 0.1 mL of trace metal grade concentrated nitric acid and 3.0 ± 0.1 mL concentrated hydrochloric acid (See Comments #4 and #5). Ensure that the sample is completely wetted by the reagents.
3. Place digestion vessel in outer body shell, cap and allow the sample and reagents to predigest for thirty (30) minutes.
4. Close vessel according to manufacturers directions and connect appropriate temperature and pressure sensors.

5. Method performance is designed to achieve a sample temperature of $175 \pm 5^\circ\text{C}$ for approximately 5.5 ± 0.25 minutes and remain at 175°C for 4.5 minutes for a total digestion time of ten (10) minutes. Adjust microwave temperature interlocks to achieve these desired limits. The pressure should peak between five (5) and ten (10) minutes for most samples. At the end of microwave digestion allow vessels to cool for five (5) minutes, remove and allow to cool to room temperature (optional - place in freezer to cool for thirty minutes).
6. Carefully uncap and vent each vessel in a chemical fume hood. Quantitatively transfer the sample to an acid-cleaned bottle. Samples containing suspended particulates will require centrifugation or filtering.
7. Quantitatively transfer the contents of the digestion vessel into the centrifuge tube, dilute to 15 mL final volume, cap centrifuge tube, invert three times and store (See Comment #8, #9 and #10).
8. Elemental analysis of soil digests can be made using atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES), or other methodologies. The method chosen will determine specific matrix modifications, calibration standards used, and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) may be facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1} .

Calculations

Report elemental constituents to the nearest 3 significant digits as mg kg^{-1} :

$$\text{Analyte Content} = \frac{(\text{Digest } \text{mg L}^{-1} - \text{Method Blank}) \times (\text{Final Digest Dilution Volume in Liters})}{(\text{Sample Mass (mg)}) \times 1000}$$

Comments

1. Microwave Calibration: Place 1.0 ± 0.1 kg of deionized water in teflon beaker and determine water temperature to the nearest $\pm 0.5^\circ\text{C}$. Microwave at 40% power for two (2.0 ± 0.01) minutes, vigorously stir solution for thirty (30) seconds and record temperature to $\pm 0.5^\circ\text{C}$. Repeat using successive microwave power settings of 50%, 60%, 70%, 80%, 90%, 95%, 100% with a fresh aliquot of deionized water each time. Absorbed power in watts (P) can be calculated using the change in temperature (ΔT according to Equation S-16.1-1):

$$P = (\Delta T) \times (34.86) \quad [\text{equ. S-16.1-1}]$$

Plot microwave calibration function of applied energy as a percent versus absorbed power in watts. Verify and check microwave calibration every three months.

2. Teflon PFA digestion vessel (liners) should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80°C . Do not brush containers to clean.
3. Check repipette dispensing volume, calibrate using an analytical balance.
4. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.
5. Some materials containing carbonates or organic matter may react violently and result in rupture seal failure. When digesting these materials reduce sample mass to 200 mg of sample material. If a vigorous reaction is noted, allow sample to predigest uncapped until reaction ceases.

6. Inspect vessel rupture seal in the cap for replacement.
7. Follow microwave manufacturer's instructions for microwave power calibration. Applying excessive microwave power may result in rupture seal or vessel failure.
8. Centrifuging may be necessary to clear the digest.
9. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.
10. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

Kingston, H. M. and L. B. Jassie. 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Anal Chem.* 58:2534-2541.

Kingston, H. M. and Lois B. Jassie. 1988. Monitoring and predicting parameters in microwave dissolution. p. 97-148. *In*: M. H. Kingston and L. B. Jassie (ed.) *Introduction to microwave sample preparation.* American Chemical Society, Washington DC.

Kingston, H. M. and L. B. Jassie, "Safety Guidelines for Microwave Systems in the Analytical Laboratory". *In* *Introduction to Microwave Acid Decomposition: Theory and Practice*; Kingston, H. M. and Jassie, L. B., eds.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1988.

Meyer, G. A. and P. N. Keller. 1992. An overview of analysis by Inductively Coupled Plasma-Atomic Emission Spectrometry. *In*: Akbar Montaser and D. W. Golightly (ed.) *Inductively Coupled Plasma-Atomic Emission Spectrometry.* VCH Publishers Inc, New York,

Soltanpour, P. N., G. W. Johnson, S. M. Workman, J. B. Jones and R. O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. p. 91-139. *In*: J. M. Bartels et al. (ed.) *Methods of soil analysis: Part 3 Chemical methods.* 3rd. ed. ASA and SSSA, Madison, WI. Book series no. 5.

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, 3rd ed; U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. U.S. Government Printing Office: Washington, DC, 1986; SW-846.

1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM, Philadelphia, PA, 1985, D1193-77.

Introduction to Microwave Sample Preparation: Theory and Practice, Kingston, H. M. and Jassie, L. B., Eds.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1988.

Kingston, H. M. EPA IAG #DWI-393254-01-0 January 1-March 31, 1988, quarterly Report.

Binstock, D. A., Yeager, W. M., Grohse, P. M. and Gaskill, A. Validation of a Method for Determining Elements in Solid Waste by Microwave Digestion, Research Triangle Institute Technical Report Draft, RTI Project Number 321U-3579-24, November, 1989, prepared for the Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC 20460.

Kingston, H. M., Walter, P. J., "Comparison of Microwave Versus Conventional Dissolution for Environmental Applications", *Spectroscopy*, vol. 7 No. 9, 20-27, 1992.

BOTANICAL SAMPLE METHODOLOGIES

DETERMINATION OF DRY MATTER CONTENT OF BOTANICAL MATERIALS P - 1.10

Gravimetric Moisture

Scope and Application

This method quantitatively determines the dry matter percentage in botanical materials based on the gravimetric loss of free water associated with heating to 105°C for a period of two hours. The method is destructive with respect to the sample. Dry matter fraction is used to correct the sample element concentration to an absolute dry matter basis. The method does not remove molecular bound water and is generally reproducible within $\pm 7\%$.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.001 g.
2. Aluminum weight dish with handle.
3. Drying oven, preheated to 105 °C.
4. Desiccator, containing a desiccating agent.

Procedure

1. Weigh approximately 2 g of air dry botanical sample material into a tared aluminum weigh pan (preweighed to nearest 0.001 g) and record moist sample weight to the nearest 0.001 g.
2. Place sample and weigh pan in drying oven for a minimum of two (2) hours.
3. Remove and place pan in desiccator for one (1) hour.
4. Weigh sample and pan on balance, weigh and record mass to nearest 0.001 g.
5. Dispose of sample (see comment #1).

Calculation

$$\text{Sample dry matter \%} = \left(1 - \frac{(\text{Sample moist wt.}) - (\text{sample dry wt.} - \text{pan tare wt.})}{(\text{Sample dry weight} - \text{pan tared weight})} \right) \times 100$$

Report dry matter content to the nearest 0.1 %.

Comment

1. Drying samples at 105°C may volatilize some carbon, nitrogen and sulfur compounds. Therefore, material used for moisture content should not be used for inorganic analysis.

Literature

Reuter, D.J., J.B. Robinson, K.I. Peverill and G.H. Price. 1986. Guidelines for collecting, handling and analyzing plant materials. p. 11-35
In: D.J. Reuter and J.B. Robinson (ed.) Plant analysis an interpretation manual. Inkata Press, Melbourne, Australia.

Smith, Morris and J. Benton Storey. 1976. The influence of washing procedure on surface removal and leaching of certain elements from trees. Hort. Sci. 14: 718-719.

Micro-Kjeldahl

Scope and Application

The Kjeldahl method quantitatively determines the amount of nitrogen (ammonium and protein) in botanical materials based on the wet oxidation of organic matter using sulfuric acid and digestion catalyst and conversion of nitrogen to ammonium (Issac and Johnson, 1976). Ammonium may be determined by distillation into boric acid and titration (Jones, 1989); spectrophotometric measurement (automated or manual); or diffusion-conductivity (Carlson, 1978). The method does not quantitatively recover nitrogen from heterocyclic rings (such as nicotinic acid) or from oxidized forms such as nitrate and nitrite. The Kjeldahl digest can be used for the determination of plant total phosphorus. The method is used to assess plant nitrogen sufficiency levels (Chapman and Pratt, 1961). The method detection limit is approximately 0.05% nitrogen (dry sample basis) and is generally reproducible within $\pm 8\%$.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.1 mg.
2. Acid fume hood and digestion heating block (400 °C).
3. Volumetric digestion tubes, 75 mL.
4. Repipette dispenser, calibrated 3.0 ± 0.1 mL.

Reagents

1. Deionized water, ASTM Type I grade.
2. Digest catalyst accelerator: prepared by mixing (100:10:1) 100 g potassium sulfate (K_2SO_4), 10 g anhydrous copper sulfate ($CuSO_4$), and 1.0 g selenium (Se) metal powder. This can be purchased as a prepared material under the brand name Kjel-tab, distributed by various chemical suppliers.
3. Concentrated sulfuric acid (H_2SO_4), reagent grade.
4. 30% hydrogen peroxide (H_2O_2); use fresh, as this material rapidly decomposes.
5. Standard calibration solutions of NH_4-N . Prepare six calibration standards ranging from 0.2 to 40.0 $mg L^{-1}$ concentration, diluted with 4% (v/v) sulfuric acid, prepared from 1000 $mg L^{-1}$ ammonium nitrogen standard solution.

Procedure

1. Determine the moisture content of the botanical material on a sub sample, Method P - 1.10.
2. Weigh 250.0 ± 5.0 mg of air dried botanical material (See Comment #1) and place in into a 75 mL volumetric digestion tube (50 ml or 100 mL digestion tubes may be substituted). Include a method blank.
3. Add Kjel-tab and 6.0 mL of concentrated sulfuric acid (See Comments #2 and #3).
4. Mix on a vortex stirrer fifteen (15) seconds to thoroughly wet the sample with acid. Note: it is essential that all dry sample material be completely moistened by acid and well mixed to insure complete digestion.
5. Place the digestion tube on a digestion block, preheated to 370°C for thirty (30) seconds or long enough to achieve complete botanical material breakup.
6. Remove from the digestion block and carefully (*slowly*) add 2-5 mL of 30% hydrogen peroxide in 1 mL increments to each digestion tube until digests begin to clear. Because this reaction takes place very rapidly, slow additions avoid excessive foaming.
7. Place the digestion tube back on the digestion block maintained at 370°C for two (2) hours. If excessive foaming occurs, remove from heat, cool two (2) minutes and add an additional 1-2 mL of hydrogen peroxide. At completion, a blue-green color may persist.
8. Remove samples from block and leave under fume hood for 5-10 minutes. Then add 10-20 mL of deionized water using a wash bottle to each tube to prevent hardening and crystal formation. Dilute digestion tubes to volume with deionized water, cap, and invert three times.