LABORATORY PROCEDURES

VIRGINIA TECH SOIL TESTING
AND
PLANT ANALYSIS LABORATORY

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INTRODUCTION

The procedures for soil analysis used in the Soil Testing and Plant Analysis Laboratory were established in the early 1950's. Although the chemical principles have not changed, procedures have been revised over the years to utilize advances in instrumentation which allow more accurate and rapid chemical determinations. The revised procedures are reported in this publication.

A routine test consisting of eleven separate analyses is performed on all samples. In addition, three separate tests are offered on a request basis. These tests are applicable only under certain conditions for which research and calibration work have been conducted. The routine and special tests consist of the following :

Routine Tests	Special Tests
pH	nitrate-nitrogen
phosphorus (P)	organic matter
potassium (K)	soluble salts
calcium (Ca)	
magnesium (Mg)	
zinc (Zn)	
manganese (Mn)	
copper (Cu)	
iron (Fe)	
boron (B)	
aluminum (Al) {reported on research samples only}	

SAMPLE PREPARATION

Soil samples are received in 1/2-pint cardboard cartons. Soil Sample Information Sheets (SSIS) are generally packaged with the samples. In a separate preparation area, the boxes are opened and placed in drying trays. Twenty-eight unknown samples plus two control samples are placed in each drying tray. The two control samples consist of one known reference sample plus either a blank or replicate sample. At this time, the sample is assigned a laboratory number which is stamped on the SSIS. Samples are numbered consecutively each calendar year, beginning with 1 on January 1.

The trays of samples are placed in a cross-flow forced air drying cabinet through which filtered air at room temperature is drawn. The air can be heated 5-8EC above ambient temperature for drying extremely wet samples. Samples remain in the drying cabinet overnight, or until air dry.

Rich, C.I., 1955. Rapid soil testing procedures used at Virginia Polytechnic Institute. Virginia Agr. Exp. Sta. Bull. 475, 8 p.

Air-dried (at 20-40EC) samples are crushed with a hammermill-type crushing machine and passed through a 10-mesh (2 mm opening) stainless steel sieve. The samples are then returned to the original sample boxes until the various subsamples are measured out.

ANALYSES - ROUTINE TESTS

pH DETERMINATION

Buffer Solutions: Color-coded buffer solutions of pH 4.0 and 7.0 are purchased from commercial sources. Alternatively, the pH 4.0 buffer can be prepared by dissolving 10.212 grams of potassium hydrogen phthalate ($HOCOC_6H_4COOK$) in 1 liter of deionized water.

Internal Filling Solution: Use Orion's 4*M* KCl, saturated Ag/AgCl, *SURE-FLOW* Reference Electrode Filling Solution, Cat. No. 900011. This clear filling solution works better than the recommended Cat. No. 610011 blue filling solution.

Procedure: From the prepared sample, scoop 10 cm^3 of soil into a 50 ml beaker. Add 10 ml of distilled water [for a 1:1 (v/v) ratio] with an automatic pipetting machine. Thoroughly mix the solution with a glass rod or mechanical stirrer and allow to sit for a minimum of 10 minutes and a maximum of 2 hours. Stir the solution again immediately before reading and while the probe is starting to equilibrate in the soil suspension. After stirring, quickly (within seconds) record the first stable pH to the nearest 0.1 pH unit.

Do a two-point calibration using fresh buffer solutions of pH 4 and 7 every 30 samples. Rinse the pH probe with distilled water between every sample; additionally, wipe or brush the end of the probe clean between samples of high clay content. If the probe response becomes sluggish or unstable, indicating that the reference junction has become contaminated, depress the electrode top cap. If that fails to correct the problem, the electrode can be disassembled and washed clean. After use, remove the reference filling solution. With distilled water, rinse the probe and replace the protective cap over the sensing surface with distilled water in it.

Note - For fine-textured soils containing a high level of organic matter it may be necessary to add an additional 20 ml of distilled water to make a suspension.
The solid state probe has an "on-chip" temperature sensor for automatic temperature compensation (ATC) at the precise point of pH measurement.

Determination of P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, Al

Extracting Solution (Mehlich No. 1 extractant - 0.05*N* HCl in 0.025*N* H_2SO_4):

Measure approximately 30 liters of deionized water into a 40-liter bottle. Add 28.0 ml of concentrated sulfuric acid (H_2SO_4) and 164.0 ml of concentrated hydrochloric acid (HCl) and make to 40-liter volume with deionized water.

Extraction Procedure:

One scoop (4 cc) of soil is measured into a 60 ml straight-walled plastic extracting beaker, and 20 ml of the Mehlich No.1 extracting solution is added with an automatic pipetting machine. The samples are shaken on a reciprocating shaker with a stroke length of 3.8 cm for 5 minutes at 180 oscillations per minute and filtered through Whatman No.2 (or equivalent), 11 cm filter paper.

Analysis Procedure:

All elements are analyzed in the same extract by an ICP (inductively coupled plasma spectrometer). Transfer of filtrate from the extraction procedure to the ICP autosampler trays is made by disposable polyethylene pipette. The transfer is a two-step procedure: the first aliquot is a rinse and the second aliquot is for the actual transfer. Pipette 4 ml of filtrate, discard into a waste beaker. Pipette another 4 ml of the same filtrate into the autosampler tray's polystyrene sample cups.

Once all sample filtrates have been transferred, cover autosampler tray with plastic wrap to prevent air borne contaminants (dust, lint, etc.) from getting into the solutions. This is very important to prevent ICP nebulizer clogging and contamination.

Samples may be stored overnight by covering with plastic wrap or capping and placing in a refrigerator. After refrigeration, samples should be allowed to equilibrate to room temperature before ICP analysis.

Elemental Analysis by ICP:

The ICAP (a simultaneous spectrometer), equipped with a Thermo Jarrell Ash autosampler, is set up to analyze approximately 30 samples for 10 elements every thirty minutes (35 seconds flush time between samples and a 4 second exposure). A quality control solution is read and verified after every tray of 30 samples.

ICP Working Standards:

The ICP is calibrated with the following series of standards (Note: atomic absorption standards are NOT pure enough for ICP standards; use only spectrally pure, plasma quality standards).

Soil #1: Final solution concentration: 0.048 *N* HCl.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 4 ml of concentrated reagent grade HCl, mix and dilute to volume with deionized water.

Soil #2: Final elemental concentration in solution: 1 $\mu g\ ml^{-1}$ Ca, 1 $\mu g\ ml^{-1}$ K, 1 $\mu g\ ml^{-1}$ Mg.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 4 ml of concentrated reagent grade HCl, mix, add 1 ml of 1000 $\mu g\ ml^{-1}$ Ca reference standard, add 1 ml of 1000 $\mu g\ ml^{-1}$ K reference standard, add 1 ml of 1000 $\mu g\ ml^{-1}$ Mg reference standard, and dilute to volume with deionized water.

Soil #3: Final elemental concentration in solution: 30 $\mu g\ ml^{-1}$ P, 2 $\mu g\ ml^{-1}$ Zn, 2 $\mu g\ ml^{-1}$ B.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 4 ml of concentrated reagent grade HCl, mix, add 30 ml of 1000 $\mu g\ ml^{-1}$ P reference standard, add 2 ml of 1000 $\mu g\ ml^{-1}$ Zn reference standard, add 2 ml of 1000 $\mu g\ ml^{-1}$ B reference standard and dilute to volume with deionized water.

Soil #4: Final elemental concentration in solution: 300 $\mu g\ ml^{-1}$ Ca, 100 $\mu g\ ml^{-1}$ K, 50 $\mu g\ ml^{-1}$ Mg, 100 $\mu g\ ml^{-1}$ Al, 10 $\mu g\ ml^{-1}$ Mn.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 4 ml of concentrated reagent grade HCl, mix, add 30 ml of 10,000 $\mu g\ ml^{-1}$ Ca reference standard, add 10 ml of 10,000 $\mu g\ ml^{-1}$ K reference standard, add 5 ml of 10,000 $\mu g\ ml^{-1}$ Mg reference standard, add 10 ml of 10,000 $\mu g\ ml^{-1}$ Al reference standard, add 10 ml of 1000 $\mu g\ ml^{-1}$ Mn reference standard, and dilute to volume with deionized water.

Soil #5: Final elemental concentration in solution: 10 $\mu g\ ml^{-1}$ Cu, 50$\mu g\ ml^{-1}$ Fe.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 4 ml of concentrated reagent grade HCl, mix, add 10 ml of 1000 $\mu g\ ml^{-1}$ Cu reference standard, add 50 ml of 1000 $\mu g\ ml^{-1}$ Fe reference standard and dilute to volume with deionized water.

ICP Quality Control Standard:

The quality control sample is prepared with spectrally pure, ICP quality standard reference solutions (Note: atomic absorption standards are NOT pure enough for ICP standards). To approximately 1000 ml of deionized water in a two-liter volumetric flask, add 8 ml of concentrated reagent grade HCl, mix and add the following amounts of each reference solution then mix and dilute to volume with deionized water:

Element	Final Concentration ($\mu g\ ml^{-1}$)	High Purity Reference Solution
P	10	2 ml of 10,000 $\mu g\ ml^{-1}$
K	50	10 ml of 10,000 $\mu g\ ml^{-1}$
Ca	200	40 ml of 10,000 $\mu g\ ml^{-1}$
Mg	50	10 ml of 10,000 $\mu g\ ml^{-1}$
Zn	1	2 ml of 1,000 $\mu g\ ml^{-1}$
Mn	10	20 ml of 1,000 $\mu g\ ml^{-1}$
Cu	1	2 ml of 1,000 $\mu g\ ml^{-1}$
Fe	5	10 ml of 1,000 $\mu g\ ml^{-1}$
B	1	2 ml of 1,000 $\mu g\ ml^{-1}$
Al	50	10 ml of 10,000 $\mu g\ ml^{-1}$

Calculation of Elemental Concentrations:

For each element, the calculation for ppm in soil is as follows:

ppm in solution * 4 = ppm in soil

where 4 is the dilution factor assuming a soil bulk density of 1.25 g/cc.

To convert from ppm to lbs/acre the equation is:

ppm in soil * 2 = lbs/acre

where the weight of an acre furrow slice (6⅔ in. depth) is assumed to be 2 million lbs.

ANALYSES-SPECIAL TESTS

ORGANIC MATTER

Reagent A: Sodium dichromate solution (0.67*M*): Dissolve 4 kg of reagent grade sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) in tap water to a volume of 20 liters.

Reagent B: Concentrated reagent grade sulfuric acid (H_2SO_4).

Procedure: The procedure is a modified Walkley-Black method. One scoop (1.5 cc) of soil is measured into a 200-ml test tube. Under a hood, 20 ml of Reagent A is added to the soil followed by 20 ml of Reagent B. The solution is allowed to cool at least 40 minutes. After cooling, 100 ml of water is added and the solution is mixed and allowed to stand overnight (or at least 8 hours). After incubation, an aliquot of the supernatant is withdrawn using a syringe-type pipette and transferred to a colorimeter vial. Readings are taken using a colorimeter equipped with a red filter (630 nm wavelength). The percentage of organic matter is determined by reference to Table 2.

Table 2. Colorimeter readings and percent organic matter for Virginia soils.[1]

Colorimeter Reading	Organic Matter, %	Colorimeter Reading	Organic Matter, %	Colorimeter Reading	Organic Matter, %
100	0.0	62	2.1	41	4.9
99-95	0.1	61	2.2	40	5.0
94-90	0.2	60	2.3	39	5.3
89-87	0.3	59	2.4	38	5.6
86	0.4	58	2.5	37	5.9
85-84	0.5	57	2.6	36	6.2
83	0.6	56	2.7	35	6.5
82	0.7	55	2.9	34	6.8
81	0.8	54	3.0	33	7.1
80-79	0.9	53	3.1	32	7.4
78	1.0	52	3.2	31	7.8
77-76	1.1	51	3.3	30	8.5
75	1.2	50	3.4	29	9.3
74-73	1.3	49	3.5	28	10.1
72-71	1.4	48	3.6	27	10.9
70-68	1.5	47	3.8	26	11.7
67	1.6	46	4.0	25	12.5
66	1.7	45	4.2	24-23	13.3
65	1.8	44	4.4	22	14.1
64	1.9	43	4.6	21	14.5
63	2.0	42	4.8	20	15.0

[1] Prepared from a curve found between colorimeter readings and organic matter determined by titration (Peach et al., 1947) in many Virginia soils.

NITRATE-NITROGEN

Reagent A: Extracting solution (0.02*N* $CuSO_4$): Dissolve 1.6 g of cupric sulfate anhydrous ($CuSO_4$) in deionized water in a 1-liter flask. Dilute to volume and mix well.

Reagent B: NO_3-N stock solution (1000 ppm N): Use a commercially prepared standard, such as ORION No. 920707. Alternatively, dissolve 6.061 g of sodium nitrate ($NaNO_3$) in 1 liter of deionized water.

Reagent C: Ionic Strength Adjustor (ISA): Use Orion's 2*M* $(NH_4)_2SO_4$ solution, No. 930711. Alternatively, prepare a 2*M* ammonium sulfate solution by dissolving 26.4 g ammonium sulfate, $(NH_4)_2SO_4$, in deionized water in a 100-ml volumetric flask.

Reagent D: Reference Electrode Filling Solution, outer chamber: Add 2 ml of ISA or a prepare 2*M* $(NH_4)_2SO_4$ solution to a 100-ml volumetric flask and dilute to volume with deionized water for a final concentration of 0.04*M* $(NH_4)_2SO_4$.

Reagent E: Reference Electrode Filling Solution, inner chamber: Use Orion's dark green double-junction electrode inner filling solution No. 90-00-02 which is saturated with AgCl.

Standards: NO_3-N Working Standards: To a 1-liter volumetric flasks add the indicated amounts of Reagent B and dilute to volume with Reagent A:

Standard No.	Reagent B, ml	NO_3-N in Standards, ppm
1	5	5
2	10	10
3	25	25
4	50	50
5	75	75
6	100	100

Procedure: One scoop (20 cc) of soil is measured into a 125 ml Erlenmeyer flask. Add 50 ml of Reagent A to the flask and shake for 10 minutes on a wrist-action shaker (at the fastest setting). Finally, filter the samples through Whatman No. 1 (or equivalent) filter paper. The NO_3-N in the standards and the extracts is determined using an ionalyzer equipped with a nitrate specific ion electrode assembly. The solutions are stirred at a constant rate while readings are taken. A standard curve is prepared by plotting millivolt reading versus ppm NO_3-N in solution. Nitrate-nitrogen in solution is read from the standard curve and ppm NO_3-N in soil is calculated with the following equation:

ppm NO_3-N in solution * 2.5 = ppm NO_3-N in soil.

Report as ppm NO_3-N in soil.

SOLUBLE SALTS

Reagent A: Use a commercially prepared NIST traceable conductivity standard of 1,000 µsiemens/cm.

or

Prepare potassium chloride standard solution (0.01 *N* KCl): Dissolve 0.7456 g of potassium chloride (KCl) in deionized water in a 1-liter volumetric flask. Mix well and dilute to volume.

Procedure: Measure one scoop (20 cc) of soil into a 50 ml beaker, add 40 ml of distilled water for a soil : water ratio of 1:2 (v/v). Stir the solution and allow the suspension to settle for at least 1 hour. The conductivity meter is checked against Reagent A. At 25EC, Reagent A has an electrical conductivity of 1.00 mmho/cm *or* 1.4118 mmho/cm. The electrical conductivity (EC) of the supernatant liquid of the soil-water solution is determined and the ppm soluble salts in soil are calculated from the following equation:

ppm soluble salts in soil = EC * 6.4 * 2

In this equation, EC represents the conductivity reading in mhos * 10^{-5}, 6.4 is the factor for converting the conductivity measurement to ppm soluble salts, and 2 represents the water volume dilution factor. Report as ppm soluble salts in soil.

Useful equations for using the units of mmhos/cm:

EC (mhos * 10^{-5}/cm) / 100 = mmhos/cm

ppm (mg salt/liter) / 1280 = mmhos/cm

1 dS/m = 1 mS/cm = 1 mmhos/cm

Resistance of a solution is the reciprocal of the electrical conductivity; therefore,

0.1 Φmhos = 10.0 Mohms

INTRODUCTION

Plant tissue analysis is an important procedure for evaluating nutrient status in plants, diagnostic purposes and environmental monitoring. By analyzing the appropriate plant part, such as the recently mature leaves, the nutrient status of plants can be determined. With a knowledge of a plants nutrient status optimal growing conditions can be established and maintained. If there is a problem with plant growth, either in natural environments or artificial environments, the diagnostic capabilities of plant tissue analysis can be used. The analysis can detect elemental deficiencies and toxicities. Once the problem is known remedial action can be taken. Where wastes are applied to land, tissue analysis can be used to monitor the effects of waste additions on the elemental status of the plants. Consequently, plant tissue analysis is an important analytical tool to help maintain the quality of our land, food sources, fiber, forage, forests and horticultural plants. The analyses performed by the Soil Testing and Plant Analysis Laboratory are as follows:

Routine Test

Calcium (Ca)	Aluminum (Al)
Magnesium (Mg)	Boron (B)
Nitrogen (N)	Copper (Cu)
Phosphorus (P)	Iron (Fe)
Potassium (K)	Manganese (Mn)
Zinc (Zn)	

SAMPLE PREPARATION

Tissue samples are received in paper bags and envelopes. Plastic containers should not be used for mailing due to tissue rotting in transit. Plant analysis information sheets are packaged with the samples. The samples are assigned a laboratory number and dried at 70E C for 24 hours or longer if necessary. Samples are numbered consecutively each calendar year, beginning with 1 on January 1.

Dried samples are ground in a stainless steel Wiley Mill to pass a 20-mesh sieve. Each sample is then mixed and stored in 30 ml glass vials with air-tight plastic stoppers.

Note - Plant samples awaiting grinding should either be left in the oven with heat on or stored in a desiccator until ground. This is essential to prevent absorption of moisture by the dried plant material.

DETERMINATION OF Ca, K, Mg, P, Al, B, Cu, Fe, Mn, and Zn

A dry ashing procedure for preparing plant tissue samples for analysis was selected over wet ashing procedures. The dry ash procedure is accurate, minimizes the use of environmentally dangerous chemicals, is relatively safe for laboratory personnel and is cost effective.

The laboratory uses a simultaneous ICP (inductively coupled plasma spectrometer) to measure

macronutrients (essential elements with percent concentrations in tissue) and micronutrients (essential elements at ppm concentrations in tissue) in the same solution. Therefore, plant tissue analysis consists of preparing the tissue to a solution which can be analyzed by ICP. Heat is used to volatilize the organic components and the resulting ash is dissolved by acid and filtered. The filtrate is then analyzed for its elemental concentration. Not all elements, such as As, Cd, N, S, and Se, can be analyzed this way since they are volatile and are lost, along with the organic components, in the ashing process.

Dry Ashing Procedure:

1) Weigh 1.000 g ∀ 0.010 g of dried, ground plant tissue into a 50 ml ceramic crucible. Record weight. Cover with a ceramic lid.

2) Ash samples at 475EC for 5 hours by placing samples in a cool muffle furnace with timer set for 6 hours, which allows one hour for the furnace to equilibrate.

3) After ashing, remove beakers and allow to cool. Dissolve the ash by adding 5.0 ml of concentrated HCl directly to each beaker. Allow to stand for 30 minutes, then add 10.0 ml of deionized water and allow to stand for 20 minutes. Finally, add 35.0 ml of deionized water to give a final volume of 50.0 ml and a 1.2 N HCl matrix. Filter through Whatman No. 42 (or equivalent) ashless filter paper. The filtrates are now ready for elemental determination.

Analysis Procedure:

All elements are analyzed in the same extract by an ICP (inductively coupled plasma spectrometer). Transfer filtrate from the dry ashing procedure to the ICP autosampler trays by pipette. The transfer is a two step procedure, the first aliquot is a rinse and the second aliquot is for the actual transfer. Set pipette to deliver 4 ml of solution. Pipette 4 ml of filtrate, discard into a waste beaker. Pipette another 4 ml of the same filtrate into the autosampler tray's polystyrene sample cups.

Once all sample filtrates have been transferred, cover autosampler tray with plastic wrap to prevent air borne contaminants (dust, lint, etc.) from getting into the solutions. This is very important to prevent ICP nebulizer clogging and contamination.

Samples may be stored overnight by covering with plastic wrap or capping and placing in a refrigerator. After refrigeration, samples should be allowed to equilibrate to room temperature before ICP analysis.

Elemental Analysis by ICP:

The ICAP (a simultaneous spectrometer) equipped with a autosampler, is set up to analyze approximately 30 samples for 10 elements every 30 minutes with a 5 second exposure. A blank and a control sample are analyzed with each set of plant samples.

ICP Working Standards:

The ICP is calibrated with the following series of standards (Note: atomic absorption standards are NOT pure enough for ICP standards; use only spectrally pure, plasma quality standards).

Plant #1: Final solution concentration of 1.2 *N* HCl.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 100 ml of concentrated reagent grade HCl, mix and dilute to volume with deionized water.

Plant #2: Final elemental concentration in solution: 400 μg ml^{-1} P, 10 μg ml^{-1} Zn, 10 μg ml^{-1} B.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 100 ml of concentrated reagent grade HCl, mix, add 400 ml of 1000 μg ml^{-1} P reference standard, add 10 ml of 1000 μg ml^{-1} Zn reference standard, add 10 ml of 1000 μg ml^{-1} B reference standard, and dilute to volume with deionized water.

Plant #3: Final elemental concentration in solution: 100 μg ml^{-1} Al, 100 μg ml^{-1} Fe.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 100 ml of concentrated reagent grade HCl, mix, add 100 ml of 1000 μg ml^{-1} Al reference standard, add 100 ml of 1000 μg ml^{-1} Fe reference standard and dilute to volume with deionized water.

Plant #4: Final elemental concentration in solution: 1000 μg ml^{-1} Ca, 1000 μg ml^{-1} K, 100 μg ml^{-1} Mg, 10 μg ml^{-1} Mn, 10 μg ml^{-1} Cu.

To approximately 500 ml of deionized water in a one liter volumetric flask, add 100 ml of concentrated reagent grade HCl, mix, add 100 ml of 10,000 μg ml^{-1} Ca reference standard, add 100 ml of 10,000 μg ml^{-1} K reference standard, add 100 ml of 1000 μg ml^{-1} Mg reference standard, add 10 ml of 1000 μg ml^{-1} Mn reference standard, add 10 ml of 1000 μg ml^{-1} Cu reference standard, and dilute to volume with deionized water.

CALCULATION OF ELEMENTAL CONCENTRATIONS

For all elements determined by the dry ash procedure the calculations of elemental concentrations in the plant tissue are as follows:

$$\frac{\mu g\ ml^{-1} \text{ in solution} * 50}{\text{weight of sample, g}} = \mu g\ ml^{-1} \text{ in plant}$$

$$\mu g\ ml^{-1} * 0.0001 = \%\ \text{concentration}$$

The plant macronutrients (Ca, K, Mg, and P) are reported as percent concentrations in tissue and the micronutrients (B, Cu, Fe, Mn, and Zn) plus aluminum (Al) are reported as $\mu g\ ml^{-1}$ ppm concentration in tissue.

TOTAL NITROGEN DETERMINATION

A micro - kjeldahl method is employed for total nitrogen determination using a custom-made heating block for the wet digestion of the plant tissue. Distillation is performed with a Kjeltec System 1004 Steam Distillation Unit.

Bulk Reagents: Sulfuric Acid, concentrated reagent grade (H_2SO_4).
Salt/Catalyst Mix - Pope Kjeldahl Formula #1 (9.9 g K_2SO_4 + 0.41 g HgO + 0.08 g $CuSO_4$ + 0.51 g Pumice).

Reagent A: Alkali Solution, (NaOH - $Na_2S_2O_3 \cdot 5\ H_2O$): Add 160 g sodium thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$) to 4 liters 50% (w/w) sodium hydroxide (NaOH) solution. Set Kjeltec unit to deliver 15 ml of alkali solution.

Reagent B: Methyl red - methylene blue mixed indicator:

Red solution: Dissolve 0.7 g of methyl red into 410 ml (335 g) of 95% (190 proof) ethanol.

Blue solution: 0.343 g in 0.2% methylene blue in 210 ml (171 g) of 95% ethanol. This solution has a shelf life of one month, therefore it must be freshly prepared each month.

Mixed solution: Mix 200 ml of the red solution with 100 ml of the blue solution (2:1 ratio of red to blue). This solution has a shelf life of one month, therefore it must be freshly prepared each month. The solution is purple in color.

Reagent C: Boric Acid Solution: To approximately 200 ml of deionized water in a 1 liter volumetric flask, add 20.0 g of boric acid (H_3BO_3) and 60 ml of the Mixed solution and dilute to volume with deionized water.

Reagent D: Potassium Biiodate Titrant Solution (0.05*N*): To approximately 200 ml of deionized water in a 1 liter volumetric flask, add 19.497 g of potassium biiodate [$KH(IO_3)_2$] and dilute to volume with deionized water. Mix with a magnetic stirrer until completely dissolved.

Reagent E: Sodium Sulfate Solution: Place 0.100 g of sodium sulfate ($Na_2SO_4 \cdot 10\ H_2O$) in a 50 ml beaker. Dissolve in 20 ml of tap water. This is added to the steam generators of the distillation unit when they are cleaned or rinsed to increase the electrical conductivity of the water.

Sample Digestion:

1) Weigh out 0.200 g $\forall$ 0.010 g of dried, ground plant tissue into a 75 ml Kjeltec test tube. Record weight.

2) Add 1.5 g of salt/catalyst mix to each test tube using a Pope Kjeldahl dispenser and mix well.

3) Add 2.5 ml concentrated H_2SO_4 to the test tube using a repipet. This procedure should be done under a hood. Swirl test tubes to mix tissue, salt/catalyst, and acid, or allow to stand overnight.

4) Place test tubes into cold heating block and turn on thermostat. Set temperature at 400EC and heat samples until they clear, then digest for 30 minutes at 400EC operating temperature (approximately 75 minutes). When the sample clears, it turns from a yellow/brown color to light aqua.

5) Remove test tubes from block immediately after digestion and allow to cool under fume hood.

Distillation:

1) Add 25 ml of Reagent C (boric acid solution) to the receiving flasks. Use a 25 ml repeater pipet to dispense boric acid solution.

2) Place a test tube containing digested sample onto each distillation unit using gloves. The steam nipple should be inside test tube and platform should be in released position such that the spring tension firmly holds test tube in place.

3) Place a receiving flask on each platform and move platforms to upper position. The glass tube outlets should be below the liquid surface.

4) Press the left "Ready/Start" button.

5) Wait several seconds and then press the right "Ready/Start" button.

6) As soon as a sample distillation is complete (about 4 min.) the corresponding green "Ready/Start" button lights up and the unit is ready for the next set of samples.

7) Remove the receiving flasks (which now contain the nitrogen to be measured) for titration. The solution in the receiving flasks is usually green at this point. Remove test tubes and place in the rack using gloves.

8) Repeat steps 1-7 until all samples are distilled.

Note - To avoid excessive cooling of the steam generators, the Kjeltec Unit should not be allowed to remain idle for more than 5 minutes between distillations.

Titration:

Receiving flasks from the distillation are used for titration.

1) Fill the burette with 0.05 N potassium biiodate acid to the 0 mark.

2) Add a magnetic stirring rod to the receiver flask and place on a magnetic stirrer.

3) Titrate to the lilac endpoint which will occur immediately after a greyish-blue color appears. Record the volume of acid to the nearest 0.1 ml.

4) Repeat steps 1-3 for each sample.

Generator Cleaning:

Clean with 25% Acetic Acid Solution overnight. To make a 25% Acetic Acid Solution - to approximately 200 ml tap water in a 500 ml graduated cylinder, add 125 ml conc. acetic acid and dilute to volume with tap water.

Calculation of N Concentrations:

1.00 ml of 0.05 *N* $KH(IO_3)_2$ = 700 μg N at the equivalence point

$$\frac{\mu g\ N}{ml} * \frac{ml\ KH(IO_3)_2}{sample\ wt} = \frac{\mu g\ N}{g\ plant\ material}$$

$$\frac{\mu g\ N}{g} = ppm$$

ppm * (0.0001) = % N

For % N in a 0.200 g sample, multiply ml of $KH(IO_3)_2$ used by 0.35.

For % N in a sample with variable weight, use :

$$\frac{0.07 * Y\ ml\ KH(IO_3)_2}{sample\ wt.,\ g} = \%\ N$$

INSTRUMENTS FOR SOIL AND PLANT ANALYSES

Soil Analysis

Analysis	Instrument
pH	ATI Orion Model 620 Digital pH meter
pH electrode	ATI Orion Model 616500, SURE-FLOW™ Solid State *pHUTURE*™ Probe
P, K, Ca, Mg, Zn, Mn, Cu, Fe & B	Thermo Jarrell Ash ICAP 61 (Inductively Coupled Argon Plasma Atomic Emission Simultaneous Spectrometer) Equipped with a TJA-300 autosampler
Organic Matter	Cenco Colorimeter
Nitrate-Nitrogen	Orion SA 720 ionalyzer with an Orion Nitrate Specific Ion Electrode
Soluble Salts	ORION model 160 conductivity meter with an ORION 016010 conductivity cell
Extraction - NO_3-N	Burrel Wrist-Action Shaker
Extraction - P, K, Ca, Mg, Zn, Mn, Cu, Fe & B	Eberbach Reciprocating, Variable Speed Shaker No. 6000
Soil Grinding	Custom Lab Equipment DC-1 HD Dynacrush
Soil Drying	Cross-flow forced air soil drying cabinet, developed at VPI&SU

Plant Analysis

Analysis	Instrument
Nitrogen	Tecator Kjeltec System 1004 Distilling Unit
Ca, K, Mg, P, Al, B, Cu, Fe, Mn, Zn	Thermo Jarell Ash ICAP 61 (Inductively Coupled Argon Plasma Atomic Emission Simultaneous Spectrometer)
Drying	Blue-M Power-O-Matic 70 Oven
Grinding	Thomas Wiley Mill Model ED-5
Ashing	Thermolyne F-A1730 Muffle Furnace

ICP PARAMETERS

The ICP is housed in an instrument room which is temperature controlled to 21EC (70EF) plus or minus 1EC (2EF) and is dehumidified. Both temperature and humidity affect the analytical results. To enhance precision the solutions are introduced to a cross flow nebulizer with a peristaltic pump. A mass flow controller was added to the ICP to improve the stability of the sample argon flow and, thereby, the reproducibility of the results. The ICP is profiled using the Hg wavelength.

The following analytical lines are used:

Element	Wavelength (nm)	Physical Channel Number	Analytical Range ($\mu g\ ml^{-1}$)
P	214.914	38	0.06 - 250
K	766.490	24	0.3 - 1000
Ca	373.690	43	0.1 - 1500
Mg	279.079	14	0.01 - 350
Zn	213.856	33	0.004 - 150
Mn	257.610	37	0.001 - 150
Cu	324.754	4	0.002 - 150
Fe	259.940	12	0.005 - 150
B	249.678	11	0.006 - 150
S	182.04	7	0.1 - 500
Na	588.995	16	0.01 - 200
Li	670.784	20	0.006 - 150
Al	396.153	41	0.025 - 500
Al	308.215	17	1.0 - 5000
Hg	546.074	13	Monitor

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