LABORATORY PROCEDURES

SOIL TESTING AND PLANT ANALYSIS LABORATORY

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SOIL AND PLANT ANALYSIS

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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 in recent 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 5 separate analyses is performed on all samples. In addition, 5 special 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 Test	Individual Special Tests
pH	zinc
phosphorous	manganese
potassium	soluble salts
calcium	nitrate-nitrogen
magnesium	organic matter

SAMPLE PREPARATION

Soil samples are received in 1/2-pint paper cartons. Soil sample information sheets are generally packaged with the sample. In the laboratory, the boxes are opened and placed in drying trays. Twenty-nine regular samples plus one control sample are placed in each drying tray. At this time, the sample is assigned a laboratory number which is stamped on the soil information sheet. Samples are numbered consecutively each calendar year, beginning with 1 on January 1.

The trays of samples are placed in a drying cabinet through which filtered air at room temperature is drawn. The air can be heated to 5-8 C. above room temperature for drying extremely wet samples. Samples remain in the drying cabinet overnight.

Dried samples are crushed with a hammermill-type crushing machine and passed through a 10-mesh (2-mm opening) stainless steel sieve. The crushed and sieved samples are placed in the original sample boxes to await measuring of subsamples for the various analyses.

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

The Soil Testing and Plant Analysis Laboratory is certified by the USDA to accept samples from quarantined areas for analysis. Samples from these areas are processed in accordance with USDA-Virginia pest quarantine regulations. Sterilization of soil samples from quarantined areas is performed in a prescribed manner after the necessary chemical analyses are performed.

ANALYSES-ROUTINE TEST

pH

From the prepared sample, one scoop (20 ml) of soil is measured into a 50-ml beaker. Twenty milliliters of distilled water are added [for a 1:1 (v/v) ratio]. The solution is stirred and allowed to sit for 15 minutes, but no longer than 2 hours. Immediately before reading, the solution is stirred again. A pH meter with a glass electrode assembly is then used for the determination. The pH meter is standardized with buffer solutions of pH 4.0 and 7.0 after each 15 determinations.

P, K, Ca, Mg - Extraction

Extracting Solution (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 H_2SO_4 and 164.0 ml of concentrated HCl and make to 40-liter volume with deionized water.

Extraction Procedure: One scoop (4 ml) of soil is measured into a 60-ml straight-walled plastic extraction beaker, and 20 ml of dilute HCl-H_2SO_4 extracting solution is added with an automatic pipetting machine . The samples are shaken on a mechanical shaker with a stroke length of 3.8 cm for 5 minutes at 180 oscillations per minute and filtered through Whatman No. 1 filter paper. The P, K, Ca, and Mg in solution are determined by the following procedures.

P Determination

Reagent A: Complexing-stabilizing stock solution: Dissolve completely 100 g of ammonium molybdate [$(NH_4)_2MoO_4$] in approximately 500 ml of deionized water in a 2-liter volumetric flask. Dissolve 2.425 g of antimony potassium tartrate [$K(SbO)C_4H_4O_4.1/2H_2O$] in the molybdate solution. Place the flask in a cold water bath and slowly add 1400 ml concentrated H_2SO_4. Mix well, cool, and make to volume with deionized water. Store in a polyethylene bottle in a

dark, cool compartment.

Reagent B: Stock reducing solution: Dissolve 176.0 g of L-Absorbic acid powder in approximately 500 ml of deionized water in a 2-liter volumetric flask. Dilute to volume with deionized water. Mix well and store in a dark bottle in a cool compartment.

Reagent C: Working solution: Dilute 20 ml of Reagent A and 10 ml of Reagent B to 1 liter with the extracting solution. Prepare fresh daily. Allow to stand at least 2 hours before adding to soil extracts. This solution is enough for approximately 25 determinations (including excess solution for rinsing of diluter). For a greater number of determinations mix reagents using the same ratio.

Reagent D: Phosphorous standard stock solution (1000 ppm P): Dissolve 4.394 g of potassium phosphate (KH_2PO_4) to approximately 500 ml of deionized water in a 1 liter volumetric flask. Bring to volume with deionized water.

P Working Standards

In 1-liter volumetric flasks, place the following amounts of Reagent D and dilute to volume with extracting solufion:

Std No.	Reagent D, ml	P in Solution ppm
1 (0 P)	0	0
2 (50 P)	5.0	5.0
3 (100 P)	10.0	10.0
4 (150 P)	15.0	15.0

Procedure: One ml of standards and extracts is diluted with 24 ml of Reagent C. After allowing 20 minutes for color development, the P in solution is determined with a spectrophotometer equipped with a direct concentration readout mode. The instrument is adjusted to 0 ppm P (0 Abs, 100% T) with Standard 1 and to 15 ppm P with Standard 4. The slope of the line is automatically set in the instrument (For instruments without this feature, a standard curve of %T vs. P, lb/A is usually prepared). Phosphorous in the soil extracts is read directly from the instrument.

K Determination

Reagent A: Potassium standard stock solution (1,000 ppm K): Dissolve 1.912 g of oven-dried KCl in 1 liter of extracting solution.

Reagent B: Lithium stock solution (1,500 meq/L): Instrumentation Laboratories Standard No. 35000.

Reagent C: Lithium working solution (18.75 meq/L): Dilute 12.5 ml of Reagent B to 1 liter with extracting solution. For a larger quantity, dilute 250 ml of Reagent B to 20 liters with deionized water.

Reagent D: Potassium 0 standard (0 ppm K): Extracting solution.

Reagent E: Potassium 50 standard (20 ppm K): Dilute 20 ml of Reagent A to 1 liter with extracting solution.

Reagent F: Potassium 100 standard (40 ppm K): Dilute 40 ml of Reagent A to 1 liter with extracting solution.

Procedure: Two ml aliquots of Reagents D, E, F and of the soil extracts are diluted with 8 ml of Reagent C. The K in the standards and extracts are determined with a flame photometer. The instrument is adjusted to zero with the diluted Reagent D, and to 100 with the diluted Reagent F. The diluted Reagent E should read 50. A standard curve is then prepared for scale reading versus lb/acre of K. Potassium in the soil extracts is determined using this standard curve.

Ca, Mg Determination

Reagent A: Lanthanum chloride diluting solution: Mix 161.0 g of lanthanum oxide (99.99% La2O3) in approximately 125 ml of deionized water in a 1-liter volumetric flask. Mix well. Place the flask in a cold water bath and SLOWLY add 250 ml of concentrated HCl. Swirl constantly as small amounts of acid are added to the flask. Make the solution to volume with deionized water and mix well. Dilute to 20 liters with deionized water for the working solution.

Reagent B: Calcium standard stock solution (10,000 ppm Ca): Fisher 10,000 ppm calcium solution.

Reagent C: Magnesium standard stock solution (10,000 ppm Mg): Fisher 10,000 ppm magnesium solution.

Reagent D: Calcium - magnesium standard solution (Ca-0 ppm; Mg-0 ppm): Extracting solution.

Reagent E: Calcium - magnesium standard solution (Ca-100 ppm; Mg-10 ppm): Dilute 10 ml of Reagent B and 1 ml of Reagent C to 1 liter with extracting solution.

Reagent F: Calcium - magnesium standard solution (Ca-200 ppm; Mg-20 ppm): Dilute 20 ml of Reagent B and 2 ml of Reagent C to 1 liter with extracting solution.

Reagent G: Calcium - magnesium standard solution (Ca-300 ppm; Mg-30 ppm): Dilute 30 ml of Reagent B and 3 ml of Reagent C to 1 liter with extracting solution.

Procedure: One ml aliquots of Reagents D, E, F and G and of the soil extracts are diluted with 9 ml of Reagent A. The Ca and Mg in the standards and extracts are determined with an atomic absorption spectrophotometer. The instrument is adjusted to zero with Reagent D and to 100 with Reagent G. Reagents E and F should read 33 and 67, respectively. Standard curves are then prepared for scale reading versus lb/acre of Ca and Mg. Calcium and Mg in the soil extracts are determined using these standard curves.

ANALYSES-SPECIAL TESTS

Zinc

Reagent A: Extracting solution (EDTA-ammonium carbonate): Dissolve 2.9225 g of EDTA and 114.10 g of ammonium carbonate in approximately 800 ml of deionized water. Adjust the pH of this solution to pH 8.6 with HCl or NH4OH as needed. Make the solution to a 1-liter volume with deionized water. Final solution is 0.01 M EDTA and 1.0M ammonium carbonate. Keep covered as much as possible.

For 20-liter quantity dissolve 58.4500 g of EDTA and 2,282.0 g of ammonium carbonate in approximately 16 liters of water. Mix well. Remove 100 ml of this solution and determine amount of HCl or NH4OH needed to adjust the solution to pH 8.6. Multiply this amount by 199 and add the acid or base to the container. Mix well, resample and check pH. Make any correction needed and make solution to volume with deionized water.

Reagent B: Zinc standard stock solution (1,000 ppm zinc): Dissolve 4.3478 g of ZnSO4.7H2O in one liter of Reagent A.

Reagent C: Diluted zinc stock solution (100 ppm Zn): Dilute 10 ml of Reagent B to 100 ml with Reagent A.

Zn Working Standards

In 500-ml volumetric flasks, place the following amounts of Reagent C, and dilute to volume with Reagent A.

Std. No.	Reagent C, ml	Zn in Solution, ppm
1	0.0	0.0
2	2.0	0.4
3	2.5	0.5
4	3.0	0.6
5	5.0	1.0
6	10.0	2.0
7	15.0	3.0

Procedure: Measure one scoop (10 ml) of soil into a 60-ml plastic extraction beaker. Add 20 ml of Reagent A with an automatic pipetting machine. Shake on a mechanical shaker with a stroke length of 3.8 cm for 5 minutes at 180 oscillations per minute. Filter through Whatman No. 42 filter paper and determine Zn in the standards and extracts with an atomic absorption spectrophotometer. Report as ppm Zn in soil.

ppm Zn in solution x 2 = ppm Zn in soil.

Manganese*

Reagent A: Extracting solution (0.05 N HCl and 0.025 N H_2SO_4): Measure approximately 15 liters of deionized water into a 20-liter bottle. Add 14.0 ml of concentrated H_2SO_4 and 82.0 ml of concentrated HCl and make to 20-liter volume with deionized water.

Reagent B: Manganese standard stock solution (1,000 ppm Mn): Dissolve 3.076 g of $MnSO_4.H_2O$ in one liter of Reagent A.

Reagent C: Diluted manganese stock solution (100 ppm Mn): Dilute 10 ml of Reagent B to 100 ml with Reagent A.

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*The extraction procedure for Mn is identical to the extraction procedure for P, K, Ca, and Mg. For samples where all 5 determinations are requested, the same extraction can be used.

Mn Working Standards

In 500 ml volumetric flasks, place the following amounts of Reagent C, and dilute to volume with Reagent A.

Std. No.	Reagent C, ml	Mn in Solution, ppm
1	0.00	0.00
2	2.50	0.50
3	5.00	1.00
4	10.00	2.00
5	12.50	2.50
6	15.00	3.00
7	20.00	4.00

Procedure: Measure one scoop (5 ml) of soil into a 60-ml extraction beaker. Add 20 ml of Reagent A with an automatic pipetting machine, shake on a mechanical shaker with a stroke length of 3.8 cm for 5 minutes at 180 oscillations per minute and filter through Whatman No. 1 filter paper. Determine Mn in the standards and extracts with an atomic absorption spectrophotometer. Report as ppm Mn in soil.

ppm Mn in solution x 4 = ppm Mn in soil

Organic Matter

Reagent A: Sodium dichromate solution (0.67M): Dissolve 4,000 g of reagent grade sodium dichromate in tap water and make to a volume of 20 liters.

Reagent B: Technical grade sulfuric acid.

Procedure: One scoop (1.5 ml) 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 solution is withdrawn using a syringe-type pipette and placed in a colorimeter vial. Readings are taken using a colorimeter equipped with a red filter. The percentage of organic matter is determined by reference to Table 1.

Table 1. Colorimeter readings and percent organic matter for Virginia soils.*

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

*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 $CuSO_4$ anhydrous in deionized water in a one-liter flask. Dilute to volume and mix well.

Reagent B: NO_3-N stock solution (1,000 ppm N): Dissolve 6.061 g of $NaNO_3$ in 1 liter of deionized water.

NO_3-N Working Standards

In 1 liter volumetric flasks, place the following amounts of Reagent B and dilute to volume with Reagent A:

Std. No.	Reagent B, ml	NO3-N in Solution, ppm
1	5	5
2	10	10
3	25	25
4	50	50
5	75	75
6	100	100

Procedure: One scoop (20 ml) of soil is measured into a 125 ml Erlenmeyer flask. Fifty ml of Reagent A are added, the solution is shaken for 10 minutes on a wrist-action shaker (at the fastest setting) and then filtered through Whatman No. 1 filter paper. The NO3-N in the standards and extracts is determined using an expanded scale pH/mv meter 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 readings versus ppm NO3-N in solution. Nitrate-nitrogen in solution is read from the standard curve and ppm NO3-N in soil is calculated with the following equation:

ppm NO3-N in solution x 2.5 = ppm NO3-N in soil.

Soluble Salts

Reagent A: Potassium cholride standard solution (0.01 N KCl): Dissolve 0.7456 g of KCl in 1 liter of deionized water.

Procedure: Add 20 ml of distilled water to the 1:1 soil to water solution used for pH determination. Stir the solution and allow to stand for at least 1 hour. The conductivity meter is standardized with Reagent A. At 25 C, Reagent A has an electrical conductivity of 0.0014118 mho/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 x 6.4 x 2

In this equation, EC represents the conductivity reading in mhos x 10 ee-5, 6.4 is the factor for converting the conductivity measurement to ppm soluble salts, and 2 represents the water volume dilution factor.

12/77 Calibration of P, K, Ca, Mg Tests

Ext. P	P - lb/A	P - ppm	P2O5 - lb/A
L-	0- 3	0- 2	0- 7
L	4- 8	2- 4	9- 18
L+	9- 11	5- 6	21- 25
M-	12- 20	6-10	28- 46
M	21- 30	11-15	48- 69
M+	31- 35	16-18	71- 80
H-	36- 55	18-28	82-126
H	56- 85	28-43	128-195
H+	86-110	43-55	197-252
VH	110+	55+	252+

Ext. K	K - lb/A	K - ppm	K2O - lb/A
L-	0- 15	0- 8	0- 18
L	16- 55	8- 28	19- 66
L+	56- 75	28- 38	68- 90
M-	76-100	38- 50	92-121
M	101-150	51- 75	122-181
M+	151-175	76- 88	182-211
H-	176-210	88-105	212-253
H	211-280	106-140	254-337
H+	281-310	141-155	339-373
VH	310+	155+	373+

Ext. Ca	Ca - lb/A	Ca - ppm	CaO - lb/A
L-	0- 240	0- 120	0- 336
L	241- 480	121- 240	337- 672
L+	481- 720	241- 360	673-1007
M-	721- 960	361- 480	1009-1343
M	961-1200	481- 600	1344-1679
M+	1201-1440	601- 720	1680-2015
H-	1441-1680	721- 840	2016-2350
H	1681-1920	841- 960	2352-2686
H+	1921-2160	961-1080	2688-3022
VH	2161-2400+	1081-1200+	3023-3358+

Ext. Mg	Mg - lb/A	Mg - ppm	MgO - lb/A
L-	0- 24	0- 12	0- 40
L	25- 48	13- 24	42- 80
L+	49- 72	25- 36	81-119
M-	73- 96	37- 48	121-159
M	97-120	49- 60	161-199
M+	121-144	61- 72	201-239
H-	145-168	73- 84	240-279
H	169-192	85- 96	280-318
H+	193-216	97-108	320-358
VH	217-240	109-120+	360-398+

INTRODUCTION

Plant analysis capabilities were incorporated into the Soil Testing and Plant Analysis Laboratory in August, 1979. Procedures consist basically of dry ashing plant tissue followed by instrumental determination except for nitrogen where wet ashing and micro-Kjeldahl procedures are employed. The analyses performed by the Soil Testing and Plant Analysis Laboratory are as follows:

Analyses Performed

Nitrogen	Copper
Phosphorus	Iron
Potassium	Manganese
Calcium	Zinc
Magnesium	

SAMPLE PREPARATION

Tissue samples are received in paper bags or envelopes. Plant analysis information sheets are packaged with the samples. In the laboratory plant tissue samples are dried at 70C for 24 hours. After drying, the samples are assigned a laboratory number. 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 8.8 g (5-dram) 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 dessicator until ground. This is essential to prevent absorption of moisture by the dried plant material.

DETERMINATION OF P, K, Ca, Mg, Cu, Fe, Mn, AND Zn

Dry Ashing

Dissolving Solution Preparation: 3.6 N (10%) HCL solution: Add 100 ml of concentrated HCl to a 1 liter volumetric flask containing approximately 500 ml of deionized water. Dilute to volume. For larger quantity, add 1800 ml of concentrated HCl to a 18 liter carboy containing approximately 10 liters of deionized water. Dilute to volume with deionized water.

Ashing Procedure:

1.) Weigh 1.000g +/- 10mg of dried, ground plant tissue into a 50 ml Kimax beaker. Record weight.

2) Ash samples at 475 C for 5 hours by placing samples in cool muffle furnace with timer set to turn on at 6 PM and off at 11 PM, and temperature set at 475 C.

3) After ashing, remove beakers and allow to cool. Add 5.0 ml of concentrated HCL, using an automatic dispenser, directly into beakers containing the ash. 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. Filter through a Whatman No. 42 filter paper. Extracts are now ready for elemental determination.

Standard Solution Preparation

Add indicated amount of the reagent from the following table to a 1-liter volumetric flask containing approximately 200 ml of 3.6 N HCL for each stock solution. Dilute to volume using 3.6 N HCl.

Element	Reagent	g/l	Concentration ---ppm--
P	potassium phosphate (KH2PO4)	21.969	5,000
K	potassium chloride (KCl)*	9.533	5,000
Ca	Calcium carbonate (CaCO3)	24.973	10,000
Mg	Magnesium sulfate (MgSO4.7H2O)	101.348	10,000
Cu	Cu metal	1.0000	1,000
Fe	Fe metal	1.0000	1,000
Mn	Manganese sulfate (MnSO4.H2O)	3.076	1,000
Zn	Zinc sulfate (ZnSO4.7H2O)	4.3478	1,000

* KCl should be dried at 105 C for two hours prior to weighing.

P Determination

Reagent A: Complexing - stabilizing stock solution: Dissolve completely 100 g of ammonium molybdate [(NH4)2MoO4] in approximately 500 ml of deionized water in a 2-liter volumetric flask. Dissolve 2.425 g of antimony potassium tartrate [K(SbO)C4H4O4.1/2H2O] in the molybdate solution. Place the flask in a cold water bath and slowly add 1400 ml concentrated H2SO4. Mix well, cool, and dilute to volume with deionized water. Store in a polyethylene bottle in a dark, cool compartment.

Reagent B: Stock reducing solution: Dissolve 176.0 g of L-Ascorbic acid powder in approximately 500 ml of deionized water in a 2-liter volumetric flask. Dilute to volume with deionized water. Mix well and store in a dark bottle in a cool compartment.

Reagent C: Working solution. Dilute 20 ml of Reagent A and 10 ml of Reagent B to 1 liter with 3.6 N HCl. Prepare fresh daily. Allow to stand at least 2 hours before adding to samples.

To a 500 ml volumetric flask containing approximately 100 ml of 3.6 N HCl add the indicated amounts of stock solution for each element then dilute to volume with 3.6 N HCl.

Standard No.	Stock Solution ml	P in Standard, ppm	P in 1:496 Dilution, ppm
1	0	0	0
2	5	50	0.101
3	10	100	0.202
4	15	150	0.302
5	20	200	0.403

Procedure: Make a 1:496 dilution of samples to Reagent C. After allowing 20 minutes for color development, the P in solution is determined with a spectrophotometer equipped with a direct concentration readout mode.

K Determination

Reagent A: Lithium stock solution (1,500 meq/L): Instrumentation Laboratories Standard No. 35000.

Reagent B: Lithium working solution (18.75 meq/L): Dilute 12.5 ml of

Reagent A to 1 liter with deionized water. This solution now contains 1,000 ppm K.

To a 500 ml volumetric flask containing approximately 100 ml of 3.6 N HCl, add the indicated amounts of stock solution in the following table and then dilute to volume with 3.6 N HCl.

Standard No.	Stock Solution, ml	K in Standards, ppm	K 1:100 Dilution, ppm
1	0	0	0
2	10	100	1.0
3	20	200	2.0
4	40	400	4.0
5	60	600	6.0
6	80	800	8.0

Procedure: Make a 1:100 dilution of sample to Reagent B. This is equivalent to two 1:10 dilutions. Determine K in standards and samples with a flame photometer.

Ca, Mg Determination

Reagent A: Lanthanum chloride diluting solution: Add 322.0 g La203 (99.99%) to approximately 250 ml of deionized water in a 2 liter volumetric flask. Mix well. Place the flask in a cold water bath and SLOWLY add 500 ml of concentrated HCl. Swirl constantly as small amounts of acid are added to the flask. Dilute to volume with deionized water. Add 1 liter of La203 solution to a 20 liter carboy containing approximately 10 liters of deionized water. Dilute to volume.

To a 500 ml volumetric flask containing approximately 100 ml of 3.6 N HCL, add indicated amounts of both Ca and Mg stock solutions. Dilute to volume with 3.6 N HCl.

Ca Stock Solution, ml	Mg stock Solution, ml	ppm in Standards Ca	ppm in Standards Mg	ppm in 1:10 Dilution Ca	ppm in 1:10 Dilution Mg
0	0	0	0	0	0
10	1	200	20	20	2
20	2	400	40	40	4
30	3	600	60	60	6
40	4	800	80	80	8

Procedure: A one ml aliquot of sample is diluted with 9 ml of Reagent

A. Calcium and magnesium are determined from the same aliquot using an atomic absorption spectrophotometer (Burner head turned 45 degrees).

Cu, Fe, Mn, Zn Determination

Cu Working Solution (50 ppm): Add 5 ml of Cu stock solution to a 100 ml volumetric flask containing approximately 50 ml of 3.6 N HCl. Dilute to volume with 3.6 N HCl.

Fe Working Solution (100 ppm): Add 10 ml of Fe stock solution to a 100 ml volumetric flask containing approximately 50 ml of 3.6 N HCl. Dilute to volume wume with 3.6 N HCl.

Mn Working Solution (200 ppm): Add 20 ml of Mn stock solution to a 100 ml volumetric flask containing approximately 50 ml of 3.6 N HCl. Dilute to volume with 3.6 N HCl.

Zn Working Solution (100 ppm): Add 10 ml Zn stock solution to a 100 ml volumetric flask containing approximately 50 ml of 3.6 N HCl. Dilute to volume with 3.6 N HCl.

To a 500 ml volumetric flask containing approximately 100ml of 3.6. N HCl add the indicated amounts of working solution for each element, then dilute to volume with 3.6 N HCl.

Standard No.	Working Solution, ml				ppm in Solution			
	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn
1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	1	2.0	0.5	2.0	0.1	0.4	0.2	0.4
3	2	4.0	1.0	3.0	0.2	0.8	0.4	0.6
4	3	6.0	2.0	4.0	0.3	1.2	0.8	0.8
5	4	8.0	3.0	5.0	0.4	1.6	1.2	1.0
6	5	10.0	5.0	6.0	0.5	2.0	2.0	1.2
7	10	20.0	6.0	7.0	1.0	4.0	2.4	1.4

Procedure: Determine Cu, Fe, Mn, Zn in the standards and samples with an atomic absorption spectrophotometer. Report concentration as ppm.

TOTAL NITROGEN DETERMINATION

For total nitrogen determination, a micro-kjeldahl method is employed using a custom-made heating block. Distillation is performed with a Kjeltec System 1004 Steam Distillation Unit.

Solution Preparation

Alkali solution NaOH - Na2S203 (sodium hydroxide, sodium thiosulfate): Add approximately 400 ml deionized water to a 1-liter volumetric flask. Add 400 g NaOH and 40g Na2S203.5H20 to the flask. Dilute to volume with deionized water and mix. Set Kjeltec unit to deliver 15 ml of alkali solution.

Methyl red - methylene blue mixed indicator:

Solution A: 669 mg of 0.2% methyl red in 410 ml (335 g) of 95% ethanol.

Solution B: 343 mg of 0.2% methylene blue in 210 ml (171 g) of 95% ethanol.

solution C: Mix 200 ml of solution A with 100 ml of solution B (2:1 ratio of A to B). This solution has a shelf life of one month. Therefore it must be freshly prepared each month.

Boric Acid Solution: Add approximately 200 ml of deionized water to a 1,000 ml volumetric flask. Add 20.0 g of boric acid and 60 ml of solution C and dilute to volume with deionized water.

Potassium Biiodate Solution (0.05 N): Add approximately 200 ml of deionized water to a 1-liter volumetric flask. Add 19.497 g of potassuim biiodate to the volumetric flask and dilute to volume with deionized water. Mix thoroughly using a magnetic stirrer.

$$0.05 \times 389.94 \text{ FW } K(IO3)2 = 19.497 \text{ g } K(IO3)2$$

Sodium Sulfate: Add 100 mg of sodium sulfate in a 50 ml beaker. Dissolve in 20 ml of tap water.

Sample Digestion

1) Weigh out 200 mg +/- 10 mg 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.

3) Add 2.5 ml of H2S04 to the test tube using repipet. This procedure should be done under hood. Swirl test tubes to mix tissue, salt/catalyst, and acid or allow to stand overnight.

4) Place test tubes into heating block. Allow samples to clear (approximately 30 min.) and then digest for 30 minutes at 400 C. Usually samples should be clear by the time the heating block reaches its 400 C operating temperature (approximatly 75 min.)

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

Distillation

1) Add 25 ml of boric acid solution to receiver flask. Use 25 ml repeater pipet to dispense boric acid solution.

2) Place a receiver flask on each platform and move platforms to upper position. The plastic tube should be below liquid surface level.

3) 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.

4) Press the left "Start" button (green "Ready/Start" lamp).

5) Wait several seconds and then press the right "start" button.

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

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

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

NOTE - The Kjeltec Unit should not be allowed to remain idle for more than 5 minutes between distillations.

If the red "Alkali Refill" light comes on there is only enough alkali left in the alkali tank for approximately 20 more tests. Refill tank with alkali as soon as possible.

Titration

1) Receiver flasks from distillation are used for titration.

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

2) Add magnetic stirring rod to receiver flask.

3) Titrate to lilac endpoint. Lilac endpoint will occur immediately after a grayish-blue color. Record ml of acid used to nearest 0.1 ml.

4) Repeate steps 1 to 3 for next sample.

Calculations:

1.00 ml of 0.05 N K(IO3)2 = 700 ug N at the equivalence point

$$\frac{\text{ug N}}{\text{ml}} \cdot \frac{\text{ml K(IO3)2}}{\text{sample wt}} = \frac{\text{ug N}}{\text{g plant material}}$$

$$\frac{\text{ug N}}{\text{g}} = \text{ppm}$$

ppm x (0.0001) = % N

For % N in a 0.200 g sample, multiply ml of K(IO3)2 used times 0.35.

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

$$\frac{0.07 \times Y \text{ ml K(IO3)2}}{\text{sample wt. g}} = \% \text{ N}$$

INSTRUMENTS USED FOR SOIL AND PLANT ANALYSIS

Soil Analysis

Analysis	Instrument
pH	Orion Model 601 Digital Ionalyzer
Phosphorus	Bausch and Lomb Spectronic 21
Potassium	Instrumentation Laboratory 143 Flame Photometer
Calcium, Magnesium	Perkin-Elmer 290 Atomic Absorption Spectrophotometer
Zinc, Manganese	Perkin-Elmer 503 Atomic Absorption Spectrophotometer
Organic Matter	Cenco Colorimeter
Nitrate-Nitrogen	Beckman Century SS-1 Expanded Scale pH Meter; Orion Nitrate Specific Ion Electrode
Soluble Salts	Solu Bridge RD 15
Extraction-NO3-N	Burrell Wrist-Action Shaker
Extraction-P,K,Ca,Mg,Zn,Mn	Eberbach Reciprocating, Variable Speed Shaker No. 6000
Soil Grinding	Custom Lab Equipment DC-1 HD Dynacrush

Plant Analysis

Analysis	Instrument
Nitrogen	Tecator Kjeltec System 1004 Distilling Unit
Phosphorus	Bausch and Lomb Spectronic 21

Potassium	Instrumentation Laboratory 143 Flame Photometer
Calcium, Magnesium	Perking-Elmer 290 Atomic Absorption Spectrophotometer
Copper, Iron Manganese, zinc	Perkin-Elmer 503 Atomic Absorption Spectrophotometer
Drying	Blue-M Power - O - Matic 70
Grinding	Thomas Wiley Mill Model ED-5
Ashing	Thermolyne F-A1730 Muffle Furnace

REFERENCES

SOIL ANALYSIS

Sample Preparation

Eik, K., E. L. Hood and J. J. Hanway. 1975. Soil sample preparation. p. 2-3. In Recommended chemical and soil test procedures for the North Central Region. N.D.Agric. Exp. Sta. Bull. 499.

pH

Handbook on reference methods for soil testing. 1974. Council on Soil Testing and Plant Analysis, Athens, Ga.

Phosphorus

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.

Handbook on reference methods for soil testing. 1974. Council on Soil Testing and Plant Analysis, Athens, Ga.

Olsen, S. R. and L. A. Dean. 1965. Phosphorus. p. 1035-1049. In C. A. Black, et. al. (eds) Methods of soil analysis. Chemical and microbiological methods. Part II. Agron. 9, Amer. Soc. Agron., Madison, Wis.

Potassium, Calcium, Magnesium

Mehlich, A 1953. Determination of P, Ca, Mg, K, Na, and NH4. North Carolina Soil Test Division (Mimeo 1953).

Handbook on reference methods for soil testing. 1974. Council on Soil Testing and Plant Analysis, Athens, Ga.

Zinc

Alley, M.M., D.C. Martens, M.G. Schnappinger, Jr., and G.W. Hawkins. 1972. Field calibration of soil tests for available zinc. Soil Sci. Soc. Amer. Proc. 36:621-624.

Manganese

Cox, F.R. 1968. Development of a yield response prediction and manganese soil test interpretation for soybeans. Agron. J. 60:521-524.

Organic Matter

Peech, M., L.T. Alexander, L.A. Dean and J. Fielding Reed. 1947. Methods of soil analysis for soil-fertility investigations. USDA Circ. 757, p. 5-7.

Allison, L.E. 1965. Organic carbon, p. 1367-1378. In C.A. Black et al. (eds) Methods of soil analysis. Chemical and microbiological methods. Part II. Agron. 9, Amer. Soc. Agron., Madison, Wis.

Nitrates

Dahnke, W.C. 1971. Use of the nitrate specific ion electrode in soil testing. Comm. Soil Sci. and Pl. An. 2:73-84.

Carson, P.L. 1975. Recommended nitrate-nitrogen tests. p. 13-15. In Recommended chemical soil test procedures for the North Central Region. N.D. Agric. Exp. Sta. Bull. 499.

Oien, A. and A.R. Selmer-Olsen. 1969. Nitrate determination in soil extracts with the nitrate electrode. Analyst 94:888-894.

Soluble Salts

Bower, C.A. and L.V. Wilcox. 1965. Soluble salts. p. 933-951. In C.A. Black et al. (eds) Methods of soil analysis. Chemical and microbiological methods. Part II. Agron. 9, Am. Soc. Agron., Madison, Wis.

Waters, W.E., W. Llewelyn, C.M. Geraldson, and S.S. Woltz. 1973. The interpretation of soluble salt procedures as influenced by salinity testing procedure and soil media. Proc. Trop. Reg. Am. Soc. Hort. Sci. 17:397-405.

U.S. Salinity Laboratory Staff. 1951. Determination of the properties of saline and alkali soils. Ch. 2 in USDA Agr. Handbook No. 60. L.A. Richards (ed) p. 7-33.

Instrumentation

Walsh, L.M. 1971. Instrumental methods for analysis of soils and plant tissue. Soil Sci. Soc. Amer., Inc., Madison, Wis. 222 p.

PLANT ANALYSIS

Dry Ashing

Isaac, R.A. and J.D. Kerber. 1971. Atomic absorption and flame photometry: Techniques and uses in soil, plant and water analysis. p. 17-37. In L.M. Walsh (ed.) Instrumental methods for analysis of soils and plant tissue. Soil Sci. Soc. Amer., Madison, Wis.

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 (eds.) Soil Testing and Plant Analysis. Soil Sci. Soc. Amer., Madison, Wis.

Nitrogen

Elmore, T. and D. D. Wolf. 1979. Unpublished data. VPI&SU Agronomy Department, Blacksburg, Va.

Mackenzie, H.S. and H.S. Wallace. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion condition - - -. temperature, catalyst, and oxidizing agent. Aust. J. Chem. 1: 55-70.

Tecator. 1976. Tecator Manual. Kjeltec System 1 (1003 Distilling Unit). Tecator, Hoganas, Sweeden.