Methods for Plant Analysis

A Guide for Conducting Plant Analysis in Missouri

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1. Kjeldahl Nitrogen and Phosphorous

Reagents and Apparatus

1. Concentrated Sulfuric Acid (H_2SO_4) reagent grade.
2. Digestion tablets (Kjeltab).
3. Glass boiling beads.
4. Aluminum digestion block with temperature controller.
5. Kimax glass tubes with 50-ml graduation mark.
6. TKN Standards.
7. Carries Solution

Procedure

1. Weigh out 0.150g of ground plant material into each 50 ml digestion tube.
2. Add one digestion tablet (Kjeltab 1.72g) and two glass boiling beads to each tube.
3. Under hood, add 3.5 ml of concentrated sulfuric acid (H_2SO_4) using an acid resistant 5-ml repipet device.
4. Include at least one blank and one check for each run.
5. Preheat aluminum digester to 180 ºC. It takes one half to one hour to reach this temperature.
6. Place tubes in block digester which has been preheated, and continue to heat to 390 +/-5ºC. Digest for two hours.
7. Remove the tubes from the heating block and allow about 20 minutes for cooling.
8. Add 10 - 15 ml of distilled water to the digestion tubes while they are still warm. Mix them to dissolve any crystals that may have formed.

9. Dilute to the 50-ml mark with distilled water and mix thoroughly after capping.
10. Filter this solution with # 2 filter paper.
11. Analyze ammonium for TKN on a Flow Injection Analyzer based on the Lachat Method No 13-107-06-2-D.

Calculations

1. To get plant TKN %, N % = reading (mg/l) x 50 / 0.150 / 10000

References

1. AOAC Official Methods of Analysis 1990. Protein (Crude) in animal feed, semi automated method No. 976.06, p72.
2. Jones, Jr., J. B., B. Wolf, and H. A. Mills 1991. Methods of Elemental Analysis (Chapter 4) pp27-38. *In*: Plant Analysis Handbook. Micro-Macro Publishing, Inc. 183 Paradise Blvd., Suite 108, Athens, Georgia.
3. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
4. QuikChem Automated Ion Analyzer Methods Manual. No. 13-107-06-2-D, December, 1996. Determination of total kjeldahl nitrogen in soils and plants by flow injection analysis (Block Digester Method), LACHAT Instruments, Milwaukee, WI.
5. QuikChem Automated Ion Analyzer Methods Manual. No. 13-115-01-1-B, December, 1996. Determination of phosphorous in soils and plants by flow injection analysis (Block Digestor Method). LACHAT Instruments, Milwaukee, WI.

2. Dry Ashing Procedure for Plant Materials

Reagents and Apparatus

1. Hydrochloric Acid, 6*N* HCl.
2. Muffle furnace.
3. 15 ml crucibles.
4. Kimax glass tubes with 50 ml graduation mark.
5. Dilutor.

Procedure

1. Weigh out 0.500g of ground and dried (75ºC) plant material into each 10 ml crucible.
2. Place the crucibles in a cool muffle furnace. Set furnace temperature to reach 500ºC (about 2-3 hours).
3. After 5 hours of muffling at 500ºC, turn off the furnace and let it cool. Do not open the door of the furnace during the ashing and cooling.
4. Add 10 ml of 6*N* HCl into each crucible to dissolve the ash.
5. Transfer all of the staff in the crucible to a Kimax glass tube, and dilute to the 50 ml mark with distilled water, and mix thoroughly.
6. Allow suspended materials to settle to the bottom of the tube, or filter with Whatman #42 filter paper.
7. The filtered solution is ready to determine K, B, Ca, Mg, Zn, Fe, Mn, and Cu, with or without further dilution.

References

1. Chapman H. D. and P. F. Pratt. 1961. Methods of Analysis for Soils, Plants, and Waters University of California, Riverside, CA.
2. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
3. Jones, Jr., J. B., B. Wolf, and H. A. Mills 1991. Preparation and Analysis (Chapter 4) pp23-26. *In*: Plant Analysis Handbook. A practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Inc. 183 Paradise Blvd., Suite 108, Athens, Georgia.
4. Jones, Jr., J. B. and V. W. Case 1990. Sampling, Handing, and Analyzing Plant Tissue Samples (Chapter 15) pp389-427. *In*: Soil Testing and Plant Analysis, 3rd ed. –SSSA book series, no. 3. 677 S. Segon Rd., Madison, WI.

3. Total K, Na, Ca and Mg

Equipment

1. Adjustable pipette
2. 20-ml glass beaker
3. Atomic Absorption Spectrophotometer

Reagents

1. 1.2 *N* HCl solution Add 103.7 ml of hydrochloric acid (sp. Gr. 1.19, 37.5%) into 1 liter bottle. Dilute with distilled water and mix well.
2. 0.105 % Lanthanum diluent Place 1.2314 g lanthanum oxide (La_2O_3), low calcium grade, in a one liter volumetric flask. Add 4 ml of 6 *N* HCl to dissolve the La_2O_3 and then dilute to one liter with demineralized water.

Procedure

1. Transfer filtrate from the dry ashing procedure into 20-ml beaker.
2. Dilute 1.0 ml of the filtrate with 9.0 ml of the 1.2 *N* HCl solutions. This is a 10 time diluted filtrate.
3. Dilute 0.5 ml of the diluted filtrate with 9.5 ml of the Lanthanum diluent.
4. Read samples on atomic absorption spectrophotometer using appropriate standards and instrument settings for Ca and Mg.
5. Flame emission spectrometers may be used for determination of K directly in the diluted filtrate.
6. This diluted filtrate is also used for determination of P.

Calibration and Standards

1. 1000 ppm K, Na, Ca and Mg stock solution
2. Working standards

   Pipette the following volumes of 1000 ppm stock solution into 1000 ml volumetric flasks and dilute to volume with 1.2 *N* HCl solutions for Ca and Mg, and with 0.12 *N* HCl solutions for K and Na:

ml of 1000 ppm stock				Final working Standards			
K	Na	Ca	Mg	K	Na	Ca	Mg
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20.0	15.0	50	5.0	20.0	15.0	50	5.0
40.0	30.0	100	10.0	40.0	30.0	100	10.0

Store in plastic bottles and keep in the refrigerator until ready to use.

Calculations

Ca and Mg % in plant = ppm in reading x 10 x 50 / 0.500 / 10000

K and Na % in plant = ppm in reading x 10 x 50 / 0.500 / 10000

References

1. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
2. Nathan, M., Stecker, J. and Y. Sun. 2006. Soil testing. A guide for conducting soil tests in Missouri. EC923. Division of Plant Sciences Extension, University of Missouri-Columbia.
3. Thomas, G. W. 1982. Exchangeable cat ions. P. 159-165. *In* A. L. Page et al. (ed). Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, Wis.
4. Warncke, D., and J. R. Brown 1998. Potassium and other basic cations. pp. 31-34. *In* J. R. Brown (ed.) recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001.

4. Micronutrient Analysis

Equipment

1. Adjustable pipette.
2. 20-ml glass beaker.
3. Atomic Absorption Spectrophotometer.

Reagents

1. 1.2 *N* HCl solution

   Add 103.7 ml of hydrochloric acid (sp. Gr. 1.19, 37.5%) into 1 liter bottle. Dilute with Distilled water and mix well.

Procedure

1. Transfer filtrate from the dry ashing procedure into 20-ml beaker.
2. Read samples with atomic absorption spectrophotometer using appropriate standards and instrument settings. Set zero with reagent blank, which is 1.2 *N* HCl solutions.
3. Report as ppm Zn, Fe, Mn or Cu in the plant.

Calibration and Standards

1. 1000 ppm Zn, Fe, Mn, and Cu stock solution
2. 100 ppm Zn, Fe, Mn, and Cu working stock solution Dilute 10 ml of 1000 ppm stock solution each to 100 ml with deionized water, respectively.

Working standards

1. Pipette the following volumes of 100 ppm working stock solution into 500 ml volumetric flasks and dilute to volume with 1.2 *N* HCI solution:

ml of 100 ppm stock				Final working Standards			
Zn	Fe	Mn	Cu	Zn	Fe	Mn	Cu
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.50	15.00	5.00	2.50	0.50	3.00	1.00	0.50
5.00	30.00	10.00	5.00	1.00	6.00	2.00	1.00

2. Store in plastic bottles and keep in the refrigerator until ready to use.

Calculations

Micronutrient ppm in plant = ppm in reading x 100

References

1. Nathan, M., Stecker, J., and Y. Sun. 2006. Soil testing. A guide for conducting soil tests in Missouri. EC923. Extension Division, University of Missouri-Columbia.
2. Chapman, H. D., and P. F. Pratt. 1961. Methods of analysis for soils, plants and waters. University of California, Riverside, CA.
3. Cox, F. P., and E. J. Kamprath. 1972. Micronutrient soil tests. P. 289-317. *In* J. J. Mortvedt et al. (ed). Micronutrients in Agriculture, Soil Sci. Soc. Amer. Inc., Madison, Wis.
4. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239
5. Lindsay, W. L., and W. A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.
6. Whitney, D. A. 1998. Micronutrients: Zinc, Iron, Manganese and Copper. pp. 41-44. *In* J. R. Brown (ed.) recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001.

5. BORON

Equipment

1. Adjustable pipette.
2. 10-ml test tubes.
3. Spectrophotometer with 420 nm of wavelength.

Preparation of Glassware

1. Boil all Pyrex glassware with a mixture of 3:1 concentrated HNO_3 and concentrated $HClO_4$ at 225°C for two hours.
2. Soak them in a 2*M* HCl acid bath overnight.
3. Rinse thoroughly with deionized water.

Reagents

1. Buffer-masking solution

   Dissolve 250 g of ammonium acetate and 15 g of ethylendiamine-tetraacetic acid disodium salt in 400-ml high quality distilled water and slowly add 125-ml of glacial acetic acid.

2. Azomethine-H solution

   Dissolve 0.45 g of azomethine-H in 100-ml of 1% (1 g/100 ml water) L-ascorbic acid solution. Let stand 24 hours prior to suing. This reagent will keep in refrigerator at 40°F for two weeks. This reagent is light sensitive and should be kept in a brown plastic bottle or a plastic bottle wrapped in aluminum foil.

Procedure

1. Pipette 1.0 ml of filtrate from the dry ashing procedure into a test tube and add 2-ml buffer-masking solution and 2 ml of azomethine-H solution. Thoroughly mix by swirling.
2. Allow the mixture to stand for 30 minutes and read on spectrophotometer at wavelength of 420 nm. Set 100% T with reagent blank, which is a 1.0 ml 1.2 N HCl solution with reagent.
3. All working standards should follow steps 1 and 2.

Calibration and Standards

1. 1000 mg B/L stock solution

   Weigh 0.5716 g of boric acid and dilute to 100 ml with deionized water.

2. 10 mg B/L working stock solution

   Dilute 1 ml of 1000 mg B/L stock solution to 100 ml with deionized water.

3. Working standards

   Pipette the following volumes of 10 mg B/L working stock solution into 50 ml

   Volumetric flasks and dilute to volume with 1.2 *N* HCI solution:

ml of 10 mg B/L	mg B/L in solution	mg B/L in plant
0.0	0.0	0
2.5	0.5	50
5.0	1.0	100

   Store in plastic bottles and keep in the refrigerator until ready to use.

Calculations

1. mg B/L in plant = mg B/L in reading x final volume / plant weight
2. mg B/L in plant = mg B/L in reading x 50 / 0.5
3. mg B/L in plant = mg B/L in reading x 100

References

1. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
2. Lohse G . 1982 Microanalytical azomethine-H method for boron determination in plant tissue. Commun. Soil Sci. Plant Anal., 13(2), 127-134.
3. McElreath, D.L. and Johnson, G.V. 1990. Soil Boron in laboratory procedures manual. Oklahoma state university soil, water, and forage analytical laboratory. AGRON 90-1.
4. Parker, D.R. and E. H. Gardner. 1981. The determination of hot-water-soluble boron in some acid Oregon soils using a modified azomethine-H procedure. Comm. in Soil Sci. Plant Anal. 12(12): 1311-1322.
5. Spouncer, L.R., R. O. Nable, and B. Cartwright. 1992. A procedure for the determination of soluble boron in soils ranging widely in boron concentrations, sodicity, and pH. Commun. Soil Sci. Plant Anal., 23(5&6), 441-453.
6. Watson, M. E. 1988. Recommended soil boron tests. pp. 23-25. *In* W.C. Dahnke (ed.) recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221 (Revised). North Dakota Agric. Exp. Stn. Bull. 499.

6. Extractable Nitrates

Reagents and Apparatus

1. Extracting solution:

   Add about 600 ml of distilled water to a 1 liter volumetric flask. Add 20.0 ml of glacial acid in the same flask. Bring to final volume with distilled water. Mix and transfer to a plastic storage bottle

2. Nitrate standard Stock Solution:

   Dry the potassium nitrate in the oven for 2 hours. Weigh 0.722 g of potassium nitrate and transfer to a 1000-ml volumetric flask. Bring to volume with the extracting solution. Label "100 ppm NO_3-N in 2 % acetic acid extracting solution."

3. Working solution:

   Set of four working NO_3^- Standards: 20.0, 10.0, 5.0, 1.0 mg N/L by volume:

   To four 100 ml volumetric flasks add, respectively, 20.0, 10.0, 5.0, and 1.0 ml of the nitrate standard stock solution. Dilute each to the mark with the working solution and invert to mix.

4. 125 ml shaking bottles.
5. 10 ml testing tubes
6. Whatman #2 filter paper without nitrates.

Procedure

1. Weigh out 0.100 g of dry ground plant material into each 125 ml shaking bottle.
2. Add 25 ml of extracting solution into each flask, insuring that all plant material has been wetted.
3. Include at least one Blank and one Check sample per run.

4. Shake vigorously for 15 minutes.
5. Filter into 30 ml of beaker using Whatman #2 filter paper without nitrates.
6. Transfer clear filtrate into 10 ml test tubes.
7. Analyze for nitrate on a Flow Injection Analyzer.

Calculations

1. NO_3-N ppm in plant = reading (ppm) x 25 / 0.100

References

1. Heanes, D. L. 1982. Determination of nitrate-N in plants by an improved extraction procedure adapted for ultraviolet spectrophotometry. Commun. Soil Sci. Plant Anal.13:805-818.
2. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
3. McElreath, D.L. and Johnson, G.V. 1990 Laboratory Procedures Manual. Oklahoma state university soil, water, and forage analytical laboratory.
4. QuikChem Automated Ion Analyzer Methods Manual. No. 13-107-04-1-A Aug. 1994. Nitrate/Nitrite, Nitrite in 2% Acetic Acid Plant Extracts. LACHAT Instruments, Milwaukee, WI.

7. Extractable Chloride

Reagents and Apparatus

1. Extracting solution

   Add about 600 ml of distilled water to a 1 liter volumetric flask. Add 20.0 ml of glacial acid in the same flask. Bring to final volume with distilled water. Mix and transfer to a plastic storage bottle

2. Chloride Standard Stock Solution

   Dissolve 0.2103 g of regain grade potassium chloride in approximately 50 ml of extracting solution. Bring up to 100-ml volume with extracting solution and label "1000 ppm Cl in 2% acetic acid extracting solution."

3. Working standards for chloride.

   Set of five working Cl- standards: 200, 160, 120, 80, and 40 mg Cl/L by volume:

   To five 100 ml volumetric flasks add, respectively, 20, 16, 12, 8, and 4 ml of chloride standard stock solution. Dilute each to the mark with the extracting solution and invert to mix.

4. 125 ml shaking bottles.
5. 10 ml testing tubes
6. Whatman #2 filter paper.

Procedure

1. Weigh out 0.100g of dry ground plant material into each 125 ml shaking bottle.
2. Add 25 ml of extracting solution into each flask, insuring that all plant material has been wetted.
3. Include at least one Blank and one Check sample per run.

4. Shake vigorously for 30 minutes.

5. Filter into 30 ml of beaker using Whatman #2 filter paper.

6. Transfer clear filtrate into 10 ml test tubes.

7. Analyze for chloride on a Flow Injection Analyzer.

Calculation

Cl ppm in plant = reading (ppm) x 25 / 0.100

References

1. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.

2. McElreath, D.L. and Johnson, G.V. 1990. Laboratory Procedures Manual. Oklahoma state university soil, water, and forage analytical laboratory.

3. QuikChem Automated Ion Analyzer Methods Manual. No. 10-117-07-1-A March. 1997. Determination of Chloride by Flow Injection Analysis Colorimetry. LACHAT Instruments, Milwaukee, WI.

8. Total Sulfur

Equipment

1. Funnels
2. Whatman No. 2 filter paper.
3. 125-ml beakers.
4. Spectrophotometer with 420 nm wavelength setting.
5. 10 ml pipette.
6. 50-ml Erlenmeyer flasks.

Reagents

1. Buffer and $BaCl_2$ solution:
2. Dissolve 5 g of gum arabic in about 500 ml of hot, D. I. Water and filter if cloudy.
3. Add 50 g of $BaCl_2 2H_2O$ and 450 ml of glacial acetic acid and dilute to 1 liter.

Procedure

1. Pipette 10 ml of filtrate from ashing procedure into a 50 ml Erlenmeyer flask.
2. Add 10 ml of the gum arabic-$BaCl_2$-acetic acid solution.
3. Shake for 10 minutes.
4. Read samples on spectrophotometer with 420 nm wavelength using appropriate standards.

Calibration and Standards

1. 100 ppm S standard solution

   Dissolve 0.544 g of oven dried (105°C) K_2SO_4 in about 500 ml of D. I. Water, add 10 ml of acetic acid as a preservative, and dilute to 1 liter with D. I. Water.

2. Working standards

Pipette 0, 4, and 8 ml of 100 ppm standard solution into 100 ml volumetric flasks. Add 25 ml of a 2000 ppm-P and 8N acetic acid solution (8.12 g of $Ca(H_2PO_4)_2H_2O$ plus 460 ml of glacial acetic acid diluted to 1 liter) and dilute to volume with D. I water. Store in plastic bottles and keep in the refrigerator until ready to use.

References

1. Chapman, H. D. and P. F. Pratt. 1961 Methods of Analysis for Soil, Plants and Waters. University of California, Riverside, CA.
2. Combs, S., J. Denning, and K. D. Frank. 1998. Sulfate - Sulfur. pp. 35-40. *In* J. R. Brown (ed.) recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001.
3. Jones, J.B. Jr. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. Ch 3: p191-239.
4. Nathan, M., Stecker, J., and Y. Sun. 2006. Soil testing. A guide for conducting soil tests in Missouri. EC923. Division of Plant Science Extension, University of Missouri-Columbia.
5. Official Methods of Analysis, AOAC 1990. p330 - 331.