elemental analysis procedures used by the Auburn University Department of Agronomy and Soils

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TABLE OF CONTENTS

Elemental Analysis Procedures Used by the Auburn University Department of Agronomy and Soils

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Introduction

This is a collection of elemental analysis procedures that have been found useful for analysis of Alabama plants and soils. All procedures are intended for use on existing equipment in the Department of Agronomy and Soils or in the Soil Testing Laboratory. Some of the procedures in this collection were developed over a long period of time by several people and most of the procedures are modifications of previously published methods.

The authors wish to thank the following people in the Department of Agronomy and Soils for their assistance in the preparation of this document: William W. Wills, Research Technician VII, and Cynthia D. Hunter, Research Technician IV, who helped compile the ICAP procedures and reviewed the manuscript; and Elizabeth A. Guertal, Assistant Professor, and Gregory L. Mullins, Professor, who reviewed the manuscript.

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General Laboratory Procedures

Courtesy

Most of the laboratories are not common use areas. Ask the person in charge of a laboratory before using equipment or chemicals. What looks like trash may be someone's research.

Safety

Federal, state, local, and Auburn University safety regulations will be followed. The following general safety procedures will be used in our laboratories.

1. A first aid kit should be maintained in each main laboratory.
2. Permanent technicians should attend first aid, CPR, and laboratory safety classes conducted by Auburn University.
3. Eye protection and appropriate protective clothing should be used in chemical laboratories.
4. Eye washes and fire extinguishers should be kept clear so they will be available in an emergency.
5. An inventory of all chemicals stored in the laboratory must be kept.
6. Material Safety Data Sheets (MSDS) must be collected for all chemicals used and stored in the laboratory. Material Safety Data Sheets may be obtained from the Office of Safety and Environmental Health.

Waste Disposal

Waste disposal guidelines are given in "A Guide to the Handling and Disposal of Hazardous Waste at Auburn University," which may be obtained from the Office of Safety and Environmental Health. Most of the procedures in this manual generate acidic wastes (pHs < 3) that must be neutralized before they can be poured down the drain.

Plant Sample Preparation

Washing, Drying, Grinding, and Storing

1. If the plant is contaminated with nutrient spray or if Fe is to be measured, wash the sample in 0.1% Tirton X-100 solution while the material is fresh and rinse in deionized water. The washing must be done quickly to avoid leaching of K and Ca from the tissue.
2. Dry the plant material at 65°C for 24 hours or until dry. Do not dry plant material in a 110°C drying oven or in a gravity convection oven. Do not store dried samples in the plant driers. Dried samples will stay dry until ground if stored in a closed plastic garbage bag.
3. For ease of manipulation and uniformity of composition, plant samples must be ground. Grinding becomes very critical if less than 0.5 g of the sample is to be used in the analysis. All the grinding mills cause some contamination of the samples with trace elements. Trace element contamination seems to be less with the Udy mills that use a carborundum grinding ring.
4. Plant material should be redried for 24 hours at 65°C to remove water picked up during grinding.
5. Samples should not be stored at room temperature in Whirl-Pak bags for more than two months before being analyzed. Samples that need to be kept for longer periods of time should be frozen in sealed containers.

Dry Ashing of Plant Samples

1. Principle of the Method:

The carbon in a known weight of dried plant material is burned off by combustion with atmospheric oxygen in a muffle furnace. The compounds in the ash are further oxidized using nitric acid, brought into solution using hydrochloric acid, and made to a known volume with deionized water. Silica is filtered out of the solution before analysis.

2. Apparatus:
    a. Muffle furnace
    b. 50 mL beakers with watch glass covers
    c. Filter stand, funnels, and Whatman #40 filter paper
    d. 100 mL volumetric flasks
    e. Storage bottles
3. Reagents:
    a. Deionized water
    b. 1 N HCl (83 mL concentrated HCl per liter)
    c. 1 N HNO_3 (64 mL concentrated HNO_3 per liter)
4. Procedure:
    a. Weigh 0.5 g of previously dried and ground plant material into a 50 mL beaker.
    b. Cover the beakers with watch glasses and place in a muffle furnace. Heat at 450°C for 4 hours or overnight. Turn the furnace off, open the furnace door, and allow the samples to cool before removing them from the furnace.
    c. Add 10 mL of 1 N HNO_3 and evaporate slowly to dryness on a hot plate in the fume hood. Take just to dryness and do not bake.
    d. Add 10 mL of 1 N HCl to dissolve the residue. The silica will not dissolve.
    e. Warm nearly to boiling and transfer to a 100 mL volumetric flask. Wash the beaker three times with small amounts of deionized water.
    f. Bring the contents of the flask to 100 mL volume using deionized water and filter the contents into a storage bottle. Do not wash the volumetric flask or filter paper during this step.

5. Remarks:
    a. Always carry a blank through the procedure.
    b. This ashing procedure is not suitable for N, B, Hg, Si, or Se.
    c. If trace elements are to be determined, increase the sample size to 1 g.
    d. The watch glass covers in step 4.b. are critical if Ni is to be determined.
    e. The muffle furnaces set at 450°C will actually be about 485°C.
    f. Step 4.c. is critical for P and trace element determinations.
    g. If Ca, Mg, and K are to be determined on the AA spectrophotometer, dilute a portion of the digest 1:10.
6. Safety:
    a. Obtain Material Safety Data Sheets (MSDS) for nitric and hydrochloric acids and read them.
    b. Wear eye protection, a rubber or plastic apron, and gloves while carrying out this procedure.
    c. Use great care in step 4.b. The muffle furnace is very hot.

Wet Ashing of Plant Samples

1. Principle of the Method:

The carbon in a known weight of dried plant material is oxidized using nitric and perchloric acids. The digest is stabilized by adding hydrochloric acid and made to a known volume using deionized water. Silica is filtered out of the solution before analysis.

2. Apparatus:
   a. Digestion block in perchloric acid fume hood
   b. 250 mL digestion tubes and rack
   c. Filter stand, funnels, and Whatman #40 filter paper
   d. 100 mL volumetric flasks
   e. Storage bottles
3. Reagents:
   a. Deionized water
   b. Acid Mix (700 mL concentrated HNO_3 + 300 mL concentrated $HClO_4$)
   c. 1 N HCl (83 mL concentrated HCl per liter)
4. Procedure:
   a. Weigh 0.5 g of previously dried and ground plant material into a 250 mL digestion tube.
   b. Add 10 mL of Acid Mix and allow the tubes to stand overnight at room temperature in a fume hood.
   c. Heat the tubes in a block digestor at 190°C until the digestion is complete. Add more Acid Mix as needed to keep the tubes from going dry. The digestion is complete when the contents are light yellow and there is less than 3 mL of liquid in each tube.
   d. Remove the tubes from the block and cool in the perchloric hood.
   e. Add 10 mL of 1 N HCl to each tube and transfer the contents to a 100 mL volumetric flask using deionized water.
   f. Bring the contents of the volumetric flasks to 100 mL using deionized water and filter the contents into a storage bottle. Do not wash the volumetric flask or filter paper during this step.
   g. Wash down the perchloric acid fume hood.

5. Remarks:
    a. Always carry a blank through the procedure.
    b. This ashing procedure is not suitable for N, B, Hg, Si, or Se.
    c. If trace elements are to be determined, increase the sample size to 1 g.
    d. Step 4.b. can be done in a conventional fume hood.
    e. Steps 4.c. and 4.d. must be done in the perchloric acid fume hood in FS292.*
    f. When the digestion is complete in step 4.c., the samples will be light yellow because of Mn compounds.
    g. If Ca, Mg, and K are to be determined on the AA spectrophotometer, dilute a portion of the digest 1:10.
6. Safety:
    a. Obtain Material Safety Data Sheets (MSDS) for nitric, perchloric, and hydrochloric acids and read them.
    b. Wear eye protection, a rubber or plastic apron, and gloves while carrying out this procedure.
    c. **Use great care when handling perchloric acid. Dry perchloric acid is explosive. Organic materials treated with perchloric acid and dried are explosive. Samples allowed to go dry in step 4.c. may explode.**
    d. The perchloric acid fume hood should be washed down daily to remove dried perchloric acid.

*FS292 = FUNCHESS HALL ROOM 292.

Soil Sample Preparation

Soil Testing Laboratory Procedure

1. Dry soils in a forced air oven at 55°C until dry. Oven drying will affect K and Mn extraction. The ovens in FS225* are not temperature controlled.
2. Dried samples are ground to pass a 10-mesh sieve. The Braun pulverizer in FS225 will grind pieces of rock that are in the sample. Samples containing rock will need to be hand pulverized with a mortar and pestle before they are screened.

Air-dried Samples

1. Air-dry samples by spreading them on trays in the laboratory.
2. Dried samples that do not contain rocks can be ground using the Braun pulverizer in FS225. If the pulverizer is set too close it will grind mineral grains and change the extractable nutrients. Dried samples that contain rocks should be carefully pulverized with a mortar and pestle and screened with a 2 mm sieve to remove the rocks.

Moist Samples

Moist samples can be analyzed if they are thoroughly mixed and if a subsample is taken for moisture determination.

*FS 225 = Funchess Hall Room 225.

THE ICAP SPECTROMETER

Principle of Operation

The Jarrell-Ash ICAP 9000 spectrometer in FS271A* has been upgraded by replacing the controller board, external computer, and software to a Thermo-Jarrell-Ash ICAP 61. The ICAP (or ICP-AES) uses a radio frequency generator to turn a stream of argon gas containing droplets of a sample into a plasma by induction heating. Compounds in the plasma are reduced to ground state atoms by the high temperature. Elemental emission lines are separated by a polychromator and measured using photomultiplier tubes. The ICAP 9000 has a set of exit slits and a photomultiplier for each emission line measured (simultaneous spectrometer).

Samples for ICAP determination must be in aqueous solution. Organic solvents cannot be used unless the RF generator is retuned. Solutions containing very high levels of salt cannot be aspirated properly and will damage the torch. Samples containing radioactive tracers cannot be analyzed using the ICAP 9000. The torch ventilation system in FS271A is not certified for radioactive materials. The following elements are the only ones that can be determined on the ICAP 9000 without adding more exit slits and photomultiplier tubes.

ELEMENTS DETERMINABLE ON ICAP 9000

Atomic #	Element	Symbol	Wavelength *nm*	Order
5	boron	B	249.770	2
11	sodium	Na	588.995	1
12	magnesium	Mg	279.079	2
13	aluminum	Al	308.215	2
14	silicon	Si	288.160	2
15	phosphorus	P	214.914	3
19	potassium	K	766.491	1
20	calcium	Ca	317.933	1
22	titanium	Ti	334.941	1
24	chromium	Cr	267.716	1
25	manganese	Mn	257.610	2
26	iron	Fe	271.441	2
27	cobalt	Co	228.616	2
29	copper	Cu	324.754	1
30	zinc	Zn	213.860	2
42	molybdenum	Mo	202.030	2
56	barium	Ba	493.409	1
80	mercury	Hg	546.074	1
82	lead	Pb	220.350	2

*FS217A = FUNCHESS HALL ROOM 217A.

The following are the specifications published by Jarrell-Ash for the ICAP 9000 spectrometer in their product brochure:

Optics: 0.75 m Rowland circle; Paschen-Runge mount

Grating: 1510 lines/mm

Spectral Range: 190 - 800 nm (air spectrometer)

Linear Dispersion:

0.92 nm/mm, first order
0.46 nm/mm, second order
0.31 nm/mm, third order

Resolution:

0.045 nm, first order
0.023 nm, second order
0.015 nm, third order

Detection Limits and Upper Limits of the Linear Range:

Atomic #	Symbol	Detection limit	Upper limit of linear range
		ppm	*ppm*
5	B	0.004	150
11	Na	0.01	200
12	Mg	0.001	50
13	Al	0.025	500
14	Si	0.005	200
15	P	0.06	250
19	K	0.3	1,000
20	Ca	0.001	20
22	Ti	0.002	150
24	Cr	0.005	150
25	Mn	0.001	150
26	Fe	0.005	150
27	Co	0.003	150
29	Cu	0.002	150
30	Zn	0.004	150
42	Mo	0.005	200
56	Ba	0.001	100
80	Hg	(No data for this wavelength)	
82	Pb	0.025	200

RF Source: 2500 watts at 27.12 MHz

Starting the ICAP

1. Make sure that the circuit breakers on the RF generator are on. These breakers are left on during the week but are turned off during weekends and holidays.
2. Make sure that the torch is centered in the induction coils, then close the torch access door.
3. Aspirate fresh deionized water during the warm up period.
4. Flip the automatic power control (APC) lever down into the manual mode.
5. Rotate the power knob counterclockwise to OFF.
6. Open the argon main valve on the tank. Turn on the coolant and plasma gas streams by flipping the toggle switches up. Open the needle valve to the sample gas stream by rotating the knob counterclockwise. At this point there should be a mist in the premix chamber, the blue light should be on, and the red light off. The argon regulator pressure should be 40 psi and the coolant gas flow should be 20 lpm.
7. Aspirate water for 3 minutes to purge air from the system. Turn off the sample gas stream by turning the needle valve clockwise. The mist should disappear from the premix chamber. Wait 3 minutes to clear water from the torch.
8. Push R.F. on. Both the blue and red lights should now be on.
9. Alternate turning the power up with the power knob and pushing the ignitor button until the plasma lights. Do not press and hold the ignitor button. Operating with the power in the red zone of the power meter will melt the torch. If the torch glows red after ignition, immediately push R.F. off. The torch may be over heating.
10. Adjust the power to 1.4 on the power meter using the power knob.
11. Penetrate the plasma by turning the sample needle valve slowly counterclockwise. The plasma plume should be hollow and there should be mist in the premix chamber.

12. Flip the automatic power control up to the automatic setting. The power level should drop to about 1.1.
13. Wait 30 to 45 minutes for the temperature to stabilize inside the torch chamber.
14. While aspirating 1000 ppm yttrium (Y), position the red tongue in the plasma from 0 to 2 mm above the top of the torch by rotating the plasma gas control knob.
15. Turn the computer on using the switch on the standby power system.
16. Profile the spectrometer with the Hg lamp in the verticle position (in the beam path). Aspirate water during this procedure.
17. After profiling the spectrometer, rotate the Hg lamp out of the beam path. Be careful not to drop the access door onto the lamp.
18. Select a method appropriate for your samples.
19. Standardize the ICAP and run samples.

Shutting Down the ICAP

1. Aspirate water for 1 minute after final sample.
2. Push the RF OFF (blue) button.
3. Turn the Power knob to zero.
4. Flip the Automatic Power Control (APC) switch down to MANUAL.
5. Close the argon main valve on the tank.
6. Let the gas lines bleed. The flowmeter balls will drop to zero.
7. Flip the Coolant and Plasma gas levers down to turn them off.
8. If you are the last operator scheduled on Friday afternoon, turn the two main power breakers on the RF Generator off. Skip this step if it is not Friday or if there is someone scheduled after you.
9. Turn the computer off using the switch on the standby power system.

Emergency Procedures

1. If the aspirator sucks up air and the alarm goes off, do not panic.
    a. Push the RF OFF (blue) button.
    b. Turn the Power knob to zero.
    c. Push the O.L. Reset (red) button on the RF Generator.
    d. Flip the Automatic Power Control (APC) lever down to manual.
    e. Re-light the plasma.
    f. Re-standardize.
    g. Continue sample analysis.
2. If there is a power surge or a main power outage, the ICAP will have to be reset.
    a. Push the Reset button on the back of the ICAP near the main power breakers. This will reset the power supply.
    b. Push the Reset button on the controller panel on the front of the ICAP. This will reset the internal computer.
    c. Re-light the plasma.
    d. Re-standardize.
    e. Continue sample analysis.

ELEMENTAL ANALYSIS PROCEDURES

As in Plant Samples

1. Principle of the Method:

Arsenic in plants is low enough that it must be run using the graphite furnace atomic absorption technique (GFAA). Plant samples should be wet ashed.

2. Standards: 0, 0.01, 0.05, 0.10, 0.25, and 0.50 ppm As in 0.1 N HCl.
3. Matrix modifier: to 5 mL of each sample add 500 µL of 0.5% (w/v) $Ni(NO_3)_2 \cdot 6H_2O$ + 10% concentrated HNO_3; to 10 mL of each standard add 1 mL of 0.5% (w/v) $Ni(NO_3)_2 \cdot 6H_2O$ + 10% concentrated HNO_3.
4. IL251:
    a. Lamp: As at 8 ma; D_2 at 25 ma
    b. Wavelength: 193.7 nm
    c. Slit width: 640
    d. Integration: Pk Ht at 1/16 sec
    e. Mode: (SB)A-B
    f. Focus for maximum intensity
    g. HV: adjust until intensity is between 2 and 8
5. IL655 (with IL755 temperature sensor):
    a. Coolflow: on at 20°C
    b. Purge gas: argon at 45 psi and 30/5 SCFH
    c. Mode: auto
    d. Temperature feedback: on
    e. Clean: off
    f. Program:

Step	Temperature	Time	Integrate Start
	°C	*X5 sec.*	
1	150	0	
2	150	1	
3	750	4	
4	1000	4	
5	2300	0	*
6	2300	2	

6. IL254:
    a. Nebulizer flow: air at 40 psi and 2.8 LPM
    b. Delay: 06
    c. Deposit: 020
    d. Repeat: 1

B In Plant Samples

1. Principle of the Method:

The carbon in a known weight of dried plant material is burned off by atmospheric oxygen in a muffle furnace. The compounds in the ash are brought into solution using hydrochloric acid and made to a known volume using deionized water. Silica is filtered out of the solution before analysis on the ICAP spectrometer.

2. Apparatus:
    a. Muffle furnace
    b. Porcelain evaporating dishes
    c. Plastic funnels and Whatman #40 filter paper
    d. 100 mL plastic volumetric flasks
    e. Plastic storage bottles
3. Reagents:
    a. Deionized water
    b. HCl solution (dilute 83 mL concentrated HCl to 100 mL with deionized water)
4. Procedure:
    a. Weigh 1.0 g of previously dried and ground plant material into a porcelain evaporating dish.
    b. Place the evaporating dishes in a muffle furnace and heat at 450°C for 4 hours or overnight. Turn the furnace off, open the furnace door, and allow the samples to cool before removing them from the furnace.
    c. Moisten the cool samples with distilled water and add 1 mL of HCl solution.
    d. Transfer the sample to a 100 mL plastic volumetric flask using deionized water and a plastic stirring rod. Make the flask to volume with deionized water and allow the silica to settle out.
    e. Filter part of the contents of the volumetric flask into a plastic bottle for storage. Do not wash the volumetric flask or filter paper during this step.

5. Remarks:
    a. Always carry a blank through the procedure.
    b. This ashing procedure is not suitable for N, P, Hg, Si, or Se.
    c. The final concentration of HCl in the digest is 0.1 N.
    d. The muffle furnace set at 450°C will actually be about 485°C.
6. Safety:
    a. Obtain a Material Safety Data Sheet (MSDS) for hydrochloric acid and read it.
    b. Wear eye protection, an apron or lab coat, and gloves while carrying out this procedure.
    c. Use great care in step 4.b. The muffle furnace is very hot.

Ca in Mehlich I Soil Extract

1. Principle of the Method:

Calcium in Mehlich I Soil Extract (double acid extract) is concentrated enough to analyze using the flame atomization technique on the atomic absorption spectrophotometer. Because of interferences by phosphorus, the nitrous oxide-acetylene flame must be used.

2. Standards: 0, 2, 4, 6, 8, and 10 ppm in Mehlich I extracting solution.
3. Dilution: topsoil samples will need to be diluted 1/50, 1/100, 1/200, 1/1000, or 1/2000.
4. IL251:
    a. Lamp: Ca at 7 ma; no D_2 lamp
    b. Burner: nitrous oxide-acetylene parallel to and 1/2 inch below beam
    c. Wavelength: 422.7 nm
    d. Slit Width: 320
    e. Integration: auto at 4 sec
    f. Mode: (SB)A
    g. Focus for maximum intensity
    h. HV: adjust for intensity between 2 and 8
    i. Oxidant: air at 40 psi and 20 scfh; nitrous oxide at 30 psi
    j. Fuel: acetylene at 15 psi and 8 scfh to light; after switching to nitrous oxide, adjust fuel for a red feather of 3/4 inch when not aspirating water
    k. Nebulizer: set for aspiration rate of 1 to 1.5 mL in 15 sec

Cd in Plant Samples

1. Principle of the Method:

Cadmium in plants is low enough that it must be run using the graphite furnace atomic absorption technique (GFAA). Plant samples may be digested using either wet or dry ashing.

2. Standards: 0, 0.005, 0.01, 0.02, 0.03 ppm Cd in 0.1 N HCl
3. Matrix modifier: To 5 mL of each sample add 50 µL of concentrated HNO_3 and 50 µL concentrated H_3PO_4. To 10 mL of each standard add 100 µL of concentrated HNO_3 and 100 µL of concentrated H_3PO_4.
4. IL251:
    a. Lamp: Cd at 3 ma; D_2 at 25 ma
    b. Wavelength: 228.8 nm
    c. Slit width: 320
    d. Integration: Pk Ht at 1/16 sec
    e. Mode: (SB)A-B
    f. Focus for maximum intensity
    g. HV: adjust until intensity is between 2 and 8
5. IL655 (with IL755 temperature sensor):
    a. Coolflow: on at 20°C
    b. Purge gas: argon at 45 psi and 30/5 SCFH
    c. Mode: auto
    d. Temperature feedback: on
    e. Clean: off
    f. Program:

Step	Temperature °C	Time *X5 sec.*	Integrate Start
1	150	0	
2	150	1	
3	225	3	
4	225	4	
5	2250	0	*
6	2250	2	

6. IL254:
    a. Nebulizer flow: air at 40 psi and 2.8 LPM
    b. Delay: 06
    c. Deposit: 020
    d. Repeat: 1

K in Mehlich I Soil Extract

1. Principle of the Method:

Potassium in Mehlich I Soil Extract (double acid extract) is concentrated enough in topsoil extracts to analyze using the flame emission mode on the atomic absorption spectrophotometer.

2. Standards: 0, 10, 20, 30, 40, and 50 ppm K in Mehlich I extracting solution.
3. Dilution: Topsoil samples can probably be run undiluted.
4. IL251:
    a. Lamp: none
    b. Burner: air-acetylene perpendicular to and 1/2 inch below beam
    c. Wavelength: 766.5 nm
    d. Slit width: 320
    e. Integration: auto at 1 sec
    f. Mode: FE
    g. HV: 460
    h. Oxidant: air at 40 psi and 20 scfh
    i. Fuel: acetylene at 15 psi and adjust flow for blue flame
    j. Nebulizer: set for aspiration rate of 1 to 1.5 mL in 15 sec

Mg in Mehlich I Soil Extract

1. Principle of the Method:

Magnesium in Mehlich I Soil Extract (double acid extract) is concentrated enough in topsoil extracts to analyze using the flame atomization technique on the atomic absorption spectrophotometer.

2. Standards: 0, 2, 4, 6, 8, and 10 ppm in Mehlich I extracting solution.
3. Dilution: topsoil samples will need to be diluted 1/10 or 1/50 with Mehlich I extracting solution.
4. IL251:
    a. Lamp: Mg at 3 ma; no D_2 lamp
    b. Burner: air-acetylene perpendicular to and 1/2 inch below beam
    c. Wavelength: 285.2 nm
    d. Slit width: 320
    e. Integration: auto at 1 sec
    f. Mode: (SB)A
    g. Focus for maximum intensity
    h. HV: adjust for intensity between 2 and 8
    i. Oxidant: air at 40 psi and 20 scfh
    j. Fuel: Acetylene at 15 psi and adjust flow for blue flame
    k. Nebulizer: set for aspiration rate of 1 to 1.5 mL in 15 sec

Ni in Plant Samples

1. Principle of the Method:

Nickel in plants is low enough that it must be run using the graphite furnace atomic absorption technique (GFAA). Plants samples may be digested using either wet or dry ashing procedures.

2. Standards: 0, 0.01, 0.03, 0.05, 0.07, 0.10 ppm Ni in 0.1 N HCl
3. Matrix modifier: To 5 mL of each sample add 50 µL of concentrated HNO_3. To 10 mL of each standard add 100 µL of concentrated HNO_3.
4. IL251:
    a. Lamp: Ni at 10 ma; D_2 at 30 ma
    b. Wavelength: 232.0 nm
    c. Slit width: 80
    d. Integration: Pk Ht at 1/16 sec
    e. Mode: (SB)A-B
    f. Focus for maximum intensity
    g. HV - adjust until intensity is between 2 and 8
5. IL655 (with IL755 temperature sensor):
    a. Coolflow: on at 20°C
    b. Purge gas: argon at 45 psi and 30/5 SCFH
    c. Mode: auto
    d. Temperature feedback: on
    e. Clean: off
    f. Program:

Step	Temperature	Time	Integrate Start
	°C	*X5 sec.*	
1	150	0	
2	150	1	
3	475	4	
4	600	4	
5	2250	0	*
6	2250	2	

6. IL254:
    a. Nebulizer flow: air at 40 psi and 2.8 LPM
    b. Delay: 06
    c. Deposit: 020
    d. Repeat: 1

Pb in Plant Samples

1. Principle of the Method:

Lead in plants is low enough that it must be run using the graphite furnace atomic absorption technique (GFAA). Plant samples may be digested using either wet or dry ashing.

2. Standards: 0, 0.01, 0.03, 0.05, 0.07, 0.10 ppm Pb in 0.1 N HCl
3. Matrix modifier: To 5 mL of each sample add 50 µL of concentrated HNO_3. To 10 mL of each standard add 100 µL of concentrated HNO_3.
4. IL251:
    a. Lamp: Pb at 5 ma; D_2 at 25 ma
    b. Wavelength: 283.3 nm
    c. Slit width: 320
    d. Integration: Pk Ht at 1/16 sec
    e. Mode: (SB)A-B
    f. Focus for maximum intensity
    g. HV: adjust until intensity is between 2 and 8
5. IL655 (with IL755 temperature sensor):
    a. Coolflow: on at 20°C
    b. Purge gas: argon at 45 psi and 30/5 SCFH
    c. Mode: auto
    d. Temperature feedback: on
    e. Clean: off
    f. Program:

Step	Temperature	Time	Integrate Start
	°C	*X5 sec.*	
1	150	0	
2	150	1	
3	350	3	
4	600	4	
5	2200	0	*
6	2200	2	

6. IL254:
    a. Nebulizer flow: air at 40 psi and 2.8 LPM
    b. Delay: 06
    c. Deposit: 020
    d. Repeat: 1

Se in Plant Samples

1. Principle of the Method:

Plants growing on Alabama soils are extremely low in selenium. The following fluorometric method (a modification of an AOAC procedure) can be used to determine total Se in plant tissue. The wet ashing procedure given here must be used for digestion of the plant material. Use of the standard wet ashing procedure will result in loss of Se by volatilization.

2. Apparatus:
   a. 250 mL digestion tubes and rack
   b. 50°C water bath
   c. 50 mL Erlenmeyer flasks
   d. Block digestor in perchloric acid fume hood
   e. 125 mL separatory funnels
   f. 15 mL centrifuge tubes
   g. Centrifuge
   h. Fluorometer
3. Reagents:
   a. Acid mix (700 mL concentrated HNO_3 + 300 mL concentrated $HClO_4$)
   b. 5 N NH_4OH (34 mL concentrated NH_4OH + 66 mL H_2O)
   c. EDTA solution: Add 20 mL H_2O to 1.9 g EDTA (acid form). Slowly add 5 N NH_4OH with stirring until the EDTA just dissolves. Dissolve 6 g hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in 100 mL H_2O. Combine the solutions and dilute to 250 mL with H_2O.
   d. Cresol red indicator: Dissolve 0.1 g cresol red in 10 mL H_2O and add one drop of saturated NaOH solution (50%). Dilute to 50 mL with H_2O.
   e. 1000 ppm Se standard
   f. Decalin (decahydronaphthalene)
   g. 0.1 N HCl: Dilute 8.3 mL concentrated HCl to 1000 mL with H_2O
   h. 20% HCl (20 mL concentrated HCl + 80 mL H_2O)

i. DAN (2,3-diaminonaphthalene) solution:
Prepare the DAN solution under yellow lights. Add 100 mL 0.1 N HCl to 0.1 g DAN. Heat in 50°C water bath for 15 minutes. Cool to room temperature and extract twice with 20 mL decalin. Shake the flask vigorously and discard the decalin. Filter the DAN solution through Whatman #40 filter paper saturated with water. Do not expose the DAN solution to white light. The DAN solution must be made fresh daily.

4. Standards:

Standards containing 0, 0.2, 0.4, and 0.6 μg Se should be prepared by diluting the 1000 ppm Se standard. The standards are carried through the same digestion procedure as the samples. The 0 μg Se standards also serve as reagent blanks.

5. Procedure:

a. Weigh 1 g of previously dried and ground plant material into a 250 mL digestion tube. Add 10 mL of acid mix and stopper the digestion tubes by inverting 50 mL Erlenmyer flasks into their tops. Allow the tubes to set overnight at room temperature in a conventional fume hood.

b. Heat the digestion tubes in a 50°C water bath for 2 hours. Leave the tubes covered during this step.

c. Remove the 50 mL Erlenmyer flasks from the digestion tubes and heat the samples at 200°C using the heating block in the perchloric acid fume hood. Continue heating until about 1 mL of perchloric acid is left in the tubes.

d. Remove the tubes from the heating block and add 1 mL of 20% HCl. Stopper the tubes with inverted 50 mL Erlenmeyer flasks and return them to the heating block for 5 minutes. Remove the tubes from the block and allow them to cool.

e. Add 5 mL of EDTA solution and 2 drops of cresol red indicator to each tube. Neutralize the solutions to yellow using 5 N NH_4OH and then acidify the solutions to orange-pink using 20% HCl.

f. This and all the following steps must be carried out under yellow lights. Prepare the DAN solution.g. Add 5 mL of DAN solution and 20 mL of 0.1 N HCl to each tube.

h. Heat the samples by setting the tubes in the 50°C water bath for 25 minutes. Remove the tubes from the water bath and cool by setting them in a pan of room temperature water.

i. Pour the contents of the digestion tubes into separatory funnels containing 10 mL of decalin. Shake the funnels and discard the water layer.

j. Wash the contents of the separatory funnels twice using 25 mL of 0.1 N HCl each time.

k. Pour the organic layer from the separatory funnels into 15 mL centrifuge tubes and centrifuge at medium speed for 2 minutes.

l. Pipette 3 ml of the organic layer from the centrifuge tubes into fluorometer cuvettes and determine the fluorescence. The fluorometer should be set to illuminate the sample at a wavelength of 369 nm and to determine the fluorescence at a wavelength of 525 nm.

m. Compare the fluorescence of the unknowns to the fluores cence of the standards carried through the procedure to determine Se in the unknowns.

Se in Soil Samples

1. Principle of the Method:

Soils in Alabama, with the exception of calcareous Black Belt soils, are very low in plant available selenium. This procedure is a modification of the boiling water extraction procedure for plant available Se. Calcium chloride solution is used instead of deionized water as an extracting agent because some Alabama soils disperse in deionized water. Selenium in a large volume of extract is concentrated by evaporation.

2. Apparatus:

a. 250 mL digestion tubes and rack
b. 50 mL Erlenmeyer flasks
c. 500 mL Erlenmeyer flasks and reflux apparatus
d. 50°C water bath
e. AA spectrophotometer with hydride generator
f. 150 mL fleakers
g. 500 mL fleakers

3. Reagents:

a. 0.01 M $CaCl_2$ (1.47 g $CaCl_2 \cdot 2H_2O$ per 1000 mL of solution)
b. Acid Mix (700 mL concentrated HNO_3 + 300 mL concentrated $HClO_4$)
c. Concentrated HCl
d. 0.3% $NaBH_4$ in 1% NaOH: Add 1 g NaOH to 100 mL water in a 500 mL fleaker and mix. Then add 0.3 g $NaBH_4$ and mix.
e. 1 M $CaCl_2$ (14.70 g $CaCl_2 \cdot 2 H_2O$ per 100 mL of solution.)
f. 1000 ppm Se standard

4. Standards:

Standards containing 0, 0.1, 0.2, 0.3, and 0.4 µg of Se are prepared in 150 mL fleakers by diluting the 1000 ppm Se standard. To each fleaker, add 2 mL of 1 M $CaCl_2$ and 33 mL of concentrated HCL. Make the final volumes in the fleakers to 100 mL with H_2O. Blanks should be carried through the entire extraction procedure.

5. Procedure:

a. Place 60 g of previously dried and ground soil into a 500 mL Erlenmeyer flask. Add 300 mL of 0.01 M $CaCl_2$ and reflux the suspension for 30 minutes. Filter the extract through Whatman #40 filter paper into a beaker.

b. Transfer 200 mL of the filtrates to 250 mL digestion tubes, add 10 mL of acid mix, stopper the tubes with inverted 50 mL Erlenmeyer flasks, and heat the tubes in a 50°C water bath for 2 hours. The water bath should be in a conventional fume hood.

c. Unstopper the tubes and transfer them to the block digestor in the perchloric acid fume hood. Evaporate the extract and digest the sample by heating the tubes at 300°C until the final volume is about 1 mL.

d. Remove the tubes from the block, add 33 mL of concentrated HCl, stopper the tubes with inverted 50 ml Erlenmeyer flasks, and place the tubes back on the block. When the acid starts to boil, remove the tubes from the block and let them cool.

e. Transfer the contents of the digestion tubes to 150 mL fleakers and make the final volume in the fleakers 100 mL with water.

f. Selenium is determined in the extracts, standards, and blanks using the IL251 AA spectrophotometer with the IL440 hydride generator accessory.

6. IL251:

a. Lamp: Se at 10 ma; no D_2 lamp

b. Burner: special air-acetylene with attached quartz cell

c. Wavelength: 196.0 nm

d. Slit Width: 320

e. Integration: Pk Area for 16 sec

f. Mode: (SB)A

g. Focus for maximum intensity

h. HV: adjust for intensity between 2 and 8

i. Oxidant: Air at 40 psi and 20 scfh

j. Fuel: Acetylene at 15 psi and adjust flow for yellow flame.

k. Nebulizer: Set for aspiration rate of 1 to 1.5 ml in 15 sec.: aspirate water at all times.

7. IL440:

a. Inert gas: argon at 20 psi and 6 to 7 lpm

b. Reagent: 0.3% $NaBH_4$ in 1% NaOH

c. Reagent amount: 5

d. Reaction time: 0.5 min.

BIBLIOGRAPHY

AA Spectrometer Procedures

Emmel, R.H., J.J. Sotera, and R.L. Stux. 1977. Atomic absorption methods manual. Instrumentation Laboratory, Inc., Wilmington, MA.

General Analysis Procedures

American Public Health Association. 1992. Standard methods for the examination of water and wastewater. 18th ed. Am.Public Health Assoc., Washington, DC.

Helrich, K. (ed.). 1990. Official methods of analysis of the Association of Official Analytical Chemists. Assoc. of Official Analytical Chemists, Inc., Arlington, VA.

Hue, N.V., and C.E. Evans. 1986. Procedures used by the Auburn University Soil Testing Laboratory. Alabama Agri. Exp. Stn., Dept. of Agron. & Soils, Dept. Series 106.

Jackson, M.L. 1958. Soil chemical analysis. Prentice-Hall, Inc. Sixth printing, 1970, by the author, Dept. of Soil Sci., Univ. of Wisconsin, Madison, WI.

Page, A.L. (ed.). 1982. Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Walsh, L.M. (ed.). 1971. Instrumental methods for analysis of soils and plant tissue. SSSA, Madison, WI.

Walsh, L.M., and J.D. Beaton (eds.). 1973. Soil testing and plant analysis. Revised ed. SSSA, Madison, WI.

ICAP Spectrometer Procedures

Jarrell-Ash Div./Fisher Scientific Co. 1982. Jarrell-Ash ICAP-9000 plasma spectrometer. Jarrell-Ash Div./Fisher Scientific Co., Waltham, MA.

Thermo Jarrell Ash Corp. 1991. ICAP 61E plasma pectrometer operator's manual. Thermo Jarrell Ash Corp., Franklin, MA.