NCDA&CS Methods for Plant Tissue Analysis

Plant/Waste/Solution/Media Laboratory

Agronomic Division

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http://www.ncagr.gov/agronomi/uyrplant.htm

Plant Tissue Analysis

Plant tissue analysis is used to measure the nutrient content (total or ionic) of foliar and petiole plant materials and to identify nutrient deficiencies and toxicities. The report provides recommendations for monitoring and adjustment of crop fertilization programs. The NCDA&CS Agronomic Division does not perform any testing for microbial pathogens (i.e., disease) or organic contaminants (e.g., pesticides, herbicides) on plant material.

The standard plant tissue analysis includes measurement of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), sodium (Na), and aluminum (Al). Interpretation indexes are provided for most crops where the crop, correct growth stage and plant part are reported.

Standard tissue analysis for **N.C. residents**	$5.00
Standard tissue analysis for **non-N.C. residents**	$25.00
ǂStandard tissue analysis for **N.C. researchers**	$12.00
ǂStandard tissue analysis for **non-N.C. researchers**	$25.00

ǂ A completed NCDA&CS Research Project Agreement is **required prior to submission** of research samples. *Please contact Dr. Kristin Hicks at Kristin.Hicks@ncagr.gov to set up a Research Project Agreement. The NCDA&CS Cooperative Research Agreement can be found at: http://www.ncagr.gov/agronomi/documents/Research_Project_Agreement_PWSM.pdf*

In addition to the standard analysis, certain tests are available by request for an additional fee per sample.

Additional tests available by request:

- Molybdenum (Mo) + $2
- Nitrate-nitrogen (NO_3-N) + $2
- Chloride (Cl^-) + $2
- ǂ Heavy Metals: cadmium, nickel, lead, arsenic, chromium, selenium + $20

(ǂNote: heavy metals analysis is available only for research purposes with a valid research agreement from a university, government agency or private scientific research institute. NO EXCEPTIONS.)

Additional tests automatically assigned to specific crops:
In addition to the standard analysis, certain crops are automatically assigned additional tests based on established crop nutrient needs or best management practices. Due to the high Mo needs of ***Brassicas*** **(broccoli, Brussels sprouts, cabbage, cauliflower, collards, kale, turnip greens, and rapeseed), spinach, alfalfa and poinsettia**, these crops are automatically analyzed for Mo content.

Strawberry and cotton samples automatically receive a NO_3-N analysis on the petiole. Petiole NO_3-N levels are used to monitor real-time nitrogen status of these crops throughout the growing season and is standard practice in much of the Southeast.

While NCDA&CS recommends that these tests be included in a research project for these crops, researchers have the option to request that these additional tests not be included in the research agreement and analysis. These tests will be automatically included for all the above-mentioned crops at an additional $2 fee per sample except by written request on the research agreement to exclude them.

For detailed plant sampling instructions, see:
http://www.ncagr.gov/agronomi/pdffiles/plantguide.pdf

Quick Guides to plant tissue sampling by crop are available here:
http://www.ncagr.gov/agronomi/uyrplant.htm

The Sample Submission form for growers can be found here:
http://www.ncagr.gov/agronomi/pdffiles/isplant.pdf

The Sample Submission form for researchers can be found here:
http://www.ncagr.gov/agronomi/pdffiles/Plant_Tissue_Sample_Submission_Form_Research.pdf

Minimum Sample Masses

To obtain a representative sample, NCDA&CS strongly recommends that clients follow the recommendations listed for each crop in the NCDA&CS Plant Tissue Analysis Guide: http://www.ncagr.gov/agronomi/pdffiles/plantguide.pdf. A good rule of thumb for a suitable amount of sample is two full handfuls of plant material. Where this is not possible, please note the minimum dried, ground plant material required to perform each analysis (Table 1).

Table 1. Plant tissue methods summary with minimum material required for method.

Plant Tissue Samples Method Summary			
Sample Test	**Minimum mass**	**Analytical Method**	**Reference**
N, C	20 mg	Oxygen combustion (Dumas method)	AOAC 972.43; Campbell 1992
P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Na, Al	0.20 g	Acid digestion; ICP-OES	Donohue and Aho 1992; EPA 200.7
Mo, As, Cd, Cr, Ni, Pb, Se	0.50 g	Acid digestion; ICP-OES	Donohue and Aho 1992; EPA 200.7
NO_3-N	0.10 g	Acetic acid extraction; Continuous Flow Analysis	Miller 1998; EPA 353.1; Skalar 2018b
Cl^-	0.10 g	Acetic acid extraction; Continuous Flow Analysis	Miller 1998; Skalar 2018a

Analytical Methods

Sample Processing & Storage

Upon receipt, samples are examined for condition (e.g., mold, inadequate mass for analysis) and correct plant part. Depending upon the crop, sufficiency ranges may be based on leaf blade only, petiole only or the whole leaf (blade plus petiole). Where sufficiency ranges are based on the leaf blade only or petiole only, it is recommended that the petiole be detached in field so that nutrients are not moving from the petiole into the leaf blade tissue during shipment to the lab. If the client has not done so for crops where it is recommended to detach petioles, the lab detaches the petioles upon receipt.

Prior to homogenization of plant material by grinding, samples are dried overnight (12–24 hr) at 80 ^{o}C. Each sample is then processed through a stainless-steel grinder with a 20-mesh (1 mm) screen (Campbell and Plank 1992). Most samples are ground on a cutting-grinding mill (IKA Works, Inc.; Wilmington, NC), but large volume and/or coarse samples are ground on a Fritsch Pulverisette 19 cutting mill (Fritsch USA, Inc. Idar-Oberstein, Germany). Small mass samples (< 5 g, wet weight basis) are ground on a tube mill without a screen (IKA Tube Mill 100; IKA

Works, Inc.; Wilmington, NC). The dried, ground plant material is stored at room temperature in a 7-dram plastic snap cap vial (~26 cm^3) until analysis. Research samples are stored for one calendar year and grower samples for six weeks from date of sample receipt. For researchers only, if you would like your samples returned to you after analysis, please note this on the research agreement.

N, P, K, Ca, Mg, S, Na and Cl are reported in % and all other elements (e.g., Fe, Mn, Zn, Cu, B, Al, Mo, As, Cd, Cr, Ni, Pb, and Se) and NO_3-N are reported in parts per million (ppm) [equivalent to mg kg^{-1}]. All results are reported on a dry weight basis.

Total nitrogen (N) and carbon (C)

Total nitrogen and total carbon are determined by oxygen combustion gas chromatography with subsequent quantification by thermal conductivity detector (AOAC 1990b; Campbell 1992). Total Nitrogen is analyzed using a Thermo Scientific FlashSmart EA Combustion Nitrogen/Protein Analyzer (CE Elantech Instruments; Lakewood, NJ) on a 49-51 mg aliquot of dried, ground plant material. For samples also requiring Total C, Total N and Total C are determined using a Thermofinnigan Flash EA1112 (CE Elantech Instruments; Lakewood, NJ) on an 8-10 mg aliquot of dried, ground plant material. Nitrogen content is analyzed as part of the standard analysis. Carbon is measured only by request. Results are reported as % N and %C.

Nitrogen and Carbon Quality Control:
Method detection limits (MDL) are determined when a new instrument or method is put into use and verified annually. **The MDL for N only is 0.065%. For samples requiring C, the MDL for N is 0.073% and the MDL for C is 0.387%.**

Dried samples are quantified using five (N) or six (C) calibration standards. Four internal and external reference samples are analyzed at the start of the day to verify the calibration. An internal plant reference material is analyzed every 12 samples and at the end of each batch for continuing calibration verification.

Phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sodium (Na), aluminum (Al), nickel (Ni), cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), selenium (Se), and molybdenum (Mo)

Total concentrations of P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Na, Al, Ni, Cd, Pb, As, Cr, Se, and Mo are determined with Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos EOP and Arcos II EOP, Spectro Analytical: A Division of Ametek; Mahwah, NJ) (Donohue and Aho 1992; adapted USEPA 2001), after closed-vessel nitric acid (HNO_3) digestion in a microwave digestion system (MARS 6 Microwaves; CEM Corp.; Matthews, NC).

For most elements, a 0.5 g dried/ground aliquot is digested in 10 mL 15.6N HNO_3. When heavy metals (Ni, Cd, Pb, As, Cr, Se) or Mo are requested, a 1.0 g aliquot of dried/ground sample is digested in 15 mL or 10 mL HNO_3, respectively. Samples are digested for 30 minutes at 200 °C in

a microwave (Plant Materials method; CEM), and the digested sample volume is brought to 50 mL with deionized water and then filtered through pre-folded Whatman #2 filter paper (Folded Filter Paper, Albuquerque, NM). Elements are measured at the wavelengths listed in Table 2.

Table 2. Wavelengths used to quantify total elemental concentrations in plant materials by ICP-OES.

Element	Wavelength (nm)
Aluminum (Al)	396.152
Arsenic (As)	189.042
Boron (B)	208.959
Cadmium (Cd)	214.438
Calcium (Ca)	183.801, 315.887, 318.128
Chromium (Cr)	267.716, 357.869
Copper (Cu)	324.754
Iron (Fe)	259.941
Lead (Pb)	220.353, 405.778
Magnesium (Mg)	279.079
Manganese (Mn)	257.611
Molybdenum (Mo)	202.095
Nickel (Ni)	341.476
Phosphorus (P)	178.287
Potassium (K)	404.721, 766.491
Selenium (Se)	196.090
Sodium (Na)	330.237, 589.592
Sulfur (S)	182.034
Zinc (Zn)	213.856

Results are expressed as a percentage (%) for P, K, Ca, Mg, S and Na and in parts per million (ppm) [equivalent to milligrams per kilogram (mg/kg)] for all other elements on a dry-weight basis.

ICP-OES Quality Control:
Elements are measured using a curve with at least five calibration points.

A method blank and internal reference sample are digested and analyzed with each batch of 40 samples. A second internal reference sample is digested and analyzed once per day. For heavy metals, a matrix spike and an external reference are also digested and analyzed with each batch. An interference check is also analyzed with each heavy metal analysis.

A calibration verification solution and calibration blank are run after the daily calibration, after every 10 samples and at the end of each run. An independent calibration verification solution is

analyzed at the beginning and end of each run. The method detection limits (MDLs) for each analyte are listed in Table 3.

Table 3. Method detection limits (MDL) of total elemental concentrations in plant materials by ICP-OES.

Element	MDL (ppm)
Aluminum (Al)	1.26
Arsenic (As)	0.150
Boron (B)	1.24
Cadmium (Cd)	0.100
Calcium (Ca)	118.08
Chromium (Cr)	0.200
Copper (Cu)	0.31
Iron (Fe)	2.35
Lead (Pb)	0.300
Magnesium (Mg)	52.96
Manganese (Mn)	0.15
Molybdenum (Mo)	0.06
Nickel (Ni)	0.200
Phosphorus (P)	1.10
Potassium (K)	9.66
Selenium (Se)	0.200
Sodium (Na)	32.85
Sulfur (S)	2.41
Zinc (Zn)	0.50

Nitrate-nitrogen (NO_3-N)

Nitrate-nitrogen (NO_3-N) is extracted from plant tissue with 2% acetic acid (25 mL) on a 0.25 g dried/ground aliquot of sample (Miller 1998). The extract is filtered, and NO_3-N is determined by hydrazine reduction, where nitrate is reduced to nitrite with hydrazinium sulfate catalyzed by Cu^{2+}, under alkaline conditions and at elevated temperature (Kempers and Luft 1988). The NO_2-N concentration (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with α-naphthyl-ethylenediamine dihydrochloride to form a highly-colored azo dye which is measured at 540 nm (modified Griess reaction) (USEPA 1978b; Kempers and Luft 1988; Skalar Analytical 2018b).

NO_3-N is quantified by continuous flow analysis using an auto-flow spectrophotometric analyzer (San^{++} Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands). Nitrate-nitrogen (NO_3-N) and nitrite-nitrogen (NO_2-N) are reported as NO_3-N in parts per million (ppm)

[equivalent to mg L^{-1}] on a dry-weight basis. Nitrate-nitrogen is analyzed on all strawberry and cotton samples and on other crops by request.

Nitrate-nitrogen Quality Control:
Method detection limits (MDL) are determined when a new instrument or method is put into use and verified annually. **The MDL for NO_3-N in plant materials is 45.4 ppm.**

Samples are quantified using nine calibration standards. A method blank and two internal reference materials are extracted and analyzed with each batch of 12 samples. On days where more than one extraction batch is analyzed, one of the internal reference samples is extracted again with each additional batch. A spiked duplicate is analyzed with each batch. A calibration verification solution and calibration blank are analyzed at the beginning and end of each batch and after every 10 samples. Four independent calibration verification solutions are analyzed at the beginning and end of each run. Drift checks are analyzed at the beginning and end of each run and every 20 samples. Two nitrite checks (NO_2-N) are analyzed to verify the completeness of the nitrate reduction reaction at the beginning and end of each run.

Chloride (Cl^-)

Chloride is extracted from plant tissue with 2% acetic acid (25 mL) on a 0.25 g dried, ground aliquot of sample (Miller 1998). The extract is filtered and then used for chloride determination by the thiocyanate displacement method by the formation of soluble mercuric chloride. The liberated thiocyanate forms a red colored complex with ferric iron ions also present in solution (USEPA 1978a; Zall et al. 1956; Skalar 2018a). This complex is measured at 470 nm on a segmented flow analyzer (San^{++} Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands). Results are expressed in parts per million (ppm) [equivalent to mg/L].

Chloride Quality Control: Method detection limits (MDL) are determined when a new instrument or method is put into use and verified annually. **The MDL for Cl^- in plant materials is 126.52 ppm.**

Samples are quantified using nine calibration standards. A method blank and two internal reference materials are extracted and analyzed with each batch of 12 samples. On days where more than one extraction batch is analyzed, one of the internal reference samples is extracted again with each additional batch. A spiked duplicate is analyzed with each batch. A calibration verification solution and calibration blank are analyzed at the beginning and end of each batch and after every 10 samples. Four independent calibration verification solutions are analyzed at the beginning and end of each run. Drift checks are analyzed at the beginning and end of each run and every 20 samples.

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