Plant Tissue Analysis Methods



AGRONOMIC
SERVICES

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Introduction

The NCDA&CS Plant Tissue Analysis Lab performs the following analyses based on standard industry methods:

- Nitrogen (N) and carbon (C)
- Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo), sodium (Na), aluminum (Al), arsenic (As), cadmium (Cd) chromium (Cr), nickel (Ni), lead (Pb) and selenium (Se)
- Nitrate-nitrogen (NO_3-N) and chloride (Cl^-)

Minimum Sample Masses

No matter how precise and accurate an analytical method is, meaningful results are only as good as the sample itself. The most significant cause of poor statistical results in plant analysis is due to imprecise sample collection and preparation rather than analytical measurement.

Clients should endeavor to submit the recommended amount of plant material (Table 1). Research samples automatically receive quality control rechecks on 10% of their samples at no additional charge, but only if the recommended masses are submitted. Also, human error occasionally leads to the need to re-analyze a sample. Without sufficient material, this results in data loss for the researcher.

Where recommended amounts are 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 each method.

Sample Test	Minimum mass	Recommended mass	Analytical Method	Reference
N, C	20 mg	50 – 100 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	0.5 g	Acid digestion; ICP-OES	Donohue and Aho 1992; EPA 200.7
Mo, As, Cd, Cr, Ni, Pb, Se	0.50 g	1.0 g	Acid digestion; ICP-OES	Donohue and Aho 1992; EPA 200.7
NO_3-N	0.10 g	0.25 g	Acetic acid extraction; Flow Injection Analysis	Miller 1998; EPA 353.2; FIA NO3-W-1-1
Cl^-	0.10 g	0.25 g	Acetic acid extraction; Flow Injection Analysis	Miller 1998; EPA 325.2; FIA Cl-W-1-1

Sample Processing—Grower Samples Only

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 the 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).

Sample Storage

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.

Analytical Methods

Method detection limits (MDL) are determined annually and when a new instrument or method is put into use.

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 mg kg^{-1}. All results are reported on a dry weight basis.

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 %.

Nitrogen and Carbon Quality Control. Dried samples are quantified using five (N) or six (C) calibration standards. Four internal and external reference samples are analyzed after calibration, and three 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.

Elemental Analysis

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 Advantec #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

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.

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 cadmium reduction, where nitrate is reduced to nitrite with copperized cadmium, under alkaline conditions. The NO_2-N concentration (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-Naphthyl) ethylenediamine dihydrochloride to form a magenta-colored azo dye which is measured at 520 and 600 nm (USEPA 1993; FIA NO3-W-1-1). NO_3-N is quantified by flow injection analysis (FIAlyzer-1000, FIA Lab; Seattle, WA).

Nitrate-nitrogen Quality Control. Samples are quantified using nine calibration standards. A method blank (DI water, filtered) is analyzed with each batch. A duplicate aliquot of a filtered solution sample is spiked and analyzed for analytical recovery with each batch. A second method blank (2% acetic acid extractant, filtered) 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 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. 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, which is measured at 480 nm (Standard Methods; FIA CL-W-1-1). Cl is quantified by flow injection analysis (FIAlyzer-1000, FIA Lab; Seattle, WA).

Chloride Quality Control. Samples are quantified using nine calibration standards. A method blank (DI water, filtered) is analyzed with each batch. A duplicate aliquot of a filtered solution sample is spiked and analyzed for analytical recovery with each batch. A second method blank (2% acetic acid extractant, filtered) 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

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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