PROTOCOL: Quantification of Phosphorous, Base Cations and Metals in Dry Leaf Tissue by ICP-OES Using Microwave Digestion.

(i) **Equipment**

- Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometer
- Analytical balance (0.001 g)
- CEM MARS Xpress Microwave Digester, vessels with plugs and caps, vessel racks
- 5 ml pipettor

(ii) **Consumable materials**

- Translucent polypropylene test tubes (13 mm × 100 mm)
- Concentrated nitric acid (68–71% w/w HNO_3. Trace Metal grade)
- E-pure (or equivalent) water
- Acid resistant gloves, goggles, and apron
- Multi-element primary standard set (see Standard Preparation)
- Aardvark Straws for sample transfer
- 20 ml plastic Scintillation vials

(iii) **Sample preparation**

- Label dry, acid-washed Teflon vessels.
- Weigh 0.4 g (± 0.005 g) dried ground leaf material and transfer it into a microwave digester vessel.
- Include one blank and one to two reference standards (i.e. NIST peach) per run (40 total vessels).
- Add 10 ml HNO_3 to each vessel with sample.
- Add plug and cap to each vessel, remove label, insert sequentially into cavity on microwave digester carousel.
- Digest according to CEM MARS Xpress parameters (1600 W, 100%, ramp 30 min, 200 ^{0}C, hold 25 min).
- Once digested, transfer sample to 20 ml scintillation vile and let sit for at least 30 min for particulates to settle.
- Transfer 1 ml of digested sample to 3 ml E-pure water in 8 ml polystyrene test tubes and place in ICP rack.

(iv) **Measurement procedure**

- Total elemental concentration of B, Ca, Fe, K, Mg, Mn, P, and Zn in each digested solution is determined through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) on a Thermo Jarrel Ash IRIS instrument.
- The instrument is calibrated using multi-point linear fitting with high and low concentration working standard solutions containing all the elements of interest (detailed procedure in "Standard Preparation Procedure" below) and blank solutions.
- The ICP values are validated with standardization blanks, NIST reference material (peach leaves), drift control samples, and quality control samples.

(v) **Standard Preparation Procedure**

- Prepare high and low concentration working standard solutions from single element primary standard solutions as listed below.
- Use a background matrix of 3% trace metal grade HCl solution for the standards and the blank solution.
- Measure each stock solution by weight into a graduated cylinder assuming a density of stock solution as 1.0 g/ml unless otherwise converted.
- Record actual concentrations in the prepared solution if they differ from the calculated values.

- Pour solution into a 1 L volumetric flask.
- Carefully bring to volume, add a stopper and invert 10 times to homogenize the solution.
- Transfer solution to a properly labeled Nalgene container for storage.
- Be certain to calibrate the new standard with old standards to ensure continuity across analyses.

	High Concentration Standard		Low Concentration Standard	
Element	**Conc. (ppm)**	**Solvent**	**Conc. (ppm)**	**Solvent**
B	10	DI Water	5	DI Water
Ca	50	2% Nitric Acid	25	2% Nitric Acid
Fe	1	2% Nitric Acid	0.5	2% Nitric Acid
K	70	DI Water	35	DI Water
Mg	30	2% Nitric Acid	15	2% Nitric Acid
Mn	10	2% Nitric Acid	5	2% Nitric Acid
P	10	DI Water	5	DI Water
Zn	1	5% Nitric Acid	0.5	5% Nitric Acid

(vi) **Data preparation and finalization**

- Correct data in terms of standardization blanks, NIST reference standards, drift correction, and dilution factor application.
- Quality assurance and quality control checks are performed on the data in accordance to the specifications of Dr. Guangchao Li, EM-1 Lab, Stanford CA.
- Calculate elemental concentrations on a dry weight basis and present them on a mass or percentage basis using the following equations:

$$\frac{\mu g\ \text{element}}{g\ \text{dry sample}} = \frac{\text{ICP data } (\mu g\ ml^{-1}) * 8\ ml * 10\ ml}{2\ ml * \text{wt.g dry sample}}$$

$$\%\ \text{of element} = \mu g/g\ \text{calculation} * (1g/\ 1{,}000{,}000\ \mu g) * (100)$$

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