Plant Foliar % P

•Ellen Esch, Feb 2020, adapted from Ogdahl, M, Buyarski, C., and PrometheusWiki contributors. 2013. "Plant Phosphorus Protocol - Sulfuric acid digestion." /tiki-pagehistory.php?page=Plant Phosphorus Protocol - Sulfuric acid digestion&preview=11

Make solutions:

10N H_2SO_4 (check under hood to see if any is already made!)

- In a clean 500 mL volumetric flask, add ~200 mL Nanopure, place in an ice bath
- Then add 138.9 mL H2SO4 and fill to 500 mL with Nanopure.

Molybdate reagent (check in fridge to see if any is made!)

- In a clean 1 L volumetric flask dissolve:
  - 0.208 g Antimony Potassium - Tartrate
  - 9.6 g Ammonium Heptamolybdate 4-hydrate
  - Fill to 1 L with nanopure

Ascorbic acid ** Make fresh daily**

- 0.5 g ascorbic acid into 50mL nanopure

Apple Standard (NIST 1515); have on hand! 0.159% P

Protocol - combustion:

1. Place *borosilicate* test tubes in a *stainless steel* test tube rack (very important regarding glass and metal type, or else they might melt! Obviously no lids here)
2. Weigh out the apple leaf standard into tared test tubes
   i. Weigh out 8-10 different weights in the range of 0.1 to 6 mg (of oven-dried and cooled sample) into the pre-tared test tubes. **note this is VERY LITTLE standard…keep in mind the jar cost >$1,000! Please try to minimize waste, but NEVER put any excess back into the container!
3. Weigh out the samples, and place a tin foil "lid" over them to indicate they are done
   a. 1-4 mg of sample into the pre-weighed and recorded test tubes should suffice
4. Make sure to include some blanks too (no sample or standard material)
5. Ash samples, standards and blanks in muffle furnace at 550°C for 2 hours. Ideally, place into a cool muffle furnace and raise the temp up to 330°C first, hold it there for 30 minutes, and then bump it up to 550°C for the next 2 hrs.
6. Remove from furnace, and let cool to room temp.

Protocol - colorimetry:

1. To the ashed test tubes, add 0.4 mL 10 N H_2SO_4 and vortex
2. Add 5 mL nanopure water and vortex
3. Cap test tubes, and autoclave on a liquid cycle at 121°C with 30 min exposure, according to the total P protocol (cap tubes, and put test tubes in water bath, etc).
4. Into a microplate, pipette in:
   a. 135 µL of the sample
   b. 25 µL of the molybdate reagent
   c. 20 µL of the ascorbic acid solution
   d. 70 µL of nanopure water
5. Measure samples at 880 nm after 1 hour, but before 3 hours.
6. Use the plant standards to calculate %P in the unknown samples
   a. x-axis = avg. absorbance, y-axis = known mg P in standard (apple standard weight * 0.159).
   b. Use the resulting regression line to figure out the "calculated mg P" in your samples.
   c. And then the "calculated mg P" divided by the sample weight gives the "% P in sample"