A & L GREAT LAKES LABORATORIES, INC.
Standard Operating Procedure

SOP:	NEO-10-020	Revision:	0	Effective Date:	04/20/2023	Page:	1 of 10

TITLE: Mineral Analysis of Microwave Plant Tissues

AUTHOR / JOB TITLE: Amanda Lehman / ICP Laboratory Technician
Beth Meyer / ICP Laboratory Technician
Steven Piercy / ICP Laboratory Technician

PURPOSE OR SUMMARY: To ensure that the preparation, microwave digestion and analysis of plants are done in a uniform and professional manner in order to provide accurate and reproducible results.

SCOPE / APPLICATION: This SOP is to be used for NEON project specific samples for microwave plant analyses on the ICP after they have been digested.

DISTRIBUTION: Quality Assurance Officer, Agriculture Department Manager, four (4) copies for Ag Division Manuals

APPROVAL		
Name	Job Title	Date
	Director of Technical Services	04/19/2023
	Quality Assurance Coordinator	04/17/2023
	ICP Laboratory Technician	04/17/2023

HISTORY	
Supercedes	Reason for change

Copy:	1 of 1

I. REPORTING LIMIT

A. K=0.2%, Ca=0.05%, Mg=0.01%, P=0.01%, S=0.04%, and Na=0.05%

B. Al=10ppm, Fe=4.9ppm, Mn=11ppm, Zn=1.0ppm, B=2.6ppm and Cu=1.20ppm

II. APPLICABLE MATRIX OR MATRICES

A. Plants and Feeds

III. DEFINITIONS

A. N/A

IV. INTERFERENCES

A. N/A

V. SAFETY

A. Follow normal laboratory safety guidelines. Use caution when dealing with acids and bases. Consult MSDS sheets.

B. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably possible.

C. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS: Hydrogen peroxide, Nitric acid

D. Use caution when dealing with acids.

VI. EQUIPMENT AND SUPPLIES

A. ICP racks with 21 spaces (3x7 configuration)

B. ICP-OES with auto-sampler, computer, appropriate hardware and software

C. Teflon bottles

D. Class A pipets

E. Volumetric Flask

VII. REAGENTS AND STANDARDS

A. Reagents

1. ICP Rinse Solution 5% Nitric Acid
   a) 400 mL Nitric Acid (HNO_3) diluted to 8 L with de-ionized water.
   b) Store in a plastic carboy in the instrument room.
   c) Refill the ICP rinse stations as necessary.
2. De-ionized water
3. Nitric Acid, concentrated **((CAUTION))**
4. ICP PT STANDARD STOCK in 5% HNO_3 : Certified stock purchased from outside source.

   and

   ICP PT CONTROL STOCK in 5% HNO_3 : Certified stock purchased from outside source, with a different lot number than the Standard Stock to fulfill "different source requirement".
   a) 10,000 µg / mL Potassium (K)
   b) 5,000 µg / mL Calcium (Ca)
   c) 1,000 µg / mL Magnesium (Mg), Phosphorus (P) and Sulfur (S)

d) 100 µg / mL Aluminum (Al) and Sodium (Na)

e) 50 µg / mL Iron (Fe)

f) 20 µg / mL Manganese (Mn) and Zinc (Zn)

g) 5 µg / mL Boron (B) and Copper (Cu)

B. Working Standards: Blank and 3 Standards are used to calibrate the ICP.

1. The mineral concentrations in each working standard can be found in **Exhibit A**.

2. **Plant High (micro)**: Fill a 1 L volumetric flask half-way with de-ionized water. Using class A pipette, add 100 mL of PT Standard Stock and 100 mL HNO_3. Bring to volume with de-ionized water and mix thoroughly then transfer to a Teflon bottle.

3. **Plant Medium (micro)**: Fill a 1 L volumetric flask half-way with de-ionized water. Using class A pipette, add 50 mL of PT Standard Stock and 100 mL HNO_3. Bring to volume with de-ionized water and mix thoroughly then transfer to a Teflon bottle.

4. **Plant Low (micro)**: Fill a 1 L volumetric flask half-way with de-ionized water. Using class A pipette, add 5 mL of PT Standard Stock and 100 mL HNO_3. Bring to volume with de-ionized water and mix thoroughly then transfer to a Teflon bottle.

5. **Blank (micro)**: In a 1L volumetric flask add 100 mL HNO_3. Fill the flask half-way with de-ionized water. Swirl in an ice bath until the flask is cool to the touch. Dilute the solution to 1 L with de-ionized water and immediately transfer to a Teflon bottle.

C. Control Solution: The Plant Control Solution is analyzed immediately after the standard calibration and after every 12 samples analyzed (called Flex Control). It is also analyzed as a sample in the run (called Internal Control).

1. **Plant Control Solution (micro)**: Fill a 1 L volumetric flask half-way with de-ionized water. Using class A pipette, add 25 mL of PT Control Stock and 100 mL HNO_3. Bring to volume with de-ionized water and mix thoroughly then transfer to a Teflon bottle.

VIII. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

A. N/A

IX. QUALITY CONTROL

A. A&L Feed Check – run approximately every 20 samples.

B. Blank – run approximately every 20 samples.

C. NBS Soybean (purchased) – run approximately every 20 samples except for Thursday.

D. NBS Peach Leaves (purchased) – run once every day.

E. Petiole Check – replaces the A&L Feed Check in runs that contain petiole samples.

F. Flex Control is run after every 12 samples.

G. Internal Control is run approximately every 50 samples and takes place of the Reference used for the microwave digestion.

X. CALIBRATION AND STANDARDIZATION

A. N/A

XI. PROCEDURE

A. START UP

1. Turn on the exhaust hood for the ICP's located on the wall inside the entry to the ICP room.

2. Turn on the computer and monitor.

3. Login to the computer.

a) Use "ICPAG" or "ICPLAB" as your login name.

b) Use "PASSWORD" as the password.

c) Double click on the "ESI SC" icon. This will open the software for the auto-sampler and initialize it.

d) Double click on the "iTEVA" icon. This will open the ICP software. Select the appropriate username or use ADMIN if one is not applicable. Press the "OK" button.

e) Allow the ICP to initialize.

4. In iTEVA Control Center, click the icon in the bottom right corner that resembles the ICP plasma.

a) This will bring up the "Plasma Status" box.

b) Secure the peristaltic pump tubing for both the carrier line and the waste line.

(1) The carrier line consists of the ICP Rinse Solution 5% Nitric Acid.

c) In the Plasma Status box select box select "Plasma On".

d) Leave the torch on for a minimum of 15 minutes before running an analysis.

B. ICP FILE SET UP

1. Plant files are imported through the network weighing program.

a) Open the PlantWtExport program.

b) Type in the correct Julian date.

c) Type in the year (YYYY).

d) Click top (from sample #) "Browse" button, click on first Sample ID number of the file (i.e.1). Escape.

e) Click bottom (to sample #) "Browse" button, click on the last Sample ID number of the file (i.e. 100). Escape.

f) Select "Export". A screen will come up saying the weights have been successfully exported, click "OK".

g) Exit the program.

2. Make sure the correct loop is installed on the ESI valve.

a) The "FAST-Plants" method is used for microwave plants and requires a 500 µL loop.

(1) If the correct loop is not installed:

(a) Loosen the peristaltic pump tubing for the carrier line.

(b) Disconnect the tube on the valve by unscrewing ports 1 and 4.

(c) Screw the 500 µL loop into ports 1 and 4.

(d) Secure the peristaltic pump tubing for the carrier line.

(e) In the ESI software, select "FAST". This will open the "FAST METHOD CONTROL". Then press "LOAD1" then "INJECT1" several times to load the loop with carrier.

3. Under the ESI software, click on the rack boxes to bring up the "Rack Setup". Choose the rack labeled "LR21 (3x7)". This is the 21 spot ICP plant rack.

a) Click Save.

b) Repeat the same step for all three remaining rack boxes if necessary.

4. Under the ESI software, select "FAST". This will open the "FAST Method Control".
   a) Under "File", select "Open Text File".
   b) Select "FAST-Plants". This is the ESI method used for Microwave Plant Analysis.
   c) Close the box.
5. Select the iTEVA Control Center. Click on the icon labeled "Analyst".
6. A box will appear labeled "Select a method".
   a) Select the " Plant_Microwave".
   b) All the methods are preceded by a letter to indicate which ICP (A, B, etc.) the results are from. (i.e. A_Plant_Microwave)
   c) Click OK.
7. Select the "Sequence" tab on the bottom left hand side of the screen.
8. Select "Auto-Session" from the upper left menu. Then select "New Autosampler".
9. A box will appear labeled "New Automation Session".
   a) Select the "New" button.
   b) Select the "Import from Delimited Text File".
      (1) Use the drop-down box and select "weights THERMO".
      (2) Select OK.
      (3) This will import the sample list into your sequence file. The weight, sample ID, and Julian Date will all be imported to the correct locations.

      Note: The concentration of each element reported is calculated using the actual sample weights and final volumes. The results from the ICP software then show the concentration (either ppm or %, depending on the element) for each element in the original dry sample.
10. In the top left corner under the menu, select the icon "List View".
    a) The icon looks like a chart.
    b) The icon can be used to toggle between the map and list view of the samples.
11. In the large white box on the left-hand side of the screen, there is a highlighted line of text titled "Untitled (Cetac by ESI SC4)". There should also be an icon resembling an auto-sampler in front of the text.
    a) Right click on the text line.
    b) Click "Auto-Locate All".
    c) Numbers will appear in the first four columns of the list view. If your toggle back to the map view, you will see the test tubes have been shaded.
    d) This will locate all your standards and samples in the ESI auto-sampler. The auto-locate feature always starts with the first tube in the first rack and fills numerically.
    e) Samples past the last rack need to be manually located. Auto-locate will only locate the first 84 samples when the 3x7 racks are in use.
12. All of the QC Internal Controls in the run will need their correction factor changed to 100 from 125.
13. Expand the "Untitled (Cetac by ESI SC4)" box by clicking on the "+".

14. Expand the group below it.

    a) This will be labeled "S_X_Plant_Microwave" depending on the ICP. The "X" will be filled with the letter corresponding to the ICP (A, B, etc...) The version number on the method will also be included on the line.

15. Expand the line labeled "Samples".

    a) A list will appear of all samples and their position ID's.

C. ANALYSIS

1. Obtain the plant microwave samples. They will be in 50 mL Screw Cap Tubes in a 6x6 blue 36 place rack.
2. Move microwave samples from the blue racks into a LR21 (3x7) tray. The first sample should go in the front right corner and follow back numerically. Leave empty spot(s) for the "reference" samples.

    a) A test tube containing control solution replaces all the tubes marked as "reference".This occurs approximately every 50 samples.

3. Place the trays on the autosampler in the correct spots according to the software map.
4. When ready to start the run, right click the first sample in the expanded "Samples" line.

    a) Select "Start AutoSession Run at This Sample".

    b) A box will appear titled "Method's Initial Actions". Calibration and QC should be checked. Select "OK".

    c) The calibration will take place before running the samples.

5. A control solution is run after every 12 samples.
6. Monitor checks and controls as they are analyzed and recalibrate as necessary. Each QC check sample and control solution have specific quality control parameters.

    a) Under the Sequence tab, click the red square labeled "Abort Auto Session" to stop the run.

    b) In the list view, highlight the rows and columns of the samples right before the point at which you want to recalibrate.

    c) Cut the highlighted samples by pressing the "Remove Samples" icon.

    d) When ready to restart the run, right click the first sample in the expanded "Samples" line.

        (1) Select "Start AutoSession Run At This Sample".

        (2) A box will appear titled "Method's Initial Actions". Calibration and QC should be checked. Select "OK".

        (3) The calibration will take place before the run.

D. ICP DATA TRANSFER

1. All data is automatically transferred to a text file on the desktop named "PResults".

    a) The data is exported as the samples are run.

2. Cut the file labeled "PResults" by right clicking it.
3. Paste the file into the shortcut folder on the desktop named "X_Plants".

    a) X will equal the letter that corresponds with the ICP (A, B, etc...)

4. Select the "LIMS" icon.

a) Type in "ICP".
b) "TAB" once.
c) Type in "ICP" and press "ENTER"
d) Select "PLANTS".
e) Select "LAB DATA TRANSFER"
f) Select "TRANSFER ICP DATA."
g) Highlight the appropriate ICP instrument initial (A, B, etc...).
h) Select "TRANSFER ICP".
i) When the transfer is completed, click on "OK".
j) Select "EXIT".

5. Check the printout for missing data and, if necessary, rerun any samples that were missed.
   a) Any samples that over ranged on data can be run as a 1:10 dilution and printed out.

E. SHUT DOWN

1. After completion of the analyses the following shut down procedure will be followed:
   a) Rinse the ICP with de-ionized water at least one minute.
   b) Click the "Plasma Status" icon. Select "Plasma Off".
   c) Release the pressure on the pump tubing from the peristaltic pump.
   d) Close down the iTEVA and ESI software.
   e) Turn off the monitor and computer.

XII. GENERAL COMMENTS

A. Watch for Zinc contamination from gloves.

B. µg / mL = ppm

C. Standards and controls should have different lot numbers to fulfill the "different source" requirement. The different lot numbers of the standard solution and control solution confirms that the stock solutions and working standards and controls have been prepared properly.

XIII. DATA ANALYSIS AND CALCULATIONS

A. The ICP software calculates the concentration of each element in ppm or % in the original dry sample by multiplying the concentration in solution by the final volume and dividing by the actual weight (g) used.

B. ppm / 10000 = % or mg/L / 10000 = %

XIV. METHOD PERFORMANCE

A. N/A

XV. POLLUTION PREVENTION AND WASTE MANAGEMENT

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the USEPA recommends recycling as the next best option.

B. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

C. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits is also required.

XVI. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

A. Pt Control Stock Flex Control Acceptance Criteria is within 10% for routine analysis. Project requirements may vary depending on project specifies. Neon project 2018 Quality Assurance requirements are listed below in Table 2:

Table 2. QA Requirements for Major/Minor/Trace Element Analysis

QA Check	Frequency	Acceptance Criteria	Corrective Action	Insufficient Mass for Corrective Action
Blank	Minimum 1 per run/batch	Within the lab's standard acceptance criteria	Blank values should be subtracted from reported results.	N/A
QA material with known element concentrations, traceable to a national standard such as those provided by North American Proficiency Testing Program (NAPT) or NIST	Minimum 1 per run/batch	All analytes within 20% of known values	Maintenance and/or recalibration, then re-run samples; re-digest run/batch if QA material still fails acceptance criteria.	*Report sample data, include appropriate quality flag*
QA standard solution with known element concentrations	5-10% of total run	All analytes within 5% of known values	Maintenance and/or recalibration, then re-analyze samples in run/batch	N/A

B. Quality Control materials within 20% of the known values.

1. If this criteria is not met for any of the required analytes, corrective action will be taken as listed in Table 2. If the problem persists, the issue will be reported using the analyte-specific quality flag variables and the entry '**standard value out of range**' for all records in that batch.

C. NBS (peach and soy) Acceptance Criteria – reference the Plant Analysis Quality Control sheet.

D. A&L Feed Check Acceptance Criteria – reference the Plant Analysis Quality Control sheet.

E. Petiole Check Acceptance Criteria – reference the Plant Analysis Quality Control sheet.

F. Additionally, all Neon samples will be checked against established thresholds for plant traits (see below), with follow-up actions if samples are identified as outliers:

1. Sample data will be reviewed to determine if there were any analytical issues.
2. If possible, the digest solution will be re-run to confirm results.
3. If results are confirmed and the issue pertains to an entire set of samples, a few will be re-digested and re-analyzed. If the issue is random/sporadic, move to the next step.

4. If all analytical issues are ruled out but values are still outliers, the data will be reported using analyte-specific quality flag variables for each outlier record.

   a) The entry "**sample value outlier**" will be used to report outliers in all elements.

5. Each Element Thresholds are listed below:

Macros (%)	Micros (mg/kg)
Foliar Phosphorus Concentration < 0.5	Foliar Manganese Concentration < 2000
Foliar Potassium Concentration < 4	Foliar Iron Concentration < 350
Foliar Calcium Concentration < 5	Foliar Copper Concentration < 40
Foliar Magnesium Concentration < 1	Foliar Boron Concentration < 120
Foliar Sulfur Concentration < 0.55	Foliar Zinc Concentration < 150

6. If there are no data quality issues to report, element-specific quality flags will report "**OK.**" If there is some other issue with data quality not covered here, an entry of "**other**" will be used with more detail provided in remarks.

XVII. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

A. If the Flex Control or Quality Control Check(s) data is out-of-control the ICP will be recalibrated and the samples will be re-run. Samples may require being re-digested.

XVIII. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

A. Reference A&L Great Lakes Laboratories, Inc. Quality Assurance Manual

XIX. REFERENCES

A. iTEVA Software Help

XX. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

A. Exhibit A – Mineral Concentrations in Solution

Exhibit A

MINERAL CONCENTRATIONS IN SOLUTION

	HIGH	MED	LOW	CONTROL SOLUTION
Manganese	2 ppm	1 ppm	0.1 ppm	0.5 ppm
Iron	5 ppm	2.5 ppm	0.25 ppm	1.25 ppm
Phosphorus	0.01%	0.005%	0.0005%	0.0025%
Copper	0.5 ppm	0.25 ppm	0.025 ppm	0.125 ppm
Zinc	2 ppm	1 ppm	0.1 ppm	0.5 ppm
Boron	0.5 ppm	0.25 ppm	0.025 ppm	0.125 ppm
Aluminum	10 ppm	5 ppm	0.5 ppm	2.5 ppm
Calcium	0.05%	0.025%	0.0025%	0.0125%
Potassium	0.1%	0.05%	0.005%	0.025%
Magnesium	0.01%	0.005%	0.0005%	0.0025%
Sodium	0.001%	0.0005%	0.00005%	0.00025%
Sulfur	0.01%	0.005%	0.0005%	0.0025%