STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF CHLOROPHYLL *a* AND PHEOPHYTIN *a* BY THE FLUOROMETRIC METHOD FOR THE NEON PROGRAM

CHM 2041

Method Reference: Standard Methods for the Examination of Water and Wastewater, 22nd Edition, Method 10200H

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Training Statement:
I have read, understand, and agree to follow this SOP.

Signature_____ Date _____

Printed Name _____

Changes made in this version:

- Identified that the SOP is specific to the analysis of samples received from the NEON Program.
- Added information describing use of the Turner Designs Trilogy fluorometer with acidification module.
- Updated the MDLs for the Sequoia Turner fluorometer and added the MDL for the Turner Designs Trilogy fluorometer with acidification module.
- Updated the names of fields populated in the client receipt form during sample login.
- Deleted use of a barcode scanner to login samples.
- Replaced the chlorophyll analysis data sheets for the Sequoia Turner and Turner Designs fluorometers with a sheet that can be used for both instruments (Attachment 4).
- Updated the workbook used to generate reports.
- Specified that samples should be shipped by regional samplers within 7 days of collection and extracted within 14 days of collection.

TABLE OF CONTENTS

I. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the procedure for the fluorometric determination of chlorophyll *a* and pheophytin *a* in water samples for the National Ecological Observatory Network (NEON) Program. The analyst may choose from two different fluorometers for analysis: the Sequoia Turner or the Turner Designs Trilogy with acidification module.

1.2 The Method Detection Limit (MDL) for the Sequoia Turner method is 0.013 µg/L and for the Turner Designs Trilogy method is 0.015 µg/L, based on the filtration of a 100 mL water sample and a final extract volume of 7 mL. The MDL will vary with the sample volume filtered and the final extract volume. For instance, with the Sequoia Turner, if 50 mL is filtered and the final extract volume is 7 mL, then the MDL is 0.026 µg/L. The most current MDLs are filed on the GLEC server in folder S:\Nutrient Chemistry\QAQC\Annual QAQC activities\MDLs_LOQ Verfi.

1.3 A Reporting Limit (RL) of 0.20 µg/L is used for both instruments.

1.4 This SOP is intended for use by analysts trained and skilled at standard chemistry laboratory operations and procedures, and who have completed hands on training with an analyst proficient in this specific method and passed a demonstration of capability study.

II. SUMMARY OF METHOD

2.1 Phytoplankton pigments are extracted off a filter, from the field filtration of a water sample, using acetone and maceration. Chlorophyll *a* and pheophytin *a* in the extract are measured by fluorescence before and after acidification. The concentrations of chlorophyll *a* and pheophytin *a* are calculated from the fluorometer results.

2.2 This SOP is based on Standard Methods for the Examination of Water and Wastewater, 22nd Edition, Method 10200 H.

III. DEFINITIONS

3.1 Analytical batch – A set of 20 or fewer field samples plus the appropriate QC samples (i.e., a laboratory reagent blank [LRB], laboratory fortified blank unfiltered [LFBUF], and laboratory duplicate [LD]) that are extracted and analyzed together.

3.2 Calibration Standards – A series of four solutions prepared in acetone from a stock standard solution (also in acetone) with a known concentration of the analyte. Used to determine the concentration of analyte in the samples.

3.3 Chemical Abstracts Service (CAS) registry number – A unique numerical identifier for a chemical, assigned by CAS, a division of the American Chemical Society.

3.4 Chlorophyll *a* – A photosynthetic pigment found in plants, including phytoplankton. It constitutes about 1 to 2% of the dry weight of planktonic algae. Therefore, the total phytoplankton biomass of a water sample may be estimated based on the chlorophyll *a* concentration.

3.5 Control Limits – Minimum and maximum acceptability criteria for analytical batch QC sample results (i.e., the recovery of the analyte in LFBUFs). Upper control limits = the average of a set of 20 or more data points + (standard deviation x 3). Lower control limits = average of a set of 20 or more data points – (standard deviation x 3). Control limits are calculated using data accumulated over multiple years.

3.6 Deionized (DI) water – Water that has had its mineral ions removed. It is produced by purifying tap water by reverse osmosis (RO) followed by passing it through carbon and de-ionization cartridges. It is a physical process using ion exchange resins which bind to and filter out the mineral salts from water.

3.6.1 Traverse City Toxicology Laboratory water system: When the resistivity drops to 17.9 MΩ-cm or less and does not return to 18 or higher within 30 minutes, order replacement cartridges. Continue to use the DI water while waiting for the replacement cartridges to arrive, unless the resistivity drops to 15 MΩ-cm or less. Install the replacement cartridges upon receipt.

3.6.2 Nutrient Chemistry Laboratory water system: Since this system does not have a digital resistivity meter to use as a maintenance indicator, inspect the DI cartridges every one to two months, depending on use. Replace all cartridges if beads have separated and created head space in any of the three cartridges. Discontinue use of this DI system while waiting for replacement cartridges to be installed.

3.7 Demonstration of Capability (DOC) – A study conducted by the analyst for the purpose of establishing his/her ability to generate results with acceptable accuracy, precision, and sensitivity.

3.8 Laboratory fortified blank unfiltered (LFBUF) – Due to the fact that the matrix for the chlorophyll *a* stock standard is acetone, the chlorophyll *a* in an LFB (i.e., DI water fortified with the stock standard) would not be retained on the filter used for field samples. Therefore, no value can be derived from processing an LFB through the entire procedure, as is the practice for most analytical chemistry

methods. In order to determine whether the instrument is calibrated correctly, and the analyst is capable of making accurate and precise measurements using the method, the fluorescence reading for a calibration standard is processed through the calculation. The fluorescence reading for the 25µg/L calibration standard is entered as an unknown, and the concentration is calculated. A minimum of one LFBUF is included in each analysis batch. The LFBUF is equivalent to the NEON QC Standard.

3.9 Laboratory Reagent Blank (LRB) – An aliquot of reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, and reagents used in the procedure. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the equipment. A minimum of one LRB is included in each analytical batch. This may also be called the method blank (MB).

3.10 Lab Solution Number (LS Number) – A unique identifier assigned to each new reagent prepared in the laboratory.

3.10.1 For prepared reagents, the LS number follows this format: 'PrepMMddYY[rgt identifier]', where 'Prep' designates that it is a prepared reagent and is followed by the date and a shorthand identifier for the particular reagent name. Prepared solution containers must be labeled with: the solution and concentration, the date prepared, the preparer's initials, the expiration date, and the LS number. Prepared reagents are recorded in the Google doc called Reagent Prep Log.

3.10.2 For prepared standards, the LS number follows the format STDmmddyy[std identifier]. If prepared standards are to be held and used on more than one day, they must be labeled with the: LS number; name of the standard; date of preparation; expiration date; initials of the person who prepared them; and GHS requirements for hazardous chemicals, if appropriate. Prepared standards are recorded in the Google doc called Standard Prep Log.

3.10.3 Purchased reagents, standards, and QC standards are identified as CHEMmmddyy[chem identifier], STDmmddyy[std identifier], and QCmmddyy[QC std identifier], respectively. Purchased reagents, standards, and QC standards are labeled with the LS number and the receipt date. The receipt of purchased reagents, standards and QC standards are recorded in the Inorganic Standard and Chemical Receipt Log found on the GLEC server at S:\Nutrient Chemistry\Inorganic logs.

3.11 Macerate – To become soft or separate and disintegrate as a result of soaking and agitation.

3.12 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination and accurate quantitation is not expected between the MDL and the RL. This may also be called the Limit of Detection (LOD).

3.13 Pheophytin *a* – The breakdown product of chlorophyll *a*. Upon acidification, the chlorophyll *a* molecule loses a magnesium atom, thereby degrading to pheophytin *a*.

3.14 Quality Control (QC) – Procedures used to identify and control sources of error. QC samples include: one LRB and one LFBUF for every batch.

3.15 Quality Control (QC) Standard – A solution containing the method analyte at a known concentration that is obtained from a source external to the laboratory and different from the source used to prepare the calibration standards. It is used to check calibration standard integrity. A QC Standard is prepared and analyzed to check each calibration.

3.16 Reporting Limit (RL) or Limit of Quantitation (LOQ) – The minimum quantifiable concentration of the analyte in a sample. The RL is based on the concentration of the lowest calibration standard, adjusted to account for the concentration factor resulting from sample preparation. This may also be called the Limit of Quantitation (LOQ).

3.17 Safety Data Sheet (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, flammability, and reactivity, including how it should be stored, handled, and disposed of.

IV. INTERFERENCES AND CAUTIONS

4.1 All glassware and cuvettes must be clean and acid-free. Wash all glassware and equipment according to SOP LAB 1020, Chlorophyll *a* Wash Section.

4.2 Do not expose samples to light. Even brief exposure to light during storage, extraction and analysis will alter chlorophyll values. Perform this procedure in a dark room, dimly illuminated by a green-filtered lamp.

4.3 The presence of chlorophyll *b* can cause an underestimation of chlorophyll *a* when using the standard fluorometric acidification method.

V. HEALTH AND SAFETY

5.1 U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) has adopted requirements for labeling hazardous chemicals as part of its revision to the Hazard Communication Standard, 29 CFR 1910.1200. These requirements align the U.S. with the United Nations' Globally Harmonized System (GHS) of classification and labeling of chemicals. They apply to anyone who handles, uses, stores, or transports hazardous chemicals in any amount. Labels must include: company name, address, and telephone number; chemical identifier; a signal word; a hazard statement(s); precautionary statement(s); and pictograms(s). For more information, see the documents saved on the GLEC server in folder S:\GLEC Health & Safety\OSHA Hazardous Chemical labeling. If you require a label for a container with hazardous material that you are storing, shipping, or using for longer than one shift, contact Erica Schneider, Environmental Health & Safety Officer, at 231-525-0520 or eschneider@glec.com.

5.2 SDSs for the chemicals referenced in this SOP are available in GLEC's Traverse City, MI library. Review the applicable SDSs prior to using this SOP for the first time and periodically thereafter to become familiar with the chemicals' toxicity, health hazards, physical properties, flammability, and reactivity. Each chemical must be stored, handled, and disposed of in the prescribed manner.

5.3 Regard each chemical as a potential health hazard and minimize exposure.

5.4 Wear a laboratory coat, disposable gloves, and safety glasses while preparing reagents, conducting the method, disposing of test materials, and cleaning glassware and equipment.

5.5 Perform extractions under a ventilated fume hood.

VI. EQUIPMENT AND SUPPLIES

Note: Ordering information for most consumable supplies used in the inorganic chemistry lab can be found on the GLEC server purchase order (PO) file at S:\PO Database.

6.1 Aluminum foil – used to protect extracts from light.

6.2 Ampules – 15 milliliter [mL] capacity, glass.

6.3 Beaker – 50 mL capacity, glass.

6.4 Centrifuge – refrigerated, Beckman J2-21, or equivalent, with a rotor capable of holding 15 mL centrifuge tubes.

6.5 Centrifuge tubes - 15 mL glass, graduated, screw cap.

6.6 Cuvettes – for the Sequoia Turner fluorometer, with 1 centimeter [cm], 4 cm, and 10 cm path lengths.

6.7 Cuvettes – for the Turner Designs Trilogy fluorometer, Trilogy.

6.8 Fluorometer

6.8.1 Sequoia Turner Model 450 modified for chlorophyll *a* analysis. The emission filter is a Turner SC665 and the excitation filter is a Turner NB440; or

6.8.2 Turner Designs Trilogy, with acidification module.

6.9 Forceps – for handling filters.

6.10 Lamp - green filtered.

6.11 Pasteur pipettes - disposable glass, and a Pasteur pipette bulb.

6.12 Pipettes – glass, graduated, 10 mL capacity: and a pipette bulb.

6.13 Propane torch.

6.14 Syringes - 10 mL, solvent resistant, gas-tight.

6.15 Tissue grinder - 7 mL glass, loose (or B) pestles, and tight (or A) pestles. Do not use acid washed glassware as it will cause the chlorophyll to degrade to pheophytin.

6.16 Volumetric flasks – 25, 100, and 1000 mL.

VII. REAGENTS AND STANDARDS

7.1 All chemicals are of reagent grade unless otherwise specified. Order information for manufactured reagents and standards used in the Inorganic Chemistry Laboratory is located in the Inorganic Standard and Chemical Receipt Log on the GLEC server at S:\Nutrient Chemistry\Inorganic logs. The PO database, at S:\PO Database is used to order reagents and standards.

7.2 The container for each reagent or standard that is, or is made with, an OSHA-specified hazardous chemical must be labeled following the OSHA/GHS requirements (see Section 5.1) unless it is used within one shift. **In this section of the SOP, these reagents and standards are designated with §.**

7.3 Gases – Not Applicable. No gases are used in this procedure.

7.4 Water – Deionized (DI) water.

7.5 Reagents – See Sections 3.10.1 and 3.10.3 for instructions on labeling and documenting prepared and purchased reagents, respectively. Each time a reagent is used, the date must be recorded in a new Use Date column in the appropriate sheet of the appropriate log: Google doc Reagent Prep Log for prepared reagents or Inorganic Standard and Chemical Receipt Log found at S:\Nutrient Chemistry\Inorganic logs for purchased reagents.

7.5.1 1 N Sodium bicarbonate ($NaHCO_3$) - To prepare, dissolve 4.2 g $NaHCO_3$ (CAS# 144-55-8) in 50 mL DI water.

7.5.2 **§** 90% Aqueous buffered acetone - To prepare, combine 450 mL nanograde acetone (CAS# 67-64-1) and 50 mL DI water. Add 5 drops of 1 N $NaHCO_3$ to ensure an alkaline solution.

7.5.3 **§** 6 N Hydrochloric acid (HCl, CAS# 7647-01-0) - Purchased.

7.5.4 **§** 1 N HCl – Prepare enough volume for use on the day of analysis, approximately 12 mL. To prepare, add 2.0 mL of 6 N HCl to 10 mL of DI water in a 50 mL beaker.

7.6 Standard Solutions - See Sections 3.10.2 and 3.10.3 for instructions on labeling and documenting prepared and purchased standards, respectively. Each time a standard is used, the date must be recorded in a new Use Date column in the appropriate sheet of the appropriate log: Google doc Standard Prep Log for prepared standards, or Inorganic Standard and Chemical Receipt Log (S:\Nutrient Chemistry\Inorganic logs) for purchased standards. Prepare all standard solutions for this method in a fume hood in a dark room, illuminated by a green-filtered lamp.

7.6.1 Chlorophyll solid from spinach (CAS# 479-61-8) – Purchase 1 mg of 95% chlorophyll *a* from spinach, from Fisher Scientific, Catalog number 501786410 (Sigma Aldrich C57531MG).

7.6.2 **§** Stock solution (1 mg/L) – Use purchased solid chlorophyll *a* to prepare approximately 200 ampules of 1 mg/L standard in acetone.

7.6.2.1 Add 1 mg of solid chlorophyll to 800 mL of 90% buffered acetone in a 1 L volumetric flask. Bring to 1 L with 90% buffered acetone.

7.6.2.2 Use a glass pipet to transfer no less than 5 mL of the prepared chlorophyll standard solution to each of approximately 200 ampules. Immediately place each of the ampules on crushed ice.

7.6.2.3 Wrap each ampule in aluminum foil. This step may be completed prior to adding the standard solution.

7.6.2.4 Carefully flame-seal the ampules using a torch.

7.6.2.5 Store the ampules in a freezer at ≤ -10°C until use.

7.6.3 § Working stock solution and 50 µg/L calibration standard - Using a gas-tight syringe, transfer 5.0 mL of stock solution to a 100 mL volumetric flask. Dilute to 100 mL with 90% buffered acetone.

7.6.4 § 5 µg/L calibration standard – Using a clean gas-tight syringe, transfer 2.5 mL of the working stock solution to a 25 mL volumetric flask. Dilute to 25 mL with 90% buffered acetone.

7.6.5 § 10 µg/L calibration standard – Using the same gas-tight syringe that was used in Section 7.6.4, transfer 5 mL of the working stock solution to a 25 mL volumetric flask. Dilute to 25 mL with 90% buffered acetone.

7.6.6 § 25 µg/L calibration standard and LFBUF - Using the same gas-tight syringe that was used in Section 7.6.4, transfer 12.5 mL of the working stock solution to a 25 mL volumetric flask. Dilute to 25 mL with 90% buffered acetone.

7.6.7 § Chlorophyll *a* QC standard – Purchase from Turner Designs, Catalog number 10-850.The QC standard may be used as purchased if the concentration is within the analytical range. If not, dilute it to an appropriate concentration with 90% buffered acetone.

VIII. SAMPLE RECEIPT, STORAGE AND HOLDING TIME

8.1 Sample Receipt

8.1.1 Upon receipt, ensure all samples (foil wrapped filters) are in good condition (e.g., dry-ice still present in cooler, foil packaging not

damaged, sample identification present and legible). If samples are received in compromised condition, notify the Battelle Technical Representative within two business days.

8.1.2 Complete the client receipt form, emailed to GLEC, to document the condition of samples. Upload the completed form to the NEON Data Portal, per instructions provided on the GLEC server in S:\\NEON Battelle\NEON Algal chlorophyll_2020\Project files\Uploading files.pdf. See Attachment 1.

8.1.3 Sample Log-in – Log samples into the Nutrient Report database found on the GLEC server at S:\Nutrient Chemistry\Report Database\Nutrient Reports.mdb. Add client samples to the LOG IN table. Fields populated at log in include:

8.1.3.1 COC ID – an identifier created for the delivery batch.

8.1.3.2 sampleID – the NEON identifier for the sample.

8.1.3.3 sampleCode – the scannable barcode from the bottle label.

8.1.3.4 sampleCondition – If applicable, select a value derived from column D in the Lab Data File tab of S:\NEON Battelle\NEON Algal chlorophyll_2020\Project files\NEON algal chlorophyll_Field Descriptions.xlsx. See Attachment 2.

8.1.3.5 remarks – Remarks about the sample condition.

8.1.3.6 Login ID – GLEC's lab-generated sample ID. This becomes part of the final LabSampleID, used to populate the internalLabID field on the final ingest report. GLEC's Login ID begins with NE, followed by the numeric month and day of sample collection, followed by a four-digit number that identifies the sample beginning with 0001 and adding one for each sample received from the client. For example: NE05180001, NE05180002 for NEON samples #1 and 2, collected on May 18th.

8.1.3.7 ParamID1 and ParamID2 – CHLRPHYLA and Pheophytin, respectively. Used to populate the analyte field on the final ingest report.

8.1.3.8 collectDate – the date and time the sample was collected in YYYY-MM-DDTHH:MM format that is used to populate GLEC's SampleDate field in M/DD/YYYY format.

8.1.3.9 shipmentReceivedDate – the date the sample was received by GLEC in YYYYMMDD format that is used to populate GLEC's ReceiveDate in M/DD/YYYY format.

8.1.3.10 Matrix – Water.

8.1.3.11 Client Initials – NE.

8.1.3.12 receivedBy – email address of the receiver at GLEC.

8.1.3.13 Lab Qualifier – GLEC's internal qualifier code for sample anomalies.

8.1.3.14 filterVolume – Volume of sample filtered by NEON.

8.1.3.15 filterSize – Size of the filter, 25 mm.

8.1.3.16 sampleType – chla/pheo.

8.1.3.17 Initials of enterer.

8.1.3.18 Replicate – 1 or 2. Derived from the suffix on the NEON sampleID.

8.2 Sample storage – Store the filters containing the concentrated samples between -20ºC and -70 ºC in a freezer with an auto-notification system.

8.3 Sample holding time – Extract and analyze samples within 7 days of receipt to avoid pigment degradation. If this holding time is exceeded, proceed with analysis, flag data appropriately, and contact the Battelle Technical Representative within 48 hours of the incident. Samples should be shipped by regional samplers within 7 days of collection and extracted within 14 days of collection.

IX. QUALITY CONTROL

9.1 LRB - Analyze an LRB sample at the beginning and end of each analytical batch to determine if any interferences are present in the laboratory environment or the equipment. Prepare the LRB by filtering 100 mL of DI water using the same procedure as is used for field samples. The raw fluorescence unit reading for LRBs must be < 0.5; if it is not, reanalyze the LRB. If the result is again ≥ 0.5 raw fluorescence units, determine the source of the problem. If the corrective action fails, report data with a quality flag in the dataQF field. See Table 2.

9.2 LFBUF (equivalent to the NEON QC Standard) – For each analytical batch, use the instrument reading for a calibration standard in the expected mid-range of sample concentrations as an unknown to back-calculate the measured concentration. Calculate the percent recovery (R) using Equation 1, where D is the result for the LFBUF sample and C is the fortified concentration:

$$R = \frac{D}{C} \times 100 \qquad \textbf{Equation 1}$$

The calculated result must be within 5% of the known value. If the result falls outside of this criterion, re-prepare and reanalyze the calibration standards and recalculate the LFBUF concentration. If the corrective action fails, analyze samples and report data with a quality flag in the dataQF field. See Table 2.

9.3 Quality Control (QC) Standard - Analyze a purchased QC Standard after the calibration standards, prior to analyzing field samples, as a second source check of the calibration standards. Concentration varies by lot. Chlorophyll recovery must be within 10% of the true value to be considered acceptable. If the result is outside this criterion, determine the cause of the discrepancy. Flag the samples with a CH or CL flag. See Table 2.

9.4 Data Qualifier Codes - Use the codes listed in Table 2 to flag results with associated QC problems. Flag the results on data sheets, in databases and in all associated reports to the client.

Table 1. QC Requirements

QC Check	Frequency	Acceptance Criteria	Corrective Action	Procedure if Corrective Action Fails
LRB	1 at the beginning and end of each batch	Raw fluorescence units <0.5	Maintenance and/or recalibration until value meets acceptance criteria	Analyze samples, report data with quality flag
LFBUF	At least 1 per batch	Observed value within 5% of known value	Maintenance and/or recalibration until value meets acceptance criteria	Analyze samples, report data with quality flag

Table 2. Result Codes

Laboratory Result Remark Code	Definition
ACC	Laboratory accident resulted in no obtainable value
J	Estimated result below the RL but above the MDL
CH	QC indicated possible low recovery; actual concentration may be higher
CL	QC indicated possible high recovery; actual concentration may be lower

Laboratory Result Remark Code	Definition
HT	Holding time was exceeded before analysis
INT	Interference encountered during analysis may have affected the accuracy of the result
IST	Improper sample collection, preservation, or transport
NH	Non-homogenous sample made analysis of a representative sample questionable
QC	Quality control problems encountered
QNS	Insufficient sample quantity to perform analysis
U	Analyte not detected at a concentration >MDL.
FHT	Past holding time for filtration.

X. METHOD PERFORMANCE

10.1 Initial DOC – An initial DOC must be successfully performed by each analyst prior to analyzing any field samples. The analyst must prepare and analyze a minimum of four replicate LFBUFs, following the procedures in Section XII. Calculate percent recovery for each replicate LFBUF (Equation 1) and enter the results into the spreadsheet located on the GLEC server as S:\Nutrient Chemistry\QAQC\Annual QAQC activities\Demonstration of Capability\ yyyy\DOC yyyy.xlsx. The recovery must be within the control limits, included in the spreadsheet, which were generated using SOP 2039.

10.2 Ongoing DOC – At a minimum frequency of once per year, each analyst must successfully perform an ongoing DOC. Use the initial DOC technique, or analyze a blind sample, (i.e., a purchased PT or a sample prepared by the GLEC Laboratory Coordinator or a primary analyst). The result for the blind sample must fall within the limits of acceptability provided by the manufacturer, or within 15% of the prepared concentration, if prepared in the laboratory.

10.3 MDL Study - An MDL is used to determine the minimum concentration that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

10.3.1 MDL results and precision information are documented on the GLEC server in S:\Nutrient Chemistry\QAQC\Annual QAQC activities\MDLs_LOQ Verfi\.

10.3.2 An initial MDL (MDL_i) study was performed using the method described below.

10.3.2.1 Prepare 7 replicate standard solutions over a three-day period at a concentration estimated to be near the MDL. Prepare three one day, two the next and two the following day.

10.3.2.2 Analyze the 7 replicates using all the steps in Section XII.

10.3.2.3 Multiply the standard deviation by 3.14. The resulting value is the MDL.

10.3.3 Each year, perform an Ongoing Annual Verification by calculating an MDL_s based on accumulated LFBUF results and an MDL_b based on accumulated LRB results. If the higher of the two is 0.5 to 2.0 times the MDL, and fewer than 3% of the LRB results generated in the last calendar year are above the MDL_i, then the MDL_i is verified and may continue to be used as the MDL. Otherwise, the higher of the MDL_s and MDL_b must be used as the MDL.

10.3.3.1 Calculate the MDL_s – Use all low level LFBUF results from the past 24 months in the MDL_s calculation. MDL data points should be generated in each quarter that analysis is performed and should total at least seven per calendar year. If there are fewer than seven low level LFBUF data points, perform additional analyses of low level LFBUFs to total seven. Ensure that data points are generated on at least three different dates in each year.

10.3.3.1.1 Calculate the standard deviation of the results.

10.3.3.1.2 Subtract 1 from the number of data points collected and find the corresponding 99^{th} percentile t-value in a t-value table.

10.3.3.1.3 Multiply the standard deviation by the t-value to determine the MDL_s.

10.3.3.2 Calculate the MDL_b – Use all LRB results from the past 24 months in the MDL_b calculation. MDL_b data points should be generated in each quarter that analysis is performed and should total at least seven per calendar year. If there are fewer than seven LRB data points, perform additional analyses of LRBs to total seven. Ensure that data points are generated on at least three different dates in each year.

10.3.3.2.1 Calculate the standard deviation of the results.

10.3.3.2.2 Subtract 1 from the number of data points collected and find the corresponding 99th percentile t-value in a t-value table.

10.3.3.2.3 Multiply the standard deviation by the t-value.

10.3.3.2.4 Determine the mean.

10.3.3.2.5 If the mean is greater than zero, add it to the result of step 10.3.3.2.3 to determine the MDL_b.

10.4 RL or LOQ Verification – The RL is 0.20 µg/L.

10.4.1 Control limits for the verification of the RL were calculated from the percent recovery results of the accumulated MDL data points in the control chart workbook S:\Nutrient Chemistry\QAQC\Annual QAQC activities\MDLs_LOQ Verfi\RL Verifications\yyyy\Chlorophyll Sequoia LOQ verif.xlsx. See GLEC SOP CHM 2039 Section 12.3 for specific instructions on determining control limits.

10.4.2 The RL need not be verified annually if the MDL is verified annually.

10.5 Control Limits for LFBUF recovery– On an annual basis, update control limits by adding data for all LFBUFs generated during the previous year to the control chart workbook S:\Nutrient Chemistry\QAQC\Annual QAQC activities\Control Charts\current LFB RPD MS MSD\Chlor.xlsx. See GLEC SOP CHM 2039, Section 12.1 for specific instructions on updating these control limits.

10.6 Calibration verification - Calibration standards should have a similar fluorescence from one analysis to the next. The back calculated chlorophyll *a* concentration of the standards are typically within 10% of the expected concentration. If there is a significant change in fluorescence or the recovery of back calculated concentrations do not fall within 10%, determine the cause of this change before any samples are analyzed.

XI. CALIBRATION

11.1 On both the Sequoia Turner and Turner Designs Trilogy (Trilogy) instruments, use the chlorophyll *a* standard solution to determine the fluorometer calibration factor F_s. Use the same procedures for these measurements as for the sample extracts. Take readings before and after acidification so that r_s can be determined.

11.2 Allow the fluorometer to warm up for fifteen minutes.

11.3 Zero the instrument. Fill a clean cuvette with the 90% acetone blank solution and place it into the sample well.

11.3.1 On the Sequoia Turner, turn the 'zero' knob until instrument is zeroed.

11.3.2 On the Trilogy, touch OK to zero the instrument.

11.4 Remove the cuvette and empty it, discarding the blank solution.

11.5 Fill the cuvette with the lowest concentration standard, place it in the sample well, and record the fluorescence reading as Rb.

11.6 Acidify the standard in the cuvette using one drop from a Pasteur pipet (0.01 mL) of 1N HCl. Acidification converts any chlorophyll *a* present to pheophytin *a*. After 90 seconds, take a fluorescence reading and record it as Ra. Empty the cuvette, discarding the acidified standard.

11.7 Repeat steps in Sections 11.4 and 11.5 with each of the next three standards, progressing from low to high concentration.

11.8 Enter the calibration information into columns T through W on the Chlor-*a* tab of the calculation workbook found on the GLEC server at S:\Nutrient Chemistry\Excel Reports\Analyte\Calculations Spreadsheets – Verified\ Chlor-a Pheo-a Calculations_NEON_final.xls. See Section 13.1 for calculations.

11.8.1 Save a copy of the workbook in S:\Nutrient Chemistry\Excel Reports\Analyte\Calculation by Analyte and Year\Chlorophyll\yyyy, where yyyy is the current year, on the GLEC server by naming it with the current date in the file name (e.g., Chlor-a Pheo-a Calculations_NEON_final 061820.xlsx).

11.8.2 Enter the fluorometric reading for each standard before the addition of acid in the appropriate cell in row 10 (i.e., T10 through W10).

11.8.3 Enter the fluorometric reading for each standard after the addition of acid in the appropriate cell in row 11 (i.e., T11 through W11).

11.8.4 If necessary, enter the known concentration of each standard in the appropriate cell in row 12 (i.e., T12 through W12). The known concentration remains the same from one analysis batch to the next, unless a non-standard calibration is performed.

XII. PROCEDURE

12.1 Extraction

12.1.1 Enter all sample IDs for samples to be extracted on the chlorophyll extraction data sheet, found in S:\Nutrient Chemistry\Templates (see Attachment 3).

12.1.2 Extract samples in a fume hood in a dark room dimly illuminated by a green-filtered lamp.

12.1.3 Using forceps, place the filter containing the concentrated sample in a tissue grinder and cover it with 2 mL of 90% aqueous acetone solution. Macerate with the loose fitting (B) pestle then the tight fitting (A) pestle for about 3 minutes or, if a GF/F filter was used, until it has been converted to a slurry.

12.1.4 Transfer the extract to a screw-capped graduated centrifuge tube. Rinse the grinder with a few mLs of 90% aqueous acetone and add the rinsate to the extract. Bring all sample extracts up to the same volume (e.g., 7 mL) with 90% buffered acetone, and record this volume.

12.1.5 Wrap the test tubes with aluminum foil to prevent light exposure.

12.1.6 Let the samples steep for at least two hours, and not more than 24 hours, in a refrigerator (≤ 6°C) in the dark. Normally the samples are steeped overnight. Shake samples at least once during the steeping period.

12.1.7 Clarify the samples by centrifuging the tubes for 30 minutes at 2800 rpm.

12.1.8 Follow instructions in GLEC SOP CHM 2017 for entering extraction information into the Nutrient Reports database.

12.2 Fluorometric chlorophyll *a* analysis with acidification for pheophytin *a*, following calibration (Section XI).

12.2.1 Allow samples to come to ambient temperature.

12.2.2 Set the sensitivity level to the mid-point.

12.2.3 Complete the header information in the chlorophyll analysis data sheet in S:\Nutrient Chemistry\Templates (see Attachment 4) and record the sample IDs.

12.2.4 If the instrument was not calibrated on the same day as sample analysis, insert a solvent blank and zero the instrument. Check the zero reading periodically and adjust as needed.

12.2.5 Fill a clean cuvette with an aliquot of LRB extract. Place the cuvette in the sample well. Record the fluorometric units reading on the chlorophyll analysis data sheet as Fluorescence Before Acid (Rb).

12.2.6 Acidify the LRB extract by adding one drop from a Pasteur pipet (0.01 mL) of 1 N HCl. After 90 seconds, take a fluorescence reading and record it as Fluorescence After Acid (Ra).

12.2.7 Repeat steps in Sections 12.2.5 and 12.2.6 with the QC standard, LFBUF, each field sample extract, ending with a second aliquot of LRB extract.

12.2.8 See Section 13.1 to determine concentrations.

XIII. DATA ANALYSIS AND CALCULATIONS

13.1 For each calibration standard, calculate the fluorometer response factor (F_s) and the acidification response factor using Equations 2 and 3. This is performed automatically on the Chlor *a* tab of the workbook created in Section 11.7.

$$F_s = \frac{\text{Concentration of chlorophyll } a \text{ std } (\mu g/L)}{Rb} \quad \textbf{Equation 2}$$

Where: F_s is the fluorometer response factor
Rb is the fluorometer reading of the standard before acidification.

$$r_s = Rb/Ra \quad \textbf{Equation 3}$$

Where: r is the acidification response factor
Rb is the fluorometer reading of the standard before acidification
Ra is the fluorometer reading of the standard after acidification.

13.2 Calculate the concentrations of chlorophyll *a* and pheophytin *a* in the samples. The workbook created in Section 11.7 is set up to perform automated calculations. Open S:\Nutrient Chemistry\Excel Reports\Analyte\Calculation by Analyte and Year\Chlorophyll\yyyy\ Chlor-a Pheo-a Calculations_NEON_final mmddyy.xls (where mmddyy is the date of analysis).

13.2.1 Enter the LabSampleID in column B on the Chlor *a* tab.

13.2.2 Enter the extract volume in column AB on the Chlor *a* tab.

13.2.3 Enter the sample volume filtered in column AC on the Chlor *a* tab.

13.2.4 Enter the fluorometer reading prior to the addition of acid (Rb) in column AD on the Chlor *a* tab.

13.2.5 Enter the fluorometer reading after the addition of acid (Ra) in column AE of the Chlor *a* tab.

13.2.6 Calculate the chlorophyll *a* concentration in the sample extract (C*a*) using Equation 4. This is performed automatically in column AF of the Chlor *a* tab.

C_a (µg/L) = F_{ave} * (r_s/(r_s-1)) * (Rb - Ra) **Equation 4**

Where: F_{ave} is the average of the F_s values of the 4 standards;

r_s is the average of the acidification response factors for the 4 standards;

Rb is the fluorometer reading of the sample extract before acidification; and

Ra is the fluorometer reading of the sample extract after acidification.

13.2.7 Convert C*a* to the concentration of chlorophyll *a* in the original water sample using Equation 5. This is performed automatically in column AG of the Chlor *a* tab. The result is also reported in column C of the Chlor *a* tab.

Chlorophyll *a* (µg/L) = C*a* (µg/L) * D * V/W **Equation 5**

Where: C*a* is the chlorophyll *a* determined in Section 13.2.6;
D is the dilution factor for the sample extract (for example, if the dilution is 1:10, then D = 10). An extract is only diluted if the result for the undiluted extract is above the range of the fluorometer;
V is the volume of the sample extract (mL);
W is the volume of the water sample filtered (mL).

13.2.8 Calculate the pheophytin *a* concentration in the sample extract (P*a*) using Equation 6. This is performed automatically in column AH of the Chlor *a* tab.

P*a* (µg/L) = F_{ave} * (r_s/(r_s-1)) * (r_s *Ra - Rb) **Equation 6**

Where: F_{ave} is the average of the F_s values of the 4 standards;
r_s is the average of the acidification response factors for the 4 standards;
Rb is the fluorometer reading of the sample extract before acidification; and
Ra is the fluorometer reading of the sample extract after acidification.

13.2.9 Convert P*a* to the concentration of pheophytin *a* in the original water sample using Equation 7. This is performed automatically in column AI of the Chlor *a* tab. The result is also reported in column C of the Pheo *a* tab.

Pheophytin *a* (mg/L) = P*a* (mg/L) * D * V/W **Equation 7**

Where: P*a* is the pheophytin *a* determined in Section 13.2.8;
D is the dilution factor for the sample extract (for example, if the dilution is 1:10, then D = 10). An extract is only diluted if the result for the undiluted extract is above the range of the fluorometer;
V is the volume of the sample extract (mL);
W is the volume of the water sample filtered (mL).

13.3 Generate reports

13.3.1 Copy and paste the data from columns A through O and AB through AE of the Chlor *a* tab of the calculation workbook created in Section 11.7, into the top rows (starting with row 2) of columns A through S in S:\Nutrient Chemistry\Excel Reports\Analyte\REPORT TEMPLATE NEON CHLOR FINAL.xlsx.

13.3.2 Copy and paste the data from columns A through O of the Pheo *a* tab and columns AB through AE of the Chlor *a* tab of the calculation workbook created in Section 11.7, into the rows below the Chlor *a* data in columns A through S of S:\Nutrient Chemistry\Excel Reports\Analyte\REPORT TEMPLATE NEON CHLOR FINAL.xlsx.

13.3.3 Rename the file to the CalRefID and save it in S:\Nutrient Chemistry\Excel Reports\Analytes\yyyy, where yyyy is the year. For detailed instructions, see GLEC SOP CHM 2017.

13.3.4 Follow GLEC SOP CHM 2017 to import the spreadsheet created in Section 13.3.3 to the Nutrient Reports database and generate a report.

XIV. EQUIPMENT MAINTENANCE

14.1 Always keep an extra fluorometer lamp on hand and change the lamp as needed. Compare the fluorescence values for standards from one analysis to the next. Readings that are dropping or becoming less stable may be indicative that the lamp life is nearing its end.

14.2 Clean the windows on the instrument monthly with a cotton swab and alcohol.

14.3 Record all maintenance in the fluorometer maintenance log which is kept in the Inorganic Chemistry Laboratory near the fluorometer.

XV. QUALITY ASSURANCE

15.1 Data reports are reviewed by a qualified GLEC staff member, such as the Report Review Specialist, Laboratory Coordinator/Technical Director, or the Chemistry Lab Director, before submission to the client. This QC Review is an independent review; it is performed by someone not associated with the data generation. This review evaluates the computations performed, and the accuracy and traceability of the data as summarized in SOP CHM 2036. It is the responsibility of the person who generated the report to satisfactorily address any of the QC reviewer's comments and concerns and to generate the final report.

15.2 Records are maintained as indicated in this SOP. Hard copies of all data generated or acquired are maintained in secure files at GLEC. Any electronic data or other information are filed and stored by the project name on GLEC's computer server which is backed-up daily. Original file copies of data are always kept in-house at GLEC. Duplicate copies of information are produced if it is necessary for the data to leave the GLEC offices.

XVI. WASTE MANAGEMENT/POLLUTION PREVENTION

16.1 Let small amounts (e.g., < 10 mL) of acetone evaporate in the fume hood. Larger amounts can be poured into a designated waste container and stored in the chemical storage shed for disposal.

16.2 Dispose of hazardous material by appointment with RecycleSmart (231.941.5555) at the Department of Public Works garage (361 East Welch Court, Traverse City, Michigan 49686).

16.3 This method should be conducted with active pollution prevention as an objective, by: modifying processes to reduce or eliminate waste at the source, promoting the use of non-toxic or less-toxic substances, implementing conservation techniques, and re-using materials when possible rather than disposing of them.

XVII. DEVIATIONS

17.1 One drop (0.01 mL) of 1N HCl is used for the acidification of samples for pheophytin correction instead of 0.1 mL of 0.1N HCl, as stated in the reference method.

17.2 If it becomes necessary to deviate from the method outlined in this SOP for any reason, document the deviation on the data sheets, log books, and client reports.

XVIII. REFERENCES

18.1 29 CFR 1910.1200. Hazard Communication, 29 C.F.R. §1910.1200 (2012). https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10099.

18.2 42 CFR 493.1236. Standard: Evaluation of proficiency testing performance, 42 C.F.R. §493.1236 (2018). http://www.ecfr.gov/cgi-bin/text-idx?rgn=div8&node=42:5.0.1.1.9.11.25.27.

18.3 EPA 2016. Definition and Procedure for the Determination of Method Detection Limit, Revision 2. U.S. EPA Office of Water 821-R-16-006. December 2016.

18.4 GLEC SOP CHM 2017. Tracking Samples and Data.

18.5 GLEC SOP CHM 2036. Performing Quality Control Reviews of Nutrient Chemistry Data and Client Reports.

18.6 GLEC SOP CHM 2039. SOP for Updating Control Limits.

18.7 GLEC SOP FLD 6001. Collecting and Field Filtering Samples for Chlorophyll *a* Analysis.

18.8 GLEC SOP FLD 6004 Determination of Water Transparency by Secchi Disk.

18.9 GLEC SOP LAB 1020. Manual Cleaning of Glassware, Equipment, and Sample Containers used in GLEC-TC Laboratories.

18.10 SM Method 10200H. Chlorophyll. In Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2012. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

18.11 Turner Designs Trilogy Manual.

ATTACHMENT 1

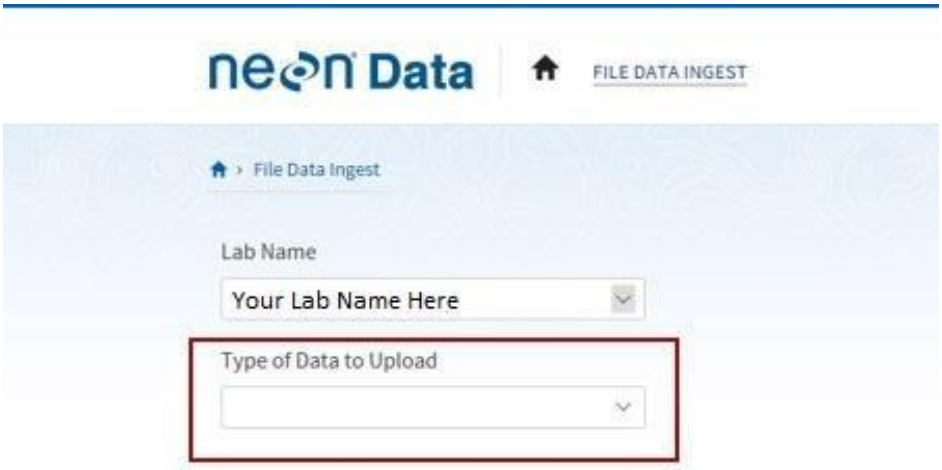
Uploading Files

Uploading Data to the NEON Data Portal

- Once account access has been provided upon contract award, upload all future files here: http://data.neonscience.org/web/external-lab-ingest/

- Once logged in, select your Lab Name: **To Be Provided Upon Contract Award**
- Most accounts will only have one option for Lab Name that is automatically selected.

- A drop-down list for "Type of Data to Upload" will then appear.



Select the appropriate option from the list below:

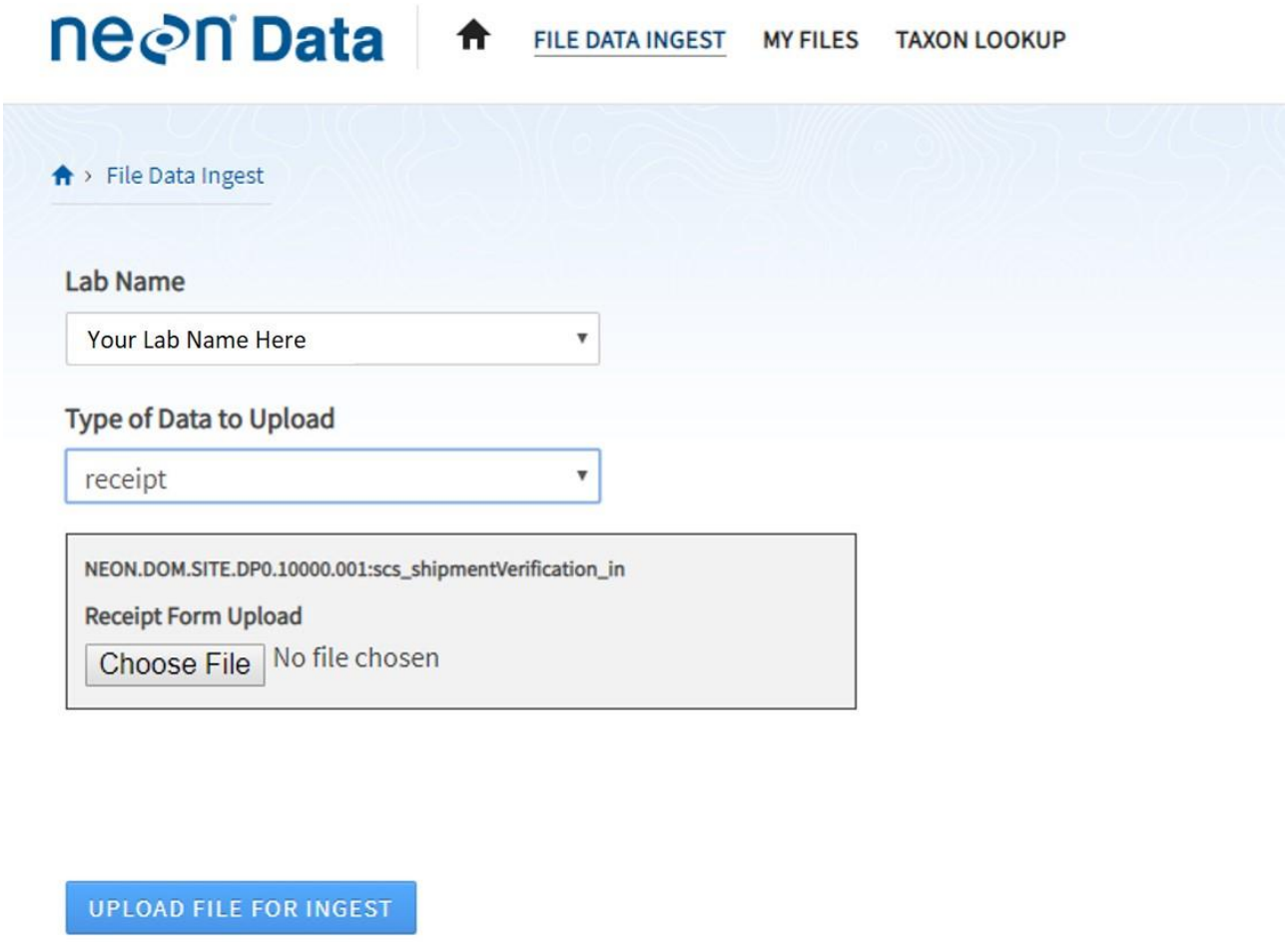
receipt – this is for return of receipt forms that are emailed to the lab when samples are shipped, to record condition upon receipt.

Particulate_Chemistry_Lab_Data – this is the return for all algal chlorophyll results in the completed data ingest sheet format, as agreed to for the ***Algal Chlorophyll*** contract.

- Grey boxes should appear with a "Browse" or "Choose File" button to select a file for various file types.

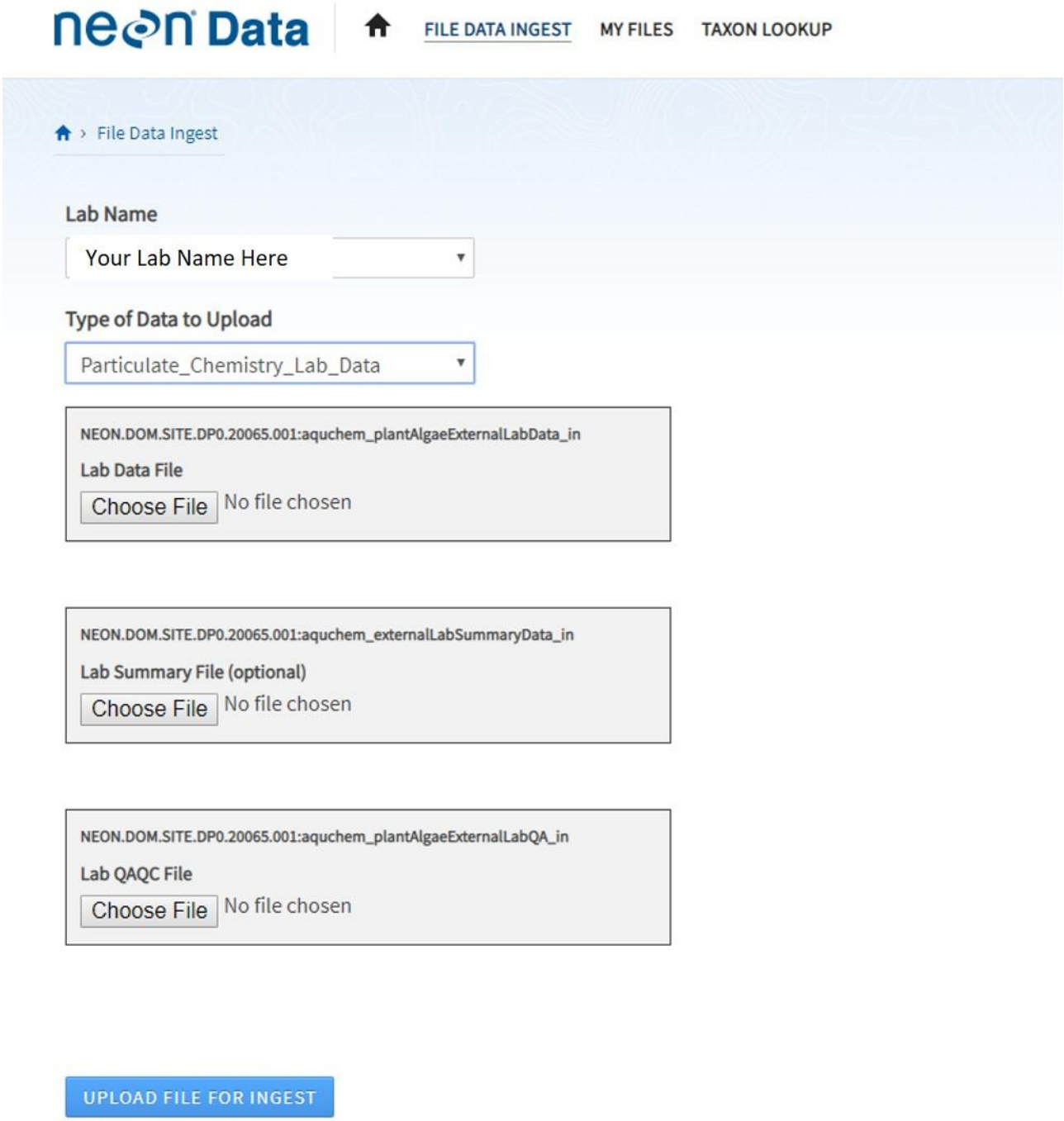
File types for receipt:

1) **Receipt Form Upload** (scs_plantAlgaeExternalLabshipment verification_in) – upload the receipt form here (example filename: receipt_form_D08201801101113231l9.csv)



File types for *Particulate_Chemistry_Lab_Data*:

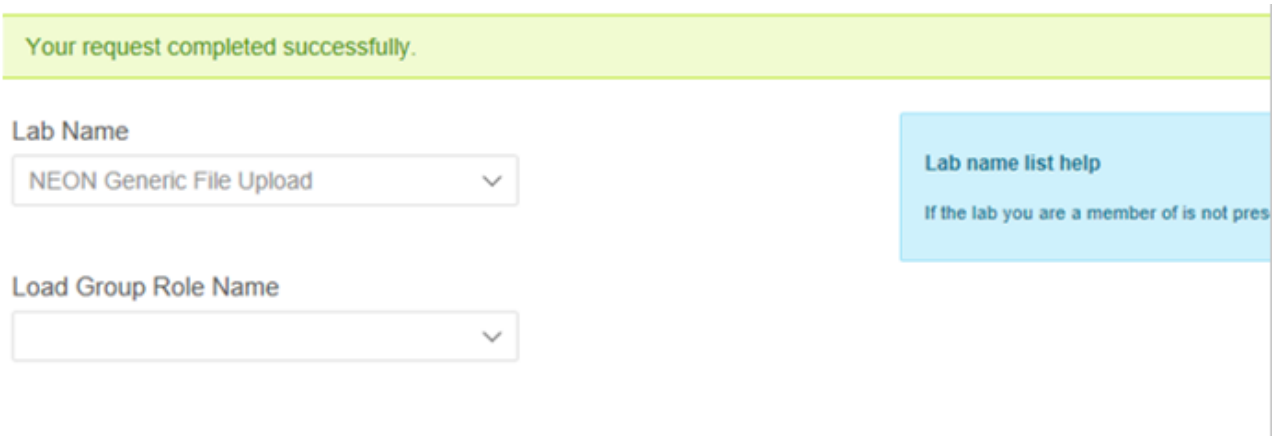
1) **Lab Data File** (aquchem_plantAlgaeExternalLabData_in) – upload sample data here (example file name: algChem_chlorophyll_POSE_20171209.csv)
2) **Lab QAQC File** (aquchem_externalLabSummaryData_in) – upload batch QA data here (example file name: algChem_chlorophyll_POSE_20180109.csv)
3) **Lab Summary File (optional)** – upload annual summary data here, this data upload is not required (example file name: algChem_chlorophyll_LABNAME_summary_20180901.csv)



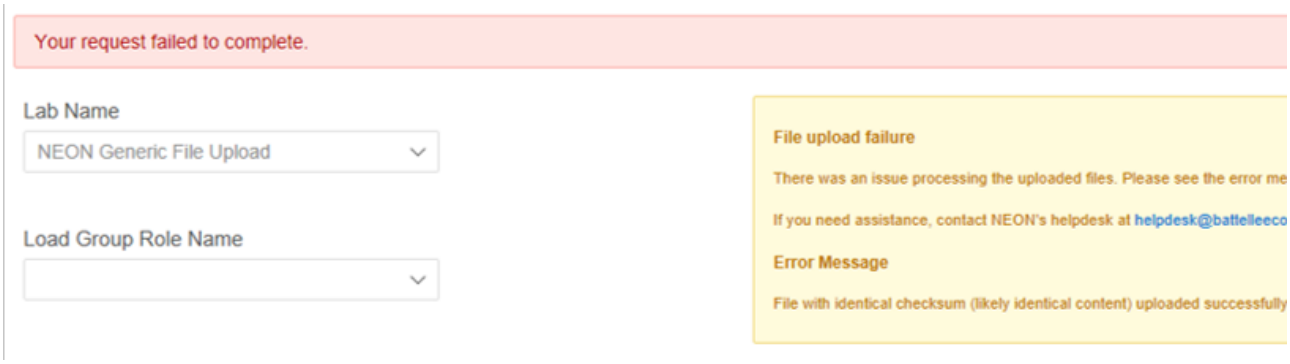
- Multiple boxes/buttons will appear for cases where there are multiple file types that can be uploaded (for example, for different sample data files, or for batchQA and external summary files). You can load the files individually or simultaneously.

- Click the blue "Upload file for ingest" button to submit the file(s). The file(s) will not be transmitted to NEON until this button is pressed.

- If the data are *accepted*, a green banner will appear with the message "Your request completed successfully". You can view successfully uploaded files at the "MY FILES" tab at the top of the page.



- If the data are *rejected*, a red banner will appear along with a description of the error. The row of data with the problem will be noted if it is a specific data error. If the remedy to the problem is not obvious, contact NEON using the information at the end of this document.



What to do if uploaded data needs to be corrected

Contact NEON using the name and details at the end of this document to change or correct data that have already been loaded successfully. DO NOT use the spreadsheet upload system to correct or re-upload already-submitted data.

Important formatting and requirements

- Data should be returned using the templates and detailed requirements for the file format included in the contract.
- A few requirements common to all files uploaded through this system:
    - Must be in comma separated variable (.csv) format.
    - Cannot have empty rows of data at the end of the file. Please delete rows as opposed to just clearing the contents if data needs to be removed from a file.
    - Letters cannot be used in numeric fields. For example, 'BDL' cannot be used where a numeric concentration is below the detection limit.
    - Double quotes "" cannot be used within double quotes.
    - Dates are formatted as YYYY-MM-DD or YYYYMMDD.
    - UTF-8 encoding should be used.
    - Empty cells should be left blank. Do not use NA or other notation.
- Please review the specific requirements relevant to your templates and data variables, since file upload may fail if any of these requirements are not met.

File Names

- For sample data: Prefix for the module ("algChem"), a string describing the NEON project site(s) analyzed, the NEON site or domain if relevenat, and the date data were uploaded (YYYYMMDD), separated by underscores ("_") and with no spaces.
    - Examples:
        - algChem_POSE_LEWI_20160628.csv

Questions

Please feel free to contact the Battelle Technical Representative with any questions regarding the data return process.

ATTACHMENT 2

NEON algal chlorophyll_Field Descriptions

Table	Field name	Description	List of values (must use values presented in this list)	Example	Data source	Required by NEON?
aquchem_plantAlgaeExternalLabData_in	batchID	Lab-assigned batch ID		*NEa-1*	external lab	N
aquchem_plantAlgaeExternalLabData_in	sampleID	NEON identifier for sample		*ARIK.20130918.epilithon.1*	NEON sample label	Y
aquchem_plantAlgaeExternalLabData_in	sampleCode	Scannable sample barcode		*A00012345*	NEON sample label	Y
aquchem_plantAlgaeExternalLabData_in	sampleCondition	Condition of sample at time of analysis. If more than one applies, pick the option that has the largest effect on the quality of the data.	condition ok Sample Received Damaged But Analyzable Sample Damaged or Lost During Storage Sample Received Not Frozen Sample Not Frozen During Storage Sample Overflow from Filter Filter Damaged other (specify in remarks)	*other*	external lab	Y
aquchem_plantAlgaeExternalLabData_in	internalLabID	Lab-assigned sample number		*01498*	external lab	N
aquchem_plantAlgaeExternalLabData_in	analysisDate	Date filter was analyzed		*2014-07-02*	external lab	Y
aquchem_plantAlgaeExternalLabData_in	analyzedBy	First initial, last name of analytical technician		*sparker*	external lab	Y
aquchem_plantAlgaeExternalLabData_in	sampleType	Type of sample as processed in lab	chl/pheo	*chl/pheo*	NEON sample label or shipping manifest	Y
aquchem_plantAlgaeExternalLabData_in	replicate	Filter or solid sample replicate (1 or 2)		*2*	NEON sample label	Y
aquchem_plantAlgaeExternalLabData_in	sampleVolumeFiltered	Volume of sample filtered in NEON domain lab from shipping inventory (mL); 'NA' for solid sample		*10*	NEON shipping manifest	N
aquchem_plantAlgaeExternalLabData_in	filterSize	Filter diameter (mm); 'NA' for solid sample		*25*	external lab	N
aquchem_plantAlgaeExternalLabData_in	percentFilterAnalyzed	Fraction of the filter sampled (%); 'NA' for solid sample		*100*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	dilutionFactor	The factor by which the sample was diluted prior to		*10*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	solventVolume	Volume of solvent used in the extraction		*10*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	responseFactor	Response factor for sensitivity setting		*0.000224*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	fluorometerSensitivitySetting	Sensitivity setting on the fluorometer		*1*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	preAcidificationFluorescence	Fluorescence read before acidification		*408.3838*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	postAcidificationFluorescence	Fluorescence read after acidification		*313.8731*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	analyte	Analyte measured at external lab	totalchla chla pheo	*chlpheo*	external lab	Y
aquchem_plantAlgaeExternalLabData_in	analyteConcentration	Concentration of analyte on filter		*8.524*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	plantAlgaeLabUnits	Units associated with reported analyte	microgramsPerLiter	*microgramsPerLiter*	external lab	Y if sample is analyzable
aquchem_plantAlgaeExternalLabData_in	testMethod	Analytical method used for analysis (name of lab SOP)	LABNAME_algalChlorophyll_V1	*LABNAME_algalChlorophyll_V1*	external lab	Y
aquchem_plantAlgaeExternalLabData_in	method	Published method		*EPA Method 440.0*	external lab	N
aquchem_plantAlgaeExternalLabData_in	dataQF	*TBD*	*TBD*	*TBD*	external lab	N
aquchem_plantAlgaeExternalLabData_in	externalRemarks	Technician notes; free text comments accompanying the record		*below detection*	external lab	N

ATTACHMENT 3

Chlorophyll Extraction Data Sheet

CHLOROPHYLL FILTRATION AND EXTRACTION

Date Filtered_____

Time Filtered_____

Date Extracted_____

Analyst Initials_____

LabSampleID	Filter Volume (mL)	Extract Volume (mL)

ATTACHMENT 4

Chlorophyll Analysis Data Sheet

CHLOROPHYLL ANALYSIS

Sequoia Turner ☐ Trilogy ☐

Date Analyzed _____

Time Analyzed _____

Analyst Initials _____

Sensitivity Setting _____

LabSampleID	Fluorescence Before Acid	Fluorescence After Acid	Std Prep ID
0.005 mg/L			
0.010 mg/L			
0.025 mg/L			
0.05 mg/L			
QC			
LRB			
LFBUF			