Horn Point Analytical Services Laboratory
Version 1.2 (Battelle Service Contract); Dec 4, 2018

CHLOROPHYLL *a*

Fluorometric methods are available for analysis of chlorophyll *a*. Chlorophyll, in a measured volume of water, is concentrated by filtering through a glass fiber filter, and the pigments are extracted in acetone. Fluorescence is proportional to chlorophyll concentration.

Methodology

Arar, E. J. and G. B. Collins. 1997. Method 445.0, Revision 1.2: In vitro determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence. National Exposure Research Laboratory, USEPA, Cincinnati, OH, 22 p.

Instrumentation

Fluorometer: Turner Designs Model 10-AU digital calibrated against a spectrophotometer using pure chlorophyll *a* from spinach (Sigma Chemical Company, 10865) or pure chlorophyll *a* (C6144). We acknowledge that the Turner Designs Model 10-AU is no longer being manufactured; however, Turner Designs will continue to support this instrument for the foreseeable future. There are 3 Turner Designs 10-AU fluorometers at Horn Point Laboratory which may be used by HPL Analytical Services in case of instrument problems.

Sample Preparation and Storage

Note: The following procedure is specific for the NEON samples (Battelle service contract). These standard operating procedures are kept on file in the Analytical Services laboratory.

1. Algae samples collected by Battelle will be filtered and frozen within 24 hours of collection. Filters used for algae (25 mm GF/F) will be frozen at -20 ºC, folded in aluminum foil packets, and shipped on dry ice. Frozen filters will be shipped overnight to the Contractor within 7 days of sampling. The majority of samples will be shipped between the months of April and October. Shipments will include a hard-copy manifest and will be preceded by an email notification of shipment containing an electronic copy of the manifest and receipt form.

2. Upon receipt of samples, HPL Analytical Services will ensure all samples are in good condition (e.g., dry-ice still present in cooler, samples not damaged, sample identification present and legible, etc.). If samples are received in compromised condition, HPL Analytical Services should notify the Battelle Technical Representative within two business days. An electronic receipt form that will be emailed with the shipping information is also to be completed to document condition of samples upon receipt. Each completed receipt form will be uploaded to the NEON Data Portal.

Sample Storage

While in HPL Analytical Services' custody, samples will be stored according to the conditions in Table 1.

Table 1. Storage Conditions, Hold Times, and Analytical Methods: Algal Chlorophyll a and Pheophytin *a*

Grouping	Analyte	Analysis Approach	Method or Standard Operating Procedure (SOP)	Required Method Detection Limit	Hold Time	Storage Conditions
Periphyton, Seston	Chlorophyll *a* (GF/F filter)*	Acetone extraction/ fluorometric detection	EPA 445.0 or similar	0.5 ug/L	14 days from date of sample collection	-20 °C**
	Pheophytin *a* (GF/F filter)*					

*Chlorophyll *a* and Pheophytin *a* will be analyzed from the same filter sample.
**Freezer should be equipped with a back-up generator or an auto-notification system to protect against failure.

To avoid pigment degradation, **samples should be analyzed within 7 days from receipt so that the holding time of 14 days from collection is not exceeded**. If this holding time is exceeded, HPL Analytical Services should proceed with analysis, flag data appropriately, and contact the Battelle Technical Representative within 48 hours of the incident.

During pigment extraction, samples are kept in a refrigerator (4°C) in the HPL Analytical Services laboratory for 2-24 hours (typically overnight).

General Analytical Procedure

1. Daily, prepare standard curve using Chlorophyll *a* stock solution (detailed below)

2. During extraction, light exposure must be minimized. The HPL Analytical Services Laboratory room where sample analysis is performed does not have windows. To further minimize light, keep samples shaded and reduce artificial lighting whenever samples are outside of freezers or refrigerators.

3. When ready for analysis, place the filter in an appropriate tube and add a small, measured amount of 90 % acetone. Extract the pigments by either grinding the filter with a Teflon pestle and glass grinding tube, soaking the filter in acetone, or macerating the filter using a sonic probe.

4. Clarify the extract either by centrifugation or filtration through either a Teflon HPLC syringe cartridge filter or a glass fiber filter.

5. Transfer a portion of the clarified sample into a glass cuvette for reading on the fluorometer. If a sample concentration exceeds the highest concentration of the chlorophyll standards used for calibration, quantitatively dilute the sample with 90% acetone and reread on the fluorometer.

6. **Instrument Quality Control**: Daily, during sample analysis to document that the instrument is performing satisfactorily, read a QC standard of known concentration and the secondary solid state standard at high and low readings, every 20 samples on the fluorometer.

Calibration of the Fluorometers

Determine the concentration of the chlorophyll *a* stock solution on the spectrophotometer. The concentration of the stock solution is calculated from a published extinction coefficient and the absorbance reading at 664 nm, corrected for turbidity with an absorbance reading taken at 750 nm. [The extinction coefficient is consistent with what investigators involved with the Joint Global Ocean Flux Study (JGOFS) and Sea-Viewing-Wide-Field-of-View Sensor (SeaWIFS) use. The spectrophotometer is checked for absorbance value accuracy using NIST traceable neutral density filters (Latasa et al. 1999). Chlorophyll *a* stock solution is prepared to read in the range of 0.1 to 0.9, in accordance with Standard Methods of the Examination of Water and Wastewater (1992). The chromatographic purity of the chlorophyll *a* standard is monitored by HPLC.] Use up to six standards, that span the range of expected sample concentration, and one blank to determine the standard curve. Calculate a linear regression and residuals of the standard curve.

Calculations:

The formulas used to calculate chl *a* and phaeophytin *a* concentrations are from Standard Methods for the Examination of Water and Wastewater (1998) Section 20200H.3 and EPA Method 445.0.

$$\text{Chl } a \text{ :µg/l seawater} = ((R_b - R_a) - \text{y intercept}) * \text{slope}^{-1}) * V_e * V_f^{-1}$$

or

$$\text{Chl } a \text{ :µg/l seawater} = F_s * (((r / (r-1)) * (R_b - R_a) * (V_e * V_f^{-1}))$$

$$\text{Phaeophytin a :µg/l} = F_s * (((r/(r-1) * (rR_a - R_b) * (V_e * V_f^{-1}))$$

$$F_s = C_a * R_b^{-1}$$

Where:
F_s = calibration factor for sensitivity setting S,
R_a = fluorescence of sample extract standard after acidification, $r = R_b/R_a$, as determined with pure chlorophyll a standard,
R_b = flurorescence of sample extract before acidification, V_e = extraction volume (in ml),
V_f = volume of water filtered onto filter (in ml), and C_a = concentration of pure chlorophyll a standard.