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DISCLAIMER This procedure was written to meet the needs of the research program at the U.S. EPA-Atlantic Ecology Division. It is not a U.S. EPA Standard Method and must not be referred to as such. Mention of Trade names or commercial products does not constitute endorsement or recommendation for use.

1.0 OBJECTIVES

This procedure is used to calculate chlorophyll a content (ug/l) in coastal and estuarine waters from phytoplankton fluorescence at 680 nm from light excited at 436 nm (Turner Designs, 1994). The method is based on the direct reading of fluorescence of extracted chlorophyll *a* using a Turner Designs Model AU-10 Digital Fluorometer with Optical Kit P/N 10-040R. The Optical Kit P/N 10-040R allows for the non-acidic analysis of chlorophyll *a* in the presence of phaeophytin *a* and other chlorophylls. This is an update of AED/MAB/DJK/2003-02-001 created by Darryl Keith.

2.0 MATERIALS AND EQUIPMENT

Turner Designs Model AU-10 Digital Fluorometer with Optical Kit P/N 10-040R
Disposable 10mL fluorometer cuvettes (13 X 100 mm borosilicate tubes) with snap-caps
Disposable 15mL polystyrene conical tubes (screw-cap)
47 mm Whatman GF/F filters (1825-047)
Hand or Electric vacuum pump
Nitrile gloves
2 liter Nalgene HDPE screw-capped bottles
Forceps
Dispensette® Dispenser (suitable for 10 mL)—or similar dispenser for organic solvents.
90% acetone/10% deionized water solution
Freezer
Test tube rack
Marking pens
Vortex mixer

3.0 PROCEDURE - NOTE: FOR EACH STEP WITH AN IDENTIFIABLE HAZARD A CORRESPONDING ENTRY MUST BE COMPLETED IN THE JOB HAZARD ANALYSIS FORM AT THE END OF THIS DOCUMENT.

3.1 Don appropriate personal protective equipment.

- Lab coat, nitrile gloves, and lab glasses are required while performing this LOP.
- Perform all operations with open acetone in the fume hood.

3.2 Extraction tube preparation.

- Label sufficient 15 mL polystyrene tubes—2 for each sample and 2 blanks.
- Dispense 10 mL of 90% acetone into each tube using the Dispensette® Dispenser. Be sure to prime the pump prior to dispensing into first tube.
- Place tubes in test tube rack.
- Place in freezer for at least one hr

3.3 Filtration of samples.

- Retrieve test tube rack of polystyrene tubes from the freezer. Place on ice in a cooler.
- Samples should be process as soon as practical—ideally the same day collected. Water samples are stored on ice in a cooler until transported to AED. Duplicate 100 mL (50 mL may be sufficient for many waters) subsamples are filtered onto 47 mm Whatman GF/F filters using an electric or hand vacuum pump in subdued light—pressure should not exceed 5 in of Hg. Duplicate deionized water samples should be prepared for blanks.
- Use forceps to roll the filter into a tube and place into one of the polystyrene tubes well below the surface of the 90% acetone. Return tube to rack in cooler.
- Record volume of seawater filtered and the tube label in data book.
- When finished filtering all of the samples, return the test tube rack to the freezer for at least 12 hr. Rack should be covered to minimize exposure to light.

3.4 Chlorophyll *a* Analysis Using the Turner Designs AU-10 Fluorometer.

- Turn instrument on 1 hour before analysis. Prior to first sample (and at the completion of all samples) insert solid standard and record the high and low values. This is also a good time to label the fluorometer cuvettes.
- Retrieve polystyrene tubes from freezer, mix each tube with vortex mixer, keep in dark and let warm to room temperature. Doing this when the instrument is first turned on should allow sufficient time for warming.
- When ready to proceed, remove, in subdued light, a polystyrene tube from test tube rack and pour off sufficient liquid to fill fluorometer cuvette at least ¾ full. Cap tube.
- Use Kimwipes to wipe off any spillage from the cuvette.
- Insert cuvette into the sample compartment of the fluorometer and read fluorescence. (When numbers first appear hit *, it will read DELAY, AVE, and record value when it reads DONE)
- Record raw fluorescence in the data book. Note, the instrument is currently set to read raw fluorescence units.
- Chlorophyll *a* concentration (μg/l) in the cuvette is calculated from a standard curve.

$$\text{Chl } a \text{ (cuvette)} = (Fs-Fb)*\text{slope}$$

Where:

Fs is the sample raw fluorescence value
Fb is the raw fluorescence value of the blank, and
slope is the slope of the calibration curve—see below.

- Chlorophyll *a* concentration in the original water sample is calculated

$$\text{Chl } a \ (\mu g/l) = \text{Chl } a \text{ (cuvette)}*v/V_f \text{ (UNESCO, 1997)}$$

Where

v = 10 mL = volume of acetone used for extraction
V_f = 0.10 liter = volume of seawater filtered

3.5 Waste Generation/Disposal

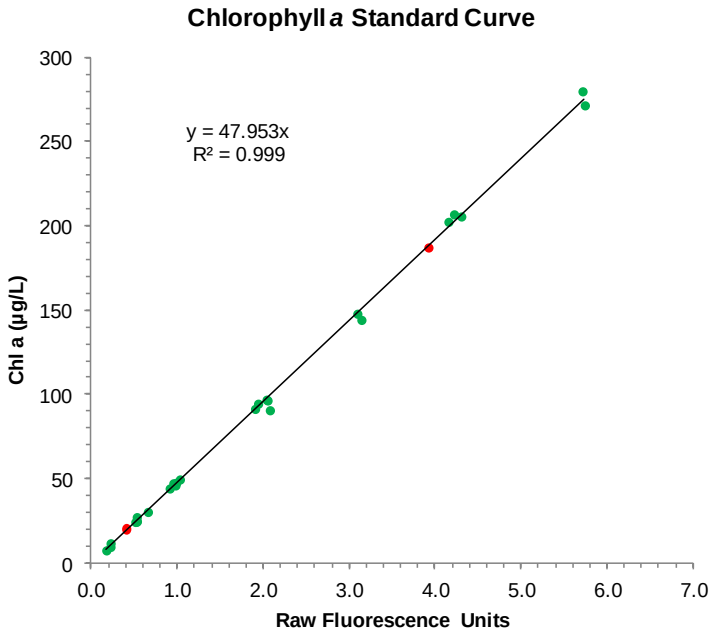
Solvent waste (90% acetone) is disposed of in the appropriate hazardous waste container. Waste from this method should be stored, managed, and disposed of in a waste container according to appropriate local and RCRA guidelines. Monitor the waste level within waste containers and when full, contact the AED SHEMP for waste removal.
Residual, as well as used filter can be tossed.

4.0 QA/QC

A standard curve verifies the accuracy of the fluorometer. Chlorophyl *a* purchased from SIGMA (CS144-1MG)—original source *Anacystis nidulansi*—was the source of the dry standard. Note, this is a cyanobacterium so chlorophyll *a* is the only chlorophyll (no b or c, etc.). A primary standard was made up in 90% acetone. Five separate secondary concentrations in 90% acetone were created from the primary standard (ranging from 7.5 to 280 ug/L). A Perkin-Elmer Lambda 35 spectrophotometer was the instrument used to measure the chlorophyll *a* concentration. The same standards were then read on the Turner fluorometer[1]. The standard curve is presented below. The green markers represent the standards as prepared above. The two red markers represent standards purchased directly from Turner (already in 90% acetone). The fact that the red markers are in alignment with our standards is a quality check on the proper function of our Lambda 35 Spectrophotometer.

Readings using Turner's solid standards should be made during each sample run at the beginning of the run and at the end. There is a high and low standard. The data from these measurements establish whether the instrument is performing as expected. Based on summer/fall 2014 measurements (N = 20) the "raw" fluorescence readings for the low solid standard average 0.145 ± 0.004m and the high, 0.708 ± 0.19. This is equivalent to 6.95 and 33.95 ug/L chlorophyll *a.* If readings deviate by more than 5% from these values then you should consider adjusting the final chl *a* values accordingly.

[1] The Turner fluorometer was calibrated on the MED range setting and set to represent approximately 50ug/L. The LOW and HIGH ranges are a factor of 10 on either sided of this.



5.0 TROUBLE SHOOTING

If the solid standard measurements deviate by more than 10 to 15% from the averages listed above, consideration should be given to whether the fluorometer should be recalibrated according to the instruments factory instructions. This process should be accompanied by the recreation of the chlorophyll *a* standard curve.

6.0 REFERENCES

Ritchie, RJ. 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosyn. Res. 89:27-41.

Turner Designs (1994). Optical Configuration Guide for the Model 10-AU-005 Field Fluorometer. Version 6. June 27.

UNESCO (1997). Phytoplankton pigments in oceanography: guidelines to modern methods. Edited by S.W. Jeffrey, R.F.C. Mantoura, and S.W. Wright. Sponsored by SCOR and UNESCO.

USEPA (1992) Method 445.0: In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence. Adapted by E.J. Arar and G.B. Collins. Version 1.1. EMSL/ORD/EPA - Cincinnati. November

Welschmeyer, N. (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr., 39(8), 1985-1992.

Atlantic Ecology Division (AED)

JOB HAZARD ANALYSIS

Hazard Types (HT)

1. Toxic Chemic
2. Flammable Chemicals
3. Corrosive Chemicals
4. Environmental
5. Explosion (Chemical Reaction)
6. Explosion (Over pressurization)
7. Mechanical / Vibration
8. Electrical (Shock, Short Circuit)
9. Electrical (Fire)
10. Electrical (Static, ESD)
11. Electrical (Loss of Power)
12. Ergonomic (Overexertion)
13. Ergonomic (Human Error)
14. Vibration
15. Fall (Slips / Trips)
16 Fall (To a Different Level)
17. Excavation (Collapse)
18. Fire, Heat, Thermal, Cold
19. Noise
20. Radiation (Ionizing / Nonionizing)
21. Visibility
22. Weather
23. Caught (In, On, Between)
24. Struck (by, against)

Job Task:

Personal Protective Equipment:

Chemicals In Use:

CRITICAL TO SAFETY (CTS)

Risk Estimation Matrix

Probability of Occurrence of Harm	SEVERITY OF HARM			
	Catastrophic	Serious	Moderate	Minor
VERY LIKELY	High	High	High	Medium
LIKELY	High	High	Medium	Low
UNLIKELY	Medium	Medium	Low	Negligible
REMOTE	Low	Low	Negligible	Negligible

* High = CTS tasks should receive engineering controls prior to assigning administrative or PPE controls.

Step #	Procedures (LOP procedure step)	Potential Hazards	HT	Check CTS	Recommended Safe Practice
3.1	Don appropriate personal protective equipment.	Exposure to and/or inhalation of acetone. Forms flammable/explosive vapor.	1, 2		Perform all operations with open acetone in the fume hood. Take precautions to prevent static discharge.
3.2	Extraction tube preparation.	Exposure to and/or inhalation of acetone Forms flammable/explosive vapor.	1, 2		Perform all operations with open acetone in the fume hood. Take precautions to prevent static discharge.
3.3	Filtration of samples.	Exposure to and/or inhalation of acetone Forms flammable/explosive vapor.	1, 2		Perform all operations with open acetone in the fume hood. Take precautions to prevent static discharge.
3.4	Chlorophyll *a* Analysis Using the Tuner Designs AU-10 Fluorometer	Exposure to and/or inhalation of acetone Forms flammable/explosive vapor.	1, 2		Perform all operations with open acetone in the fume hood. Take precautions to prevent static discharge.
3.5	Waste Generation/Disposal	Exposure to and/or inhalation of acetone Forms flammable/explosive vapor.	1, 2		Perform all operations with open acetone in the fume hood. Take precautions to prevent static discharge.

Personal Protective Equipment Recommendations			
Where engineering and administrative controls aren't feasible or sufficient for controlling hazards, PPE must be used to protect workers. The following PPE are recommended for the noted tasks:			
Eye and Face Protection			
	Safety glasses with side shields		Reflective goggles/face shield
x	Chemical splash goggles		Cutting/braising/welding eye protection
	Face shield		Other:
Head Protection			
	Hard hat, bump cap		Helmet, cowl, hood
	Welding helmet/ mask		Other:
Foot Protection			
	Safety shoes/ boot		Other:
	Chemical-resistant boots		
Body Protection			
	Apron (splash, work)		Head-reflective garments
x	Lab Coat		Sleeves (cut-resistant)
	Coveralls (work, chemical resistant) Type chemical: Type coverall:		Other:
Respiratory Protection			
	Respirator		Type of respirator:
Hand Protection			
	Rubber insulating gloves		Rubber insulating sleeves
	Rubber insulating hoods	x	Other: Nitrile gloves

PPE Hazard Assessment Form

HEALTH AND SAFETY HAZARDS		
Chemical Hazards		
	Vapors/Gases	
	Dusts/Mists/Fumes	
	Liquid Splash	
	Other	
Comments:		
Physical Hazards		
	Impact-Flying dust, particles, chips	
	Penetration/Punctures	
	Cuts/Lacerations	
	Compressions–Pinch, crush, rollover	
	Heat-Sparks, molten splash, high temperatures	
	Cold-Cyrogens, cold temperatures	
	Light (optical radiation)	
	Electrical Shock	
	Fire	
	Radiation-Ionizing, Non-ionizing	
Comments:		
Biological Hazards		
	Bloodborne Pathogens	
	Animals (Zoonotic)	
	Other	
Comments:		

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