### Trilogy Laboratory Fluorometer-Draft 2018

**Division of Watershed Management**

**Department of Environmental Protection**

**627 Main Street, Second Floor**

## Worcester, MA

# Standard Operating Procedure

**Title: Extracted Chlorophyll *a* (SM -10200 H) (USEPA Fluorometric Method 445 and 445 with the Welschmeyer modification)**

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1. **SCOPE AND APPLICATION**

# Chlorophyll is a pigment found in plants that allows the organism to use radiant energy for converting carbon dioxide into organic compounds in a process called photosynthesis. Several types of chlorophyll exist and these and other pigments are used to characterize algae. One type, chlorophyll *a*, is most widely used for biomass estimates because of its presence in all algae and because it constitutes approximately 1-2% of the dry weight of organic material . A knowledge of chlorophyll *a* concentrations can provide an estimate of biomass of the phytoplankton and the periphyton that can be used for comparative assessments of geographical, spatial and temporal variations (American Public Health Assoc., 1981). Chlorophyll *a* measurements are made from both phytoplankton samples and periphyton samples from lakes, streams, rivers, and estuarine waters. These measurements are used in several indices to determine the trophic status of lakes and rivers.

**2.0** **SUMMARY**

A modification of the EPA Method 445.0 for chlorophyll a analysis is being used with the Turner Design Trilogy Fluorometer. The modification requires no acidification and has EPA approval. The method was developed by Dr. Nicholas Welschmeyer (1994). According to Turner Design product information, the filters used with the Trilogy as well as the special blue (mercury) lamp eliminate the interference to chlorophyll a from chlorophyll b and phaeopigments. Background fluorescence is reportedly reduced with this method from interfering compounds including chlorophyll b, c, pheophytin and dissolved organic matter.

**3.0 SAFETY CONSIDERATIONS**

Normal safety procedures should be followed in the field (Lakes SOP) regarding water safety. In the laboratory, care should be taken in the use of acetone which is inflammable and can irritate skin as well as other health effects. No smoking or lighting of matches or any sparking agents should be used in the Color lab. When possible use the fume hood to make all transfers involving acetone. The fume hood is located in the Color lab. If work cannot be done under the hood because of the height considerations or logistics, then the fan located to the right of the sink should be used at low velocity pointed away from the source of the acetone. Nitrile gloves should be used when handling the acetone. **These can be found in the drawers in the Colilert section of room 117**. Goggles or safety glasses are also available for use (DEP CN 8.0 Personal Protective Equipment SOP, 1999). Placing of chairs around the lab table should be avoided as quick exit is needed in the event that fire or explosion occurs.

**4.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING**

Grab and integrated samples are the most common types of sample collection used for chlorophyll analysis from phytoplankton. Grab samples are collected in pre-cleaned 250 ml HDPE amber containers. The grab sample is obtained from a boat or by wading in-stream where possible. Boat samples should be collected from the upstream/windward side of the boat.

For lake sampling, a pre-cleaned, labeled 1000 ml HDPE container is used in the collection of an integrated sample of the water column. An integrated sample is collected using thin walled polyethylene tubing (3/8-inch internal diameter/0.9 cm). The tubing is lowered down to the desired depth (3 times the secchi disk reading), the top is crimped at the surface and then it is pulled out of the water. The tube end is held near the mouth of the sample container, but held so that the tubing does not contact the inside of the container. De-crimping allows the water to drain out; the sampling procedure is repeated until ~750 mL are collected (MA DEP, 2018). The sample should be kept in the dark and on ice until it is brought back to the lab. At the lab, the sample should be stored at 4°C and in complete darkness in the refrigerator in the microscopy lab. The samples should be processed within 24 hours of their collection. If samples cannot be analyzed within this time then the samples should be filtered and the filters frozen for future analysis (within 28 days of their collection).

Grab samples are particularly used for the shoreline samples as part of our recreational assessment or at the request of the Massachusetts Dept. of Public Health. The MassDEP samples are collected by holding the base of the amber plastic container below the surface, removing the cap and then plunging it 6 to 9 inches beneath the surface. The container neck should point slightly upward and the mouth should be directed toward the current. If there is no current, an artificial one can be created by pushing a container forward horizontally in a direction away from the hand. Before capping the container, spill a small amount out-typically to the shoulder of the container-to allow enough air space for mixing. All sample containers should be stored on ice in a cooler until delivery at CERO-Worcester.

Grab algae samples may be collected when the water body is shallow and not stratified, or there is interest in knowing about the algae in the surface waters, particularly if bloom conditions are observed.

The chlorophyll *a* samples from periphyton, attached algae, can be collected in different ways. The method used predominantly by MA DEP for periphyton collection is to scrape clean a known area of natural substrates (rocks, vegetation etc.), the loosened material is rinsed into a measured volume of water and a subset removed for chlorophyll analysis (see**:** CN: 35.0 Percent Cover and Periphyton Collection Determinations, Massachusetts DEP 2012).

Samples from periphyton can also be collected from artificial substrates (glass slides) which have been deployed in the environment for a period of approximately three weeks (Weber, 1973). The slide trays are removed in the field and are kept on ice until they are brought back to the laboratory (CERO-Worcester) where the slide is scraped into distilled water which is then filtered. The filters are then ground or frozen (American Public Health Assoc., 1981).

**5.0 APPARATUS, EQUIPMENT, MATERIALS**

* Chlorophyll *a* Calibration Standard: Turner Design, 10-850
* Secondary Standard-Turner Design
* Fluorometer – Turner Design (Trilogy).
* Fluorescence module for chlorophyll a (non acidification method)-minimum detection limit 0.025 µg/L
* Tissue grinder and tube – Thomas Tissue Grinder (Thomas Scientific, 3431J70)
* Side arm vacuum flask and pump
* Millipore filter holder
* Glass fiber filter: Whatman GF/C, 2.1 cm (VWR, Inc.)
* Centrifuge - International Equipment Co., model, Centra CL2 (Fisher Scientific)
* 15mL graduated conical end polypropylene centrifuge tubes with screw caps (Fisher Scientific-14 959 70C)
* Test tube racks
* Disposable borosilicate cuvettes –12 mm by 75 mm cuvettes (Fisher Scientific)
* Aluminum foil
* Test tube brushes – conical end
* Parafilm
* Disposable glass 1 ml pipettes
* Calibrated glass pipette (TD) 1 mL, 1/100 markings
* Glass rods
* Volumetric flasks and pipettes, 4 mL, 6 mL
* Dell laptop computer with cables to attach to Trilogy

#### 6.0 REAGENTS

Modified 445.0-no acidification

HPLC grade acetone (Fisher Scientific, A-924-4) is used to make up the 90 % solution: 90 mLs of acetone is added to 10.0 mLs of de-ionized water.

De-ionized water is prepared at DWM using a Barnstead E-pure Filtration System.

**7.0 CALIBRATION**

# The Trilogy fluorometer (Turner Design) is calibrated using pure chlorophyll *a* concentrations purchased from Turner Design and used to set the solid standard (Turner Design) concentration. Calibration is done at the start of the field season and then re-checked periodically with the solid standard.

The calibration procedure is outlined in the Turner Design Trilogy Operating Manual. The general calibration steps are as follows:

**For Direct Concentration Calibration a blank solution and at least one standard solution are required. In 2018, the Trilogy fluorometer was calibrated using chlorophyll a standards from Turner Design-lot # CAS 158-01 Two chlorophyll a concentrations included a low (20.1 µg/L) and a high (205 µg/L).**

**The following steps must be followed to calibrate the Trilogy:**

* **Turn on the Trilogy using the toggle switch in the back**
* **Insert (or check) that the non-acidification module chlorophyll is in place in the top of the Trilogy**
* **On the home screen touch “Calibrate’**
* **Select “Run New Calibration’**
* Select µg/L as the unit of measurement
* Insert the calibration ‘blank’ and touch ‘OK’
* Going from the lowest concentration to the highest, enter the concentration of the first standard
* Insert the glass borosilicate cuvette containing the standard and touch ‘OK’
* Select ‘Enter More Standards’
* Insert the cuvette with the standard and touch ‘OK’
* Save the calibration and give it a name
* Re-measure the first standard to see if value is the same or <5% differnce.
* When asked for volume of standard sample and of solvent enter 1 mL for sample and 1 for solvent respectively. The **standard sample is unique;** typically **the volume of the filtered sample will be 50 and of the solvent 10 mls-** unless different volumes were used and recorded in the lab book
* Insert the Solid Standard in the Optical Module and use the Allen wrench included with the solid standard to change the adjustment screw to get the same reading as the Standard that was previously measured. Turning the screw clockwise will produce a lower signal.
* Re-measure the standard and see if the standard reading and the solid standard reading are the same or close.

**8.0 PROCEDURE**

# Background Information

Samples are generally processed as soon as they are received at the DWM laboratory, but must be processed within 24 +/- hours of the time of collection. Samples that cannot be analyzed within 24 +/- 2 hours of collection must be filtered and the filters frozen for future analysis. Samples must be analyzed within 28 days of first filtering and freezing.

**Log Book-**Chlorophyll *a* samples are logged in using the logbook stored in the ‘chlorophyll’ drawer located in the Chlorophyll/Color laboratory (room **117)** You first need to create a lab number which will include year-batch-sample # (consecutive for that batch) e.g.18-01-01 for the first batch. The batch represents all the samples that are run on a certain day. to use you need to Check the lab log-in book to create a batch number. The batch number will be the next consecutive number. The sample numbers are then added to the batch number in consecutive order. Additional information is obtained from the Chain of Custody form including: OWMID #, name of the water body/town, sample date, sample time, initials of sample collector if ‘regular’ employee. Also included in the logbook is the amount of water filtered. **It is critical that the amount be recorded for each sample.** The filtered amount is later entered in the algorithm used to calculate the chlorophyll concentrations and must be accurate.

**Filter**-After entering the log-in information and filtering the samples, the filters need to be either frozen for future analysis or ground for analysis the following day.

**Log into computer**-Transfer the pertinent information from the log book to the 2018 Chlorophyll lab book on the computer (W:\\DWM>SOP>temp>2018data>chlorophyll a>2018\_chlorophylla\_Lab\_book\_template). When you open this file do not update it, but instead go to file:save as and enter CHL18\_XX (XX is the batch # you just created). If the samples were frozen, enter the time when **all** the samples were put in the freezer. If samples were ground, record the time when **each sample** was completed and enter it onto the bench sheet, print it out and add it to the chlorophyll black notebook.

The following day record the time the samples are read as well as the temperature on this same sheet. The chlorophyll readings that were obtained from the Trilogy are entered onto the bench sheet as well as the temperature and any dilutions that were made. Once all the samples have been processed enter the info into the on-line data file. Print out the completed sheet and have it QC’d by asking a second person to read out the pertinent data from the lab sheets for verification in the computer file. There is an associated check list to fill to verify that each part of the review was completed. When finished, the checklist should be added to the completed lab sheet and put in the box by Jane Ryder’s cubicle. The bench sheet should be returned to the chlorophyll lab book.

**Lab Procedures**

**Initial Prep**

1. Pick up the chain-of custody form from the folder in the staging area and add the appropriate signatures at the bottom. Copy the chain of custody form and give one to Joan Beskenis and the original should be put back in the folder located on the refrigerator unit in the staging room.
2. Retrieve the samples from the bio-refrigerator in the microscopy room. Compare the numbers on the samples to the chain of custody form. **Carefully check that the samples containers are the round ones for chlorophyll and not the square bottles for algae counts!**
3. Line up the samples on the bench top in Rm **117** by time collected-earliest time should be run first.
4. Fill out lab book with the information listed on the chain of custody form.
5. Every tenth sample should be a duplicate. This sample is given a consecutive lab number like the other samples, but it is denoted duplicate (LD) on the computer file.
6. The last sample in the batch is a lab blank. This sample is given a consecutive lab number like the other samples, but it is denoted blank (LB) on the computer file.
7. Fill out the computer spreadsheet (see Log Book above)
8. Organize and label the plastic 2-inch Whatman petri dish using an indelible black marker. The label should include the lab number and date the samples were collected. Aluminum foil that has been cut into ~4x4 inch squares are used to completely cover the petri dish. These are also labeled with the lab # and the sample date.
9. Set up spreadsheet. Go to W:\DWM\>SOPTemp>2018data>chlorophyll a>2018\_Chlorophylla\_Lab\_book\_template and open it. Do not hit update file, but click ‘save as’ and name the file chl-18-‘batch #’ e.g. chl-18-02.
10. Enter the name of the batch at the top of the page and if you are filtering the samples use the drop down to insert your name. Print out this page. Make sure printer is: WOR-01-RICOH-DWM-env-fp-win-003.

**Filtering Samples**

1. **After all the labeling and computer entries are completed begin work with the samples.**
2. There are two graduated cylinders to use: the one with the **red markings is for DI water only,** and the one with the **yellow collar is for** **sample water** **only.** At the beginning of the sample run the graduated cylinder (yellow one) should be washed 3 times with tap water and once with about 40 ml of sample water from the previously shaken sample jar.
3. Shake the sample gently 25-30 times.
4. Using tweezers, take a 2.1 cm Whatman GC/F, glass fiber filter and place it on the Millipore filtering flask screen. Do not touch the filter. Attach the glass tube to the filter flask using the metal clamp.
5. Measure ***50 mLs*** of sample or less using the **yellow collared graduated cylinder**. If an amount other than 50 mLs is used it should be recorded in the Chlorophyll *a* Logbook and also in the Chlorophyll *a* data file. To accurately measure 50 mls the final level can be reached using a 1 ml pipet-disposable. Read the level of the meniscus from the side of the graduated cylinder not looking down. The meniscus is read at the bottom of the liquid curvature. Do not use these pipets more than once.
6. Carefully pour approx. 15 mL of measured sample into the filtration apparatus and turn on the vacuum. The sample should pass quickly through the glass fiber filter. If the sample is not filtering through – either because too much sediment is present or the algal concentration is too high – then less than 50mL’s can be filtered or a dilution made. A notation on the amount of sample filtered must be made in the Chlorophyll *a* Logbook and the Chlorophyll *a* computer file.
7. Continue pouring sample until all is filtered.
8. Fill the designated sample cylinder (yellow collar) with ***50 mL*** DI water.
9. With the filter still in place, run the ***50 mLs*** of de-ionized water through the filter column, using the designated graduated cylinder, to wash loose phytoplankton cells off the sides of the column.

**Cleaning Between Samples**

1. Use the spray bottle to wash any remaining cells from the filter funnel.
2. Turn off pump in between filling the cylinder to also aide in washing phytoplankton off the sides of the column.
3. After all water has been filtered, unclamp the filter holder and with tweezers transfer the filter to the previously marked petri dish.
4. Cover the petri dish and wrap it in aluminum foil to keep out the light. Temporarily transfer the petri dish to the drawer until you have finished preparing all samples, then remove them and put them in the freezer in the Microscopy Lab.
5. Remove the filter funnel and wash it under the tap water. Use the small brush to loosen any cells on the inside of the funnel or at the bottom of the funnel.
6. **Put a new filter on the filter apparatus**, re-attach the funnel and rinse the filter by filtering at least ***100 mL*** DI water. Discard filter disk.
7. Clean the designated sample graduated cylinder with three tap water rinses followed by a DI rinse.
8. If at any time the graduated cylinder or other glassware looks dirty after these rinsing procedures, a small amount of washing detergent should be added to the cylinder and then scrubbed with the brush to remove any film or dirt that might have built up.
9. **Check the level of the water in the filter flask from time to time!. Make sure that it is not overflowing. After about 4-5 samples the flask will need to be emptied out. To do this take off the filtering apparatus, disconnect the tube leading from the pump. The sample plug will need to be removed in order for the collected water to be poured out into the ‘algae’ bucket located under the sink.** Repeat above steps until all samples are filtered.

**Lab QC**

1. **After the last sample a batch blank should be run**. The blank is denoted LB on the data entry sheet. After the yellow graduated cylinder has been cleaned, as in step 17 filter ***50 mL*** of DI for the blank.
2. **Every tenth sample a duplicate should be run, this is denoted LD.** This sample is given a new number, and is denoted as a duplicate in the computer file.
3. Return the sample bottle to the refrigerator if algal counts or identifications are requested. The field sheet should indicate this. If the sample is not needed further then pour it in the sink, rinse the container out with tap water three times and then put it in the recycling bag underneath the sink in the ‘staging room’.
4. Rinse the graduated sample cylinder and filter holder several times in de-ionized water.

**Freezing the Samples**

1. All of the filters should be added to the top shelf of the freezer in the Microscopy Lab-room 122.

# Analytical Procedures

# Grinding the Samples

1. When acetone is being used, turn on the fume hood and the fan by the grinding apparatus.

*Transfers of all chemicals and preparation of the 90% acetone should be done under the hood when possible. Grinding of the samples; however, should* ***not*** *be done there because of the risk of sparks which could ignite the acetone.*

1. The oscillating fan should be used when running the chlorophyll samples.
2. It should be operated at low velocity between the analyst and the acetone source but pointing away from the analysts’ face.
3. **Retrieve petri dishes containing the filters from the freezer in the macroinvertebrate microscopy lab.**
4. Remove the filter using the tweezers and add to grinding tube.
5. Push the frozen filter to the bottom of tissue grinding tube using a glass rod. The filters rapidly thaw and are malleable.
6. Using a 4 mL volumetric pipette add 90% aqueous acetone solution to the grinding tube.
7. Set clock/alarm for two minutes. Grind contents in grinding tube for 2 minutes or until the sample has been sufficiently macerated. Care must be taken not to overheat the sample which degrades the chlorophyll.
8. The contents of the grinding tube are carefully washed into a 15mL graduated centrifuge tube using a 6 mL volumetric pipette containing 90 % aqueous acetone to rinse the pestle and the grinding tube. The rinse and the filter slurry are added to the centrifuge tube.
9. Write the sample # on the ***top of the cap and on the side of the cuvette***.
10. The centrifuge tubes are sealed with screw caps, shaken vigorously and then wrapped with aluminum foil to eliminate any exposure to light. The wrapped tubes are then stored in the refrigerator in the Microscopy room at 4°C for 24 hours plus and minus 2 hours.

**Cleaning between Samples**

1. Between samples the grinder tube and the grinder pestle should be wiped with a Kimwipe and then washed with 90 % acetone to remove any remaining chlorophyll. Use the acetone wash bottle to clean the grinder tube and then pour the gathered material into the waste acetone jar. Both the acetone wash bottle and the waste acetone jar are found in the box for inflammable items.
2. Finally, the grinder tube is cleaned with a brush and then washed three times with tap water, one time with DI and then one time with acetone. After wiping the pouring spout on the grinding tube with a Kimwipe, the acetone wash bottle should be used to cleanse the sides of the grinder tube.

**Analysis**

1. After 24 hours, centrifuge tubes are then taken out of the refrigerator and put into the centrifuge. The tubes are centrifuged for 10 minutes at 1000 rpm. Note: the centrifuge must have a balanced load to work. (*the centrifuge is set to run 10 minutes at 1000 rpm)*.
2. Allow the tubes to come to room temperature (approximately 30 minutes). (*I typically spin them down and then leave them in the dark in the centrifuge and take them out one by one as they are read.*)
3. While the tubes are coming to room temperature, boot up the lap top computer.

**Reading the Samples**

1. When the computer has booted, turn on the Trilogy fluorometer and then connect it to the lap top using the USB cable. Click on the Trilogy icon and then click on connect to Excel. Save the Trilogy file on W:\\DWM>N>chlorophyll2018>date (eg. 15May18). Name the file by the date you are currently reading the sample concentrations e.g. 25May2018. The Trilogy does not store more than 20 measurements at one time. If more than 20 measurements are taken the oldest reading will be overwritten.
2. The chlorophyll readings should be done no more than 24 hours +/- 2 after grinding, if you do go over this holding time still run the samples but annotate this change from procedure. A decision will be made by the project manager whether to accept the data or not.

Use of the Trilogy Fluorometer

Set up of the Trilogy fluorometer involves connecting to the lap top first. The computer should be on as well as the fluorometer before the computer is plugged into the fluorometer and before the yellow internet plug is attached.

Once the computer has booted up and the Trilogy is connected then click on the Trilogy SIS button on the desktop. A box will open with com ports listed and Excel. Click start and the gray boxes should turn to yellow and the Excel program should open up. Now the results from the Trilogy will appear in the Excel spreadsheet.

Trilogy-After turning on the fluorometer, a message will appear on the screen asking if the correct module is selected. You should chose chl-NA and click ok which will bring you to the home screen. Click **calibrate** and then select the previously conducted calibration-May 2018. Next click Sample ID and enter the batch and lab number you are analyzing e.g. 01-01. Finally, hit measure fluorescence, you will be asked the volume of the sample and the volume of the solvent. **If it’s a phytoplankton sample enter the actual volume of sample filtered-typically 50 mls and 10 mls for the solvent. The lab book should be checked to see if other amounts were used particularly for the sample volume. If it’s a periphyton sample I put in 1 for sample volume and 1 for solvent.** Check on the screen to see if you did enter the amount filtered and the amount of solvent correctly. If you made a mistake let the instrument calculate a value, but then read it again..

1. After centrifuging and waiting for the samples to come to room temperature, pour approximately four mL of supernatant into a clean, disposable 75 mm borosilicate cuvette.
2. Then put the thermometer in the remaining sample in the centrifuge tube while you wait for the reading on the Trilogy.
3. For phytoplankton samples at the query on the Trilogy ‘what was volume of sample and solvent used’ enter 50 mLs for the sample volume and 10 for the solvent volume unless it is indicated in the lab book that other quantities were used. If periphyton samples are analyzed enter 1 ml for both the sample volume and the solvent volume.
4. Record the chlorophyll reading on the data sheet.
5. Record the temperature of the remaining sample and add it to the chlorophyll *a* data sheet.
6. When all samples are run the Trilogy Excel spreadsheet should be saved (W>DWM>SOP\_temp>2018data>chlorophyll>Trilogy readings>15May18). Name the file by the date you are currently reading the sample concentrations e.g. 15May2018).
7. Add the data sheet information to the computer file. The spreadsheet name for each date should be: CHL18\_01 (CHL\_year\_batch#).
8. Record the amount of acetone waste produced that day on the waste generated sheet attached to the post opposite the flammable cabinet

**QC Check:**

1. After completing the readings, the analyst should seek someone to assist in performing a quality control check. There is a Quality Control Check List in the front of the chlorophyll notebook for items to check (Appendix A).
2. Gather the lab book, Trilogy Excel printout, bench sheet and the final chlorophyll printout. Check that the Trilogy readings were recorded properly on the bench sheet and filtered volume obtained from the lab book was also correct..
3. Next check the computer entries against the bench sheet. If any edits are made they should be initialed and dated. The bench sheet should be kept in the chlorophyll lab folder along with the quality control check sheet. added to the notebook

**9.0 CLEANING PROCEDURE**

Typically, disposable borosilicate cuvettes are now used. If these are unavailable then regular borosilicate cuvettes are cleaned by emptying the contents into the waste jar held in the Flammable Storage Cabinet, using the cuvette brush to wash out the interior and rinsing the interior with de-ionized water. Following this the cuvettes are rinsed with acetone and then the de-ionized water again.

Other reusable glassware is soaked for four hours in laboratory grade detergent and water, rinsed with tap water and then with acetone followed by two more de-ionized water rinses.

The waste acetone container should be emptied every night into the disposal bottle that is kept in the Flammable Storage Cabinet. The waste containers, both the transfer and larger storage containers, must be labeled with proper waste labels which include the following information: “hazardous waste”, the date that the disposal jar was first used, the contents must be listed and the word “ignitable” included on the label. The labels should be facing forward in the storage cabinet.

**10.0 QUALITY CONTROL**

A laboratory duplicate is run on every tenth sample. The precision for these samples will be determined by calculating their relative percent difference (RPD).

RPD= (sample- duplicate)/(average of sample and duplicate)\*100. The RPD should not exceed 20%.

The accuracy of the sample measurements will be determined by comparing the concentration of chlorophyll check samples (Turner Design, Inc.) to the calculated concentration. The check sample values should fall within plus and minus 20% of the standard calibration readings. If this level of accuracy is not met then the source of the error must be identified before further analyses are done. If the project data objectives allow it, a larger percent error may be acceptable.

A laboratory blank is the last filter extracted of a sample set. It is used to check for contamination of the reagents or apparatus. This laboratory blank filter is extracted and analyzed similar to a sample filter. If the sample value is greater than 1 mg/m3 (1 ug/L) then the samples for that day must be re-run or their data annotated and judgment made as to whether the data objectives are still met.

The computer data file entries will be checked on at least a weekly basis against entries in the lab book, the Trilogy print-out from the fluorometer and with the hand written lab bench sheet.

Chlorophyll a results are reported as mg/m3 or ug/L The chlorophyll values are recorded to the nearest tenth if the results are below 50 ug/L and those greater than 50 ug/Lare reported as whole numbers. The MDL for the method is 1 ug/l. Readings less than 1 are reported as <1 ug/L.

1. **INTERFERENCES**

High concentrations of humic acids can cause interference because they fluoresce at the same wavelengths as the chlorophyll *a*. Other substances that fluoresce in the red region of the light spectrum may interfere with chlorophyll *a* measurement.

Pheophytin *a* (a degradation product of chlorophyll *a*)and chlorophyll *a* are measured at similar wavelengths. This can result in an overestimation of chlorophyll a measurement if you are using the acidification method for chlorophyll a analysis. A correction formula is used to adjust for this problem. These formulas are provided in Standard Methods (American Public Health Assoc., 1981).

1. **PREVENTIVE MAINTENANCE**

The oil level on the water pump used for filtering must be checked daily and oil added if necessary. The oil is kept in the bottom cabinet to the right of the fume hood.

When doing the filtering always check that the volume of water in the ‘side-arm’ flask is not more than half full. When it gets to about that level it should be emptied out and the water disposed of in the sink.

The waste acetone is recycled at WES. It must be transported there in an iced cooler and packed in such a manner that it cannot tip over. The cooler should be tied down with a bungee cord and labeled “waste acetone” on the outside.

1. **CORRECTIVE ACTIONS**

If the calibration, laboratory duplicate or laboratory blank fail to meet the criteria in section 10.0, then the analyst must re-examine the collection and analysis techniques and inspect the fluorometer for contamination or malfunction. If the problem persists the analyst must initiate a corrective action following the directions in the standard operating procedure DEP CN 5.0 Corrective Actions. The survey and project coordinators must be notified.

1. **WASTE AND POLLUTION PREVENTION**

The use of disposable borosilicate cuvettes has resulted in a dramatic decrease in the use of acetone. The use of the “no-acid” method has resulted in the elimination of the use of concentrated sulphuric acid and of the waste acid. While not immediately harmful to the environment the use of disposable cuvettes increases the waste returned to the landfills.

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1. **DEFINITIONS/ACRONYMS**

phytoplankton-algae that is not attached and is usually semi-buoyant so that it floats within the water column

periphyton-attached algae

phaeopigments-breakdown pigments from chlorophyll a

HPLC-high pressure liquid chromatography (grade of acetone)

1. **APPENDICES**

Appendix A

**Chlorophyll Quality Control Check List**

Initials QC Reviewer(s)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Batch numbers:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date reviewed:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |
| --- | --- |
|  | **Enter 🗸when completed** |
| If corrections must be made cross out value on bench sheet , initial and date the entry and make the correction on the final sheet |  |
| Do the number of mLs filtered agree between lab book and bench sheet? |  |
| When reading the chlorophyll samples using the Trilogy, was a dilution made and was it recorded? |  |
| Check Trilogy readings on Excel worksheet for each batch. Was the correct chlorophyll reading transferred from the worksheet to the bench sheet? |  |
| Was the correct chlorophyll reading transferred from the bench sheet to the final lab sheet? |  |
| Was a dilution made and recorded on bench sheet and transferred to the final sheet? |  |
| Is all information recorded on the final sheet analyst name, readers name, batch number etc? |  |
| After checking, initial the upper right hand corner of the bench sheet (s) and date it |  |
| Keep the checked and initialed bench sheet in the black loose leaf notebook.kept in the lab. |  |
| The QC check list should also be kept with the bench sheet in the notebook. |  |