APPENDIX L

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT (SCCWRP)

QUALITY ASSURANCE DOCUMENT FOR

CHLOROPHYLL A

AND

TOTAL SUSPENDED SOLIDS

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Quality Assurance Summary for the Determination Chlorophyll *a*

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT
(SCCWRP)

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SCOPE AND APPLICATION – chlorophyll a

1.1 This method provides a procedure for the fluorometric determination of chlorophyll *a* and its magnesium-free derivative, pheophytin *a* in estuarine water samples

1.2 This method is based on US EPA Method 445.0.

2.0 SUMMARY OF METHOD

2.1 Chlorophyll-a in a measured volume of sample water is concentrated by filtering at low vacuum through a glass fiber filter. The pigments are extracted from the sample in 90% acetone and allowed to steep overnight, but not to exceed 24hrs, to ensure thorough extraction of chlorophyll a. The fluorescence of the sample is measured before and after acidification with 0.1M hydrochloric acid.

3.0 DEFINITIONS

3.1 Stock Standard Solution (SSS)- A solution prepared in the laboratory using reference materials purchased from Turner Designs or Sigma. The chl *a* from this source is harvested from *Anacystis nidulans*.

3.2 Laboratory reagent blank (LRB)- An aliquot of reagent water (Milli-Q) or other blank matrices that are treated exactly the same as the sample including exposure to all glassware, equipment, solvents, reagents, internal standards and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.3 Field duplicates- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Assessment of duplicates provides a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4 Quality Control Sample (QCs) - It is a solution of known concentration obtained from a source external to the laboratory to check laboratory performance.

4.0 INTERFERENCES

4.1 Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of both chlorophyll a and pheophytin a

4.2 Spectral interferences resulting from the fluorescence of the accessory pigment chlorophyll b, and the chlorophyll a degradation product pheophytin a, can result in the overestimation of chlorophyll a concentrations. However, highly selective optical filters used in this method minimize these interferences.

4.3 Quenching effects are observed in highly concentrated solutions or in the presence of high concentrations of other chlorophylls or carotenoids. In such circumstances, samples should be diluted.

4.4 Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, Standards, LRBs and QCs must be at the same temperature to prevent errors/ low precision. Analysis of samples at ambient temperatures is recommended in this method.

4.5 All photosynthetic pigments are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20^{o}C to prevent degradation.

5.0 HEALTH AND SAFETY

5.1 Lab safety- Safety glasses are required for all laboratory analysis. Use gloves to avoid skin irritation from contact with acetone; work under the fume hood when possible. Refer to Material Data Safety Sheets (MSDS) for any other information about personnel protective equipment and other safety considerations when using acetone and hydrochloric acid.

5.2 Chemical hygiene- Refer to the MSDS datasheets for questions concerning a chemical's toxicity and the necessary safety precautions.

5.3 Waste Disposal- Dispose of waste in the acetone collection bottle. See Darrin Greenstein to notify for disposal upon request.

6.0 PERSONNEL/ TRAINING/ RESPONSIBILITIES

6.1 General Responsibilities- This method is restricted to use by or under the supervision of the analyst experienced in the method. Each analyst must be trained and able to read and understand the SOP.

6.2 Laboratory analysts: it is the responsibility of analysts/technicians to;

- 6.2.1 Read and understand the SOP and follow it as written.
- 6.2.2 Produce quality data that meets all of the laboratory requirements.
- 6.2.3 Demonstrate proficiency before performing this procedure without supervision
- 6.2.4 Repeat the initial demonstration of proficiency each time a modification is made to the method.

6.3 Laboratory managers: it is the responsibility of the laboratory manager to:

- 6.3.1 Ensure that all analysts have the technical ability and have the adequate training required to perform this procedure.
- 6.3.2 Ensure that all analysts have completed the required demonstration of proficiency before performing this procedure without supervision.
- 6.3.3 Produce quality data that meets all laboratory requirements.

7.0 APPARATUS AND MATERIALS- All reusable lab ware that comes in contact with chlorophyll solutions should be clean and acid free. Dishwashing should include soaking in laboratory grade detergent and water, rinsing with tap water then rinsing with deionized water.

7.1 Turner Designs Trilogy Fluorometer
7.2 Tissue grinder/Teflon pestle
7.3 10mL Borosilicate glass tubes with caps
7.4 Glass micro fiber filters GF/F 0.7um retention 47mm
7.5 Petri dishes (50mm)
7.6 Aluminum foil
7.7 Vacuum pump
7.8 Glass rods
7.9 Filtration unit

7.10 Flat-tipped forceps
7.11 Assorted Class A calibrated pipettes
7.12 50ml, 100ml and 1-L class A volumetric flask

8.0 REAGENTS AND STANDARDS

8.1 Acetone, HPLC grade
8.2 Hydrochloric Acid
8.3 Chlorophyll a free of chlorophyll b
8.4 Milli-Q water
8.5 0.1M HCl solution- Add 0.85 ml of concentrated hydrochloric acid to approximately 50ml of water and diluted to 100ml.
8.6 Aqueous Acetone Solution- 90% acetone / 10% Milli-Q water. Carefully measure 100ml of water into a volumetric flask and fill to the line. Transfer to a 1-L flask. Fill flask to the line with Acetone. Mix, Label and Store in Amber bottle in Flammables Cabinet.
8.7 Chlorophyll Standard Stock Solution (SSS)- Chlorophyll *a* from Turner Designs or Sigma is shipped in an amber glass ampoule. This should be stored in the freezer until use. Tap the ampoule until all of the dried chlorophyll has settled on the bottom. Working in a darkened room, carefully break the tip off the ampoule and transfer contents into a 50 ml volumetric flask and dilute to volume with 90% acetone. Transfer to a darkened bottle or wrap the flask in foil to protect from the light. Label bottle including the chlorophyll lot number. When stored in an airtight container at room temperature, the SSS is stable for at least six months.
8.8 Chlorophyll a Primary Dilution Standard (PDS) - add 1 ml of the SSS (section 9.7) to a 100ml volumetric flask and dilute to volume with aqueous acetone solution (section 9.6). If exactly 1mg of pure chlorophyll was used to prepare the SSS, the concentration of the PDS is 200 ug/L. Prepare fresh prior to use and label flask and wrap in foil.
8.9 Quality Control Sample (QCs)- Since there are no commercially available QCs, QCs are prepared from the PDS at the following concentrations:

- QC1 = 5 ug/L
- QC2 = 20 ug/L
- QC3 = 50 ug/L

9.0 SAMPLE COLLECTION. PRESERVATION AND STORAGE

9.1 For periphyton samples, aliquots of 25mL are filtered onto glass-fiber filters (0.7 um). Filters containing the algae homogenate are removed from the filtering apparatus, folded in half (or quarters) with the sample-containing side on the inside. They are kept inside 47mm Petri dishes that snap shut, and are labeled, wrapped in aluminum foil to protect against light exposure, and stored on ice in the field. The samples should be placed in the freezer immediately upon return to the lab after fieldwork. They can be kept frozen for up to 30 days before analysis.
9.2 For suspended chlorophyll a, the same procedure is followed, except 500mL of sample water (or however much is needed to leave a visible green residue on the filter) is filtered to isolate the samples. Filters are then treated the same as for the periphyton samples.

10.0 PROCEDURE

10.1 Extraction

10.1.1 If sampled filters have been frozen, remove them from the freezer but keep them in the dark. Set up the tissue grinder and have on hand tissues and squirt bottles containing water and acetone. Workspace lighting should be the minimum that is necessary to read instructions and operate instrumentation. Remove a filter from its container and place it in the glass grinding tube. The filter may be torn into smaller pieces to facilitate extraction. Push it to the bottom of the tube with a glass rod. With a volumetric pipet, add 4 mL of the aqueous acetone solution to the grinding tube. Grind the filter until it has been converted to a slurry. (NOTE: Although grinding is required, care must be taken not to overheat the sample. Good judgment and common sense will help you in deciding when the sample has been sufficiently macerated.) Pour the slurry into a 15-mL screw-cap centrifuge tube and, using a 6-mL volumetric pipet, rinse the pestle and the grinding tube with 90% acetone. Add the rinse to the centrifuge tube containing the filter slurry. Cap the tube and shake it vigorously. Place it in the dark before proceeding to the next filter extraction. Before placing another filter in the grinding tube, use the acetone and water squirt bottles to thoroughly rinse the pestle, grinding tube and glass rod. The last rinse should be with acetone. Use a clean tissue to remove any filter residue that adheres to the pestle or to the steel rod of the pestle. Proceed to the next filter and repeat the steps above. Approximately 500 mL of acetone and water waste are generated per 20 samples from the rinsing of glassware and apparatus.

10.1.2 Shake each tube vigorously before placing them to steep in the dark at 4°C. Samples should be allowed to steep for a minimum of 2 h but not to exceed 24 h. The tubes should be shaken at least once during the steeping period.

10.1.3 After steeping is complete, shake the tubes vigorously and centrifuge samples for 15 min at 675 g or for 5 min at 1000 g. Samples should be allowed to come to ambient temperature before analysis. This can be done by placing the tubes in a constant temperature water bath or by letting them stand at room temperature for 30 min. Recalibrate the fluorometer if the room temperature fluctuated ± 3EC from the last calibration date. Refer to Trilogy manual for more details on calibration.

10.2 SAMPLE ANALYSIS

10.2.1 After the fluorometer has warmed up for at least 15 min, use the 90% acetone solution to zero the instrument on the sensitivity setting that will be used for sample analysis.

10.2.2 Pour or pipet the supernatant of the extracted sample into a sample cuvette. The volume of sample required must be known so that the correct amount of acid can be added in the pheophytin a determinative step. For a

cuvette that holds 5 mL of extraction solution, 0.15 mL of the 0.1 N HCl solution should be used. Choose a sensitivity setting that yields a midscale reading when possible and avoid the minimum sensitivity setting. If the concentration of chlorophyll a in the sample is >90% of the upper limit of the LDR, then dilute the sample with the 90% acetone solution and reanalyze. Record the fluorescence measurement and sensitivity setting used for the sample. Remove the cuvette from the fluorometer and acidify the extract to a final concentration of 0.003 N HCl using the 0.1 N HCl solution. Use a Pasteur type pipette to thoroughly mix the sample by aspirating and dispensing the sample into the cuvette, keeping the pipette tip below the surface of the liquid to avoid aerating the sample. Wait 90 sec before measuring fluorescence again. NOTE: Proper mixing is critical for precise and accurate results.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 For 'corrected chlorophyll a', calculate the chlorophyll *a* in the water sample as follows:

Chlorophyll *a* (ug/L) = F_s (r/(r-1))* (R_b-R_a)*(V_e/V_s)

Where: F_s = the conversion factor of the sensitivity setting
r/ r-1 = 2.096, or r = 1.9124 (r =R_b/R_a, as determined with pure chlorophyll *a* for the instrument).
R_b = reading before acidification
R_a = reading after acidification
V_e = volume of extract, and
V_s = volume of sample.

12.0 QC/ QA CRITERIA

12.1 The QCs should be within ± 20% of the known value
12.2 Check the instrument calibration using the solid standard.
12.3 RPD for duplicate samples should be ± 25%

2540 SOLIDS

2540 D. Total Suspended Solids Dried at 103–105°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate of total suspended solids, calculate the difference between total dissolved solids and total solids.

b. Interferences: See 2540A.2 and 2540B.1. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not representative. Because excessive residue on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

2. Apparatus

Apparatus listed in Sections 2450B.2 and 2540C.2 is required, except for evaporating dishes, steam bath, and 180°C drying oven. In addition:

Aluminum weighing dishes.

3. Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. Dry in an oven at 103 to 105°C for 1 h. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

b. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.

c. Sample analysis: Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it. Stir sample by agitating the bottle. Quickly but carefully pour a measured volume from a clean graduated cylinder onto the seated glass-fiber filter. Record the entire volume that has been filtered. Use some MQ water to rinse out the bottle (as there will be probably be some settled material on the bottom) and the graduated cylinder. Pour this into the filtration funnel, as well, but do not include this in the figure reflecting the total volume filtered (only include actual sample volume.) Wash the filter with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Samples with high dissolved solids may require additional washings. Carefully remove filter from filtration apparatus and transfer it back to its original aluminum weighing dish. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

4. Calculation

$$\text{mg total suspended solids/L} = \frac{(A\text{-}B) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

5. Precision

The standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1707 mg/L in studies by two analysts of four sets of 10 determinations each.

Single-laboratory duplicate analyses of 50 samples of water and wastewater were made with a standard deviation of differences of 2.8 mg/L.