APPENDIX E

CHLOROPHYLL-*A* STANDARD OPERATING PROCEDURES

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STANDARD OPERATING PROCEDURE FOR CHLOROPHYLL-A DETERMINATION USED BY SABINE RIVER AUTHORITY

1 SCOPE & APPLICABILITY

1.1 This method is for determining chlorophyll *a* concentration in natural water samples using a spectrophotometer with a 2nm spectral bandwidth.

1.2 This method is prepared in conjunction with guidelines from EPA Method 446.0, Revision 1.2 and Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 10200H "Chlorophyll".

2 SUMMARY OF METHOD

2.1 A 200mL, or otherwise specified, aliquot of natural water is filtered in a dark room. Pigment is extracted from the filter through maceration, steeping, and centrifugation in 90% acetone. The pigment extract is then analyzed using a spectrophotometer at 664nm and 750nm, and again after acidification at 650nm and 750nm. Addition of acid results in the loss of the magnesium atom, converting chlorophyll *a* to pheophytin *a*. The change in optical density after acidification is used to determine the corrected values for chlorophyll *a*.

2.2 The lower detection limit for this method is approximately 3µg/L, when incorporating a 200mL filtered sample volume and a 20mL extract volume. When other analysis volumes are used, the lower detection limit must be calculated using the absorbance detection limit value for the spectrophotometer. This absorbance detection limit is determined through method detection limit (MDL) studies.

3 DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1 Analyte = the component of interest, in this case, "chlorophyll *a*".

3.2 Chlorophyll *a* = a photosynthetic pigment which is a component of planktonic algae, constituting 1-2% of the dry weight. Chlorophyll *a* is used extensively to estimate phytoplankton biomass.

3.3 Laboratory Pure Water = reagent water meeting purity characteristics of ASTM Type II laboratory distilled water (daily conductivity <1.0umhos/cm).

3.4 Method Blank = a sample containing no target analyte that is taken through the entire sampling and analytical procedure. The analysis of a method blank helps identify any contamination introduced in the analysis process. In this case, the method “blank” consists of 100mL of laboratory pure water that is treated as a natural water sample.

4 HEALTH AND SAFETY WARNINGS

4.1 Lab Safety - Safety glasses are required for all laboratory analysis. Use gloves to avoid skin irritation from contact with acetone; work under a hood when possible. Please refer to the MSDS file for any other information about personal protective equipment and other safety considerations.

4.2 Chemical Hygiene - Hazards of the chemicals used in this method were discussed in the previous section. Please refer to the MSDS file for any further questions concerning a chemical’s toxicity and the necessary safety precautions.

4.3 Waste Disposal – Waste is disposed of in an appropriate acetone collection bottle. Dirty 5cm spectrophotometric cells are rinsed with lab pure water and 90% acetone solution.

4.4 Pollution Prevention – If waste is disposed of correctly, no pollution problems should occur from using this method.

5 INTERFERENCES

5.1 Chlorophyll *a* can be significantly under or over-estimated using a spectrophotometer for analysis in part because of the overlap of the absorption bands of co-occurring accessory pigments and chlorophyll *a* degradation products. Using a spectrophotometer with a spectral bandwidth of 2nm or less can help prevent some of these errors.

5.2 Light, changes in temperature, and exposure to air can also interfere with the test, leading to decreases in chlorophyll *a* concentration. Use care in dealing with samples during the analysis process.

5.3 Excess sample turbidity can interfere with proper absorbance readings. 750nm absorbance results represent sample clarity/turbidity; this value should be less than 0.005 absorbance units for optimal chlorophyll *a* results. Samples with a 750nm absorbance of greater than 0.02 must be centrifuged again, except under special circumstances. See management staff if there is a question on sample clarity.

6 PERSONNEL QUALIFICATIONS AND RESPONSIBILITIES

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

6.2 Laboratory Analysts and Technicians – It is the responsibility of analyst/technicians to:

6.2.1 Read and understand this SOP and follow it as written.

6.2.2 Produce quality data that meets all laboratory and customer requirements.

6.2.3 Complete the required demonstration of proficiency before performing this procedure without supervision.

6.2.4 Enter laboratory sample and QC results into the LIMS data system for laboratory supervisor review.

6.2.5 Repeat the required initial demonstration of laboratory capability each time a modification is made to the method.

6.3 Section Leaders – It is the responsibility of the section leader to:

6.3.1 Ensure that all analysts/technicians have the technical ability and have received adequate training required to perform this procedure.

6.3.2 Ensure analysts/technicians have completed the required demonstration of proficiency before performing this procedure without supervision.

6.3.3 Produce quality data that meets all laboratory and customer requirements.

7 APPARATUS AND MATERIALS

Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance can be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.1 Milton Roy GENESYS™ 6 spectrophotometer, or equivalent, with an adaptor for cylindrical cells

7.2 5cm cylindrical cells with caps, Fisher Scientific #14-385-930E

7.3 Whatman glass microfiber filters GF/F - 47mm or equivalent

7.4 Vacuum pump

7.5 Gelman magnetic filter funnels

7.6 1000mL side-arm filtering flasks

7.7 Graduated cylinders

7.8 Yamato overhead lab stirrer for grinding filters

7.9 Plastic disposable pipettes

7.10 Kontes glass round-bottom 25mL tubes with grinding pestle attachments

7.11 35mL and 15mL graduated polypropylene centrifuge tubes with caps

7.12 ThermoIEC Centra CL2™ centrifuge or equivalent

7.13 100-1000µL Brinkmann Eppendorf™ micropipette with adjustable dispensing volume feature

7.14 Refrigerator with freezer

8 REAGENTS, GASES, AND STANDARDS

8.1 Lab Pure Water

8.2 90% Acetone Solution: Add 900mL of pure acetone to a 1L volumetric flask, and dilute to the mark with lab pure water. Shake or stir to mix. Use until empty.

8.3 Hydrochloric Acid (HCl), 0.1N: Add 8.5mL of concentrated hydrochloric acid to a 1L flask containing about 500mL of lab pure water. Cool and dilute to the mark with lab pure water. Stir to mix. Use until empty.

8.4 Magnesium Carbonate/90% Acetone Solution: Add 1.0g of solid magnesium carbonate to a 100mL flask, and dilute to the mark with lab pure water. Stir to dissolve as much magnesium carbonate as possible. Filter this mixture using a glass fiber filter and add the filtrate to 900mL of acetone in a 1L flask. Use lab pure water to dilute to the mark, if necessary. Stir to mix. Use until empty.

8.5 Turner Designs Chlorophyll *a* in 90% Acetone Standard Ampoule, concentration varies: This standard is purchased; it is used to find the lower absorbance detection limit of the spectrophotometer (MDL study) and prepare check standards. Use until expiration date, if stored in a freezer.

8.6 Turner Designs Chlorophyll *a* in 90% Acetone, Stock Standard: Transfer 15-17mL from a Turner Designs chlorophyll *a* standard ampoule to a 500mL flask and dilute to the mark with 90% acetone solution. Use chilled pipettes and volumetric flasks when making transfers. Calculate the stock standard concentration using the original concentration of the ampoulized standard. Use until expiration date of original ampoule, if stored in a freezer.

8.7 Turner Designs Check Standard: The concentration of this standard should be close to that of the method estimated detection limit, determined to be 35.4µL. Preparation instructions will vary depending on the concentration of the Turner Designs chlorophyll *a* standard ampoule. Use chilled pipettes and volumetric flasks when making transfers. Prepare fresh with each analysis batch; this check standard should be assigned a standard number.

8.8 Sigma Aldrich 1mg/L Spinach Standard: a purchased ampoulized standard that is used to prepare chlorophyll *a* dilutions for determining upper detection limit data.

9 SAMPLE COLLECTION, HANDLING, AND PRESERVATION

9.1 Aqueous samples are collected in amber jars and stored at 4 ± 2ºC in a dark environment. After samples are obtained, they should be put on ice immediately to avoid degradation.

9.2 Filtration must be completed within 48 hours after samples are collected. The sample filters can be stored frozen for no more than 14 days; filters must be analyzed within this time period.

10 METHOD CALIBRATION

10.1 No method calibration is necessary for this procedure. A spectrophotometer check is conducted quarterly with certified standards to validate instrument performance.

11 SAMPLE PREPARATION AND BATCH ANALYSIS

11.1 Aqueous Sample Preparation:

11.1.1 Conduct filtration in an area with subdued light. Rinse a glass-fiber filter with 100mL of lab pure water. Measure 200mL of well-mixed sample into a graduated cylinder and filter. A smaller sample volume can be used if samples are high in solids (turbidity). NOTE: Notify management staff before using alternate filter volumes; this will affect the reportable detection limit for chlorophyll *a*. Larger volumes can also be used to increase method sensitivity, where applicable. Filter one lab pure water blank and one duplicate for every 10 or fewer samples in a QC filtration batch.

11.1.2 After filtration, fold each filter in half, then two more times (with the residue on the inside) and place inside a plastic 15mL capped centrifuge tube. Make sure that all tubes are numbered and that the numbers and corresponding samples are recorded in the chlorophyll *a* filtration bench book. Record any necessary comments about sample appearance in the bench book.

11.1.3 If sample filters are to be analyzed immediately, go to the next step. Place sample filters in the laboratory freezer for analysis at a later date (store frozen for no more than 14 days).

11.1.4 Transfer an individual filter to a 25mL round bottom grinding test tube and add approximately 5mL of magnesium carbonate/90% acetone solution (NOTE: Maceration is more effective when grinding half of the filter at a time). Tear the filter into pieces to achieve more efficient maceration. (NOTE: Set 15mL labeled plastic tubes aside to use again later).

11.1.5 Securely attach a grinding pestle to an overhead stirring apparatus. Place the grinding pestle slowly into the grinding test tube, submerging it in the 5mL of solution. SLOWLY begin stirring, accelerating the speed to approximately 500 rpm. Stir for several minutes, moving the grinding tube up and down, to macerate the residual filter. Analytical judgment must be used in determining maceration completion. Slowly reduce the stirrer speed while removing the tube from the pestle, avoiding any splashing of tube contents. Rinse residue on pestle with 90% acetone solution into the grinding tube.

11.1.6 Carefully and quantitatively transfer grinding tube contents to a 35mL graduated centrifuge tube with multiple washings and adjust the total volume to 20mL with 90% acetone solution. (NOTE: Extraction volume may vary based on sample appearance. Notify management staff before using an alternate extract volume; this will affect the reportable detection limit for chlorophyll *a*.) Shake each capped tube individually.

11.1.7 Steep samples at a diagonal in a dark refrigerator for AT LEAST 2 hours and no more than 24 hours, shaking each tube several times during this period to allow for maximum filter/acetone contact.

11.1.8 After steeping, centrifuge the samples for 30 minutes at 4000rpm in the 35mL plastic centrifuge tubes. Carefully transfer 13mL of the clarified liquid extract into the ORIGINAL 15mL labeled plastic test tubes with a plastic disposable pipette, leaving the filter residue at the bottom. Centrifuge the 15mL tubes for 30 minutes at 4000rpm. Keep the samples in the dark until analysis.

11.2 QC Batch Analysis:

11.2.1 Turn on the GENESYS 6 spectrophotometer and let it warm up for at least 30 minutes. Use the arrow key on the instrument keypad to highlight the CHLORO #1 program, then press ENTER. Press the RUN TEST key on the screen keypad to display the analysis screen.

11.2.2 Transfer 11mL of sample extract to a 5cm pathlength cylindrical cell using a plastic disposable pipette, avoiding the introduction of solids from the bottom of the test tube. Wipe off the outside of the cell with a Kimwipe™ to remove smudges or excess sample.

11.2.3 Fill a separate 5cm cell with 90% acetone and insert it into the spectrophotometer (Cells have been set aside as "blanks" for this purpose). Press the MEASURE BLANK button. Insert your sample cell, and press MEASURE SAMPLE. The spectrophotometer will print the sample absorbances at 664nm and 750nm on the screen and on paper. The 750nm absorbance should be less than 0.02 for acceptable results; if it is not, the sample can be further centrifuged to remove turbidity.

11.2.4 Add exactly 330µL of 0.1N HCl using an adjustable volume micropipette to the sample cell and slowly rock it back and forth to mix. Be careful not to shake the cell or introduce any air. Wait 90 seconds after acidification before taking absorbance readings. Setting a timer will help.

11.2.5 Using the spectrophotometer keypad, access the "CHLORO 2" program by following the steps below:

11.2.5.1. Hit the ESC key to exit the CHLORO 1 program.

11.2.5.2. Press the STORED TESTS key on the screen keypad, and then LOAD INTERNAL TEST and ENTER.

11.2.5.3. Use the arrow key on the keypad to highlight the CHLORO #2 program and press ENTER.

11.2.5.4. Press the RUN TEST key on the screen keypad to display the analysis screen.

11.2.5.5. Repeat step 11.2.3 to measure the blank and sample at 665nm and 750nm. Save all spectrophotometer printouts.

11.2.6 A Turner Designs check standard (see the REAGENTS, GASES, and STANDARDS section) should be analyzed with each QC batch. This standard is analyzed in the same way as a batch sample, and results should be recorded in the chlorophyll *a* bench book.

11.2.7 Rinse all dirty sample cells with lab pure water and 90% acetone to rinse. Allow them to air dry. Wash all test tubes and plastic ware by hand with laboratory pure water, solid detergent soap, and acetone.

12 DATA ACQUISITION, CALCULATIONS, & DATA REDUCTION

12.1 Filtration batch data is currently recorded in a chlorophyll *a* filtration logbook. Spectrophotometric data from chlorophyll *a* analysis is currently recorded in a chlorophyll *a* analysis bench book and entered into a computer calculation program to obtain final results. Individual LIMS worklists should be pulled for filtration and analysis. Results are manually entered into the laboratory information management system (LIMS). The method lower detection limit should be calculated based on the sample volume and extract volume used, as well as the current lower absorbance detection limit.

12.2 Chlorophyll *a* Calculations:

If a computer program is not available, calculate chlorophyll *a* concentration as follows:

$$\text{Chlorophyll } a\text{, mg/L} = \frac{26.7 * (664 \text{ abs} - 665 \text{ abs}) * EV}{SV * L}$$

$$\text{Pheophytin } a\text{, mg/L} = \frac{26.7 * [1.7(665 \text{ abs}) - 664 \text{ abs}] * EV}{SV * L}$$

Where:

EV = volume of extract, L (0.020 typically)
SV = volume of whole water sample filtered, L (0.2 typically)
L = optical pathlength of cuvette, cm (5)

Note: Multiply by 1000 for results in µg/L.
750nm wavelength results can be subtracted out to correct for any clarity/turbidity issues.

When analyzing standards (ex. Turner Designs), where no filtration or maceration is required, use the following equations:

$$\text{Chlorophyll } a\text{, mg/L} = 26.7 * (664 \text{ abs} - 665 \text{ abs})/L$$

$$\text{Pheophytin } a\text{, mg/L} = 26.7 * [1.7 * ((665 \text{ abs}) - (664 \text{ abs}))]/L$$

12.3 QC Calculations:

Calculate % recovery (%R) for check standards using the following equation:

$$\%R = \frac{\text{Test Result}}{\text{True Result}} * 100$$

Where:

Test Result = the value obtained from the analysis
True Result = the actual value of the standard or LCS

Calculate the %RPD (precision and replication evaluation) for sample duplicates using the following equation.

$$\%RPD = \frac{[SR1 - SR2]}{\frac{1}{2} * (SR1 + SR2)} * 100$$

Where:
SR1 = sample result for replicate 1
SR2 = sample result for replicate 2

13 QUALITY CONTROL, ACCEPTANCE CRITERIA, AND CORRECTIVE ACTION

13.1 Method Blank:

13.1.1 Frequency: A method blank will be analyzed once per QC batch. The method blank consists of 200mL of laboratory pure water treated as a sample. This blank should be less than the calculated method lower detection limit for the analysis.

13.1.2 Acceptance Criteria: If the blank result is greater than the lower detection limit, notify management staff.

13.1.3 Corrective Action: Notify management staff if there is a problem with the laboratory pure water or GENESYS 6 spectrophotometer.

13.2 Matrix Duplicates:

13.2.1 Frequency: Matrix duplicates will be prepared and analyzed on a frequency of at least one per 10 or fewer samples in a filtration batch.

13.2.2 Acceptance Criteria: Refer to the QA book for chlorophyll *a* to obtain the latest control limits for matrix duplicate %RPD values. Limits are recalculated approximately every 40 data points. All duplicate %RPD values are to be recorded and plotted in this book.

13.2.3 Matrix/Standard Spike Recovery and Duplicate RPD Failure Corrective Action: If the duplicate %RPD does not meet the acceptance criteria, the system has to be evaluated for possible errors. All samples must be reanalyzed, or if there is insufficient sample for reanalysis or the sample holding time has expired, the samples must be reported as "No Result." In all cases, the out-of-control result should be recorded in the logbook on the QA officer's desk and the QA officer should be notified.

13.3. Turner Designs Check Standard:

13.3.1. Frequency**:** A Turner Designs check standard should be analyzed with every QC batch

13.3.2 Acceptance Criteria**:** Refer to the QA book for chlorophyll *a* to obtain the latest control limits for check standard %R. Limits are recalculated approximately every 40 data points. All %R data are to be recorded and plotted in this book.

13.3.3 Check Standard and LCS Recovery Failure Corrective Action: If the %R data does not meet the acceptance criteria, the system has to be evaluated for possible errors. In all cases, the out-of-control result should be recorded in the logbook on the QA officer's desk and the QA officer should be notified. Affected samples may be reanalyzed, or if there is insufficient sample for reanalysis or the sample holding time has expired, the samples may be reported as "No Result," or accepted by the QA officer based on other criteria.

14 REFERENCE SECTION

14.1 Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, Method 10200H "Chlorophyll"

14.2 EPA Method 446.0, Revision 1.2, September 1997, "In Vitro Determination of Chlorophylls a, b, $c_1 + c_2$ and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry."

15 METHOD PERFORMANCE

15.1 See the chlorophyll *a* QA book for details on recent method performance.

Sabine River Authority
Environmental Services Division

Chlorophyll a Standard Operating Procedure Development Special Project

Background

Results from the spectrophotometric analysis of chlorophyll *a* have historically produced variable results, often with less than satisfactory quality assurance; and published analysis methods leave many test parameters open to analyst interpretation. Consequentially, there was a need to study chlorophyll *a* methodology to determine possible areas for improvement and standardization. The purpose of this study was to determine to what extent collection and analytical technique variables affect the results of spectrophotometric analyses of chlorophyll *a* sampled from Texas surface waters.

At the request of the Texas Commission on Environmental Quality, the Sabine River Authority (SRA) conducted a series of chlorophyll *a* analyses using Method 10200-H from *Standard Methods for the Examination of Water and Wastewater, 19th Edition.* The study used the algae *Selenastrum capricornatum* cultured in-house, and the cell count was determined using a hemacytometer. Samples from the culture were analyzed for chlorophyll *a* to determine the typical absorbance range for that known cell concentration. The algae culture was then used to study the precision and accuracy of the acidification techniques, steeping duration, freezing duration, and filtering methods involved in chlorophyll a analysis.

Initial Quality Control

The linear dynamic range (LDR) and instrument detection limit (IDL) were determined for the Spectronic™ GENESYS 6™ UV-Vis Spectrophotometer[1]. An estimated detection limit (EDL) was also calculated using a set of natural phytoplankton samples.

Dilutions were made from standards of known chlorophyll *a* concentration provided by outside vendors. The dilution series was analyzed using the GENESYS 6™ equipped with a 5 cm pathlength cell and instrument absorbance response was recorded. These absorbance results were used to determine the slope and y-intercept of the calibration linear regression and to make capability determinations about the spectrophotometer. Thirteen natural water samples were collected, filtered, macerated, and analyzed according to Method 10200-H; these sample results were then used to calculate an EDL.

The results indicated that a lower detection limit of 0.01 mg/L and an upper analytical limit of 8 mg/L can be achieved for chlorophyll a analysis. A good working range was found to be 0.01 – 1 mg/l.

[1] http://www.thermo.com/com/cda/product/detail/1,1055,17245,00.html, accessed 4/24/05.

Study Procedures

For the procedures using the cultured algae, 100 mL replicate samples were obtained from the stock culture with a known cell concentration. Blank and sample replicates were filtered through Whatman® GF/F glass fiber filters in a darkened room. Samples were filtered for no more than ten minutes per filter and not filtered to dryness. After filtering, forceps were used to remove and fold the filter in half, twice longitudinally, to form a narrow packet. The folded filter was then placed in an opaque plastic 15 mL centrifuge tube. Maceration was performed using a mechanical glass tissue grinder at 400 rpm for two minutes. The filter residue was brought to a volume of 15 mL with magnesium carbonate/90% acetone solution and allowed to steep approximately one hour. Blanks and samples were then analyzed using a 2 nm bandwidth GENESYS 6™ spectrophotometer equipped with a 5 cm pathlength cell at 664, 665, and 750 nm wavelengths. The results from the absorbance readings were printed from the instrument and recorded in the bench book. Final results were calculated using Lorenzen's Pheopigment-Corrected Chlorophyll *a* and Pheophytin *a* equations found in Section 12.2 of EPA Method 446.0 for Chlorophyll *a*.

Acidification Technique

Acidification technique was evaluated using replicate 100 mL samples of cultured algae and filter blanks. The purpose was to determine in the acidification step if pipette mixing or hand mixing results in a significant difference in chlorophyll *a* results. A pipette[2] was used to add the acid to the sample in the cylindrical spectrophotometric cell. Mixing was accomplished by repeatedly withdrawing part of the solution and then slowly replacing it into the cell. The "hand-mixing" technique utilized a slow tilting of the cylindrical cell back and forth to mix the acid and sample after the addition of the acid.

The final results did not show a significant difference between the two methods of acidification, but the hand mixing technique was found to be less likely to introduce error by the addition of air to the sample cell or loss of sample.

[2] Result comparison from inter-laboratory analyses of split samples during the study revealed that delivering an exact volume of acid using a calibrated micro-pipette produced more consistent results than delivering the acid with a "eye-dropper" type pipette.

Steeping Duration

Replicate sets consisting of three 100 mL filter blanks and fifteen 100 mL samples of cultured algae were used to evaluate the effect of steeping duration on the chlorophyll *a* results. The steeping duration test results are shown in Table 1.

Table 1

	Steeping Duration		
Replicate	**2 hrs**	**18.5 hrs**	**23.5 hrs**
1	19.2	34.2	28.8
2	23.8	41.7	32.0
3	31.0	37.4	21.4
4	35.2	39.5	41.7
5	29.9	29.9	47.0
Average	***27.8***	***36.5***	***34.2***
Min	***19.2***	***29.9***	***28.8***
Max	***35.2***	***41.7***	***47.0***
StdDev	***7.6***	***3.0***	***12.9***

No relationship between steeping duration and chlorophyll *a* concentration was observed from the data.

Freezing Duration

The purpose of this analysis was to explore freezing time as a possible contributing factor to chlorophyll *a* result variability. Four 100 mL filter blanks and twenty 100 mL samples of cultured algae were filtered. Each sample filter set was prepared from the same algae culture dilution. The absorbance data and final chlorophyll *a* results obtained from the study are detailed in Table 2.

Table 2

	Freezing Duration			
Replicate	**0 Days**	**5 Days**	**14 Days**	**26 Days**
1	30.4	31.2	36.8	36.0
2	18.4	35.2	30.4	52.1
3	25.6	35.2	31.2	42.5
4	43.3	42.5	20.0	45.7
5	33.6	40.0	*No Result*	*No Result*
Average	***30.3***	***36.8***	***29.6***	***44.1***
Min	***30.4***	***31.2***	***20.0***	***36.0***
Max	***43.3***	***42.5***	***36.8***	***52.1***
StdDev	***2.3***	***6.2***	***11.9***	***6.9***

Filters were more difficult to macerate at this stage due to filter hardening. Problems were also noted with maceration involving the 0 day set, since filters were still very wet. The fewest problems with maceration were observed in the 5 to 14 days sets. No significant losses in chlorophyll *a* concentration over time were observed from the data.

Field vs. Laboratory Filtration

This portion of the study was to determine if a loss of chlorophyll *a* concentration occurs in samples that are transported for laboratory filtration (within 12 hours of collection). Samples of natural water were collected from two different sites and split into two potions for field and laboratory filtrations. The field portions were filtered into five aliquots of 420 mL using a calibrated syringe filtration apparatus. The five laboratory sample portions were divided into 500 mL opaque plastic bottles and stored on ice during transport. When the samples arrived at the laboratory, 420 mL samples were filtered from each bottle using the field syringe filtration apparatus. Results for each sample set are listed in Table 3:

Table 3

	Lab vs. Field Filtration			
Replicate	**Lab-Site 6A**	**Field-Site 6A**	**Lab-Site 6CN**	**Field-Site 6CN**
1	3.1	2.8	6.1	9.9
2	4.1	5.1	6.6	6.1
3	3.3	3.3	8.6	6.4
4	5.1	4.8	4.8	4.3
5	4.3	4.8	*9.2*	*6.1*
Average	***4.0***	***4.2***	***6.5***	***6.7***
Min	***3.1***	***2.8***	***4.8***	***4.3***
Max	***5.1***	***5.1***	***8.6***	***9.9***
StdDev	***0.8***	***1.4***	***0.9***	***4.0***

No significant chlorophyll *a* loses were observed when field sample were preserved and transported for same-day laboratory filtration; therefore, field filtration offers no quantifiable analytical advantage over laboratory filtration.

Conclusions

The study demonstrated that hand mixing after acidification was sufficient and avoided the addition of air to the sample or the loss of sample, particularly when using a 5 cm pathlength cylindrical cell.

Although each of the freezing duration times studied produced suitable results, a freezing interval of one to fourteen days was found to be optimal for proper filter maceration. Chlorophyll *a* results are highly dependant on adequate maceration of filters. Wet and overly hardened filters can create maceration problems, which affect final sample turbidity and result variability. This is especially true when using glass fiber filters.
Increased extract clarity can also be achieved by varying spectrophotometric cell size. It was also determined that a higher dilution volume prior to steeping improved sample clarity. The filter slurry was increased to 20 mL volume instead of the original 15 mL.

It was found that the use of glass fiber filters for chlorophyll *a* sample filtration improved maceration by assisting in the breaking of algae cell walls. Glass fiber filters are specified in EPA Method 446.0; however, glass fiber and membrane filters are both acceptable in Method 10200H.

No significant difference in chlorophyll a concentration was determined between ambient water samples filtered in the field or filtered in the laboratory.

Although not part of the original study plan, it was discovered that the time between acidification and obtaining final absorbance readings should be standardized. EPA Method 446 specifies a 90-second interval between acidification and final spectrophotometric analysis, but the time interval is not specified in Method 10200H. Test results show optimum results when the final absorbance readings were taken 90 seconds after acidification.