Procedure No. P-13-65
Rev. 0 (11/02)

PROCEDURE FOR SEMI-QUANTITATIVE ANALYSIS OF
SOFT ALGAE AND DIATOMS

Prepared by: <u>Karin Ponader and Diane Winter</u>

Approved by: _____   _____

Robin S. Davis   Date

Quality Assurance Manager

Procedures for Semi-quantitative Analysis of Soft Algae and Diatoms

1. PURPOSE:

   1.1.   This protocol describes a semi-quantitative procedure for analyzing the soft algal component
          of samples to estimate the relative proportions of soft algae and diatoms. As part of a
          multidisciplinary approach to stream and river research, soft algae samples are collected
          individually, or split out of diatom samples, to be analyzed for species content and
          biovolume. These samples usually represent a composite of algal samples collected from
          natural substrates (e.g., Charles et al., 2000). Samples could be collected from either
          individual substrate types (e.g., rocks) or from the majority of micro-habitats in a defined
          sampling zone, reach or station.

2. SCOPE:

   2.1.   This is a semi-quantitative procedure designed to estimate the percentage (or proportion)
          that each genus/species contributes to the total number of algal cells and the algal biovolume
          of the sample. This is a two-step method. The first step (Section 6.3) involves identifying the
          most common genera/species and estimating the relative percentage of each of these in the
          algal assemblage. In the second step (Section 6.4), the relative percentage that each
          genus/species contributes to the algal biovolume in the sample is estimated. As this is a
          semi-quantitative method, cells are not counted or measured, but a general estimate is
          arrived at which describes the relative proportions of the common genera/species observed
          in the sample through examination of several transects.

   2.2.   This procedure is applicable for analysis of soft algae in a wide variety of samples where the
          objective is a semi-quantitative estimate of the relative proportions of algal genera/species
          contributed by the most common taxa. It is not designed to provide a complete list of taxa
          present or precise algal abundance or biovolume estimates.

   2.3.   This procedure applies to personnel responsible for taxonomic analysis of soft–algae
          samples.

3. REFERENCES:

   3.1.   Bahls, L.L., 1993. Periphyton bioassessment methods for Montana streams. Montana
          Water Quality Bureau, Department of Health and Environmental Science, Helena, MT.

   3.2.   Barbour, M.T., Gerritsen, J., Snyder, B.D. and Stribling, J.B., 1999. Rapid bioassessment
          protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates

and fish, Second Edition. EPA 841-B-99-002, Washington, D.C., U.S. Environmental
Protection Agency, Office of Water.

3.3.   Firth, C. 2002. Counting procedures for algae analysis and measuring of cell biovolumes for
calculating biomass percentages. PCER Procedure P-XX-XX. Patrick Center for
Environmental Research, Academy of Natural Sciences, Philadelphia, PA.

3.4.   Charles, D., Winter, D., Hoffman, M. 2000. Field sampling procedures for the New Jersey
Algae Indicators project. PCER Procedure P-13-64. Patrick Center for Environmental
Research, Academy of Natural Sciences, Philadelphia, PA.

3.5.   Sprouffske, K., 2002. Subcontractor Soft Algae Database Guide. PCER Procedure P-
XX-XX. Patrick Center for Environmental Research, Academy of Natural Sciences,
Philadelphia, PA.

4.   DEFINITIONS:

4.1.   Biomass: the amount of algal biogenic material in a sample by weight.

4.2.   Biovolume: the amount of algal biogenic material in a sample by volume.

4.3.   Soft Algae: the non-diatom component of the algae sample.

5.   APPARATUS/EQUIPMENT:

5.1.   Research quality microscope with 10X and 40X objective.

5.2.   Microscope slides, 75 x 25 mm.

5.3.   Glass microscope cover slips, rectangular, 22 x 50 mm, #1 thickness.

5.4.   Plastic pipettes, 5.25 inch.

5.5.   Pre-printed bench sheets (attached).

5.6.   Distilled water (algae-free water of any sort).

5.7.   Computer with Microsoft Access software installed, access to a copy of the Contracted
Soft Algae Database created to allow entry of data to larger database system (Sprouffske,
2002), and internet connection for data transferal (if working away from the PCER). The

database can be found either in G:\Phycdata\DATABASE\Contracted Soft Algae DB, if at PCER, or will be sent electronically to analyst.

6. METHODS:

6.1.   Print out bench sheets, see attachment 9.1.
6.2.   Prepare the slide for analysis.
    6.2.1.   Shake each sample to dislodge epiphytic algae and randomize algal cells and colonies. Use scissors or other sharp implements (razor blade, etc.) to cut up any long filamentous algae present in the sample. Immediately after mixing, use a plastic pipette to deliver a portion (2 or 3 drops) of the sample onto a waiting slide. The exact amount of sample analyzed is not as important as is the need to represent the entire algal community. If there are large clumps of filamentous algae, snip off the tip of the pipette to enable their inclusion in the subsample. These large clumps should then be evenly dispersed over the area of the coverslip.
    6.2.2.   Place edge of coverslip at an angle of 45° to the slide surface and next to the sample suspension. Carefully allow the coverslip to fall gently onto the sample suspension.
    6.2.3.   To avoid extensive exposure to formalin fumes, if not working in a hood, have a small fan running during the preparation of the samples.
    6.2.4.   To make sure the density of algal cells in the sample is uniform between the samples, examine the slide at 10X. If too dense, dilute the sample with distilled water, and repeat from step 6.2.1. If too dilute, make sure to report this in the notes of the bench sheet and database.

6.3.   Estimate the relative abundance of cell numbers for each genus/species.
    6.3.1.   Scan the entire slide at 10X to estimate the abundance rating based on the relative number of cells for each of the larger soft algal genera/species (see 4.3) in the entire sample. Use the abundance scale below (Table 1) to report relative abundance estimates. Use the abundance rating definitions as a guideline only, if the sample is very sparse, the analyst should use his/her best judgement in assigning abundance rating categories.

Table 1: Abundance rating scale for Section 6.3.

Average Entered in Computer	Estimated % of Cell Observed	Abundance Rating
0.5	0-1	R – (rare) – Only one or two cells observed during entire scan
3	1-5	F – (frequent) - More than one cell is observed, but they appear only sporadically

12.5	5-20	C - (common) - Individual cells appear in several fields of view
30	20-40	A – (abundant) - One or two cells appear in most fields of view
55	40-70	VA – (very abundant) - Multiple cells appear in most fields of view
85	70-100	D – (dominant) - Cells greatly exceed those of other algae in numbers

6.3.2. Switch to 40X to estimate the abundance rating for the smaller algal species, including diatoms. The abundance of all diatoms is to be estimated as a single group. Randomly examine 8 - 10 fields of view, or more if necessary, to obtain a representative estimate of the percentage of the most common genera/species in the sample. Try to take only 30 minutes for both the 10X and 40X examination, not including time spent on species identification.

6.3.3. If identification to a species level will be a lengthy process, do not spend excessive time on species identification if the genus/species abundance is rare. In this case, identify only to the genus level (e.g., *Scenedesmus* spp.).

6.3.4. Record Abundance Rating abbreviation (e.g., "C") in the appropriate column for each species on the data sheet while counting (see data sheet). The average percentage assigned to each abundance rating is what will be entered in the computer database when the examination is complete (e.g., 12.5 for an abundance rating of "C").

6.4. Estimate the percentages of biovolumes of each genus/species.

6.4.1. After abundance ratings are determined, assign biovolume percentages to each genus/species observed. Re-examine the slide to aid in the estimation of the relative proportions of the algae in the slide. Do not assign percentages to species that contribute less than 10% to the total algal biovolume. Group all diatoms together in a single category.

6.4.2. Estimate percentages only of diatoms which contain chloroplast material. Switch to 40X if necessary to make this distinction.

6.4.3. There is no fixed scale for the biovolume percentage estimates, percentages should be assigned based on individual analyst opinion. Do not estimate percentage increments smaller than 5% (e.g., 10, 15, 20, etc.).

6.4.4. This estimation of biovolume should not exceed 10 minutes.

6.4.5. The main objective of this analysis is to record the most common species in the sample. Always list the proportion of diatoms in the assemblage, but do not record the genus/species of other algae which occur as less than 10% of the community.

6.4.6. Record any additional notes on the datasheet, and in the "notes" column in the Contracted Soft Algae database ("Soft algae processing data" Table). These may include the condition of the sample, the condition of the algae, the presence of macrophytes or moss, and other information deemed important. If there are

genera/species which occur in <10% of the sample, but might be useful in interpretation of the data, they should be listed in the notes, as well.

7. QUALITY ASSURANCE AND QUALITY CONTROL:

   7.1. The results of these analyses will be compared with results of analyses from samples collected in different areas. It is important that the level of effort be similar. With the exception of time spent learning new floras, this analysis should be finished within 30-45 minutes and no more than 1 hour. Samples with >90% diatoms can be analyzed very quickly. On average, ideally, 10-15 samples should be analyzed per day (7 hrs). If these time limits are being exceeded consistently, it must be reported to the Project Manager and noted on the bench sheets as a protocol deviation.

   7.2. Minor protocol deviations should be noted on the bench sheets and reported to Project Manger. Any major deviation should be reported to the Project Manager prior to proceeding.

   7.3. Minor protocol modifications should be reported to the Project Manager and noted on the Phycology Section's master copy of the protocol. Numerous and major modifications will require a protocol revision and approval by the Patrick Center Quality Assurance Officer.

   7.4. Completed electronic database will be e-mailed and paper copy of bench sheets will be sent to PCER upon completion of project samples.

8. DATA ENTRY:
   Refer to "Subcontractor Soft Algae Database Guide" (Sprouffske, 2002), which can be found at G:\Phycdata\DATABASE\Contracted Soft Algae DB\Subcontractor Instructions.doc.

9. ATTACHMENTS:

   9.1. Semi-Quantitative Analysis of Soft Algae and Diatoms Bench sheet (following page).
      9.1.1. This method takes into account only the two dimensional area taken up by the algal specimens. Due to time restrictions of this method, the third dimension cannot be taken into account. The authors are aware that the term volume is not appropriate in this case. This method is designed to provide only a rough estimate of biovolume.

Semi-Quantitative Analysis of Soft-Algae and Diatoms					
The Academy of Natural Sciences, PCER, Phycology Section					
Project:					
Sample ID:					
Analysis Date:					
Analyst:					
Taxon Name:	Estimated Cell #	Bio vol.	Taxon Name:	Estimated Cell #	Bio vol.

			Bacilleriophyta (Diatoms)		
Notes:					