STANDARD OPERATING PROCEDURE FOR COLLECTION AND FILTERING WATER COLUMN AND BENTHIC CHLOROPHYLL-A SAMPLES

WATER QUALITY

State of Utah
Department of Environmental Quality
Division of Water Quality

Revision 2.1
Effective May 2020

Foreword

Utah Division of Water Quality (DWQ) Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical experts. This document is intended primarily for internal DWQ use. This SOP should not replace any official published methods.

Any reference within this document to specific equipment, manufacturers, or supplies is only for descriptive purposes and does not constitute an endorsement of a particular product or service by DWQ. Additionally, any distribution of this SOP does not constitute an endorsement of a particular procedure or method.

Although DWQ will follow this SOP in most instances, there may be instances in which DWQ will use an alternative methodology, procedure, or process.

The methodology detailed below is the protocol followed by DWQ's monitoring staff and verified by DWQ's Quality Assurance officer.

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06/02/2020

Date

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Toby Hooker (Jun 2, 2020 10:53 MDT)

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Revision Page

Date	Revision #	Summary of Changes	Sections	Other Comments
5/21/2013	1	Draft SOP was updated to include new laboratory methods (corrected for pheophytin) and sample labels.	8 & 10	New SOP. Began document control/revision tracking.
2/5/2020	2.0	Added benthic Chl-a collection and analysis methods	All	Added benthic Chl-a procedure. Modeled after the UCASE procedure.
3/10/2020	2.1	Updated language, grammar, and structure	All	Clarified and revised sentence structure and grammar throughout the entire document.

Table of Contents

1.0 SCOPE AND APPLICABILITY

This document presents the Utah Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for field collection and processing of water column and benthic samples collected from Utah's wetlands, lakes, and rivers/streams.

Water sample collection should be performed in accordance with specific program SOPs, field manuals, or a specific project sampling and analysis plan (SAP). This SOP applies to all personnel collecting chlorophyll-*a* samples including DWQ monitors, DWQ cooperators, and volunteer monitors.

Chlorophyll-*a* is a green photosynthetic pigment and concentrations of chlorophyll-*a* are used to infer algal biomass. The data from chlorophyll-*a* sampling are used by DWQ to monitor aquatic health in the following ways:

- In lakes and reservoirs, chlorophyll-a concentrations are one component used to calculate the Carlson Trophic State Index. This is a measure of the degree of eutrophication in a lake/reservoir.
- In rivers and streams, Chlorophyll-*a* concentrations are used to support nutrient criteria development.
- In wetlands, Chlorophyll-*a* is included in a suite of water chemistry parameters evaluated as a component of a Multimetric Index (MMI) used to assess wetland condition.

2.0 SUMMARY OF METHODS

2.1 Water Column

After collecting the water sample in an amber container, a filter apparatus consisting of a hand pump, plastic tubing, a filter flask, and filter funnel with a filter stage is used to filter the water sample (Figure 1). An unused glass-fiber filter is placed on the filter stage. Sample water is poured into the filter funnel and then pumped through the filter, at a vacuum pressure not to exceed 7 psi (14 inHg). Filter as much water volume as possible to a maximum volume of 2000 mL. Generally the filtering volumes fall within the range of 500-2000 mL. Note that turbid water may clog the filter, resulting in a lower filtration volume, and this would reduce the amount of chlorophyll in the sample. The filter (containing the residue to be analyzed) is removed from the filter stage and wrapped in foil and placed into a labeled bag. The total volume filtered is recorded and the sample is frozen by placing the sample on dry ice in the field before it is transferred to the freezer. Alternatively, the sample can be stored on wet ice before placing it in a freezer, so long as this is completed by the end of the sampling day. Samples must remain frozen until delivered to the laboratory.

2.2 Benthic

The benthic chlorophyll-a sample (periphyton) is an 11-point composite sample with individual aliquots of sample collected from each of the 11 transects within the sampling area ("A" through "K"). Each aliquot of sample is collected from either the soft bottom sediments or scrubbed from

stream bed substrates (rocks, woody debris, other debris). Following the completion of site sampling activities a benthic-chlorophyll-*a* sample and an ash free dry mass sample (AFDM), also known as dry weight, are prepared by filtering the composite sample.

For some special projects the number of composite samples and the individual aliquots sampled may differ. Refer to the program specific SAP for more details.

3.0 DEFINITIONS AND ACRONYMS

DI: deionized water

inHg: inches of Mercury

mL: milliliter(s)

mm: millimeter(s)

psi: pounds per square inch

residue: the part of the sample remaining as a solid on the filter paper after the liquid passes through the filter

RPM: revolutions per minute

thalweg: the deepest and fastest part of the channel (most often), containing the most cross-sectional flow.

triple rinse: fill a sample container ½ full with native or DI water, agitate, and empty three times. Triple-rinsing is an EPA verified procedure proven to remove and/or dilute contamination

µm: micrometer(s), also called microns

4.0 HEALTH AND SAFETY WARNINGS

Hazardous conditions potentially exist at every waterbody. If unfavorable conditions are present at the time of sampling, it is recommended that the sampling be rescheduled. If hazardous conditions arise during sampling, such as lightning, high winds, rising water, or flash flood warning, personnel should cease sampling and move to a safe location.

When working in Utah and other warm climates, take steps to avoid heat induced illnesses such as heat stroke or heat exhaustion.

Use caution when working in waders as drowning hazards exist.

Take appropriate precautions when operating equipment and working on, in, or around water, as well as possibly steep and unconsolidated banks, bridges, or edges of ponds/lagoons. All field crews should follow DWQ health and safety procedures and be equipped with safety equipment such as proper wading gear, personal flotation devices (PFDs), gloves, first aid kits, cellular phone, etc.

Use caution when sampling from a bridge or boat and take appropriate actions to make the situation as safe as possible; suspend the sampling if conditions are unsafe.

5.0 CAUTIONS

No additional cautions for this procedure.

6.0 INTERFERENCES

Sample contamination from poorly cleaned equipment must be prevented. A dirty filter apparatus can lead to sample contamination. Prior to filtering at each site, any equipment coming into contact with sample water needs to be triple-rinsed to remove any contamination. If noticeable buildup is observed on the stage or funnel (*see Figure 3*) they should be soaked in soapy water (liquinox) and then scrubbed until buildup is removed.

Chlorophyll-*a* pigment is broken down by exposure to sunlight (photodecomposition). Therefore, the filtering procedure should be carried out in the lowest lighting conditions possible at the time samples are filtered, and sample containers should be amber.

Prepared samples must be stored in a cooler with dry ice or placed in a freezer immediately to prevent sample degradation. Samples can be stored on wet ice before placing them in a freezer, so long as this is completed by the end of the sampling day. Samples must remain frozen until delivered to the laboratory.

Do not touch the inside of the bottles, filtering apparatus, or graduated cylinder. It is critical to avoid sample contamination.

When the sampler is scrubbing a substrate for the benthic collection method, care must be taken to scrub the top surface of the substrate as it was in the stream. The diatoms that are being targeted are on the sun exposed (top) side of the substrate.

When using a hand pump be careful to not exceed 7 psi (14 inches of Hg) when applying vacuum pressure. High pressures can rupture algal cell walls allowing the chlorophyll-*a* pigment to pass through the filter and not be included in the analyzed sample, leading to underestimated concentrations or false negative results.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

DWQ personnel performing water sampling must be familiar with sampling techniques, safety procedures, proper handling, and record keeping. Samplers are responsible for attending refresher meetings held each spring/summer to review procedures and techniques. New staff will be trained in the field and lab by DWQ personnel.

Cooperators are required to read this SOP annually and acknowledge they have done so via a signature page (see **Appendix 1**) that will be kept on-file at DWQ along with the official hard copy of this SOP.

8.0 EQUIPMENT AND SUPPLIES

- ❑ Copy of this SOP
- ❑ Field notebook, lab sheet
- ❑ Pens, pencils
- ❑ Sample labels (**Figure 1**)
- ❑ Sample containers for filters (aluminum foil and ziptop bag is preferable, another clean opaque container is an acceptable alternative)
- ❑ Polypropylene graduated cylinder, 500 mL
- ❑ Glass fiber pre-filters (Grade GF/F, 47 mm diameter, 0.7 µm pore size)
- ❑ Plastic disposable filter forceps, length: 4 ½" (2 pair aids in filter folding)
- ❑ DI water in squeeze spray bottle
- ❑ Cooler and dry ice (if unable to put samples in freezer immediately after processing)
- ❑ Hand pump (25" Hg, 15cc pumping rate/stroke) and Tygon or similar tubing (approximately 24"), diameter needs to fit filter flask arm, **OR**
- ❑ Nalgene or similar polypropylene filter flask, 500 mL
- ❑ Nalgene analytical test filter funnels
- ❑ #7 filter flask stoppers with ½ -inch hole
- ❑ Spray/squeeze bottle
- ❑ Cooler with wet ice
- ❑ Stiff bristle brush (toothbrush, shortened paint brush, etc)
- ❑ 60 mL syringe with tip cut off
- ❑ Circular area delimeter
- ❑ Funnel
- ❑ 500 mL sample bottle

9.0 PROCEDURE

9.1 Water Column Chlorophyll

9.1.1 Sample Collection

At least 2000 mL of water should be collected in an amber container. The amber container must be triple-rinsed away from the sampling site and prior to sample collection. If sampling a stream, wade into the thalweg (generally the deepest, swiftest area of water flow) and fill the container avoiding the surface water. If sampling a lake or non-flowing body of water, fill the container and avoid surface scum and stirred up bottom sediments. See DWQ's *SOP for Collection of Water Chemistry Samples* for more information.

9.1.2 Sample Processing

This procedure can be carried out using a Hand Pump outfitted with a vacuum pressure gauge. Regardless of what pumping method is used, the same filter flask and filter holder can be used, and operating pressures must not exceed 7 psi (14 inHg). *Figure 3* provides a photograph of the entire filter apparatus assembly attached to a hand-pump.

Start each sampling trip with equipment that has been cleaned with soap (liquinox) and water (if needed), triple rinsed with DI water, and allowed to dry.

1) Attach tubing onto filter flask arm and pump.

2) Insert the funnel adapter into the #7 stopper; wetting the adapter may aid in this.

3) Insert the filter funnel assembly into the top of the filter flask until it seals tightly. See *Figure 3*.

4) Remove the top of the filter funnel from the filter stage.

5) Using the forceps, place an unused glass fiber filter on the filter stage.

6) Triple rinse graduated cylinder with DI water

7) Homogenize the sample, and pour it into a graduated cylinder in increments of up to 250 mL (smaller volume may be used if sample is turbid). It is important to not overfill. If this happens and the filter becomes clogged, the procedure will have to be re-done from the start.

8) Pour aliquots of sample into the filter funnel and use the pump to pull the sample through the filter.

9) Continue filtering measured volumes of sample, being careful not to exceed 7 psi (14 inches of Hg) of vacuum pressure during filtration. Stop when up to 2,000 mL of sample water has been filtered, or the filtration rate has slowed and sufficient sample has been filtered. Don't quit filtering too early! *This step is subjective, but very important.*

10) If the filter is clogged, the procedure *has to be restarted* in order to filter all water that enters the funnel.

NOTE: *If the sample is turbid, only filter small volumes of sample at a time. Try to estimate how much turbid water you can put through a filter by paying attention to how the pressure increases and the flow-rate decreases as you pull the water through the filter. If you overestimate the volume of water that can pass through the filter and water is left remaining on top of a clogged filter, filtering* ***must be repeated*** *with another volume of sample and a new filter.*

11) Following the last aliquot of sample water use DI water to rinse the graduated cylinder used to pour an aliquot of sample into the filter flask. Pour this rinse through the filter flask to capture any material left behind in the cylinder.

12) Rinse down the sides of the filter funnel with DI water and use the pump to pull the rinse water through the filter. You can stop filtering when all the water has passed through the filter. Remove the filter funnel being careful not to disturb the filter stage or filter.

13) Using forceps, remove the filter from the filter stage, being careful not to rip the filter or scrape off any of the sample.

14) Using forceps, fold the filter in half with the residue facing the inside.

15) Place the folded filter onto a piece of aluminum foil and fold the foil to make a package for the filter.

16) If using foil, place the foil-wrapped sample into a small ziptop bag.

17) Fill out the sample label and adhere it to the inside of the ziptop bag containing the sample. Place the sample into a larger ziptop bag and store the sample immediately on dry ice, wet ice, or place in the freezer.

18) Deliver the sample(s) to the laboratory as soon as possible. Samples must be analyzed within 3 weeks (21 days) of collection.

9.2 Benthic Chlorophyll

The procedure below details the process for collecting the benthic chlorophyll-a sample at 11 stream transects. It involves collecting 11 subsamples to create one ~500 mL composite sample that chlorophyll is then filtered from. In addition to the basic procedure it is important that samplers keep the following in mind:

- If other sampling work is being performed at the site (physical habitat, benthic macroinvertebrates, etc) remember to collect the sample slightly above or below the transect line to avoid substrates which have already been disturbed.
- When selecting samples at each transect be mindful to distribute your sample collections across the width of the stream. The easiest way to do this is to determine a rotating method at transect A starting at either the left or right bank or center and alternate left, right, center as you move to each transect.

9.2.1 Sample Collection

1. Starting with Transect "A" (most downstream location), collect a single sample using the procedure below.

    a) Identify a suitable substrate (rock, wood, etc.) that is big enough (>15 cm diameter) and can be easily removed from the stream and is located at a depth of less than 0.5 meters. Place the substrate in a plastic funnel which drains into a 500 mL sample container with volume graduations marked on it.

    b) Place the area delimiter on the upper surface of the substrate to define a 12-cm^2 area. Dislodge attached periphyton from the substrate within the delimiter into the funnel by brushing with a stiff bristled brush. Take care to ensure that the upper surface of the substrate is the surface that is being scrubbed and that the entire surface within the delimeter is scrubbed.

    c) Rinse the dislodged sample from the substrate, funnel, and brush with a spray bottle filled with DI. While rinsing the sample into the 500 mL container, pay close attention to the 45 mL markings on the container. Each sample collected needs to be as close to 45 mL as possible so the aliquots are equal.

    d) Put the sample container in a small cooler with ice while you travel between transects and collect the subsequent samples (the sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite).

    e) If no coarse sediment (cobbles or larger) or suitable substrate are present:
        - Use the area delimiter to define a 12-cm^2 area of soft sediments.
        - Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60 mL syringe. Fill the syringe with 45 mL of sample.
        - Empty the syringe into the same 500 mL container as above.

2. Repeat step 1 for Transects "B" through "K" (moving upstream to obtain all 11 subsamples). Place the subsample collected at each transect into the single 500 mL container to produce the composite sample with a maximum total volume of 495 mL.

9.2.2 Sample Processing

Following the completion of sampling activities keep the sample container in a cooler on ice and limit the exposure to sunlight. To process the sample for chlorophyll-a and AFDM follow the procedure above in **Section 9.1** with the following exceptions:

- Limit the sample volume for both the chlorophyll-a and AFDM samples to 25 mL.
- Place each sample into its own foil wrapper and labeled bag.

9.3 Equipment Cleaning

To clean sampling and processing equipment for the water column chlorophyll-*a* samples do the following: triple-rinse the graduated cylinder, filter funnel, filter flask, and forceps with DI water and shake dry as best as possible. If time allows, let the equipment air dry.

To clean the sampling and processing equipment for the benthic-chlorophyll-*a* samples do the following: triple rinse the 500 mL sample container, brush, funnel, and syringe. If fine sediment or sample residue remains on the equipment perform a liquinox wash and lightly scrub clean. Following cleaning, allow for the equipment to air dry.

9.4 Laboratory Analysis

A variety of analytical methods are available for determination of chlorophyll-a concentrations. Most commonly, filters are extracted and analyzed for chlorophyll-*a* using Standard Method 10200 H (SM 10200H; homogenization followed by spectrophotometry). In addition to SM 10200H, the Environmental Chemistry laboratory at Utah Public Health Laboratory (UPHL), also offers a higher-sensitivity method based on chromatographic separation of plant pigments; this method is referred to as "HPLC" on UPHL lab sheets. Similar methods available include modifications of SM 10200H using a fluorescence detector, or EPA methods 445 and 446. Methodology and quality assurance and quality control procedures for these analyses are on-hand at DWQ, but can also be obtained from:

Unified State Laboratories: Public Health, Utah Department of Health
4431 South 2700 West
Taylorsville, UT 84119
(801) 965-2400
UPHL@utah.gov

10.0 DATA AND RECORDS MANAGEMENT

The volume of sample water filtered must be recorded in two places: on the lab sheet and in the field notes. In addition, the field method ("WATER" for water column samples; "BENTHIC" for benthic samples) *must be circled on the sample label* (**Figure 1**). Once the laboratory has received the samples, the lab sheets are photocopied and copies sent back to DWQ. In addition, the volume of water filtered should be recorded in the electronic field data file for the sampling trip.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

Field quality control samples for chlorophyll-*a* sampling include blanks and replicates.

For replicates, perform the entire sampling procedure again with a unique DWQ site ID.

For blanks, clean the filtering apparatus as described in **Section 9.0**. Filter 500 mL of DI water through the filter apparatus and process as a regular sample. At a minimum one blank should be performed per sampling trip or per every 10 samples to check for cross-contamination between samples. If your program or project-specific quality assurance plan or SAP requires a different frequency for collecting QA/QC samples, follow that program plan or SAP.

12.0 REFERENCE MATERIALS

Hambrook Berkman, J.A., and Canova, M.G., 2007, Algal biomass indicators (ver. 1.0): U.S. Geological Survey Techniques of Water-Resources investigations, book 9, chap. A7, section 7.4, August, accessed__*date*__ from http://pubs.water.usgs.gov/twri9A/.

Lazorchak, James M., Donald J. Klemm, and David V. Peck. 1998. Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods For Measuring the Ecological Condition Of Wadeable Streams. Washington D.C.: U.S. Environmental Protection Agency.

Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2017, Method 10200H "Chlorophyll".

USEPA. 2010. Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes. EPA 905-R-05-001. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, Illinois. (http://www.epa.gov/glnpo/monitoring/sop/index.html)

USEPA. 2017. National Rivers and Streams Assessment 2018/19: Field Operations Manual –

Wadeable. EPA-841-B-17-003a. U.S. Environmental Protection Agency, Office of Water

Washington, DC.

Related DWQ SOPs:

Utah Comprehensive Assessment of Stream Ecosystems (UCASE) Field Operations Manual

Standard Operating Procedure for Collection of Lake Water Samples

Standard Operating Procedure for Collection of Water Chemistry Samples

13.0 FIGURES

Figure 1: **Chlorophyll-*a* sample label.**

U:\WQ\PERMITS\MONITORS\Labels

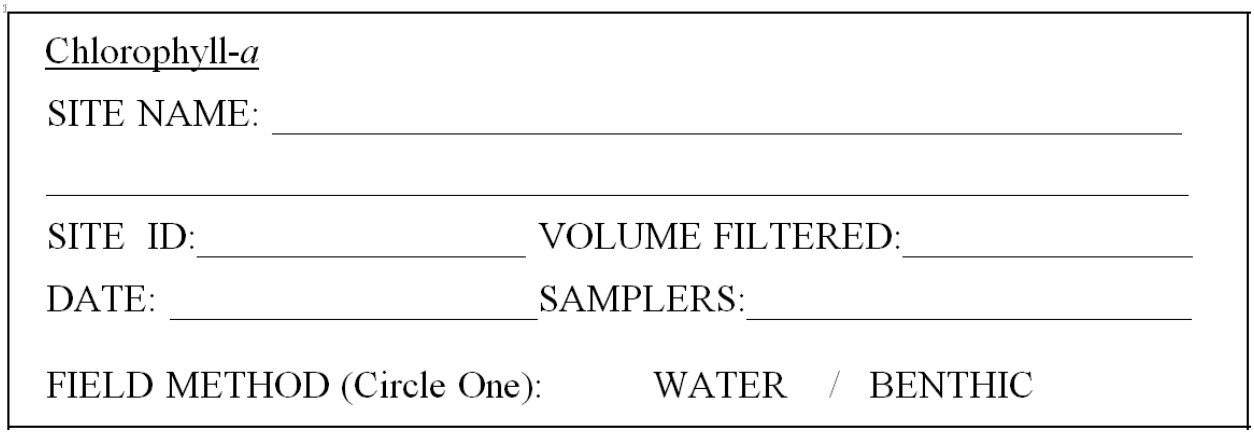


Figure 2: **Ash-Free Dry Mass Sample Label**

U:\WQ\PERMITS\MONITORS\Labels

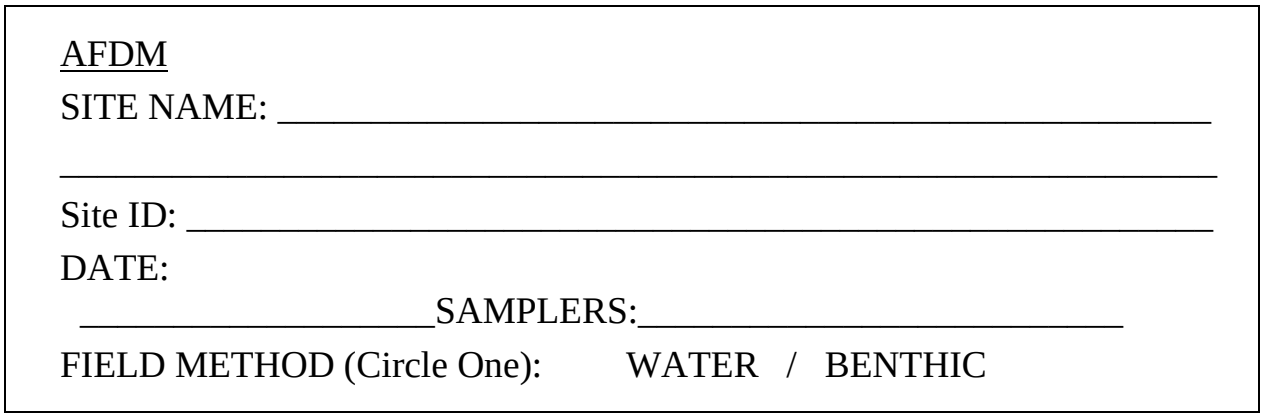
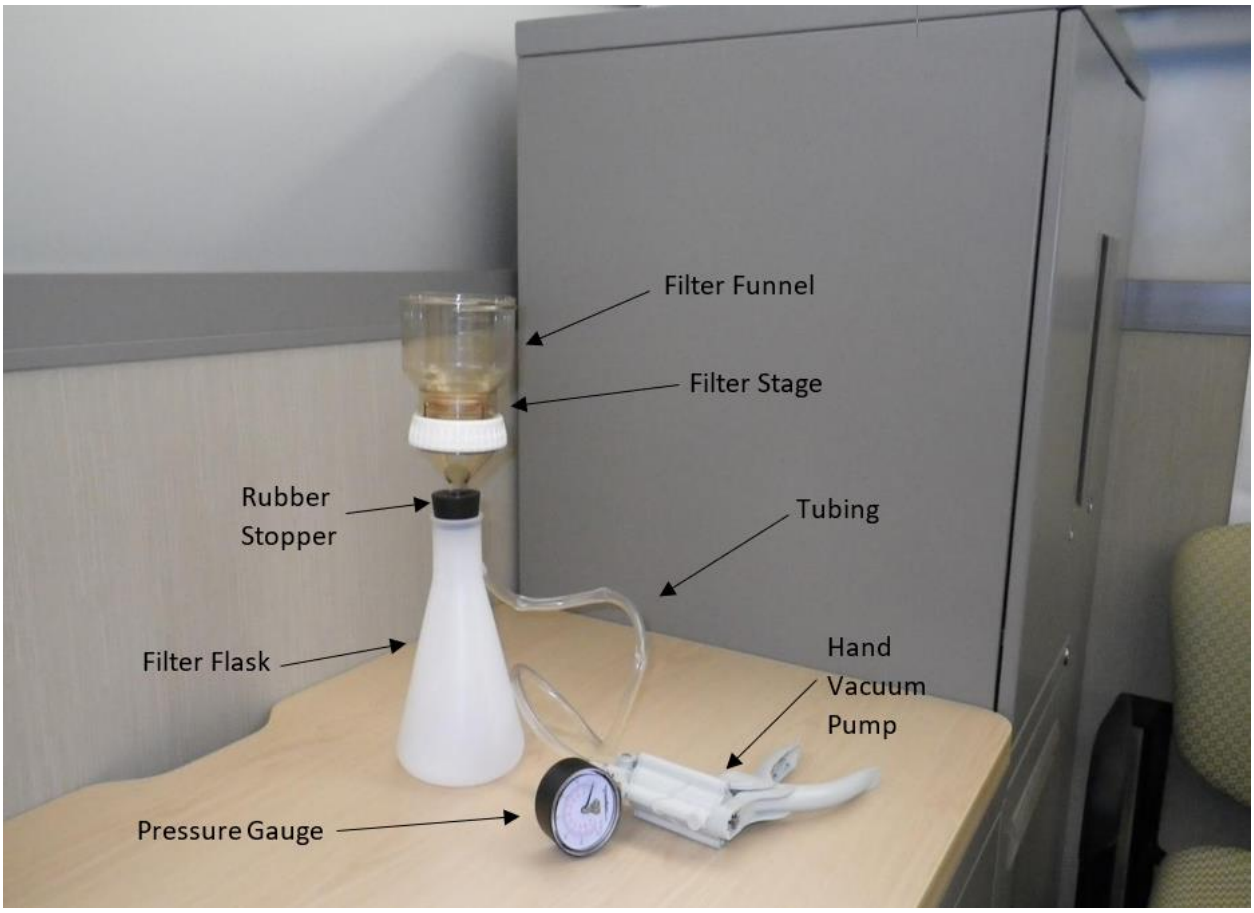


***Figure 3*: Filter apparatus with a hand pump.**



14.0 APPENDIX

APPENDIX 1 - SOP ACKNOWLEDGMENT AND TRAINING FORM

SOP Acknowledgement Form

This SOP must be read and acknowledged annually, and this form must be kept on file at DWQ.

Document Title:	
Document Revision Number:	
Document Revision Date:	

Please sign below in accordance with the following statement:

"I have read and understood the above referenced document. I agree to perform the procedures described in this SOP in accordance with the document until such time that it is superseded by a more recent approved revision."

Date	Printed Name	Signature

SOP Training Form

Training is required for all new samplers and Refreshers are required annually.

Trainee: Sign below to acknowledge that training on this SOP was received, understood, and all questions/concerns were addressed by the trainer.

Trainer: Sign below to acknowledge that training on this SOP was completed for the individual listed and that trainee is competent to perform the procedures described within.

Date	Trainee Printed Name	Trainee Signature	Trainer Printed Name	Trainer Signature
Management Approval				
Printed Name:				
Signature:				
Date:				