Appendix B

Excerpts from Central Michigan University
proposal dated 12/15/05

Quality Assurance / Quality Control Manual

The Michigan Water Research Center
Water Quality Laboratory

Quality Assurance/ Quality Control Manual

Prepared by the Staff of the Michigan Water Research Center
Central Michigan University

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Table of Contents

Introduction
Purpose and Scope
Organization and Responsibility
Training
Safety
Parameters Monitored and Associated Sampling/Analytical procedures
    Physical
    Chemical
Instrument Calibration Procedures
Chain of Custody
Quality Control Checks
Data Quality Objectives/Requirements
Preventative Maintenance
References
Appendixes
    Water analyses ID Sheet
    Chain of Custody Sheet
    Training Documentation Sheet

THE MICHIGAN WATER RESEARCH CENTER
Central Michigan University

Quality Control/ Quality Assurance
Water Quality Laboratory

Introduction

Quality-assurance and quality-control (QA/QC) practices for the operation of MWRC are described in this manual. The Laboratory Director, Laboratory Supervisor, and laboratory and field staff are responsible for implementing QA/QC procedures. This includes correctly following methods of analysis, media and reagent preparation and storage, and analytical quality-control procedures. A sample management and documentation system involves the use of service request forms and login ID's for each sample. A laboratory information management system has been implemented to store sample login information and results. Laboratory equipment maintenance and calibration records are also maintained.

Purpose and Scope

The purpose of this manual is to identify and document practices and standard operating procedures for those activities of the MWRC that affect quality of data. The manual provides MWRC personnel and customers with general descriptions of quality practices and goals to aid in the interpretation of data. This manual is intended to be an unpublished document that will be frequently updated as laboratory activities expand or change.

Organization and Responsibility

1. **Management**
   Scott McNaught, PhD, Director of the Michigan Water Research Center, will oversee the entire project, hire qualified personnel, interpret results, and write the final report.

2. **Field Responsibilities**
   Field Collection teams will be composed of 2 or students each, 1graduate student and 1 undergraduate student. The responsibilities of each student team are listed below: The Graduate Student will work with and supervise the undergraduate student. The students will equip the site with all needed collection and measurement devices and will collect the water samples for total phosphorus, total dissolved phosphorus, ammonia, nitrate, nitrate + nitrite, total residue, total dissolved solids and dissolved boron. Working with the undergraduate, the stream morphology will be measured. The Undergraduate Student is under the supervision of Graduate Student and will be responsible for keeping the chain of custody log, field notes, measurement of air temperature, water temperature, pH, conductivity, stream water velocity and discharge, oxygen measurements, and light measurements.

3. **Laboratory Responsibilities**

The laboratory that will conduct all of the laboratory analyses is the Michigan Water Research Center, 150 Brooks, Central Michigan University, Mt. Pleasant, Michigan 48859.
The laboratory staff is:
Jenny Estabrook
Laboratory Supervisor
A graduate student

4. **Corrective Action**
   Field: Each graduate student of the teams will be responsible for seeing that the samples are properly handled in the field and will use his judgement in re-collection or rejection of a sample.
   Laboratory: The laboratory supervisor will assure that the samples are handled and stored correctly in the lab and reject samples with broken seals, or see to the re-analyses of samples than do not meet the QA/QC requirements.

Training

The Laboratory Director and Laboratory Supervisor are responsible for ensuring that laboratory employees receive proper training in analytical methods and laboratory procedures and for documenting any training received. In particular, laboratory employees will be trained in sterile technique before handling samples for microbiological analysis. A new employee will receive orientation and skills training. New or established employees may receive training on new methods given by the method developer. The Laboratory Supervisor will maintain training records for microbiological methods on file by employee; this includes on-the-job training as certification of proficiency in microbiology.

The Laboratory Supervisor provides safety orientation to new employees and safety education to all employees. The employee orientation covers general safety issues, emergency procedures, standard-safety operations, the chemical-hygiene plan, hazardous-waste management, waste disposal, and location of safety equipment

Safety

Detailed laboratory safety practices and responsibilities are described in the Chemical Hygiene Plan. Safety activities include safeguards to avoid electric shock; prevent fire; prevent accidental chemical spills; and minimize microbiological dangers, facility deficiencies, and equipment failures.

Laboratory personnel that are isolating microorganisms from natural sources must be made aware that pathogens may be present in environmental samples. Technicians are to wear disposable gloves and lab coats when handling samples that are likely to contain

pathogens. Safety glasses are worn if there is a chance of projectiles, aerosols, or other foreign matter entering the eye.

Safety equipment is tested at regular intervals. Safety showers and eyewash stations are tested annually and recorded in the log book. Fire extinguishers are inspected annually. The Chemistry Stockroom (L. James Stock III) maintains a list of chemicals and arranges for disposal of hazardous waste.

Parameters Monitored and Associated Sampling/Analytical procedures

Physical

Solar radiation is measured with a Licor photometer.

Air temperature is measured with NIST traceable mercury thermometer or with a thermister electrical thermometer according to Standard Methods 2550 B.

Water temperature is measured with NIST traceable mercury thermometers or with a thermister electrical thermometer according to Standard Methods 2550 B.

Conductivity is measured according to Standard Methods 2510.
pH will be measured with the Hydrolab according the manufacturer's instructions.

Channel morphology is measured according the method of Robert G. Wetzel and Gene E. Likens in *Limnological Analyses, 2nd Ed.* with meter tapes and will include:

Length
Slope
Cross-section

Stream water is measured with a calibrated flow meter and will measure:

Velocity
Discharge

Chemical

All *collection, storage, preservation* of **water samples** is handled by EPA approved method found in *Standard Methods for the Examination of Water and Wastewater, 20th Edition.* 1998. American Public Health Association. Washington, D.C. The method is 1060.

All *collection, storage, preservation* of **sediment core** samples is handled by methods found in a text by Rump, H.H., H. Krist. 1988. *Laboratory Manual for the Examination of Water, Waste Water and Soil.* VCH Publishers. New York, New York.

All analyses of water samples are handled by EPA approved methods found in *Standard Methods for the Examination of Water and Wastewater, 20th Edition.* 1998. American Public Health Association. Washington, D.C.

All ***extraction*** of phosphorus, ammonium, nitrate and boron from sediment samples will be handled by the methods found in a text by Rump, H.H., H. Krist. 1988. *Laboratory Manual for the Examination of Water, Waste Water and Soil.* VCH Publishers. New York, New York. After extraction has occurred, methods for analysis are the same EPA approved methods as for water.

Ammonia samples are collected according to Standard Methods 1060 and analyzed according to Standard Methods 4500-NH_3-G

Nitrate and nitrite samples are collected according to Standard Methods 1060 and analyzed according to Standard Methods 4500-NO_3-F

Phosphorus samples are collected according to Standard Methods 1060 and analyzed according to Standard Methods 4500-P A, B & E.

Dissolved Oxygen samples are analyzed with the Hydrolab using the manufacturer's instructions, or as a back up, collected according to Standard Methods 1060 and analyzed using Standard Methods 4500-O C.

Total residue (total suspended solids) samples are collected according to Standard Methods 1060 and analyzed according to Standard Methods 2540 D.

Total dissolved residue samples are collected from according to Standard Methods 1060 and analyzed according to Standard Methods 2540 C.

Dissolved boron samples are collected from various sites according to Standard Methods 1060 and analyzed according to Standard Methods 4500-B, B-C.

Instrument Calibration Procedures

Autoclave

The autoclave is located in Brooks 131. Instructions for the use of the autoclave are posted on the side. Please sign in. Maintenance and calibration are provided by the Biology Department. Spore strips are autoclaved and examined for viability monthly. Printouts of the autoclave runs are produced each time recording temperature, pressure, time, and date.

Bran + Luebbe Autoanalyzer

Upon powering up, the autoanalyzer goes through a self-calibration step to check to electronics. The analyzer will not operate if all electronic parameters are not met. The lamp, pump, filter and tubing are inspected before each assay and replaced if worn. The

bubble pattern must be in line for an accurate analysis. A log is located on top of the autoanalyzer to record any maintenance.

Balances are located in Brooks 150.

OHAUS Analytical balance
OHAUS Precision +
Sartoris
Cahn Automatic Electrobalance

All balances are calibrated yearly by a factory representative and checked monthly with NIST traceable weights. A log is kept in the laboratory for recording the monthly readings. Be sure the balance is level. Check the small leveling bubble.

Freezers

Two upright freezers are located in Brooks 150 and Brooks 151. Mercury thermometers (±1.0 °C) are located in each. The temperatures are recorded in the log books on top of the refrigerators weekly.

Hydrolab

The temperature and battery are set at the factory. Compare to a NIST traceable mercury thermometer and when the temperatures do not agree, return to manufacturer for repair. In the field, check temperature against several readings with a thermistor or mercury thermometer.

The pH meter will be standardized using a two buffer system (pH 7 and 10 standard units). The meter will be standardized at least daily and the slope should be between 95 to 105%. A slope outside of this range means re-calibration. In the field, check pH against several reading with a calibrated pH stick.

Dissolved oxygen maintenance is usually required only when calibration becomes impossible or when the membrane covering the cell becomes damaged. Perform calibration according to the manufacturer's instructions before each collection trip. Take at least two samples for Winkler titration for comparison. If the Hydrolab does not function properly, do not use. Rely on back up measures. (Winkler titration for oxygen, pH sticks and reversing thermometers.) A notebook for notation of the calibration is in the drawer with the Hydrolabs.

Ovens are located in Brooks 150.

The Baxter/Scientific Products Muffle Furnace AFM-21 has an electronic digital readout. Note the temperature before opening the door.

The Soil Text Laboratory Oven has a NIST traceable mercury thermometer which should be consulted before opening the door.

Glass drying oven has a NIST traceable mercury thermometer which should be consulted before opening the door.

pH meter

Accumet Research AR-25 pH meter by Fisher Scientific is located in Brooks 150. The pH meter is standardized using a three buffer system (pH 4, 7 and 10 standard units). The meter will be standardized at least daily and the slope should be between 95 to 105%. A slope outside of this range means re-calibration.

Refrigerators

Two refrigerators are located in Brooks 150 and 151. Mercury thermometers (±1.0 °C) are located in each section of the refrigerators. The temperatures are recorded in the log books located on top of the refrigerators at least once a week.

Spectrophotometer

Beckman Model DU-640 is located in Brooks 150. A log is located on the shelf above the spectrophotometer. Instructions and supplies are located in the drawers directly below the instrument. As the spectrophotometer is powered up, the machine goes through an automatic electronic check of the systems. A screen will appear indicating if all systems have passed. All must pass or the machine can not be used.

The spectrophotometer is calibrated individually for each assay performed.

Nanopure Water Purification System

Distilled water is obtained from the Nanopure system located in Brooks 150. The resistance usually ranges from 15 to 18 μohms. The system is connected to the house distilled water. An emergency cutoff valve is located by the left rear side. The overflow tube must remain in the sink to prevent flooding. The Nanopure system is normally in the standby position when not in use. To operate push the start button and wait for the flush. The machine will go through an automatic calibration step and will indicate if the resistance is below normal. Do not use the water if the resistance is below 17.5 μohms. Consult the manufacture's instruction manual. The resistance will quickly rise to 17-18 μohms resistance. Record the resistance and the amount of water used in the log on top of the machine. Place in standby when not in use.

Chain of Custody

The chain of custody log is initialed by the collector and accompanies the samples back to the lab. Transfers of custody are recorded on the appropriate section of the chain of custody record. In the lab, the samples are logged into the computer, and sub-samples are filtered for chlorophyll a, nitrogen, and phosphorus. The technician performing the sample preparation and/or analysis initials the log as each procedure is completed. Samples are rejected if the bottle or seal is broken, and if proper temperature is not maintained.

Quality Control Checks

Method blanks are included in all assays for phosphorus, and nitrate. If phosphorus contamination (absorption @ 885 nm greater 0.0050) is found, the assay must be repeated. If nitrate contamination (baseline greater than 5% above or below the zero value), the assay must be repeated.

Field blanks are included with each field collection. Clean bottles are transported to the field and returned to the laboratory. In the laboratory, they are filled with double distilled water and assayed for phosphorus and nitrate.

Field duplicates are collected and assayed in the laboratory. Variance of ±5% results in repeating the assay or collection.

NIST traceable standards are used in the phosphorus and nitrate assays.

Standard curves are run for each phosphorus assay using NIST traceable standards, diluted to the appropriate range to include the samples. Standard curves are only acceptable if the R^2 is equal to or greater than 0.99__. If the standard curve is not acceptable, it must be repeated before the samples can be read. Samples are run duplicate or triplicate and repeated if they exceed the maximum standard or deviate by ±5%.

The Bran + Luebbe Autoanalyzer is calibrated each time by running a standard curve with a range that encompasses most of the samples. When the standard curve is calculated, the R^2 and the correlation coefficient must be equal or greater than 0.99__. If the standards are not up to this value, they must be re-assayed before the run can continue or the run repeated. After every 5 to 10 samples, an internal standard is inserted to correct for drift. The software will use these drifts to correct the final nitrate values. If the drift exceed ~ 5%, the results of the samples following the drifts are discarded and the samples are repeated. Samples are run duplicate and repeated if they exceed the maximum standard or vary by ±5%. Internal NIST traceable nitrate standards are assayed with every run.

Data Quality Objectives/Requirements

Solar radiation is measured with a Licor photometer during periods of constant cloud cover (0% or 100%). Depth profile data will be rejected if repetitive readings deviate by ±5% from the mean.

Air temperature is measured with NIST traceable mercury thermometers or with thermisters electrical thermometer according to Standard Methods 2550 B. Data will be rejected if repetitive readings deviate by ±5% from the mean.

Water temperature is measured with NIST traceable mercury thermometers or with thermisters electrical thermometer according to Standard Methods 2550 B. Data will be rejected if repetitive readings deviate by ±5% from the mean.

Conductivity is measured according to Standard Methods 2510. Data will be rejected if repetitive readings deviate by ±5% from the mean.

pH samples is determined using Accumet Research AR-25 by Fisher Scientific according to manufacturer's instructions. The pH meter is standardized using a three buffer system (pH 4, 7 and 10 standard units). The meter will be standardized at least daily and the slope should be between 95 to 105%. A slope outside of this range means re-calibration. Data will be rejected if replicates deviate by ±5% from the mean.

Stream water is measured with a calibrated flow meter and will measure velocity and discharge. Data will be rejected if repetitive readings deviate by ±5% from the mean.

Ammonia (Standard Methods 4500-NH_3-G): When the standard curve is calculated, the R^2 and the correlation coefficient must be equal or greater than 0.99. If the standards are not up to this value, they must be re-assayed before the run can continue or the run repeated. Data with lower values must be rejected.

Nitrate/Nitrite (Standard Methods 4500-NO_3-F): When the standard curve is calculated, the R^2 and the correlation coefficient must be equal or greater than 0.99. If the standards are not up to this value, they must be re-assayed before the run can continue or the run repeated. Data with lower values must be rejected.

Phosphorus (total phosphorus & total dissolved phosphorus) (Standard Methods 4500-P A, B & E): Standard curves are only acceptable if the R^2 is equal to or greater than 0.99. If the standard curve is not acceptable, it must be repeated before the samples can be read. Samples are run duplicate or triplicate and repeated if they exceed the maximum standard or deviate by ±5%.

Dissolved Oxygen samples is analyzed with the Hydrolab using the manufacturer's instructions, or as a back up, analyzed using Standard Methods 4500-O C. Hydrolab results will be rejected if there is an instrument failure. Back up, using a modified method of Winkler Titration will be used.

Total residue (total suspended solids)(Standard Methods 2540 D) will be repeated or discarded if the replicates deviate by ±5% from the mean.

Total dissolved residue (Standard Methods 2540 C) will be repeated or discarded if the replicates deviate by ±5% from the mean.

Dissolved boron (Standard Methods 4500 B, B-C) will be repeated or discarded if the replicates deviate by ±5% from the mean.

Preventative Maintenance

Autoclave
Maintenance and calibration are provided by the Biology Department. Spore strips are autoclaved and examined for viability monthly. Steam line and valves are checked annually the Central Michigan maintenance staff.

Balances
All balances are calibrated yearly by a factory representative and checked monthly with NIST traceable weights. Leveling is done by checking the small leveling bubble and adjusting the leveling screws if necessary.

Bran + Luebbe Autoanalyzer
The autoanalyzer is maintained according to manufacturer's instruction in Section 6 of the Bran + Leubbe Autoanalyzer Operations Manual.

Freezers
All freezers are cleaned and defrosted after each semester or when a spill occurs. Temperature logs are kept and monitored for fluctuation in temperature.

Hydrolab
The Hydrolab is maintained according to manufacturer's instruction in the Hydrolab Operations Manual

Ovens
All ovens are cleaned after each semester or when a spill occurs. Temperature monitored before samples are dried.

pH meter
The Accumet Research AR-25 pH meter by Fisher Scientific is maintained according to manufacturer's instruction in the Accumet Research AR-25Operations Manual.

Refrigerators
All refrigerators are cleaned and defrosted after each semester or when a spill occurs. Temperature logs are kept and monitored for fluctuation in temperature.

Spectrophotometer
The Beckman Model DU-640 is maintained according to manufacturer's instruction in the Beckman Model DU-640 Operations Manual.

Nanopure Water Purification System
The Nanopure system is normally in the standby position when not in use. To operate push the start button and wait for the flush. The machine will go through an automatic calibration step and will indicate if the resistance is below normal. Do not use the water if the resistance is below 17.5 μohms. Consult the manufacture's instruction manual.

The Nanopure Water Purification System is maintained according to manufacturer's instruction in the Nanopure Water Purification System Instruction Manual.

Corrective Action

Field: The team manager, a graduate student, will assume the responsibility of being the trouble-shooter in the field. He/she will recommend corrective measures for instrument breakdown, problems in sample collection, preservation of samples, and transportation of samples. Corrective actions will be documented on the field data sheets.

Laboratory: The laboratory supervisor will recommend corrective measures for instrument breakdowns, contamination of samples, preservation of samples, and repetition of assays. Corrective actions will be documented on the lab data sheets.

Management: The project manager will discuss and analyze any matters brought to his attention and direct repetition or elimination of problem data points.

Data Management, Analyses and Reporting Procedures

Data Base Software management

Computer-generated forms ensure that all data and methodological problems are recorded during analysis. Electronic files are backed up on a password protected Zip disk.

- MS Excel will be used for calculations and graph production.
- MS Access will be used to store and collate final data.
- MS Word/Excel will be used for preliminary and final reports.
- GIS map-producing center software (Arcview, Mapview) will be used for map production.
- TAOS software controls the autoanalyzer and computes results for Nitrate and Ammonium in tabular and graphic form.

References

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater, 20th Edition.* American Public Health Association et al. Washington, D. C.

Lind, Owen T. 1979. *Handbook of Common Methods in Limnology, 2nd Edition.* Kendall/Hunt Publishing Co. Dubuque, Iowa.

Rump, H.H., H. Krist. 1988. *Laboratory Manual for the Examination of Water, Waste Water and Soil.* VCH Publishers. New York, New York.

Wetzel, Robert G. and Gene E. Likens. 1991. *Limnological Analysis,* 2nd Ed. .Springer-Verlag, New York.

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Appendix

1. Water analyses ID Sheet
2. Chain of Custody Sheet
3. Training Documentation Sheet

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Water Analyses ID Sheet

Sample Origin: _____ **Lab ID#** _____

Results to: _____ **Date collected:**

_____

Please fill out the back of the sheet too!

Request (✓)	Parameter	mls required	Status
	Total Phosphorus ($\mu g \cdot L^{-1}$)	150	
	Total Dissolved Phosphorus ($\mu g \cdot L^{-1}$)	150	
	Soluble Reactive Phosphorus ($\mu g \cdot L^{-1}$)	150	
	Nitrate+ Nitrite ($\mu g \cdot L^{-1}$)	100	
	Nitrite only ($\mu g \cdot L^{-1}$)	70	
	Ammonium ($\mu g \cdot L^{-1}$)	100	
	Soluble Reactive Silica ($mg \cdot L^{-1}$)	150	
	Chlorophyll *a* ($\mu g \cdot L^{-1}$)	1000	
	Chlorophyll *a,b,c* ($\mu g \cdot L^{-1}$)	1000	
	pH	100	
	alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)	125	
	Ca^{++} ($mg \cdot L^{-1}$)	125	
	color ($mg \cdot L^{-1}$ Pt)	50	
	turbidity (NTU)	50	
	Conductivity ($\mu S \cdot cm^{-1}$)	100	
	Total Dissolved Solids ($mg \cdot L^{-1}$)	1000	
	Total Suspended Solids ($mg \cdot L^{-1}$)	1000	
	Dissolved oxygen ($mg \cdot L^{-1}$)	---	
	BOD ($mg \cdot L^{-1} \cdot d^{-1}$)	---	

File=ID_Sheet.doc

Page 13 of 16

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Record sample container numbers below

BOTTLE NUMBERS

ID#	Site	Depth	other		500 mL	500 mL	500 mL	125 mL	125 mL	125 mL

File=ID_Sheet.doc

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Chain of Custody Document

Survey				Samplers			
Station Number	Station Location	Date	Time	Sample Type	ID No.	No. of containers	Analysis required

Relinquished by:	Received by: Date:
Relinquished by:	Received by: Date:
Relinquished by:	Received by: Date:
Relinquished by:	Received by mobile laboratory for field analysis:

Dispatched by:	Date/Time:	Received for Laboratory by:
Method of shipment:		

Page 15 of 16

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Training Documentation

Title: (insert name of assay)
Date:

Revision History/Distribution/Training

Revision Date Responsible Person Description of Change

Distribution List
1. Laboratory SOP Manual
2. Lab manager's computer

Training:
The following laboratory staff has read this SOP and has been trained in its use.

Analyst's Name	Date

Page 16 of 16

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Alkalinity (SM-2320)

Background:

Alkalinity is the capacity of a solution to accept protons or the quantity and the kinds of compounds present that collectively shift the pH to the alkaline side of neutrality. Three kinds of alkalinity comprise total alkalinity: hydroxide (OH^-), normal carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-), but total alkalinity may include small amounts of the chlorides, sulfates and nitrates of calcium, magnesium, sodium and potassium, as well as borates, phosphates, and silicates (generally negligible in natural waters). The lethal effects of acids and alkalis appear at a pH less than 5.5 or greater than 9.5, although the toxic effects of extreme pH levels for most organisms is considerably narrower. Thus the buffering capacity of aquatic ecosystems can be very important in the maintenance of life.

Alkalinity of natural waters fluctuates readily in aquatic systems, generally resulting from influxes of carbon dioxide and rainwater from external sources. Carbon dioxide (CO_2) dissolves in water, forming carbonic acid, which lowers the pH of the system. However, this weak carbonic acid solution increases the systems capacity to dissolve minerals as it flows through the watershed, which in turn increases the alkalinity of the water, and thus the systems ability to buffer the pH of the system.

$$CO_2\,(air) \Leftrightarrow CO_2\,(dissolved) + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}$$

Bicarbonate alkalinity is the most common type found in aquatic systems, primarily because both gaseous and dissolved CO_2 are common is fresh waters, and because large portions of the Earth have carbonate present in the soils. However, when the pH is high, carbonate may be present, and when the pH is very high, hydroxides may be present.

Alkalinity can be described as an "imbalance" between the H^+ protons and weak anions (CO_2, HCO_3^- and CO_3^{2-}). It represents the number of protons that can be neutralized by the anions where the protons already present have been eliminated. This equation is written as:

$$\text{Alkalinity} = [HCO_3^-] + 2\,[CO_3^{2-}] + [OH^-] - [H^+]$$

Principle:

Alkalinity is determined by titrating water samples with a standard solution of strong acid. The equivalency or end points of the titrations are selected as the inflection points in the titration of Na_2CO_3 with H_2SO_4. These points will vary slightly with temperature, ionic concentration and free carbon dioxide concentration. End points may be determined empirically by titrations and are the pH where the derivation of pH/ ml titrant is the greatest. Points are usually taken as pH 8.3 for carbonate end point and pH 4.5 for the bicarbonate endpoint.The following reactions occur during titrations (Rainwater & Thatcher, 1960.):

$CO_3^{-2} + H^+ \rightarrow HCO_3^-$ (titration to pH 8.3)

HCO_3^- (from CO_3^-) + $H^+ \rightarrow H_2O + CO_2$

HCO_3^- (natural) + $H^+ \rightarrow H_2O + CO_2$ (titration to pH 4.5)

Sampling and Storage:

Collect 300 mL in acid washed polyethylene bottles. Store samples at low temperatures—on ice in field. Fill bottles completely and cap tightly. Process samples within 3 hours (soft water).

Detection limits: 1 mg/L

Apparatus:

Magnetic stirrer
50 ml borosilicate glass burette
- 1 L polyethylene bottles, acid washed and distilled rinsed 7 times

200-300 ml beaker or flask
pH meter with units of 0.05
pH buffers for standardization of meter (pH 4, 6,7, 8 & 10)

Reagents:

1. *Carbon dioxide-free water*
   Boil for 15 minutes and cool to room temperature; pH must be greater than 6.0.
2. *Standard sulfuric acid solution*
   a) Purchased 0.02N sulfuric acid may be used.
   b) Or dilute 200 ml 0.1 N stock solution to 1000 ml with CO_2 free H_2O to give 0.02 N solution. For accurate work this must be standardized against Na_2CO_3 (APHA, 1976).
3. *Carbonate standard*:
   Prepare a 0.05 N solution of Na_2CO_3 by drying 3 to 5 grams of primary standard Na_2CO_3 at 250°C. For 4 hours. Cool in a dissector. Weigh 2.5 ± 0.2 grams (to the nearest milligram); transfer to a one-liter volumetric flask. Fill flask to line with distilled water and dissolve and mix reagent. **Do not keep longer than 1 week.**

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Procedure:

1. Collect 250 ml or greater samples without aeration in glass or polyethylene bottles.
2. Allow sample to warm to room temperature.
3. Standardize pH meter.
4. Gently pour out 50 ml of sample and place in a 125 ml Erlenmeyer flask, if indicators are to be used. Use 80-100 ml beaker if pH meter is used.
5. Measure pH of sample after 2 minutes *without stirring*.
6. Place the 50-mL sample on a magnetic stir plate and add stir bar. Place prepared pH electrodes in sample. Stir samples slowly and titrate with acid solution. Record pH when meter stops drifting.
7. If initial pH was greater than 8.3, titrate to a pH of 8.3 in 0.05 ml increments.
8. Record the amount of acid used. This volume will be used to calculate carbonate alkalinity.
9. If the pH is less than 8.3 or following the titration to 8.3, continue to titrate to 4.5 and record the amount of acid used. This second volume will be used to calculate bicarbonate alkalinity.
10. Total alkalinity is the sum of carbonate and bicarbonate alkalinity.

Calculations:

HCO_3^-(bicarbonate) alkalinity as mg $CaCO_3$/ liter $= \dfrac{A \times \text{normality of acid} \times 10^3}{\text{ml sample}}$

Total Alkalinity as mg $CaCO_3$ $= \dfrac{B \times \text{normality of acid} \times 10^3}{\text{ml sample}}$

where:
A = ml titration to pH 8.3 (phenolphthalein or bicarbonate)
B = ml total titration from start to pH 4.5
N = Normality of acid

Quality Assurance/Control:

1. Use only acid washed glassware with accurate gradations.
2. Alkalinity of soft water is susceptible to change between time of collection and analysis. *Problem*--Loss of CO_2 from solution resulting in the conversion of bicarbonate to CO_2.

Alkalinity--page3

Analysis must be done within a few hours of collection and sample must be kept sealed.

3. Standardize H_2SO_4 titrant quarterly or when new titrant is used.

To Standardize 0.1 N H_2SO_4

1. Prepare acid: Carefully pour 28 ml of concentrated H_2SO_4 into an one liter volumetric flask containing approximately 900 ml distilled water; stir. Bring volume up to the line.
2. Standardize against 40.00 ml of 0.05 N Na_2CO_3 solution with about 60 ml water in a beaker by titrating potentiometrically to pH of 5.
3. Lift out electrodes; rinse into same beaker and boil gently for 3 to 5 minutes under a watch glass cover.
4. Cool to room temperature, rinse cover glass into beaker; and finish titrating to pH inflection point.
5. Calculate normality.

$$N = \frac{A \times B}{53.00 \times C}$$

Where:
A = grams Na_2CO_3 weighed into 1 liter flask
B = ml Na_2CO_3 solution taken for titration
C = ml acid used (about 18 -19 ml for 0.1 N)

Use measured normality in calculations or adjust normality
1 ml 0.100 N solution = 5 mg $CaCO_3$

References:

American Public Health Association et al. 1995. *Standard Methods for the Examination of Water, and Wastewater*, 19th Edition. American Public Health Association. Washington, D. C. pp.2-25-2-28.

Lind, Owen T. 1979. *Handbook of Common Methods in Limnology, 2nd Edition.* Kendall/Hunt Publishing Co. Dubuque, Iowa. pp.62-66.

Wetzel, Robert G. and Gene E. Likens. 1991. *Limnological Analysis*, 2nd Ed. .Springer-Verlag, New York. pp. 111-129.

Original: 3 Feb 99
Revised: 13 Sept 99
24 Jan 00
29 Mar 04

Alkalinity--page4

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Ash Free Dry Weight (SM-2540 E)

Background: The residue of total solids that was dried to 105°C is ignited to a constant weight at 550 °C. The remaining solids represent the fixed total, dissolved or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater or water.

Apparatus:

Polyethylene or resistant glass acid washed bottles
Evaporating dishes of 100 mL capacity
Drying oven at 103 °C
Muffle over at 550 °C
Desiccator
Analytical balance
Filter apparatus

Collection and Storage of Samples:

Collect water samples in 1-liter acid washed Nalgene bottles. Store samples at 5 °C and analyze within 48 hours of collection.

Range of Assay: 0.1 mg to 200 mg

Procedure:

1. Dry glass fiber filters and dishes by placing them in an oven at 103°C for 16 hours or overnight. Cool at room temperature for 25 minutes or in a desiccator and record weight of dish.
2. Place glass fiber filter in dish and record the weight of the dish and the filter.
3. Filtering: Measure water with a graduated cylinder. If turbidity of the water is low (less than 20 NTU), filter 1 to 2 liters of water through a glass fiber filter or until the water flowing through the filter has slowed down and sediment appears on the filter. Record the volume of water filtered. If the turbidity is high, use less water. Run duplicates or triplicates.
4. Weight the filter after the water has passed through it and record the weight.
5. Carefully place dishes in oven set at 103°C and leave for 16 hours so that evaporation of the water can occur. Cool at room temperature for 25 minutes (or in a desiccator) and reweigh dishes. Record the weight.
6. Cover the dishes and carefully place the dishes in the muffle oven at 550°C and leave for 16 hours. A flash may occur in the first fifteen minutes producing a smell or smoke.

AFDW--page1

7. Remove the dishes from the oven and allow them to cool in the air until most of the heat has dissipated. Transfer to a desiccator for final cooling in a dry atmosphere.
8. Weigh the dish and filter.

Calculation:

$$\text{mg volatile solids/L} = \frac{(A-B)\times 1000}{samplevolume, mL}$$

$$\text{mg fixed solids/L} = \frac{(B-C)\times 1000}{samplevolume, mL}$$

where:

A = weight of residue + filter + dish before ignition, mg.
B = weight of residue + filter + dish after ignition
C = weight of dish + filter, mg. before filtering

Interferences:

Negative errors in the volatile solids may be produced by loss of volatile matter during drying. Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subjects to error. In such cases, measure for suspect volatile components by another test, for example, total organic carbon. Highly alkaline residues may react with silica in sample or silica-containing crucible.

Quality Control/Quality Assurance:

1. Exclude large, floating particles or submerged agglomerates of non-homogeneous material from the sample if it is determined that their inclusion is not desired in the final results.
2. Use clean, acid-washed glassware with accurate gradation.
3. Check accuracy of balance periodically using standard weight set.

References:

American Public Health Association, et al. 1995. *Standard Methods for the Examination of Water and Wastewater, 19th Edition.* American Public health Association. Washington, D. C. pp.2-53-2-59.

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AFDW--page2

The Michigan Water Research Center
Central Michigan University

Calcium (SM 3500-Ca B) EDTA Titrimetric Method

Background:

Calcium is necessary in plant and animal nutrition and is an essential component of bones, shell, and plant structures. The presence of calcium in water supplies results from passage over deposits of limestone, dolomite, gypsum and gypiferous shale.

Calcium is the third element in Group IIA of the periodic table; it has an atomic number of 20, an atomic weight of 40.08, and a valence of 2. The average abundance of Ca in the earth's crust is 4.9%; in soils it is 0.07 to 1.7%; in streams it is about 15 mg/L; in groundwater it is from 1 to > 500 mg/L. The common forms of calcium are calcium carbonate and calcium-magnesium carbonate. Calcium carbonate solubility is controlled by pH and dissolved CO_2. The CO_2, HCO_3^-, and CO_3^{2-} equilibrium is the major buffering mechanism in fresh waters.

Sample size required: 100 mL in glass or polyethylene bottle

Detection Limits: 0.5 mg/L

Interference:

Under conditions of this assay, the following concentrations of ions cause ***no interference*** with calcium determination: Cu^{2+} < 2mg/L; Fe^{2+} < 20 mg/L; Fe^{3+} < 20 mg/L; Mn^{2+} < 10 mg/L; Zn^{2+} < 5mg/L; Pb^{+2} < 5 mg/l; Al^{3+} < 5 mg/L and Sn^{4+} < 5mg/L.

Orthophosphates precipitate calcium at the pH of the test. Strontium and barium give a positive interference and alkalinity in excess of 300 mg/L may cause an indistinct end point in hard waters.

Apparatus:

Magnetic stirrer
50 ml borosilicate glass burette
125mL polyethylene bottles, acid washed and distilled rinsed 7 times
200-300 ml beaker or flask
pH meter with units of 0.05
pH buffers for standardization of meter (pH 4, 7, & 10)

Reagents:

Sodium hydroxide, NaOH, 1 N

a) may be purchased or
b) Weigh out 40.00 grams of NaOH and add slowly to about 750 mL distilled water. Stir until dissolved. Qs to 1000 mL.

Indicator Eriochrome Blue Black R

a) may be purchased or
b) Grind together in a mortar 200mg powdered dye and 100 g solid NaCl to 40 to 50 mesh. Store in a tightly stoppered bottle. Use 0.2 g of ground mixture for the titration. During the titration the color changes from red through purple to bluish purple to a pure blue with no trace of reddish or purple tint.

Standard EDTA titrant, 0.01M.

a) 0.01M EDTA titrant may be purchased or
b) Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate in distilled water and dilute to 1000 mL.
c) Prepare standard EDTA titrant and standardize against standard calcium solution.

Standard Ca solution

a) Standard $CaCO_3$ may be purchased or
b) Weigh 1.000 g anhydrous $CaCO_3$ powder into a 500 mL Erlenmeyer flask. Place a funnel in the flask neck and add a little at a time, 1 + 1 HCl until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3 N NH_4OH or 1 + 1 HCl as required. Transfer quantitatively and dilute to 1000 mL with distilled water. 1 mL = 1.00 mg $CaCO_3$.

Procedure:

Because of the high pH used in this procedure, titrate immediately after adding alkali and indicator.

1. Pour 50 mL of sample into a clean beaker. One can analyze a smaller sample by diluting to 50 mL so that the calcium content is about 5 to 10 mg. Analyze hard waters with alkalinity higher than 300 mg CaCo3/L by taking a smaller portion and diluting to 50 mL.
2. Add 2.0 mL NaOH solution or a volume sufficient to produce a pH of 12 to 13. Stir.
3. Add 0.1 to 0.2 g indicator mixture selected (1 or 2 drops if a solution is used).
4. Titration: Add EDTA titrant slowly, with continuous stirring until indicator dye turns blue (no trace of red).

Calculations:

$$\text{mg Ca/L} = \frac{A \times B \times 400.8}{\text{mL sample}}$$

$$\text{Calcium hardness as mg } CaCO_3\text{/L} = \frac{A \times B \times 1000}{\text{mL sample}}$$

where: A = mL titrant for sample
B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant at indicator endpoint

Quality Assurance/Control:

1. Use clean, acid-washed glassware with accurate gradation.
2. Check accuracy of balance periodically using standard weight set.
3. Check effectiveness of titrant, indicator and titration procedure quarterly or when using a new titrant and/or indicator. Prepare a standard series (0, 25, 50, 100, 200 mg $CaCO_3$/L) using standard $CaCO_3$ solution. Titrate triplicate 50 ml samples of each dilution. Calculate mean and coefficient of variation (std.dev./mean*100) and compare with expected concentration.
4. For a more complete test of procedure effectiveness, prepare a standard series (0, 25, 50, 100, 200 mg $CaCO_3$/L) using standard $CaCO_3$ solution. Compare measurement results of an unknown sample using single titration procedure and standard curve.

References:

American Public Health Association et al. 1995. *Standard Methods for the Examination of Water, and Wastewater*, 19th Edition. American Public Health Association. Washington, D. C. pp.3-63-3-65

Lind, Owen T. 1979. *Handbook of Common Methods in Limnology, 2nd Edition.* Kendall/Hunt Publishing Co. Dubuque, Iowa.

Wetzel, Robert G. and Gene E. Likens. 1991. *Limnological Analysis*, 2nd Ed. .Springer-Verlag, New York.

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2 April 2, 2004

Sops/calcium

CHEMICAL OXYGEN DEMAND

Method 410.1 (Titrimetric, Mid-Level)

STORET NO. 00340

1. Scope and Application
   1.1 The Chemical Oxygen Demand (COD) method determines the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of oxidizing agent, temperature, and time.
   1.2 Since the test utilizes a specific chemical oxidation the result has no definite relationship to the Biochemical Oxygen Demand (BOD) of the waste or to the Total Organic Carbon (TOC) level. The test result should be considered as an independent measurement of organic matter in the sample, rather than as a substitute for the BOD or TOC test.
   1.3 The method can be applied to domestic and industrial waste samples having an organic carbon concentration greater than 50 mg/1. For lower concentrations of carbon such as in surface water samples, the Low Level Modification should be used. When the chloride concentration of the sample exceeds 2000 mg/1, the modification for saline waters is required.
2. Summary of Method
   2.1 Organic and oxidizable inorganic substances in the sample are oxidized by potassium dichromate in 50% sulfuric acid solution at reflux temperature. Silver sulfate is used as a catalyst and mercuric sulfate is added to remove chloride interference. The excess dichromate is titrated with standard ferrous ammonium sulfate, using orthophenanthroline ferrous complex as an indicator.
3. Sampling and Preservation
   3.1 Collect the samples in glass bottles, if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.
   3.2 Biologically active samples should be tested as soon as possible. Samples containing settleable material should be well mixed, preferably homogenized, to permit removal of representative aliquots.
   3.3 Samples should be preserved with sulfuric acid to a pH <2 and maintained at 4°C until analysis.
4. Interferences
   4.1 Traces of organic material either from the glassware or atmosphere may cause a gross, positive error.
      4.1.1 Extreme care should be exercised to avoid inclusion of organic materials in the distilled water used for reagent preparation or sample dilution.
      4.1.2 Glassware used in the test should be conditioned by running blank procedures to eliminate traces of organic material.

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4.2 Volatile materials may be lost when the sample temperature rises during the sulfuric acid addition step. To minimize this loss the flask should be cooled during addition of the sulfuric acid solution.

4.3 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion flask to complex the chlorides, thereby effectively eliminating the interference on all but brine and estuarine samples.

5. Apparatus

5.1 Reflux apparatus: Glassware should consist of a 500 ml Erlenmeyer flask or a 300 ml round bottom flask made of heat-resistant glass connected to a 12 inch Allihn condenser by means of a ground glass joint. Any equivalent reflex apparatus may be substituted provided that a ground-glass connection is used between the flask and the condenser.

6. Reagents

6.1 Distilled water: Special precautions should be taken to insure that distilled water used in this test be low in organic matter.

6.2 Standard potassium dichromate solution (0.250 N): Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103°C for two hours, in distilled water and dilute to 1000 ml.

6.3 Sulfuric acid reagent: Conc. H_2SO_4 containing 23.5g silver sulfate, Ag_2SO_4, per 4.09kg bottle. With continuous stirring, the silver sulfate may be dissolved in about 30 minutes.

6.4 Standard ferrous ammonium sulfate (0.25 N): Dissolve 98.0 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 ml of conc. H_2SO_4 (6.8), cool and dilute to 1 liter. This solution must be standardized daily against standard $K_2Cr_2O_7$ solution (6.2).

6.4.1 Standardization: To approximately 200 ml of distilled water add 25.0 ml of 0.25 N $K_2Cr_2O_7$ (6.2) solution. Add 20 ml of H_2SO_4 (6.8) and cool. Titrate with ferrous ammonium sulfate (6.4) using 3 drops of ferroin indicator (6.6). The color change is sharp, going from blue-green to reddish-brown.

$$\text{Normality} = \frac{(\text{ml } K_2Cr_2O_7)(0.25)}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

6.5 Mercuric sulfate: Powdered $HgSO_4$.

6.6 Phenanthroline ferrous sulfate (ferroin) indicator solution: Dissolve 1.48 g of 1–10 (ortho) phenanthroline monohydrate, together with 0.70 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of water. This indicator may be purchased already prepared.

6.7 Silver sulfate: Powdered Ag_2SO_4.

6.8 Sulfuric acid (sp. gr. 1.84): Concentrated H_2SO_4.

7. Procedure

7.1 Place several boiling stones in the reflux flask, followed by 50.0 ml of sample or an aliquot diluted to 50.0 ml and 1 g of $HgSO_4$ (6.5). Add 5.0 ml conc. H_2SO_4 (6.8); swirl until the mercuric sulfate has dissolved. Place reflux flask in an ice bath and slowly add, with swirling, 25.0 ml of 0.25 N $K_2Cr_2O_7$ (6.2). Now add 70 ml of sulfuric acid-silver

sulfate solution (6.3) to the cooled reflux flask, again using slow addition with swirling motion.

Caution: Care must be taken to assure that the contents of the flask are well mixed. If not, superheating may result, and the mixture may be blown out of the open end of the condenser.

7.1.1 If volatile organics are present in the sample, use an allihn condenser and add the sulfuric acid-silver sulfate solution through the condenser, while cooling the flask, to reduce loss by volatilization.

7.2 Apply heat to the flask and reflux for 2 hours. For some waste waters, the 2-hour reflux period is not necessary. The time required to give the maximum oxidation for a wastewater of constant or known composition may be determined and a shorter period of refluxing may be permissible.

7.3 Allow the flask to cool and wash down the condenser with about 25 ml of distilled water. If a round bottom flask has been used, transfer the mixture to a 500 ml Erlenmeyer flask, washing out the reflux flask 3 or 4 times with distilled water. Dilute the acid solution to about 300 ml with distilled water and allow the solution to cool to about room temperature. Add 8 to 10 drops of ferroin indicator (6.6) to the solution and titrate the excess dichromate with 0.25 N ferrous ammonium sulfate (6.4) solution to the end point. The color change will be sharp, changing from a blue-green to a reddish hue.

7.4 Blank–Simultaneously run a blank determination following the details given in (7.1) and (7.2), but using low COD water in place of sample.

8. Calculation

8.1 Calculate the COD in the sample in mg/l as follows:

$$\text{COD, mg/liter} = \frac{(A - B)N \times 8{,}000}{S}$$

where:

A = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the blank,

B = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the sample,

N = normality of the $Fe(NH_4)_2(SO_4)_2$ solution, and

S = milliliters of sample used for the test.

9. Precision and Accuracy

9.1 Eighty-six analysts in fifty-eight laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 270 mg/l COD. The standard deviation was ±17.76 mg/l COD with an accuracy as percent relative error (bias) of -4.7%. (EPA Method Research Study 3).

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 550, Method 508 (1975).
2. Annual Book of ASTM Standards, Part 31, "Water", Standard D1252–67, p 473 (1976).

The Michigan Water Research Center
Central Michigan University

Chlorophyll *a* (Chl *a*)

Background:

Chlorophyll *a* concentration is an estimate of the phytoplankton biomass. All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of phytoplankton. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins, and carotenes. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides, and pheophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups.

Sample size required: 1 to 2 liters of water in a Brown Nalgene Bottle

Detection Limits: 0.1 µg/L

Apparatus:

1 to 2 liter brown Nalgene bottles
Graduated cylinders
Millipore or other filter apparatus
HAWP Millipore filters, 4.25 cm or GF/C filter or Gelman A/E filter
plastic petri dishes
forceps
Spectrophotometer

Reagents:

1. 90% Acetone: To 90 mL Reagent grade acetone add 10 mL d-H_2O.
2. 2 N HCl: Add 186 mL concentrated HCl to 830 mL of distilled water.

Sample Collection and Preparation:

1. Collect chlorophyll *a* samples in opaque (brown) 1 to 2 L Nalgene bottles and filter within 4 hours. Filter 50 to 500 ml of lake water, depending on cell density, through glass fiber filters. Analyze samples within 2 weeks of collection.
2. Shake sample bottle vigorously before starting to filter.
3. Place a 0.45 µm Millipore HAWP filter on filter assembly.
4. Using a graduated cylinder (50 ml, 100 ml or 250ml), filter enough sample so filter is faintly colored (green, yellow, brown). Record volume filtered. Filter until no water in visible on filter. If the water is spilled while being poured into the funnel, re-do the sample. Wash down the sides of the filter funnel with a small amount of distilled water. Wait for the filter to dry.

5. Using forceps, remove and fold filter. Place in a plastic petri dish with tight fitting lid. Record information on lid: ID number, lake, depth, date, and volume filtered. Wrap dishes in one foil parcel and place in freezer for up to 14 days.
6. If the sample clogs the filter, and less than 25 ml have been filtered, then do as many as six replicates per sample. Always do at least duplicates—triplicates are preferred.

Analysis

1. Place the folded filters in 20ml scintillation vial; add 10 ml of 90% acetone. Stopper and shake.
2. Allow extraction to proceed in a dark refrigerator (4 to 5°C). An overnight extraction is preferable.
3. Use a blank of acetone containing the same number of filters used for the sample, and measure absorbance of sample and blank at 750 and 664 or 665 nm for chlorophyll *a* using matched curvettes. The 750 reading is the turbidity correction. If the absorbance reading at 664/665 is greater than 0.800, the sample must be diluted.
4. If correcting for chlorophyll *b* and/or chlorophyll *c*, also measure absorbance of sample and blank at 649 and 630 nm.
5. If correcting for phaeophytin *a*, add 30 µL 2N HCl to 10 ml sample after reading samples for chlorophyll *a*. Shake well. The pH should be 2.4 to 2.6. Re-read samples 5 to 30 minutes later at 665 and 750 nm.

Calculations:

There are many ways to calculate the concentration of chlorophyll *a*. Each formula corrects for interference from a molecule that absorbs light at a wavelength similar to that absorbed by chlorophyll *a* (ex: phaeophytin *a*, chlorophyll *b*, chlorophyll *c*). One formula does not exist to correct for all possible interferences.

The basic equation is:

Chlorophyll *a* concentration of original lake water (µg/L or mg/m^3) =

$$\frac{\text{Chlorophyll in extract (µg/liter) x Extract Volume (ml)}}{\text{Filtered volume (Liters) x Light path length (cm)}}$$

1. **Phaeophytin *a* correction:** Standard Methods 1998 (SM 10200 H-2)

$$\text{Chlorophyll } a \text{ (µg/L)} = \frac{26.7[(A664b - A\ 750b) - (A\ 665a - A750a)] \times V_1}{V_2 \times L}$$

Chlorophyll--page2

Where:

26.7 is the absorbance coefficient for chlorophyll and equals A X K where:
A = absorbance coeffiencent for chlorophyll a at 664 = 11.0 and
K = ratio expressing correction for acidification. (2.43)
A750 = absorbance at 750 nm and corrects for turbidity
b = before acidification
a = after acidification
V_1 = volume of solvent (mL)
V_2 = volume of sample filtered (L)
L = light path length (cm) = 1cm

2. **Phaeophytin *a* correction:** Monochromatic Method (Lorenzen, 1967)

$$\text{Chl } a \ (\mu g/L \text{ or } mg/m^3) = \frac{26.7\,[(A665b-A750b)-(A665a-A750a)] \times V_1}{V_2 \times L}$$

- Similar to Standard Methods except chlorophyll *a* wavelength is same before and after addition of acid.
- Phaeophytin *a* concentration may be calculated as:

Phaeo a (µg/L) = **20.8[(A665a-A750a)(V1/(V2*L))]** –Chl *a*

3. **Chlorophyll b correction:** Webb et al. 1992

$$\text{Chl a } (\mu g/L) = \frac{13.7\,(A665-A750) - 5.76\,(A649 - 750)}{V} \times 1.5$$

Where: 1.5 corrects for the volume of ethanol used and equals 15/10 (1.5)
V = volume (L) of water sample
A = absorbance

4. **Chlorophyll *b* and chlorophyll *c* correction:** Trichromatic method

$$\text{Chlorophyll } a = \frac{[11.85\,(A_{664}-A_{750})-1.54\,(A_{647}-A_{750})-0.08(A_{630}-A_{750})]\,v}{V}$$

$$\text{Chlorophyll } b = \frac{[\,21.03\,(A_{647}-A_{750})-5.43(A_{664}-A_{750})-2.66(A_{630}-A_{750})]v}{V}$$

$$\text{Chlorophyll } c = \frac{[24.52(A_{630}-A_{750})-1.67(A_{664}-A_{750})-7.60(A_{647}-A_{750})]\,v}{V}$$

Where: v = volume of acetone (mL)
V = volume(L) of water sample
A = absorbance

Chlorophyll--page3

Quality Control/Quality Assurance

1. Filter the sample as soon as possible to prevent loss of pigment.
2. Samples can be held on ice or at 4°C if processing must be delayed.
3. Protect water samples from light and exposure to acid.
4. Filtered samples from water with pH 7 or higher may be placed in airtight plastic bags and frozen for three weeks.
5. Samples from acidic waters should be processed promptly after filtration to prevent possible chlorophyll degradation from residual acidic water on the filter.
6. Use glassware and cuvettes that are clean and acid-free.
7. Wear gloves (acid on fingers).
8. Pigment extraction should be conducted with chlorophyll extracts in subdued light or yellow light to avoid degradation (opaque containers or containers wrapped with aluminum foil).
9. Pheopigments absorb near the same wavelength as chlorophyll *a*. Therefore, chlorophyll *a* may be overestimated. The sample should be acidified carefully to a final molarity of not more than 3×10^{-3} *M* to prevent certain accessory pigments from changing to absorb at the same wavelength as pheophytin *a*.
10. The volumes of extract and acid and the time after acidification are critical for accurate, consistent results.
11. Because the optical density of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, stay as close as possible to the 90 parts acetone:10 parts water formula for pigment extraction.

References:

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wasterwater, 20th Edition.* American Public Health Association et al. Washington, D. C.

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26 Feb 04
Sops/chlorophyll a

The Michigan Water Research Center
Central Michigan University

Nitrate + Nitrite (NO_3-N + NO_2-N)

Background: The automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite at pH 8.00 in a copper-cadmium reductor. The nitrite then reacts under acidic conditions with sulfanilamide to form a diazo compound that then couples with NEDD to form a reddish-purple azo dye that is measured at 550nm.

Sample Storage and Collection: Nitrate-nitrite is determined on samples which have been collected in 1 liter Nalgene polyethylene bottles and filtered through prewashed 0.45 µm HAWP Millipore membrane filters. Store samples at 5°C and analyze within 48 hours of collection. Samples can be analyzed on the Autoanalyzer or by the cadmium reduction method of Stainton et al. (1977) which reduces the nitrate to nitrite; the reagents added are the same as for a nitrite determination.

Detection Limit: 5 µg/L ***Range***: 0-54 to 0 – 448 µg/L as N
Range Switch: 0 – 196 to 0 – 1680 µg/L as N

Interferences: Sample turbidity may interfere. Remove by filtration before analysis. Sample color that absorb in the photometric range used for analysis also will interfere.

Apparatus:
Bran+Luebbe Technicon Autoanalyzer
Autoanalyzer cups
Digital Pipetters
Volumetric flasks
Filter Apparatus
0.45µm HAWP Millipore filters
GF/C or GF/F filters

Reagents:

1. *Triton X-100, 50% solution*

Triton X-100	50 mL
Methanol	50 mL

Combine 50 mL of Triton X-100 with 50 mL of methanol and mix well.

2. *Stock Copper Sulfate Solution "A", 0.01 M*

Copper sulfate	2.5 grams
Distilled water, q.s.	1000 ml

3. *Imidazole*

Imidazole	4.00 grams	1 gram
Hydrochloric acid	2 mL	0.5mL
Copper sulfate " A". 0.01 m	0.5 Ml	0.125 mL
Distilled water q.s	1000 mL	250 mL
Triton X-100, 50% solution	1 mL	0.25 mL

Dissolve imidazole in about 900 ml distilled water. Add hydrochloric acid and

copper sulfate. Dilute to one liter with distilled water, add 50% Triton X-100 and mix thoroughly.

4. *Sulfanilamide*

Sulfanilamide	10 grams	2.5 grams
Hydrochloric acid	100 mL	25 mL
Distilled water q.s.	1000 mL	250 mL
Triton X-100, 50% solution	2 ml	0.50 mL

5. *NEDD*

NEDD	1.0 gram
Hydrochloric acid	10 mL
Distilled water q.s.	1000 mL
Triton X-100, 50% solution	2 mL

6. *Standards*
   a. Stock Standard A, 100 mg/L as N (Stock A = 1 x 10^4 µg/L)
      Potassium nitrate 0.7214 grams
      Distilled water, q.s. 1000 mL
   b. Stock Standard B, 10 mg/L (Stock B = 1 x 10^3 µg/L)
      Stock Standard A 10 mL
      Distilled water, q.s. 100 mL
      Dilute 10 mL of Stock Standard A to 100 mL with distilled water and mix thoroughly.
   c. Working Standards

mL Stock B	µg/L as N
10	100
5	50
3	30
1	10

      Pipette Stock B into 100 mL volumetric flask. Dilute to 100 mL with sampler solution. Prepare fresh daily.

7. Wash water
   0.5 mL of 50% Triton X 100 (reagent #1) in 1 liter distilled water

Operation of the Bran+Luebbe Autoanalyzer

Updated March 1999

PRELIMINARY CHECKLIST

Day Before Assay:
1. Write out data book (see attached example)
2. Carboy of fresh DDW (18 ohm)
3. Prepare standards
4. Prepare reagents
5. Enter Sample Table into the computer

To select an existing table, click Spl || Otherwise go on to next step
Click sample table icon
Edit, Edit
First entries MUST be the following:

#	**Sample ID**
1	H (high std)
2	CO (DDW)
3	L (DDW)
4	S1 (high std)
5	S2
6	S3
7	S4 (low std)
8	S1
9	S2
10	S3
11	S4
12	S1
13	S2
14	S3
15	S4

'H' tells the computer that the run has begun. Stds. are run in TRIPLICATE and back to back

****note:**

- run samples in duplicate or triplicate.
- **designate a wash (W) between every cup (except** stds.) to **instruct** sampler to run DDW.
- run a mid-standard every 4-6 samples to allow computer to account for baseline Drift. **Label it 'D' followed by the number that corresponds to its** standard number (i.e. D3=S3).
- **label at least 6 sample spaces at end of run as RR1, RR2 etc. for rerunning off scale samples.** If you don't need them, just insert cups of DDW.
- the last cup to be sampled must be a high standard.

Save As your filename. You may want to use ID numbers in the filename (i.e. amm27-36.spl).
Exit.

Day of the Assay

1. Remove a sufficient number sample cups from the acid bath and rinse 5x **in DDW**.
2. Prepare cadmium coil for **nitrate** analysis
   Reagents:
   In the small labeled containers:
   a. Acetone
   b. Distilled water
   c. 6 N HCl (5 mL concentrated HCl + 5 mL distilled water
   d. Distilled water
   e. 1%(1 gram) $CuSO_4$ + 5 ml H_2SO_4 q.s. to 100 mL with distilled water
   f. Imidazole buffer from reagents

With a 20mL syringe with a 10cm length of 0.056" ID Tygon tubing, pump the solution through the coil by gently pushing and pulling in the order listed below:

Acetone (3 times)
Distilled water
6 N HC (three times)
Distilled water
$CuSO_4$
– push and pull until copper colloid appears in the beaker.
Imidazole buffer from reagents for total method—Remove all of the copper colloid; then aspirate imidazole into the coil and stop, leaving the coil full of the Imidazole buffer.

3. Draw tubing in **fresh** DDW containing Triton X (0.5 ml/liter).
4. Modify tube configuration: if high standard is ≤100 ug/L - tubing 7 and **2** to sampler
   if high standard is >100 ug/L - tubing 7 and **4** to sampler
5. Turn on nitrogen (nitrate) flow; allow the line to flush.
6. Insert correct lamp filter (550 nm - nitrate).
7. Tighten tubes and platen.
8. Attach nitrogen line; gas tubes in clickers.
9. Adjust sampler to mid-cup height.
10. Turn on computer, printer, AA (switch is in the back) and TRAAC sampler (switch is on the right end).

TAOS SETUP

Double click on ANALYSIS.
Double click on TAOS.
Brun + Luebbe OK.
Expand CFDE window.
Click manual icon (the hand).
Turn on pump to fast speed for 15 minutes.
Click DONE. While waiting, start setting up methods.

A. Sampler
Setup Sampler 1.
Ensure channel 1 and SCTRAACS are selected, control is ON, aspirate time~34, cycle time~40.
Click OK.

B. Methods
Click Mth button. | |
Select high (≤500 ug/L), mid (≤100) or low (≤50) methods file (depends on standards).
Click OK.
Click "Mth" icon.
Check standards - correct? Linear (1st order) fit.

Methods table should look like this:

Peaks per screen		10	Peak Search Parameters	
Decimal Places		2	Threshold	15
Percent full scale		100	Threshold slope	1
Chart speed		50	Plateau slope	1
			Plateau	10
Start ignore time		180	Correction	3
Initial baseline		40	Range	5
Final baseline		40		
Filter level		2		
Units	ug/L		CORRECTIONS	X
			Inverse chemistry	---

Save changes and click OK.
Ensure that the correct methods file is selected by the button. | |

C. Recall Sample File by clicking Spl. | |

D. Data

Click Data button. | |
Type in filename (same filename as sample file [i.e. amm27-36.dat]) or select from list.

RUNNING TAOS

A. Reagent absorbance

Click Manual icon (hand).
Turn pump to normal speed for 5 minutes, then check for a uniform bubble pattern
Exit manual menu.
Click "STANDBY" on top left so that it changes to "ON" and STANDBY goes to bottom left.
Click Manual icon (hand).
Set gain to 13 and baseline to 50 (channel 1, Send).
Click Realtime.
DDW absorbance (%) should read ~5-7% (5% is the best). If not, adjust baseline.
Note final base, gain and % in your data book.
Turn pump to fast speed.
Lift each drawtube out of the DDW/Triton X solution, wipe with a Kim Wipe, and place into its appropriate solution, lifting it in and out a few times to allow air to be drawn.
Run pump at high speed for 10 minutes. **Nitrate:** at 5 min. turn off pump, attach Cd coil (input side first). Continue at high speed for 5 more min.
Turn pump to normal speed for 5 min., then check for a uniform bubble pattern.
Click Realtime.
Record new % (ideally 8-10%). Difference from DDW % is the reagent absorbance.

B. Setting Baseline

Manual menu (hand).
Set gain to 90 and baseline to 50 (channel 1, Send).

Click Realtime.
After about 5 minutes, note %.
Adjust baseline (channel 1, Send, Realtime) until it is ~5%.

C. Setting Fullscale

Leave base and gain settings alone.
Fill a sample cup with your high standard and place in position 1.
Send sampler to position 1. After 3 minutes, send it back to rest (delete 1, Send).
Click Realtime.
When peak comes through, quickly adjust the gain such that you get a % fullscale of 93-96% (max gain = 250).
Check your baseline after the peak. Make adjustments as necessary (may have to repeat above procedure a few times) so that you have baseline ~5% and fullscale ~95%.
Exit Manual menu.

D. Running Samples

1. Fill at least the first 16 sample cups (rinse each cup twice with DDW and once with your sample or standard) and place in TRAACS as planned in your data book.
2. Click "ON" icon on top left. The sampler will begin to run your samples.
3. Click split-screen icon to watch your data being generated.
4. Continue to fill cups.
5. After the standards have been run, check and record the ?correlation coefficient. Watch for poor replicates or off scale data. Dilute if required and rerun as RR1 etc. at the end.
6. ?Flags are defined on Section 6, pg. 79 of the black binder.
7. Record all data in data book. Manually calculate concentrations of any diluted RR samples.

SHUTDOWN

1. **For nitrate**, turn off pump (manual icon), detach Cd coil (output end first). Ensure tubing is full of imidazole for storage.
2. Turn pump to fast.
3. Remove each draw tube from reagent jar; wipe with Kim Wipe, place in DDW/Triton X, allowing tube to draw air 2-3 times.
4. After 12 min. in DDW at fast speed, lift tubes out of DDW and draw air until all tubing and coils are dry.
5. Pump off.
6. Exit manual menu (Commands→ Exit Saves automatically).
7. Exit TAOS.
8. Turn off computer, printer, AA and TRAAC sampler.
9. Rinse sample cups and place in acid bath.
10. Turn off air or nitrogen flow.
11. Remove lamp filter; store in dessicator jar.
12. Loosen tubes and remove platen.
13. Detach nitrogen line and remove gas tubes from clickers.
14. Empty waste into labeled jug.
15. Put all reagents and Stock A standard in fridge; dispose of standard solutions.
16. Rinse all glassware and place in the acid bath; rinse nitrate tubes 5 times with distilled water; metal scoops MUST go in the soap bath, NOT in the acid bath.

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Total Dissolved Nitrogen (TDN)

Using GF/C or GF/F filters, filter samples and store at 5°C until analyzed.
To analyze:
Place 100 mL GF/C filtered sample in quartz tube.
Add 400 µL 4 N H_2SO_4 (55.5 mL concentrated H_2SO_4 made up to 500 mL) and 400µL 30% hydrogen peroxide to each tube.
Follow UV digestion method (see instrument use) then analyze irradiated sample for NH_4. If only 25mL sample is used for digestion, then decrease amount of reagents to 100µL.

References:

American Public Health Association, et al. 1995. *Standard Methods for the Examination of Water and Wasterwater, 19th Edition.* American Public Health Association. Washington, D.C. pp.4-75-4-96.

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Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. *The Chemical Analysis of Fresh Waters. 2nd Edition.* Misc. Spec. Publ. Dept. Environment Canada 25. 166pp.

Wetzel, Robert G. And Gene E. Likens, 1991. *Limnological Analyses, 2nd Edition.* Springer-Verlag. pp.81-89.

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Sops/Nitrate

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Percent Water in Sediments
Ash Free Dry Weigh in Sediments

Background: Water in sediment occupies pore spaces that arise from the physical arrangement of the particulate solid phase concurrently with the soil gas phase. This water is a reservoir and serves as a buffer sometime enabling plant and animal growth. Measurement of this is important as a prerequisite step for other measurements. Ash free Dry Weigh is useful because it offers a rough approximation of the amount of organic matter present in the solid fraction of the sediment.

Apparatus:

Ponar dredge
Polyethylene gallon bags
Evaporating dishes of 100 mL capacity
Lids for evaporating dishes
Drying oven at 103 °C
Muffle oven at 550 °C
Desiccator
Analytical balance

Collection and Storage of Samples:

Collect sediment samples using a dredge and place in polyethylene storage bags. Store samples at 5 °C and analyze within 72 hours of collection.

Range of Assay: 0.01 mg to 200 mg

Procedure:

1. Dry dishes by placing them in an oven at 103°C for 16 hours or overnight. Cool at room temperature for 25 minutes or in a desiccator and record weight of dish.
2. Using a clean spatula add sediment samples (25 to 50 grams) to dishes. Weigh the sediment and dish and record the weight. Run duplicate or triplicates.
3. Carefully place dishes in oven set at 103°C and leave for 16 hours so that evaporation of the water can occur. Remove the dishes from the oven and cool at room temperature for 25 minutes (or in a desiccator) and reweigh dishes. Record the weight.

Calculation for percent water:

$$\% \text{ water} = \frac{A - B}{C - B} \text{ X } 100$$

Percent water in sediment--1 of 3

where:

A = weight of solids + dish, mg. after drying @ 103°C
B = weight of dish
C = weight of solids +dish, mg. before drying @ 103°C

4. Cover the dishes and carefully place the dishes in the muffle oven at 550°C and leave for 16 hours. A flash may occur in the first fifteen minutes producing a smell or smoke.
5. Remove the dishes from the oven and allow them to cool in the air until most of the heat has dissipated. Transfer to a desiccator for final cooling in a dry atmosphere.
6. Weigh the dish and filter.

Calculation for Volatile and fixed solids:

$$\%\text{volatile solids} = \frac{(A-B)}{A}$$

$$\%\text{ fixed solids} = \frac{(B-C)}{B}$$

where:

A = weight of residue + dish before ignition, mg.
B = weight of residue + dish after ignition
C = weight of dish mg.

Quality Control/Quality Assurance:

1. Exclude large, floating particles or submerged agglomerates of non-homogeneous material from the sample if it is determined that their inclusion is not desired in the final results.
2. Use clean glassware with accurate gradation.
3. To ensure a draft free environment for weighing, close the laboratory doors.
4. Check accuracy of balance periodically using standard weight set.

References:

Miller, Raymond W. and D. T Gardiner. 2001. *Soils in Our Environment.* Prentice-Hall,

Inc. Upper Saddle River, New Jersey.

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Phosphorus Analysis (SM 4500 P - E)

Background:

Phosphorus occurs naturally in lakes and streams and can be present in high concentration in wastewater. Phosphorus can be present in particles or dissolved in the water. Particulate phosphorus includes detritus, living aquatic organisms, and silt with bound phosphorus. Dissolved phosphorus includes inorganic orthophosphates and condensed phosphates as well as organic molecules with phosphate such as nucleic acids and phospholipids.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. Small, dissolved, inorganic phosphorus molecules such as orthophosphate (PO_4^{-3}) are most easily absorbed by algae and bacteria.

Principle:

Since phosphorus occurs in so many forms, it is simplest to measure Total Phosphorus (TP). However, dissolved phosphorus molecules are more reactive and readily available to organisms in the water. Therefore, it is often useful to filter particles from the water sample and measure Total Dissolved Phosphorus (TDP). Sometimes it is important to know what portion of TDP is orthophosphate. Orthophosphate is readily available to aquatic organisms and is referred to as Soluble Reactive Phosphorus (SRP).

Phosphorus analysis consists of two major steps: 1) conversion of phosphorus (particulate, dissolved, or total) to dissolved orthophosphate and 2) colorimetric determination. Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in acid medium to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid. The intensity of blue color can be measured with a spectrophotometer. Phosphorus concentration is eventually determined by constructing a standard curve.

Collection and Storage of Samples:

1. Collect samples using acid washed bottles only.
2. If soluble reactive phosphorus (SRP) is to be measured, samples must be filtered immediately after collection.
3. If total dissolved phosphorus (TDP) is to be measured, store at 4°C for no more than 8 hours before preparation.
4. If only total phosphorus (TP) is to be measured, sample may be frozen until analysis is possible.

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Apparatus:

Spectrophotometer
Autoclave
Acid washed volumetric flasks
Acid washed screw top test tubes
Filtering apparatus and HAWP 0.45 um Millipore filters

Reagents:

1. *Stock solution (10^6 PO_4-P/L)*
   Dissolve 0.439 g KH_2PO_4 (monobasic) in a small amount distilled water in a one liter volumetric flask. Add 1 ml concentrated H_2SO_4 and dilute to mark.

2. *Secondary stock solution (10^4 PO_4 - P/L)*
   Dilute 10 ml of stock solution to 1 liter with distilled water in a volumetric flask.

3. *If using a purchased standard:*
   Purchased standard: (1mL of solution = 1 mg P)
   One liter would contain 10^6 µg P/liter.
   Secondary standard: (10^4 µg P/liter)
   Make a 1:100 dilution by adding 10 mL of the 10^6 standard to a 1-liter volumetric flask, adding DI water and mixing.
   Working solution: (10^3 µg P/liter)
   Make a 1:10 dilution of the secondary standard by adding 100 mL of the 10^4 solution to a 1 liter volumetric flask, adding DI water, and mixing. Use this solution to make the standards in the range of the samples to be tested.

4. *Premix Reagent–Prepare at least one day before it is to be used.* Protect from light and store in refrigerator.
   a. Carefully add 140 ml concentrated H_2SO_4 to 450 ml distilled water in a 1-liter beaker. Allow to cool.
   b. Dissolve 15 grams ammonium molybdate and 0.034 grams potassium antimony tartrate in 1.2 liters distilled water in a 2-liter reagent flask.
   c. Add cooled acid solution to the 2-liter flask.

5. *Mixed Reagent*
   Dissolve ascorbic acid in distilled water. Be sure ascorbic acid is completely dissolved before adding premix. ***Prepare only as much mixed reagent as will be used daily.*** It does not keep. Mix well and protect from light.

Wt. ascorbic acid	distilled water	Pre Mix	Final Volume
0.258 g	5 ml	20 ml	25 ml
0.517 g	10 ml	40 ml	50 ml
1.034 g	20 ml	80 ml	100 ml
1.548 g	30 ml	120 ml	150 ml
2.068 g	40 ml	160 ml	200 ml
2.585 g	50 ml	200 ml	250 ml
3.102 g	60 ml	240 ml	300 ml
3.619 g	70 ml	280 ml	350 ml
4.136 g	80 ml	320 ml	400 ml
4.653 g	90 ml	360 ml	450 ml
5.179 g	100 ml	400 ml	500 ml
5.696 g	110 ml	440 ml	550 ml
6.213 g	120 ml	480 ml	600 ml
6.730 g	130 ml	520 ml	650 ml
7.247 g	140 ml	560 ml	700 ml
7.774 g	150 ml	600 ml	750 ml
8.281 g	160 ml	640 ml	800 ml

6. *Ascorbic Acid* needs to be checked for phosphate contamination.

   When a bottle is nearly empty, open a new bottle of ascorbic acid and prepare a small amount of Mixed Reagent by dissolving 0.52 g ascorbic acid in 10 ml distilled water and add 40 ml pre-mix. Add this solution to some distilled water blanks (do not digest) and read in the usual manner. The readings should be comparable to previous values. Mark the absorbance on the bottle to indicate it has been checked.

7. *Potassium persulfate* needs to be checked for contamination.

   When an old bottle is almost finished, prepare 3 distilled water blanks and add the new persulfate to these tubes. Process the normal way and read on the spectrophotometer. The readings should be comparable to previous values. Mark on the bottle that the persulfate has been checked. Do not check persulfate and ascorbic acid on the same sample. If the reading is high, check one other bottle. If this reading is also high, then likely that particular batch number of reagent is contaminated.

Expected limitation of assay: 0.3 μg/L

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Preparation and analysis of samples

1. Shake sample
2. For **TDP** or **SRP**, set up filtering apparatus with HAWP 0.45 um Millipore filters. Filter small amount of sample, rinse filter flask and discard. Filter approximately 200 mL of the sample water. To clean duplicate tubes, fill with a small amount of filtered sample, rinse sides of tubes and lids, and discard. Fill 50 ml cut off graduated cylinder with filtered sample and pour using a funnel into the duplicate tubes.
3. For **TP do not filter**.
4. For **TP** and **TDP**, add 0.4 grams potassium persulfate to each tube. Mix by inverting.
5. For **SRP**, do not add persulfate. Add reagent, mix, and read immediately.
6. Prepare standards: Standards and blanks do not need to be filtered but require potassium persulfate. Tighten caps. Tubes may be stored at 5° C for up to 14 days. When making standards, i.e. dilutions, rinse pipette with standard and discard the standard. Low standards are 0, 25, and 50 µg / L. Unknown standards are 0, 25, 50, 100, 125, and 250 µg / L. Treat standards the same as samples. Add 0.4 grams potassium persulfate; place in autoclave and digest @ 1.4 kg / cm² for 45 minutes on slow exhaust. Cool to room temperature by placing in a sink of cold water.
7. For samples, standards and blanks: rinse tubes 2 times with distilled water before using. Add 50 ml of sample, standard or blanks, then 0.4 grams potassium persulfate; place in autoclave and digest @ 235-240ºC, 15-20 psi, (1.4 kg / cm²) for 45 minutes on slow exhaust. Cool to room temperature by placing in cold water.
8. Colorimetric assay:
   a) Using repeater pipette, add 5 ml mixed reagent to cooled, digested samples, standards and blanks.
   b) Let stand at room temperature for 20 minutes for color reaction to occur. The reaction involves formation of a blue potassium-molybdate complex, the intensity of which corresponds to amount of phosphorus in the original sample.
   c) Measure absorption at 885 nm. Readings for greater than 0.725 are no longer linear and need to be diluted. Zero the spectrophotometer using distilled water. Be sure that samples and standards are read using the same cells.

Calculations

1. Plot absorbance of standards against known standard concentrations.
2. Use regression analysis to determine the formula of a line that best fits the data points.
3. Calculate unknown phosphorus concentrations using slope and intercept of the best fit line and the following formula:

$$P = \frac{\text{absorbance units - intercept}}{\text{slope}}$$

Quality Control/Quality Assurance:

1. If dissolved phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below –10 °C.
2. Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphate may be adsorbed onto the walls of plastic bottles.
3. Soak all glass containing low concentrations of phosphorus in 5% HCl, then rinse seven times with distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.
4. Standard curves need to be established for each set of phosphorus samples. Using the appropriate range for samples, select standards that bracket the expected values of the samples. Run triplicates of the standards and plot standard curve using Excel or QuattroPro. Look for a R^2 value > 0.99. If the R^2 is lower, re-run standards making new dilutions.
5. Read absorbance within 2 hours of adding mixed reagent. Run phosphorus standards at the beginning and end of long runs (> 1 hour) to determine if blue color intensity has changed over time.
6. Allow the spectrophotometer lamp to warm up for at least 1 hour. Run blank of distilled water to zero the machine.
7. Use a long path length cell (5 or 10 cm) to measure absorbance of water samples suspected to contain low concentrations of phosphorus (< 10 µg/L).
8. Ascorbic acid potassium persulfate should be tested using known phosphorus concentrations, testing absorbance of each sample. See steps 5 and 6 in reagent preparation.
9. Premix reagent may be made and stored in an amber bottle in the refrigerator. See step 3 in reagent preparation.
10. Mixed reagent is made from the premix reagent and must be made daily.
11. Wear latex gloves to reduce phosphorus contamination from fingers.

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References:

American Public Health Association et al. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. American Public Health Association. Washington, D.C. p4-139-4-144.

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PLANKTON COUNTING PROCEDURE

Phytoplankton

Equipment: Utermohl settling chamber, inverted microscope, vacuum grease, 2 ml pipette

Procedure:

1. Assemble Utermohl settling chambers.
   a. Put small amount of vacuum grease on base around depression.
   b. Place cylinder on base and rotate to smear vacuum grease; then align inside of cylinder and base.
2. Fill settling chamber.
   a. Thoroughly mix phytoplankton sample.
   b. Remove 1-2 ml with pipette and add to chamber.
   c. Fill chamber with dH_2O until meniscus forms at surface.
   d. Cover chamber with Plexiglas square excluding all air bubbles.
   e. Set undisturbed for 24 hours.
   f. Over a large beaker, use Plexiglas square to slide upper part of chamber away from base thereby removing excess water.
3. Under inverted microscope at 400 x magnification, count phytoplankton in entire chamber or 3 random transects.
4. Discard contents of chamber after counting is completed.

Rotifers

Equipment: Sedgewick-Rafter cell, compound microscope, 44 μm sieve, disposable pipette

Procedure:

1. Pour sample through 44 μm sieve and rinse with dH_2O to remove formalin.
2. Dilute to 500 ml or more depending on density of plankton.
3. Lay cover glass on Sedgewick-Rafter cell at an angle so that one corner of cell is open.
4. Using pipet, add 1 ml of well-mixed sample to Sedgewick-Rafter cell. Adjust cover glass to fully cover cell.
5. Count 1-3 transects or entire cell for a total of 100 or more individuals of dominant taxa.

Microcrustaceans

Equipment: Bogorov tray, stereomicroscope, 44 µm sieve, 5 ml pipette

Procedure:

1. Pour sample through 44 µm sieve and rinse with dH_2O to remove formalin.
2. Dilute to 200 ml or more depending on density of plankton.
3. Mix well; using pipet, add 5 ml to Bogorov or circular counting tray.
4. Pipet 5 ml dH_2O into tray to clean pipet and spread out sample.
5. Add 2-3 drops of dilute soap solution to reduce water surface tension.
6. Count 3-4 five-ml samples for a total of 100 or more individuals of dominant taxa.

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Silica, Soluble Reactive (SM-4500-Si)

Background:

Silicon ranks next to oxygen in abundance in the earth's crust. It appears as the oxide (silica) in quartz and sand and is combined with metals in the form of many complex silicate minerals, particularly igneous rocks. Degradation of silica-containing rocks results in the presence of silica in natural waters as suspended particles, in a colloidal or polymeric state, and as silicic acids or silicate ions. Volcanic and geothermally heated waters often contain an abundance of silica.

The silica (SiO_2) content of natural water most commonly is in the 1 to 30 mg/L range, although concentrations as high as 100 mg/L are not unusual and concentrations exceeding 1000 mg/L are found in some brackish waters and brines.

Sample Collection and Storage:

Collect samples in bottles of **polyethylene** or **other plastic** or **hard rubber**, especially if there is a delay between collection and analysis. Freezing to preserve samples for analysis of other constituents can lower soluble silica values by as much as so to 40% in waters that have a pH below 6. Do not acidify sample for preservation because silica precipitates in acidic solutions. Usually, silicon is determined on unfiltered samples, collected and stored in polyethylene bottles. Keep samples at 5°C until analyzed within 48 hours.

Expected Range of Analysis: 10µg/L to 4000ug/L

Apparatus:

Plastic volumetric flasks
Plastic culture tubes
Plastic graduated cylinders
Pipetters with plastic tips
Spectrophotometer

Quality Control/Quality Assurance:

1. **When making reagents, remember to use plastic and silicon free water!**
2. 0.05 N Stannous chloride must be fresh—made the day you intend to use it.

Reagents:

1. *5% Ammonium molybdate*
   Dissolve 50 grams ammonium paramolybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1 liter silica-free distilled water.

2. *1 N HCl*
   Add 83 ml concentrated HCl to distilled water; bring up to 1 liter.
3. *Mixed molybdate*
   Mix 2 volumes 5% ammonium molybdate reagent with 3 volumes 1 N HCl.
4. *10% Tartaric acid*
   Dissolve 100 grams tartaric acid in 1 liter silica-free distilled water.
5. *3.5 N Stannous chloride stock solution*
   Dissolve 40 grams $SnCl_2 \cdot H_2O$ in 100 ml 50% HCl (1 part concentrated acid to 1 part water.)
6. *0.05 N Stannous chloride solution*
   Prepare fresh daily by diluting stock solution. The normality of the final reducing solution should be between 0.04 and 0.06 N.
   1.42 mL stock q.s. to 100 mL or
   0.71 mL stock q.s to 50 mL
7. *Commercially available Silica Standard* is at 1000 mg/L (10,000 µg/L).
   Prepare stock of 10^4 Si/L: add 10 mL of standard and q.s. to 1 liter with distilled water.
   Prepare standards of 250, 500, 1000, 2000 and 4000 µg/L :

µg/L	Dilution	ml 10^4 stock	q.s. volume
250	1:40	25	1000 mL
500	1:20	50	1000 mL
1000	1:10	100	1000mL
2000	1:5	200	1000mL
4000	1:2.5	200	500 mL

Preparation and analysis:

1. Prepare fresh standards daily, using polyethylene volumetric flasks. Dilute 1000mg/L commercial standard to 250, 500, 1000, 2000 and 4000 µg/L. Also include a blank.
2. Prepare the mixed molybdate and stannous chloride solution before starting.
3. Place 3 ml mixed molybdate reagent in a dry culture tube and then add 50 ml distilled water, silica standard or sample and mix. The acidic mixed molybdate must be added to the tube before the sample is added. Wait 15 minutes.
4. Add 2 ml tartaric acid and mix. Wait 2 minutes.
5. Add 1 ml 0.05 N stannous chloride reductant and mix.
6. Between 5 minutes and 1 hour later, read at 820 nm.
7. Construct standard curve and read samples from curve. There is a program on the computer in the lab and in the office to help with the calculations.

References:

American Public Health Association, et al. 1995. *Standard Methods for the Examination of Water and Wastewater, 19th Edition.* American Public Health Association. Washington, D.C. pp.4-115-4-120.

Lind, O. T. 1985. *Handbook of Common Methods in Limnology, 2nd Edition.* Kendal/Hunt Publishing Co. Dubuque, Iowa. pp.88-91.

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Sops/Silicon

The Michigan Water Research Center
Central Michigan University

Total Phosphorus in Soil Samples

Background:

Phosphorus occurs naturally in waters, wastewaters, and in the soil almost solely as phosphates. These are classified as orthophosphates, condensed phosphates and organically bound phosphates. They occur in solution, in detritus or in the bodies of aquatic organisms.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water.

The phosphorus analyses consists of two major steps:

a) conversion of the phosphorus form of interest to dissolved orthophosphate
b) colorimetric determination

Principle:

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

Collection and Storage of Samples:

Undisturbed samples are taken from the earth by means of a cutting cylinder in such a way that their original condition is maintained. After collection, the samples are packed in plastic bags. Each sample is provided with an identification.

Expected limitation of assay: 0.3 µg/L

Apparatus:

Drying oven capable of maintaining 105°C
Glass Rods
Spectrophotometer
Autoclave
Acid washed volumetric flasks
Acid washed screw top test tubes

Reagents:

1. *Stock solution (10^6 PO_4-P/L)*
   Dissolve 0.439 g KH_2PO_4 (monobasic) in a small amount distilled water in a one liter volumetric flask. Add 1 ml concentrated H_2SO_4 and dilute to mark.
2. *Secondary stock solution (10^3 PO_4 - P/L)*
   (1000 ug PO_4 - P/L) by diluting 10 ml to 1 liter with distilled water in a volumetric flask.
3. *Premix Reagent–Prepare at least one day before it is to be used.* Protect from light and store in refrigerator.
   a. Carefully add 140 ml concentrated H_2SO_4 to 450 ml distilled water in a 1 liter beaker. Allow to cool.
   b. Dissolve 15 grams ammonium molybdate and .034 grams potassium

antimony tartrate in 1.2 liters distilled water in a 2 liter reagent flask.

c. Add cooled acid solution to the 2 liter flask.

4. *Mixed Reagent*

Dissolve ascorbic acid in distilled water. Be sure ascorbic acid is completely dissolved before adding premix. *Prepare only as much mixed reagent as will be used daily.* It does not keep. Mix well and protect from light.

Wt. ascorbic acid	Distilled water	Pre Mix	Final Volume
0.258 g	5 ml	20 ml	25 ml
0.517 g	10 ml	40 ml	50 ml
1.034 g	20 ml	80 ml	100 ml
1.548 g	30 ml	120 ml	150 ml
2.068 g	40 ml	160 ml	200 ml
3.102 g	60 ml	240 ml	300 ml
4.136 g	80 ml	320 ml	400 ml
5.179 g	100 ml	400 ml	500 ml

4. *Ascorbic Acid* needs to be checked for phosphate contamination. When a bottle is nearly empty, open a new bottle of ascorbic acid and prepare a small amount of Mixed Reagent by dissolving 0.52 g ascorbic acid in 10 ml distilled water and add 40 ml pre-mix. Add this solution to some distilled water blanks (do not digest) and read in the usual manner. The readings should be comparable to previous values. Mark the absorbance on the bottle to indicate it has been checked.
5. *Potassium persulfate* needs to be checked for contamination. When an old bottle is almost finished, prepare 3 distilled water blanks and add the new persulfate to these tubes. Process the normal way and read on the spectrophotometer. The readings should be comparable to previous values. Mark on the bottle that the persulfate has been checked. Do not check persulfate and ascorbic acid on the same sample. If the reading is high, check one other bottle. If this reading is also high, then likely that particular batch number of reagent is contaminated.

Method:

1. Dry samples at 105° C for 24 hours.
2. Pulverize and homogenize samples with clean glass rods.
3. Transfer tared sub-samples of 100 to 125 mg to screw cap tubes and add 50 mL of d-H_2O. Shake tube.
4. Add 0.4 grams potassium persulfate to each tube. Shake.
5. Prepare standards: Standards and blanks require potassium persulfate. Tighten caps. Tubes may be stored at 5° C for up to 14 days. When making standards, i.e. dilutions, rinse pipette with buffer and discard the buffer. Low

standards are 0, 25, and 50 µg / L. Unknown standards are 0, 25, 50, 100, 125, and 250 µg / L. Treat standards the same as samples, except there is no soil. Add 0.4 grams potassium persulfate.

6. For standards and blanks: rinse tubes and lids 2 times with distilled water before using. Add 50 ml to standard or blanks, then 0.4 grams potassium persulfate; place in autoclave and digest @ 235-240°C, 15-20 psi, (1.4 kg / cm^2) for 45 minutes on slow exhaust. Cool to room temperature by placing in cold water.
7. After cooling, shake the sample and place a 12-15 mL aliquot in a centrifuge tube.
8. Centrifuge at 2000 rpm for 10 minutes.
9. Using the supernatant, dilute with d H_2O to ratio of 1:5 before using. (Use 10 mL of supernatant + 40 mL d-H_2O.)
10. Colorimetric assay:
    a) Using the repeater pipette, add 5 ml mixed reagent to cooled, digested samples, standards and blanks.
    b) Let stand at room temperature for 20 minutes for color reaction to occur. The reaction involves formation of a blue potassium-molybdate complex, the intensity of which corresponds to amount of phosphorus in the original sample.
    c) Measure absorption at 885 nm. Readings for greater than 0.725 are no longer linear and need to be diluted. Zero the spectrophotometer using distilled water. Be sure that samples and standards are read using the same cells.

Calculations

$$P = \frac{\text{absorbance units - intercept}}{\text{slope}}$$

There is a computer program to help with the calculations.

References:

American Public Health Association et al. 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th Edition. American Public Health Association. Washington, D.C.

Rump, H. H., and H. Krist. 1988. *Laboratory Manual for the Examination of Water, Waste Water and Soil.* VCH Publishers, New York, New York.

Wetzel, Robert G. and Gene E. Likens. 1991. *Limnological Analysis, 2nd Edition.* Springer-Verlag, New York. p336-338.

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SOPS/phosphorus in soils

Phosphorus in soils--page3

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Total Dissolved Solids (SM-2540B)

Background:

Solids refer to matter suspended or dissolved in water or wastewater. Total solids include total suspended solids, the portion of total solids retained by a filter, and total dissolved solids, the portion that passes through the filter. Solids may affect water or effluent quality adversely in a number of ways. Waters with high total dissolved solids are of inferior quality because they are salty and may induce an unfavorable physiological reaction in the consumer. For these reasons, a limit of 500 mg dissolved solids/L is desirable for drinking water.

Apparatus:

Polyethylene or resistant glass acid washed bottles
Evaporating dishes of 100 mL capacity
Drying oven at 103 °C
Desiccator
Analytical balance
Magnetic Stirrer
Filter apparatus
Graduated cylinders

Collection and Storage of Samples:

Collect water samples in 1-liter acid washed Nalgene bottles. Store samples at 5 °C and analyze within 48 hours of collection.

Range of Assay: 0.1 mg to 200 mg

Procedure:

1. Pre-weigh dishes used for TDS by placing them in an oven at 103°C for 16 hours or overnight. Cool at room temperature for 25 minutes or in a desiccator and record weight.
2. If the conductivity of the sample is more than 500 µmhos/cm, use 50 mL sample; otherwise use 100 mL.
3. Filtering: Using a 0.45µm Millipore filter, filter slightly more than required amount of sample. Measure with a cut off graduated cylinder into pre-weighed evaporating dish. Run duplicates.
4. Carefully place dishes in oven set at 103°C and leave for 16 hours so that evaporation of the water can occur. Cool at room temperature for 25 minutes (or in a desiccator) and reweigh dishes.

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Calculation:

$$\text{mg/L TDS} = \frac{[(\text{mg wt. of dish \& residue}) - (\text{mg wt. of dish})] \times 1000}{\text{mL sample}}$$

Quality Control/Quality Assurance:

1. Highly mineralized water with a significant concentration of calcium, magnesium, chloride and/or sulfate may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.
2. Exclude large, floating particles or submerged agglomerates of non-homogeneous material from the sample if it is determined that their inclusion is not desired in the final results.
3. Disperse visible floating oil and grease with a blender before withdrawing a sample portion for analysis.
4. Use clean, acid-washed glassware with accurate gradation.
5. Check accuracy of balance periodically using standard weight set.
6. Check effectiveness of TDS procedure quarterly or when using new equipment. Prepare a standard series (0, 25, 50, 100, 200 mg $CaCO_3$/L) using standard $CaCO_3$ solution. Filter, dry and weigh triplicate 50 ml samples of each dilution. Calculate mean and coefficient of variation (std.dev./mean*100) and compare with expected weight.

   Standard Ca solution

   a) Standard $CaCO_3$ may be purchased or
   b) Weigh 1.000 g anhydrous $CaCO_3$ powder into a 500 mL Erlenmeyer flask. Place a funnel in the flask neck and add a little at a time, 1 + 1 HCl until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3 N NH_4OH or 1 + 1 HCl as required. Transfer quantitatively and dilute to 1000 mL with distilled water. 1 mL = 1.00 mg $CaCO_3$.

References:

American Public Health Association, et al. 1995. *Standard Methods for the Examination of Water and Wastewater, 19th Edition.* American Public health Association. Washington, D. C. pp.2-53-2-54.

Lind, O. T. 1985. *Handbook of Common Methods in Limnology. 2nd Edition.* Kendal/Hunt Publishing Co. Dubuque, Iowa. pp.95-99.

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13 Sept 99
2 April 04

Total Dissolved Solids--page2

ORGANIC CARBON, TOTAL

Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680
Dissolved 00681

1. Scope and Application
   1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.
   1.2 The method is most applicable to measurement of organic carbon above 1 mg/1.
2. Summary of Method
   2.1 Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion or wet chemical oxidation. The CO_2 formed can be measured directly by an infrared detector or converted to methane (CH_4) and measured by a flame ionization detector. The amount of CO_2 or CH_4 is directly proportional to the concentration of carbonaceous material in the sample.
3. Definitions
   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:
      A) soluble, nonvolatile organic carbon; for instance, natural sugars.
      B) soluble, volatile organic carbon; for instance, mercaptans.
      C) insoluble, partially volatile carbon; for instance, oils.
      D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.
   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

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Issued 1971
Editorial revision 1974

4. Sample Handling and Preservation

4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples.
NOTE 1: A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.

4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified (pH ≤ 2) with HCl or H_2SO_4.

5. Interferences

5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.

5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.

6. Apparatus

6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.

6.2 Apparatus for total and dissolved organic carbon:

6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.

6.2.2 No specific analyzer is recommended as superior.

7. Reagents

7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.

7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.
NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.

7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.

7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as Bias, %	Accuracy as Bias, mg/liter
4.9	3.93	+15.27	+0.75
107	8.32	+ 1.01	+1.08

(FWPCA Method Study 3, Demand Analyses)

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 2574–79, p 469 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 532, Method 505, (1975).

The Michigan Water Research Center
Central Michigan University
Chain of Custody Record

Survey				Samplers			
Station Number	Station Location	Date	Time	Sample Type	ID No.	No. of Containers	Analysis Required
*							

Relinquished by:		Received by: Date:
Relinquished by:		Received by: Date:
Relinquished by:		Received by: Date:
Relinquished by:		Received by: Date:
Dispatched by:	Date/Time	Received by mobile lab. For field analysis:
Method of shipment:		Tracking Numbers:

*Use back of page for more samples

The Michigan Water Research Center
Central Michigan University
Chain of Custody Record

Station Number	Station Location	Date	Time	Sample Type	ID No.	No. of Containers	Analysis Required

2

Appendix C

Pricing Agreement

Article 1, Attachment A
Pricing

3-year project total = $238,375

Budget: 3/1/06 - 2/28/07		
Budget Category		Cost
Administration		
Project Director salary and benefits (2 weeks, full time)	$	4,729
Laboratory Analyses		
Water chemistry	$	41,545
Sediment chemistry	$	2,360
Zooplankton	$	5,753
Phytoplankton	$	13,423
Subtotal	$	63,080
Special Research Projects		
Graduate Research Assistant salary and benefits (6 mo.)	$	11,172
TOTAL	$	78,980

Budget: 3/1/07 - 2/28/08		
Budget Category		Cost
Administration		
Project Director salary and benefits (2 weeks, full time)	$	4,870
Laboratory Analyses		
Water chemistry	$	41,545
Sediment chemistry	$	2,360
Zooplankton	$	5,753
Phytoplankton	$	13,423
Subtotal	$	63,080
Special Research Projects		
Graduate Research Assistant salary and benefits (6 mo.)	$	11,588
TOTAL	$	79,538

Budget: 3/1/08 - 2/28/09	
Budget Category	Cost
Administration	
Project Director salary and benefits (2 weeks, full time)	$ 5,016
Laboratory Analyses	
Water chemistry	$ 41,545
Sediment chemistry	$ 2,360
Zooplankton	$ 5,753
Phytoplankton	$ 13,423
Subtotal	$ 63,080
Special Research Projects	
Graduate Research Assistant salary and benefits (6 mo.)	$ 11,760
TOTAL	$ 79,856

Laboratory sample costs

Parameter	# Samples	Detection Limit	Unit Cost	Total Cost
Total Phosphorus	4400	0.3 ppb	$ 7.67	$33,748.00
Total Dissolved Phosphorus	200	0.3 ppb	$ 9.44	$ 1,888.00
NO2+NO3	200	0.5 ppb	$ 12.39	$ 2,478.00
Chlorophyll a	175	0.1 ppb	$ 14.75	$ 2,581.25
TDS	40	0.001 g	$ 5.90	$ 236.00
Calcium	40	0.5 ppm	$ 9.44	$ 377.60
pH & alk	40	0.1 unit, 1 ppm	$ 5.90	$ 236.00
Phytoplankton	175	---	$ 76.70	$13,422.50
Zooplankton	75	---	$ 76.70	$ 5,752.50
% water	80	0.001 g	$ 11.80	$ 944.00
Sed TP	80	0.3 ppb	$ 17.70	$ 1,416.00
			Total	$63,079.85
			water chem	$41,544.85
			sediments	$ 2,360.00