Handbook of Analytical Methods

Compiled by the Technical Committee of the Pond Dynamics/Aquaculture Collaborative Research Support Program

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Preface

The analytical methods included in this handbook are those recommended for use by the Technical Committee of the Pond Dynamics/Aquaculture CRSP. Selection of methods to be included in the handbook is based on their use by participating researchers, and on their comments and observations. The selections are not based on actual comparisons between existing methods and no claim is made as to the characteristics of the methods included with respect to other methods. The methods have been compiled from standard references and these are indicated throughout the handbook.

We ask that you inform the Materials and Methods Subcommittee of new methods that you may be using, so that they may be included in future updates to the handbook. If you have any additional comments or suggestions please address them to:

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At the time of the first compilation of this handbook in 1990-91, the subcommittee consisted of the following members:

Raul. H. Piedrahita, *University of California Davis (Chairman)*
Claude Boyd, *Auburn University*
James Szyper, *University of Hawaii*

The methods are organized in five sections representing the type of samples that are analyzed. Within each section, the methods are listed alphabetically. Pages are not numbered and the handbook is being distributed in loose-leaf form to facilitate updating and revising methods as techniques and equipment evolve.

We wish to emphasize that this document is intended for internal use within the PD/A CRSP and is not for general distribution.

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Section One

FISH/SHRIMP

Fish/Shrimp Group Weight

Fish/Shrimp Mean Length per Ind.

Fish/Shrimp Mean Weight per Ind.

Fish/Shrimp Production

Tilapia Reproduction

VARIABLE Fish/Shrimp Group Weight

DATE October 1990 **VERSION** 1

SAMPLE Fish/Shrimp **REPORTING UNITS** kg, No. Individuals

METHOD CRSP.

REFERENCE CRSP Work Plans.

INSTRUMENTATION

Scale accurate to 1 g.

PROCEDURE

Collect a cast net (or seine net) sample equivalent to 10% of the initial stock from each pond and weigh as a group. Indicate number of individuals in the sample.

If substantial variation is observed or if reproduction is suspected, divide the sample into centimeter groups, count and weigh each group. Sex the fish and remove all female tilapia observed and replace them with males of similar weight. Any animals collected other than those stocked should be counted, weighed, measured and discarded. Record observations on reproduction or fish health.

VARIABLE Fish/Shrimp Mean Length per Ind.

DATE October 1990 **VERSION** 1

SAMPLE Fish/Shrimp **REPORTING UNITS** cm

METHOD CRSP.

REFERENCE CRSP Work Plans.

INSTRUMENTATION

Ruler or calipers accurate to 0.1 cm.

PROCEDURE

For a representative 10% subsample of the sample (equivalent to approximately 1% of initial stock) used for determination of the group weights, measure the fork length and count individuals. Express as mean length per individual.

VARIABLE Fish/Shrimp Mean Weight per Ind.

DATE October 1990 **VERSION** 1

SAMPLE Fish/Shrimp **REPORTING UNITS** g

METHOD CRSP.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

Scale accurate to 0.1 g.

PROCEDURE

For a representative 10% subsample of the grab sample (equivalent to approximately 1% of initial stock) used for determination of group weights, weigh and count individuals. Express as mean weight per individual.

VARIABLE Fish/Shrimp Production

DATE October 1990 **VERSION** 1

SAMPLE Fish/shrimp **REPORTING UNITS** # Individuals; kg

METHOD Count, weight.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

Scale accurate to 1 g.

PROCEDURE

Fish and shrimp stocks will be weighed as a group at stocking and harvest. A 10% sample will be weighed and counted to calculate the total number of individuals stocked or harvested. Any fish other than tilapia will be counted by species then weighed.

VARIABLE Tilapia Reproduction

DATE October 1990 **VERSION** 1

SAMPLE Fish/Shrimp **REPORTING UNITS** g, No. Individuals

METHOD CRSP.

REFERENCE CRSP Work Plans.

INSTRUMENTATION

Scale capable of weighing to 1 g.

PROCEDURE

Concurrent with assessment of growth of stocked fish, note the number and collective weight of any fry collected. These fry are to be discarded and not returned to the experimental ponds.

Section Two

POND

Evaporation

Inflow

Map

Pond Depth

Seepage

Surface Area

Volume

VARIABLE Evaporation

DATE October 1990 **VERSION** 1

SAMPLE Pond **REPORTING UNITS** $mm \cdot d^{-1}$

METHOD Evaporation pan.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

Floating evaporation pan.

PROCEDURE

Floating evaporation pans are expected to provide the best estimate of the evaporation rate in the ponds. The pans should be of clear acrylic glass (plexiglass), polycarbonate (lexan), or polypropylene. The inside dimensions of the pans should be approximately 0.70 m long, 0.70 m wide and 0.25 m deep with vertical sides. The pan should be floated and tethered on the pond surface so that it stays leveled.

Water should be added to the pan and changes in water depth should be monitored over time by noting the initial and final water depth in the pan. The average evaporation rate for the period can be estimated from the change in water depth and the duration of the time interval to obtain mm/d. Three pans should be installed and the results from them averaged. The pans should be maintained clean of excessive biological fouling that may darken the pan walls.

In the event of rainfall, correct readings using the rain gauge measurements.

VARIABLE Inflow

DATE October 1990 **VERSION** 1

SAMPLE Pond **REPORTING UNITS** $m^3 \cdot d^{-1}$

METHOD Various

REFERENCE Wood, J. W. 1974. Diseases of Pacific Salmon: Their Prevention and Treatment. State of Washington, pp. 71-77.

INSTRUMENTATION

Various, as described below.

PROCEDURE

Surface Inflow/Outflow should be determined using procedures described by Wood, or comparable methods.

INFLOW:

There are several ways to calculate the inflow into a pond. The following represent several methods of determining the rate of water inflow.

Direct Measurement – This may be done by simply placing a container of known volume under the spout and determining the time it takes to fill the container.

Example: A 50-gallon drum is filled in 25 seconds. 50 gals/25 secs x 60 secs = 120 gpm

Measurement from Table "Flow from Pipes" – This table (Table 2) was prepared by our engineers for 3 inch and 4 inch diameter pipes running full of water. For efficient use of this table, you should make up a wooden square, much like a carpenters square, with one leg (the Y leg) being either 9 or 16 inches long; the inside edge of the other leg (the X leg) should be marked off in 1 or ½ inch increments and be long enough to measure any water flow that you encounter at your hatchery.

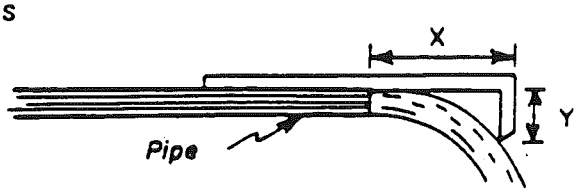
In use, the X leg of the square is placed on top of the pipe in a position where the end of the Y leg just touches the top of the extruding "jet" of water. Next, refer to the table under the correct column listing the diameter of pipe and length of Y that you used; go down the column until you find the X reading closest to the one that you measured; then read across to the appropriate gpm column to determine flow in gpm.

Example: The diameter of the pipe is 4 inches and the length of the Y leg of the square is 16 inches. When the end of the Y leg just touches the discharging water, the X leg extends out 24 inches. Referring to the appropriate column, it may be seen that the flow is between 240 and 250 gpm.

PROCEDURE Cont. **VARIABLE Inflow**

Table - 2

FLOW FROM PIPES



Formula – $G = \frac{2.56\ D^2\ X}{\sqrt{Y}}$ $X = \frac{G\sqrt{Y}}{2\ 56\ D^2}$

D = DIA. OF PIPE IN INCHES

G = GALLONS PER MINUTE (gpm)

4" Pipe Y = 16"		*3" Pipe Y = 16"*		*4" Pipe Y = 9"*		*3" Pipe Y = 9"*	
X"	*gpm*	*X"*	*gpm*	*X"*	*gpm*	*X"*	*gpm*
4.88	50	8.68	50	3.66	50	6.51	50
5.86	60	10.42	60	4.39	60	7.81	60
6.84	70	12.15	70	5.13	70	9.11	70
7.81	80	13.89	80	5.86	80	10.42	80
8.79	90	15.62	90	6.59	90	11.72	90
9.77	100	17.36	100	7.32	100	13.02	100
10.74	110	19.10	110	8.06	110	14.32	110
11.72	120	20.83	120	8.79	120	15.23	120
12.70	130	22.57	130	9.52	130	16.93	130
13.67	140	24.30	140	10.25	140	18.23	140
14.65	150	26.04	150	10.99	150	19.53	150
15.62	160	27.78	160	11.72	160	20.83	160
16.60	170	29.51	170	12.45	170	22.13	170
17.58	180	31.25	180	13.18	180	23.44	180
18.55	190	32.98	190	13.91	190	24.74	190
19.53	200	34.72	200	14.65	200	26.04	200
20.51	210	36.46	210	15.38	210	27.34	210
21.48	220	38.19	220	16.11	220	28.64	220
22.46	230	39.93	230	16.85	230	29.95	230
23.43	240	41.66	240	17.58	240	31.25	240
24.41	250	43.40	250	18.31	250	32.55	250
25.39	260	45.14	260	19.04	260	33.85	260
26.37	270	46.87	270	19.77	270	35.15	270
27.34	280	48.61	280	20.51	280	36.46	280
28.32	290	50.34	290	21.24	290	37.76	290
29.30	300	52.08	300	21.97	300	39.06	300
1" x = 10.2 gpm		1" x = 5.89 gpm		1" x = 13.7 gpm		1" x = 7.7 gpm	

X in every case equals inches and parts of inches.

PROCEDURE Cont. **VARIABLE Inflow**

Pipe Larger or Smaller than 3 or 4 Inches – The table is made up for only 3 inch and 4 inch diameter pipes but the formula at the top of the page may be used for calculating the discharge from any diameter pipe or for X measurements in excess of those listed in the table.

Example: A pipe is 6 inches in diameter and running full of water. A square with a Y leg 16 inches long just touches the top of the discharging water when the X leg extends out 30 inches.

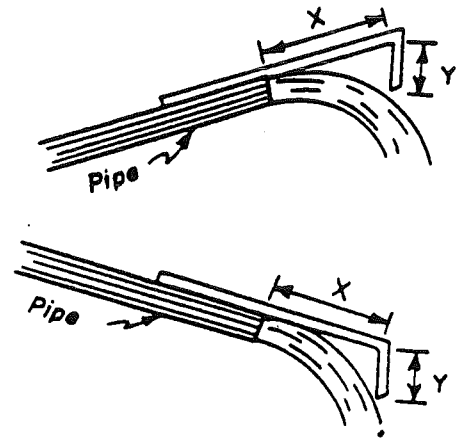
D = 6 inches
X = 30 inches
Y = 16 inches
G = Flow in gpm

$$G = \frac{2.56\,D^2\,X}{\sqrt{Y}} = \frac{(2.56)\,(6 \times 6)\,(30)}{\sqrt{16}} = \frac{(2.56)\,(36)\,(30)}{4}$$

G = 691 gpm

Note: A 6 inch pipe running full will deliver 4 times the flow of a 3 inch pipe running full at the same X and Y.

Pipes not Level – The "Flow from Pipes" table (Table 2) may be used even if the pipe is not horizontal (level). To be used correctly, however, you cannot use a square; the X leg is placed on top of the pipe but the Y leg must extend directly down to the top of the water jet as in the diagrams below. The X reading is determined and the formula or table is used in the same manner as for a level discharge pipe.



Pipes not Full – The table (Table 2) and formula are only applicable for direct use if the pipe is running full of water. They may still be used with a pipe running partly full if a factor is used to compensate for the reduced flow resulting from the pipe only being partially filled. The following table, entitled "Effective Area Factor" table (Table 3), is used for any size pipe to find the effective area factor (EAF) after determining the ratio of the freeboard (F) to the diameter (D) of the pipe. Freeboard, in this case, is the measurement in inches of that portion of the diameter of the pipe that is not full of water. Determine X and Y in the usual manner, find the theoretical flow (as if the pipe was running full) from the "Flow from Pipes" table or formula; then multiply the theoretical flow by the effective area factor (EAF) to find the actual flow.

Example: A pipe is 4 inches in diameter and running with only 3 inches of water, Y is 16 inches and X is 24 inches. The free board (F) is 1 inch (4 inches minus 3 inches); therefore, the ratio of F to D (F/D) is 0.25. Looking at the "Effective Area Factor" table (Table 3) you can see that the EAF for an F/D of 0.25 is 9.805. In the "Flow from Pipes" table (Table 2), the flow for X = 24 inches and Y = 16 inches would be about 246 gpm if the pipe was full.

246 gpm X 0.805 = 198 gpm

Table 3
"Effective Area Factor" Table. For use in the calculation of flows thru pipes running partially full of water.

Ratio F/D	*EAF*	*Ratio F/D*	*EAF*	*Ratio F/D*	*EAF*
0.00	1.000	0.23	0.826	0.37	0.664
0.10	0.948	0.24	0.816	0.38	0.651
0.11	0.939	0.25	0.805	0.39	0.639
0.12	0.931	0.26	0.793	0.40	0.627
0.13	0.922	0.27	0.782	0.41	0.614
0.14	0.914	0.28	0.770	0.42	0.602
0.15	0.905	0.29	0.759	0.43	0.589
0.16	0.896	0.30	0.747	0.44	0.577
0.17	0.886	0.31	0.735	0.45	0.564
0.18	0.877	0.32	0.723	0.46	0.551
0.19	0.867	0.33	0.712	0.47	0.538
0.20	0.858	0.34	0.700	0.48	0.526
0.21	0.847	0.35	0.688	0.49	0.513
0.22	0.837	0.36	0.676	0.50	0.500

Note: For 6 inch pipes, the following factors may be used instead of Table 3 for obtaining flows from the 3 inch pipe table:

Full 6" pipe – 4 times 3" pipe full
5/6 Full 6" pipe – 3.5 times 3" pipe full
3/4 Full 6" pipe – 3.4 times 3" pipe full
2/3 Full 6" pipe – 2.8 times 3" pipe full
1/2 Full 6" pipe – 2.0 times 3" pipe full

PROCEDURE Cont. **VARIABLE Inflow**

Table 4 DISCHARGE IN CUBIC FEET PER SECOND (cfs) AND GALLONS PER MINUTE (gmp) OVER SHARP-CRESTED WEIRS, BY THE FRANCIS FORMULA: $Q = 3.33\ H^{3/2}$

(Adapted from King's "Handbook of Hydraulics", 4th ed., Table 36)

Depth on Crest *(inches)*	Discharge per Foot of Weir Crest *cfs*	*gpm*	Depth on Crest *(inches)*	Discharge per Foot of Weir Crest *cfs*	*gpm*
1/2	.03	13	6	1.18	528
3/4	.05	24	1/4	1.25	562
1	.08	36	1/2	1.33	596
1/4	.11	50	3/4	1.41	631
1/2	.15	66	7	1.48	665
3/4	.19	83	1/4	1.56	702
2	.23	102	1/2	1.65	738
1/4	.27	122	3/4	1.73	776
1/2	.32	142	8	1.81	814
3/4	.36	164	1/4	1.90	853
3	.42	187	1/2	1.98	890
1/4	.47	211	3/4	2.07	930
1/2	.53	236	9	2.16	971
3/4	.58	262	1/4	2.25	1,012
4	.64	287	1/2	2.35	1,053
1/4	.70	315	3/4	2.44	1,096
1/2	.77	343	10	2.53	1,136
3/4	.83	372	1/4	2.63	1,179
5	.90	402	1/2	2.73	1,223
1/4	.97	433	3/4	2.82	1,268
1/2	1.03	463	11	2.92	1,312
3/4	1.10	495	1/4	3.03	1,358
			1/2	3.12	1,401
			3/4	3.23	1,448
			12	3.33	1,495

The above table is to be used for measuring the discharge of water over damboards or other similar weirs. For practical purposes the width of a damboard fits the description of a sharp-crested weir. The discharge must be free falling to use this table. In practice the depth on the weir crest is measured to the top of the curl (see diagram) on the leading edge of a yardstick when the yardstick is placed on the leading edge of the top damboard. After measuring the depth on the crest, refer to the table and multiply the flow in gpm by the length of the weir in feet.

Example: A weir is 41 inches long and the depth on the crest is 1 3/4 in.
The flow is:
3.42 X 83 = 284 gpm

(41 inches = 3.42 feet)

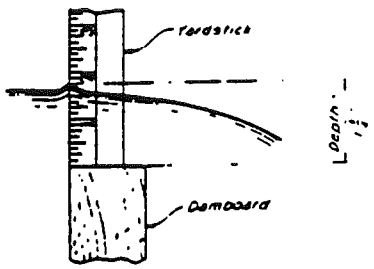


TABLE 4.

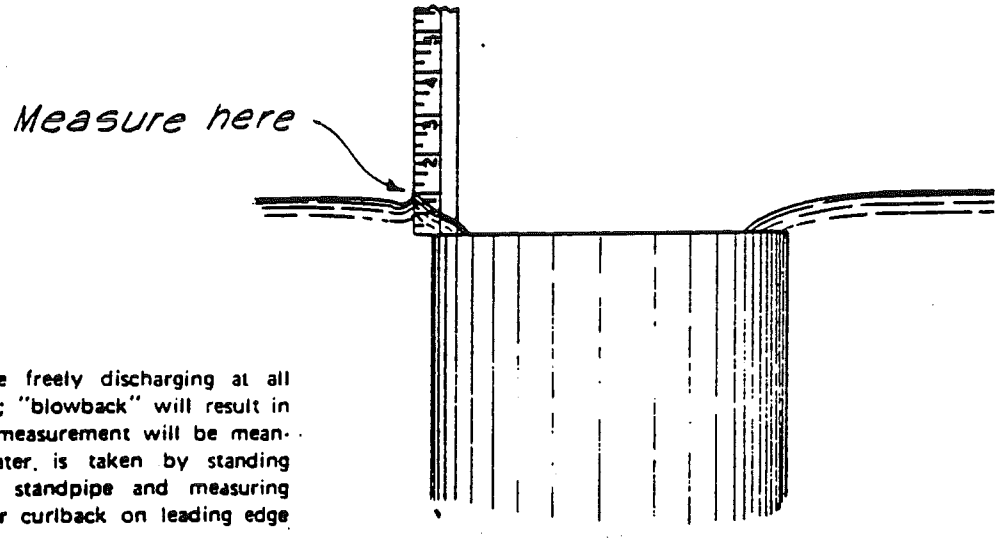
PROCEDURE Cont. **VARIABLE Inflow**

WATER DISCHARGE DOWN DRAIN SUMPS

12" diam = 3.14' crest
10" diam = 2.62' crest
8" diam = 2.09' crest
6" diam = 1.57' crest

Flow in gallons per minute

Depth on Crest *(inches)*	*12" Sump*	*10" Sump*	*8" Sump*	*6" Sump*
1/2	42	35	28	21
3/4	70	59	47	35
1	113	94	75	57
1/8	141	118	94	70
1/4	155	129	103	78
3/8	183	153	122	92
1/2	211	176	141	106
5/8	240	200	160	120
3/4	268	224	179	134
7/8	296	247	197	148
2	324	271	216	162
1/8	352	294	235	176
1/4	381	317	254	190
3/8	423	353	282	211
1/2	451	376	301	225
5/8	479	400	320	240
3/4	522	435	349	261
7/8	564	470	376	282
3	592	494	394	296



Note: Water must be freely discharging at all times down the sump; "blowback" will result in increased depth and measurement will be meaningless. Depth of water is taken by standing yardstick on lip of standpipe and measuring depth to top of water curlback on leading edge of yardstick.

TABLE 5

PROCEDURE Cont. **VARIABLE Inflow**

Measurement in Open Channels and Streams – An approximation of fair accuracy of the flow in a channel or stream can be made with relatively little effort. You will need to measure off a length of the stream, preferable 100 ft; determine the average depth through this section (consider the entire length and width of the stream); and, determine the average width of this section of stream. You will then need to time the passage of a wood chip or float through the length of this section. The following formula is then used to calculate the volume of flow in cu ft/sec (cfs).

$$Q = \frac{WDaL}{T}$$

Where: Q = volume of flow in cubic feet/sec (cfs)
W = average width of stream section in feet
D = average depth of stream section in feet
a = constant factor depending on type of stream bottom, use:
a = 0.9 for smooth sand, etc.
a = 0.8 for rough rocks, etc.
L = length of stream section measured
T = time in seconds for wood chip or float to travel the measured distance

Example: Suppose we find only a 50-foot length of stream that appears to have a uniform channel. By a series of measurements we determine the average depth to be 2 feet and the average width to be 15 feet. The bottom of the stream is mostly rocks and coarse gravel. We drop a wood chip at the upper end of the 50-foot section and find that it takes 65 seconds for the chip to reach the lower end of the section. Therefore:

W = 15 ft
D = 2 ft
a = 0.8
L = 50 ft
T = 65 secs

$$Q = \frac{15 \text{ ft} \times 2 \text{ ft} \times 0.8 \times 50 \text{ ft}}{65 \text{ secs}} = \frac{1200 \text{ cu ft}}{65 \text{ secs}}$$

= 18 cfs (approx)

Measurement from Pond Refill Time – The rate of water inflow to a pond may also be measured by determining the time necessary to refill a portion of the pond's volume. The method is valid providing no water leaves the pond during the refill period; if the pond leaks, you can't use this method. This method, however, may be the only one available to you if the water enters the pond through the upwelling chamber or through a flume or ditch at ground level.

It is necessary that the surface area of the pond be calculated. The pond is then drained down to a predetermined level below the top of the standpipe then allowed to refill. From the volume of water necessary to refill the discharged water, and the time necessary to do so, it is easy to calculate the rate of inflow.

Example: In a standard pond 80 ft long by 20 ft wide, the water is drained down 1 ft below the top of the standpipe. It takes 35 minutes to replace this top 1 ft of water.

Area of pond: 80 ft x 20 ft = 1,600 sq ft

Volume of top 1 ft: 1 ft x 1,600 sq ft = 1,600 cu ft; or, 7.48 gals/cu ft x 1,600 cu ft = 11,968 gals

Rate of inflow: 11,968 gals/35 mins = 342 gpm

VARIABLE Map

DATE October 1990 **VERSION** 1.

SAMPLE Pond **REPORTING UNITS**

METHOD Plane table.

REFERENCE Lind, O.T. 1979. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis.

INSTRUMENTATION

Plane table, compass, drawing equipment, measuring tape, stakes.

PROCEDURE

The detailed procedure is described by Lind and is attached. The plane table method should be used if the pond shape is irregular. If the ponds are square or rectangular, map preparation can be done from measurements obtained with tapes and surveying equipment such as a transit.

PROCEDURE Cont. **VARIABLE Map**

MAPPING

Mapping a pond or small lake by plane table method

The mapping of lakes is usually beyond the capability of the limnologist, who must rely on maps made by well-equipped survey teams. Often aerial photographs with known scale will suffice. However, small ponds (generally less than 5 hectares) may be accurately mapped with a minimum of equipment. Whenever possible, mapping in winter on sound ice is preferable. Welch (1948) includes an extensive section on map methods from simple to complex.

Apparatus

Plane table and tripod
Alidade (crude alidade may be made by setting pins in opposite ends of wooden ruler for "sights")
Compass
Ruler, graduated in millimeters
Map paper
Hard-lead pencils
Round-headed map pins
Steel measuring tape, approximately 30 to 50 m long
2 steel stakes, approximately 0.5 m long
Wooden stakes, approximately 1 m long (25 to 100, depending on size of pond)
Sledgehammer
Stadia rod or other painted pole at least 2 m long
Metal-core clothesline rope, of sufficient length to reach across pond, marked in 1 and 5 m increments
Small boat or canoe
Depth sounding line or electronic depth sounder

Procedure

1. Select the longest relatively straight section of shoreline for establishing a base line.

2. Drive steel stake near shore for one end of base line. Most of pond should be visible from this point. Repeat for other end of base line at a point at least 30 m from first stake and approximately the same distance from shore as the first. Make the base line as long as possible (Fig. 1).

3. Drive a wooden stake at water's edge at every major change in shoreline configuration. Maximum distance between any two wooden stakes should probably not exceed 20 m. Drive wooden stakes just deep enough to stay in place for mapping. Steel stakes on base line are permanent and should be driven so only 2 to 5 cm protrude. Tie a cloth "flag" to top of every fifth stake to facilitate counting.

4. Attach map paper to plane table. Set up tripod directly over one metal stake at end of base line, and plumb table center to the stake. Level table, and align one edge with true north-south line. Lock table in place. Draw true north arrow in corner of map; also indicate magnetic north (Fig. 2).

PROCEDURE Cont. **VARIABLE Map**

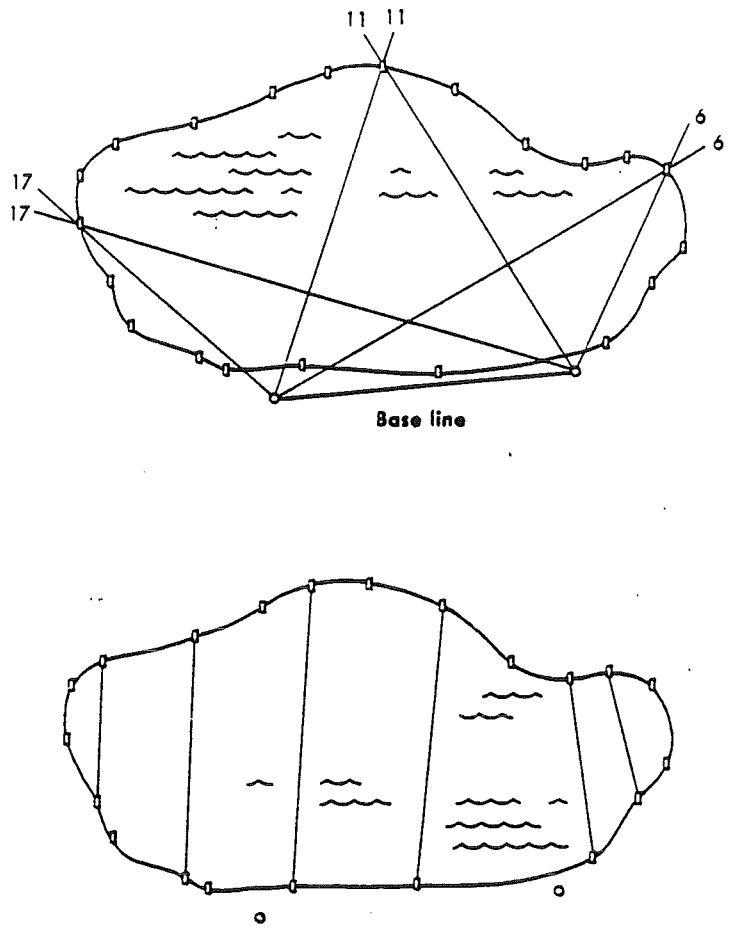


Fig. 1. Plane table map *(top)* showing position of numbered shoreline stakes and the base line. Distances for three shoreline points as determined by triangulation from the ends of the base line are shown. In a small lake or pond, bottom contours may be determined by sounding along a calibrated rope stretched between approximately opposite shoreline stakes *(bottom)*.

5. Judge shape of pond and length of base line, and determine appropriate scale. Record scale on map near north-south arrow.

6. Determine position on map that represents end of base line where table is set up. Mark this on map by firmly setting map pin in table. The pin must not move. Check again to be certain that all parts of pond will fall on map according to scale selected and position of pin.

7. Have flagman set stadia rod vertically on steel rod at other end of base line. Place zero mark on alidade against pin, and sight through alidade down base line to line up with stadia rod (Fig. 2). Draw base line on map along edge of alidade for the appropriate length as determined by scale. *Be careful not to bump the plane table at any time.*

PROCEDURE Cont. **VARIABLE Map**

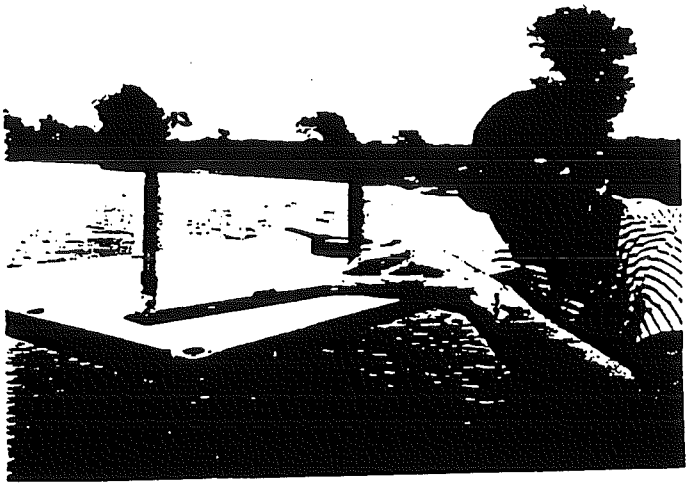


Fig. 2. Student using an open-sight alidade and a plane table to map a small pond. A compass is used to ascertain north orientation of the map. The edge of the alidade at the end nearest the student is always placed against a map pin firmly set in the table. This pin is the map position corresponding to the base line stake over which the table is centered.

8. Now in a similar manner, progressively sight alidade around the pond on each wooden stake (flagman holds stadia rod just behind the stake), and draw line along edge of alidade. Number each line consecutively. The fifth line and every multiple of 5 should be sighted on a stake with flag. Any deviation indicates a missed stake. After each flagged wooden stake has been sighted and the line drawn, resight on other end of base line. If this sighting does not coincide with original line, the table has been moved and another series must be taken.

9. After a complete circuit has been made, move and reset table over stake at other end of base line. Set pin in map at this end of base line and align table by sighting back down to other end of base line. Repeat step 8.

10. The point at which corresponding numerical lines intersect is the position of each wooden stake marking the shoreline. Connect these points with a smooth line. By doing this in the field, you are able to include minor changes in shoreline. Also indicate position of obvious features in the water (logs, weed beds, and others).

11. It is more difficult to map bottom contours for the purpose of morphometric calculations. The problem is the accurate plotting on the map of the position of the sounding team. On smaller ponds, mapping bottom contours may be done as follows: starting at one end of the pond, stretch the calibrated rope between any two known wooden stake locations. Represent this by a light line on the map.

PROCEDURE Cont. **VARIABLE Map**

12a. The sounding team in the boat moves along this line, taking soundings at appropriate intervals from one shore to the other (intervals vary depending on amount of change in bottom contours). With the use of the scale, a point is placed at the proper position on the line, and the measured depth written in at this point (Fig. 1).

12b. An alternate procedure, more suitable for larger bodies of water but less precise so far as exact position on the lake is concerned, is the use of timed echo soundings. Echo sounders are becoming increasingly available, especially as "fish finders." The boat's pilot makes a line of sight between two stakes on opposite banks and sets the boat at a constant low speed. It is important that he make every effort to maintain a straight line and constant speed between the two points. A second person calls out regular time intervals—for example, every 30 seconds—and a third person reads and records the depth shown on the echo sounder at that time. The total time taken to transect the lake is recorded. Thus the total time for distance is known, and time for any timed increment can be calculated. Although this procedure is less precise, it has a compensating advantage in that it allows many more soundings to be taken, which more accurately gives the shape of the lake basin and allows for the plotting of more closely spaced bottom contours. This increases the accuracy of the lake volume calculation.

13. Repeat the chosen sounding procedure for a parallel series of lines across the pond.

14. Determine degree of bottom development to be shown (1 m intervals are often used, but intervals may be more or less). Draw in contour lines by connecting the appropriate points and the lines. For shape between points, parallel the shoreline.

15. Label map and include date and names of map crew. Roll up map (do not fold).

16. Return map to laboratory; retrace shoreline and contours in permanent ink. Clean up other working lines with soft eraser.

Direct measure modification of plane table method

The direct measure modification of the plane table method is suited for ponds of regular outline and open water. It is simple, and depth soundings may be taken simultaneously if the work party is of sufficient size.

Apparatus

Same as for plane table method plus the following:

Good quality ruler; meter stick cut off at 60 to 70 cm is convenient length

Steel-core clothesline rope, of sufficient length to reach across pond's longest dimension, marked in 1 and 5 m increments.

If approximate size of pond is known before going to field, previously prepared table of scale conversions of meters to millimeters will facilitate time spent in field

A small boat or canoe may be necessary if obstructions are present

PROCEDURE Cont. **VARIABLE Map**

Procedure

1. Select a base point that is relatively open of vegetation and from which all parts of the pond may be seen. Drive iron stake and center drawing board over this stake. All work will be done from this location.
2. Prepare board for drawing as in plane table procedure.
3. Set out shoreline stakes as in plane table procedure.
4. Select point on paper to correspond with plane table setup over iron stake, and set map pin.
5. Use alidade and take sightings on first stake to left. Draw light line along alidade from base point out to near edge of paper.
6. Place zero end of calibrated line on iron stake, and measure distance to the sighted stake. Convert this distance to scale in millimeters; using ruler, measure out from base point the corresponding distance on the line, and mark point. Thus location and distance of first stake have been determined.
7. Repeat step 6 for all remaining stakes.
8. Connect the points, filling in detail from observation.
9. If the map party is of sufficient size, a boat sounding team may work alongside the measuring rope, taking soundings and distance between base point and stake. Depths called out may be marked on map at proper distance from base point (see following section on morphometry).

VARIABLE Pond Depth

DATE October 1990 **VERSION** 1

SAMPLE Pond **REPORTING UNITS** m

METHOD

REFERENCE

INSTRUMENTATION

No type specified.

PROCEDURE

Install staff gauge in each pond and read to the nearest 0.5 cm before restoring a recommended pond depth.

VARIABLE Seepage

DATE October 1990 **VERSION** 1

SAMPLE Pond **REPORTING UNITS** $cm \cdot d^{-1}$

METHOD Water balance.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

None required.

PROCEDURE

Determine seepage from a 24 h water balance, when there is no rainfall, and no inflow or outflow into or from the pond:

Seepage (cm/d)={ (Initial Depth (cm) - Final Depth(cm)) - Evaporation(mm/d) • (0.1 cm/mm) }

Where:

Initial Depth = water depth at the initiation of the 24 h period over which infiltration is being measured.

Final Depth = water depth at the end of the 24 h period over which infiltration is being measured.

VARIABLE Surface Area

DATE October 1990 **VERSION** 1

SAMPLE Pond **REPORTING UNITS** m^2

METHOD Planimeter.

REFERENCE Lind, O.T. 1979. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis.

INSTRUMENTATION

Polar Planimeter.
Scale

PROCEDURE

If the pond is of regular shape (i.e. rectangular or square), the area may be obtained by measuring the length (m) and width (m) at the various depths and multiplying the values.

Area estimates can be made otherwise from maps prepared for different water depths. The simplest method of accurately establishing the contours is to adjust the water level of the pond to the desired contour depth for taking the measurements and drawing the maps. The areas can be measured with the planimeter. If a planimeter is not available, the map may be traced on good quality paper, the outline of the pond contours may be cut out carefully, and the paper weighed. The contour weight can be compared to the weight of a known area. A third method consists of drawing the contours on a square-grid paper, counting the number of squares covered by each contour, and converting to the appropriate scale by multiplying by the scaled area of each square.

PROCEDURE Cont. **VARIABLE Surface Area**

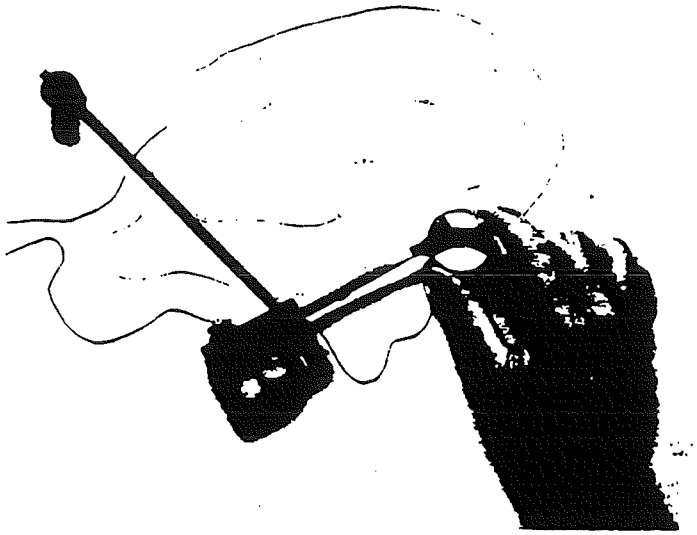


Fig. 3. Compensating polar planimeter used to determine areas. Here the planimeter is tracing the shoreline to determine the total area of a pond drawn by the plane table method.

Area by polar planimeter

Whenever instrumentation is available, the polar planimeter method is preferred. The polar planimeter (Fig. 3) is a delicate instrument, and care must be taken whenever it is used. Read over the directions supplied by the manufacturer for the instrument in use. Be sure to observe instructions regarding direction of tracing (usually clockwise) and minimum and maximum allowable angles of the tracing arm.

1. Prepare map by placing it on a hard, smooth surface. Tape it in place.
2. Most maps will be too large to be covered by one cycle of the planimeter. These maps must be ruled off into halves or smaller segments, and the areas of each summed for the total.
3. Check the calibration of your instrument on the map paper being used. Use calibration device supplied by manufacturer, or carefully rule off a known area and trace with planimeter three times.
4. Proceed with actual planimetry by tracing outline of lake or bottom contours, following carefully the manufacturer's directions.

Area by cut and weight

1. Lightly trace map outline with bottom contours onto a good grade of paper.
2. From an area outside traced area, cut out a square of known area (a 10 cm × 10 cm square is usable) and weigh this piece of paper. Calculate weight per square centimeter.
3. Cut out outline of entire lake and weigh. Calculate area by dividing weight of entire lake by weight of 1 square centimeter.
4. Repeat step 3 for each successive bottom contour.

VARIABLE Volume

DATE October 1990 **VERSION** 1.

SAMPLE Pond **REPORTING UNITS** m^3

METHOD Calculation from contours.

REFERENCE Lind, O.T. 1974. Handbook of common methods in limnology. C.V. Mosby Company, Saint Louis.

INSTRUMENTATION

PROCEDURE

The pond volume may be estimated from the area estimates for the various depths. The volume for each depth increment is:

VolumeIncrement = h/3 (A1 + A2 + Square Root (A1 A2))

Where: h = depth of volume increment (m)
A1 = area of surface of volume increment (m^2)
A2 = area of bottom of volume increment (m^2)

The total volume of the pond can be obtained by summing all the volume increments.

Section Three

SOIL/SEDIMENT

Soil Sample Collection Procedure

Aluminum - Exchangeable

Base Saturation

Benthos Composition

Cation Exchange Capacity

Exchangeable Hydrogen

Iron - Exchangeable

Lime Requirement

Nitrogen - Total

Organic Matter

pH

Phosphorus

Zinc - Exchangeable

Pond Soil Sample Collection

Unless otherwise specified for a given analysis, soils samples are to be collected as follows:

1. Use a 5 cm diameter core.

2. Collect a 5 cm deep core.

3. Record the total sample mass (g).

4. Record the dry bulk density of the sample ($kg \cdot m^{-3}$) from the total dry sample mass and the core volume ($9.82 \bullet 10^{-5}$ m^3 for the 5 cm diameter, 5 cm deep core).

VARIABLE Aluminum, Exchangeable

DATE October 1990 **VERSION** 1

SAMPLE Soil /Sediment **REPORTING UNITS** $mg \cdot kg^{-1}$

METHOD KCl extraction and colorimetric analysis with aluminon

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Shaker
Centrifuge
Colorimeter

PROCEDURE

Follow the procedures as described in the Methods of Soil Analysis (attached).

PROCEDURE Cont. **VARIABLE Aluminum, Exchangeable**

PRINCIPLES

Exchangeable or extractable Al ions are most commonly displaced with an unbuffered salt solution, such as 1*N* KCl, $CaCl_2$, or $BaCl_2$. Many of these procedures are used in the determination of Al for exchange equilibria studies, whereas others are employed in the determination of lime requirements for certain crop species (Kamprath, 1970; Reeve & Sumner, 1970; Farina et al., 1980). Most mineral soils with pH values of 5 or less contain appreciable amounts of salt-extractable Al. It is a common practice to lime these soils to pH values that reduce the exchangeable Al content and provide adequate conditions for plant growth. The type of extractant chosen will often depend on the nature of the experiment and for what specific purpose the exchangeable Al data are to be used. Extraction techniques employing unbuffered salt solutions are probably best suited for estimating truly exchangeable Al, at least to the first approximation. Other complexing agents and acid salt solutions may extract Al from both exchangeable and nonexchangeable sources, including structural hydroxy polymeric interlayers, organically bound species, and other noncrystalline metastable forms. It can be argued that some of the nonexchangeable metastable forms represent a plant-active "Al pool," which should be considered in lime recommendations (Arai, 1975; Juo & Kamprath, 1979). Many of these claims may be quite accurate; however, the methods employed in the estimation of such active Al pools are soil specific and require extensive calibration before they can be effectively implemented.

16-3.2.2 METHOD

16-3.2.2.1 Reagent.

1. Potassium chloride (KCl), 1*N*.

16-3.2.2.2 Procedure.

Weigh out 5 g of soil, sludge, sediment, etc. into a 100-ml polyethylene centrifuge tube or some other appropriate container. Add 50 ml of 1*N* KCl, stopper, and shake for 30 min. If centrifuge tubes are used, centrifuge 10 min at 2,500 rpm, and then filter the supernatant solution through Whatman no. 42 filter paper to remove floating organic matter and soil particles. An alternate approach is to filter the supernatant KCl extract from the soil using Whatman no. 42 and a Büchner funnel apparatus under low suction. The filtrates may be transferred to appropriate containers for Al analyses listed in section 16-5 or 16-6.

16-3.2.3 COMMENTS

If the soil, sludge, sediment, etc. samples are suspected to be high in exchangeable Al, it is often desirable to use less sample in the initial extraction step. Likewise, for materials low in exchangeable Al, larger sample sizes can be employed. It is important, however, to maintain a consistent sample/solution ratio (e.g., as in the above procedure 1:10). This may help avoid errors and time-consuming dilutions needed to bring the Al concentration within the detection range of the particular analytical method being used in Al determination (section 16-5 or 16-6).

PROCEDURE Cont. **VARIABLE Aluminum, Exchangeable**

In light of the many problems involved in determining "exchangeable" Al, it is not unusual to find various researchers employing different sample/solution ratios, shaking periods, and even concentrations of displacing unbuffered salt solutions. In most instances, these parameters will depend on the specific purpose or nature of the study being conducted and to a certain degree on the individual preference of the researcher. For example, in routine Al determination, the 30-min extraction period would be sufficient, whereas for a study involving careful Al exchange measurements, 2, 3, or even 24 hours may be appropriate (Bache & Sharp, 1976b). These parameters are also sometimes based on mineralogical considerations. Thus, no single procedure is best for all situations; being consistent and stating clearly which methods are being used are of prime importance.

frustrations resulting from the inability to delineate when and how these species form and to what degree they are involved in "true" surface exchange processes.

16-5 COLORIMETRIC METHODS FOR ALUMINUM ANALYSIS

16-5.1 Introduction

Several methods have been published for Al determination using colorimetric techniques. It is not our intention to present an exhaustive listing of these methods, but the Aluminon and 8-hydroxyquinoline methods are discussed in detail. In addition, we briefly discuss three alternative methods (section 16-5.4). Each method has certain advantages as well as limitations. The Aluminon method has been modified by several persons to reduce interferences and increase the sensitivity. Some of the more pertinent modifications are elaborated on in the comments section that follow each method.

16-5.2 Aluminon (Hsu, 1963; Jayman & Sivasubramaniam, 1974)

16-5.2.1 SPECIAL APPARATUS

1. Boiling water bath or steam hot plate.
2. Colorimeter (or spectrometer).

16-5.2.2 REAGENTS

1. Aluminon-acetate buffer: Dilute 120 ml of glacial acetic acid to about 900 ml with deionized water. Add 24 g of sodium hydroxide (NaOH), mix, and dissolve 0.35 g of Aluminon in the resulting solution. Dilute to 1 liter, and mix. The pH of this solution should be 4.2. When 10 ml of the buffer is added to a test solution containing 2.5 to 4.0 ml of 1*N* hydrochloric acid (HCl) and diluted to 50 ml, the pH should be from 3.7 to 4.0. The Aluminon buffer solution is stable for at least 6 months.
2. Aluminum standards, 500-ppm solution: Dissolve exactly 0.5000 g of electrically prepared metallic Al or wire in 15 ml of 6*N* hydrochloric acid (HCl) in a 1-liter volumetric flask. Dilute this solution to 1 liter with deionized water, and mix the contents thoroughly.

   To prepare a 5-ppm solution, pipette 10 ml into a 1-liter volumetric flask. Make to volume with deionized water, and mix thoroughly.

PROCEDURE Cont. **VARIABLE Aluminum, Exchangeable**

3. Complementary acid or extractant solution: Prepare an acid or extracting solution at the same concentration as the solution in which Al is being determined. For example, in the digestion of a sample in perchloric acid-hydrofluoric acid ($HClO_4$-HF), the Al would be in a 0.3*N* hydrochloric acid (HCl) solution and would be adjusted to a pH of 2 or 3 with sodium hydroxide (NaOH) (or with HCl).
4. Hydrochloric acid (HCl), 1*N*.
5. Ascorbic acid, 0.50%.

16-5.2.3 PROCEDURE

1. Pipette into a 50-ml graduated Folin-Wu tube or volumetric flask an aliquot containing 10 to 60 μg of Al, the pH of which has been previously adjusted to 2 to 3.0. For KCl extracts (section 16-3.2), add 2 to 4 ml of 1*N* HCl, which should bring the pH within the proper range. Add appropriate volumes of the complementary acid or extractant solution to each volumetric flask so that the unknown solutions have the same ionic strength; hence, this step is needed only when aliquot sizes vary appreciably as is the case for the standard solutions (see step 5 of this section). Add deionized water to bring the volume to at least 10-ml volume.
2. Add 1 ml of 0.5% ascorbic acid, and heat at least 30 min on a steam hot plate at 80 to 90°C.[3]
3. Cool the solutions, and dilute to about 35 ml with deionized water. Add 10 ml of the Aluminon-acetate buffer, make up to volume, and mix thoroughly. The pH should be 3.7 to 4.0 for systems that contain HCl, and it is desirable to check the pH on random samples.[4]
4. Measure the color intensity after 2 hours with colorimeter at 530 nm.
5. Prepare a standard by using aliquots (0, 2, 4, 6, and 8 ml) of the dilute (5 ppm) standard Al in 50-ml volumetric flasks. Add 2 ml of 1*N* HCl and appropriate volumes of deionized water to bring all flasks to about 10 ml. Treat the standards as described in steps 2 through 4 above. A plot of absorbancy vs. Al concentration should be linear.

16-5.2.4 COMMENTS

Hsu (1963) reported that each batch of Aluminon reagent should be checked before use, because he observed wide variations in sensitivity. The color intensity of the Aluminon complex varies with pH, and a range of 3.7 to 4.0 is best for HCl systems. Aluminon will precipitate below pH 3.7.

Wang and Wood (1973) report that for HNO_3-H_2SO_4 systems, the pH range should be increased to 4.1 to 4.3 to minimize the pH effect. This is accomplished by increasing the pH of the Aluminon-acetate buffer by adding 37 g of NaOH in place of the 24 g listed as reagent 1 of the Aluminon method.

Interfering ions such as phosphate and silicate were minimized by Hsu (1963) by heating the acidified Al solution before the addition of the Aluminon-acetate buffer.

Jayman and Sivasubramaniam (1974) found that interference of Fe(III) was minimized by the use of ascorbic acid. They found that 1 ml of 0.5% ascorbic acid was sufficient to eliminate up to 500 μg of Fe in the presence of 50 μg of Al in a 50-ml volume. They also found that the use of thioglycollic acid not only eliminated the interference due to Fe(III) but also bleached the color of the Aluminon reagent, thereby reducing the sensitivity.

[3] Jaymann and Sivasubramaniam (1974) reported that ascorbic acid will remove Fe. This step is necessary to break down complex Al ions that may have been present in the solution or were formed in localized zones resulting from the dropwise addition of base in the pH adjustment.

VARIABLE Base Saturation

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS**

METHOD Calculation.

REFERENCE

INSTRUMENTATION

PROCEDURE

VARIABLE Benthos Composition

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** No. $\cdot m^{-2}$

METHOD CRSP.

REFERENCE CRSP Work Plans.

INSTRUMENTATION

Sieve, U.S. Standard No. 30 (Nominal sieve opening is 0.595 mm)
Dissecting microscope
Coring device

PROCEDURE

Collect at least three cores of mud per pond. The cores may be collected with a 5 cm diameter PVC pipe, or with other suitable corer. Process samples through a No. 30 sieve, sort organisms and fix in 10% formaline or a 70% ethanol solution. Identify specimens to the order level and count the number of organisms.

VARIABLE Cation Exchange Capacity

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** meq · 100 g^{-1}

METHOD "Arid land" or "acid" soils.

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Atomic absorption spectrophotometer.
Centrifuge.
Ultrasonic disperser.
Reciprocating shaker.

PROCEDURE

Follow the procedure as described in the Methods of soil analysis. Select a method appropriate for your soil. In most cases, the "arid land" soils method is recommended for non-acid (alkaline) soils.

PROCEDURE Cont. **VARIABLE** **Cation Exchange Capacity**

8-3 CATION EXCHANGE CAPACITY OF ARID.LAND SOILS

The method of Polemio & Rhoades (1977) is particularly suited to arid land soils, including those containing carbonates, gypsum, and zeolites. The two-step procedure involves (i) saturation of cation exchange sites with Na by "equilibration" of the soil with pH 8.2, 60% ethanol solution of 0.4*N* NaOAc-0.1*N* NaCl; and (ii) extraction with 0.5*N* $MgNO_3$. Total Na and Cl are subsequently determined in the extracted solution so that the soluble Na from the excess saturating solution, carried over from the saturation step to the extraction step, may be deducted from the total Na to obtain exchangeable Na, which is equivalent to the CEC.

8-3.1 Apparatus

1. Atomic absorption spectrometer.
2. Centrifuge.
3. Round-bottom, narrow-neck centrifuge tubes, 50 ml.
4. Ultrasonic disperser with microtip focusing horn.
5. Reciprocating shaker.

8-3.2 Reagents

1. Saturating solution, 0.4*N* NaOAc-0.1*N* NaCl, 60% ethanol, pH 8.2 solution: Combine 544.32 g of sodium acetate trihydrate ($NaOAc \cdot 3H_2O$), 58.44 g of sodium chloride (NaCl), and 6 liters of ethanol, and

dilute to 10 liters with distilled water. Adjust the pH by stirring and dropwise addition of 6*N* sodium hydroxide (NaOH). Determine the Na/Cl ratio of this solution.

2. Extracting solution, 0.5*N* magnesium nitrate [$Mg(NO_3)$]: Weigh out 641.1 g of $Mg(NO_3)_2 \cdot 6H_2O$, and dilute to 10 liters.

8-3.3 Procedure

Weigh out samples of 4 to 5 g of air-dry soil (correct to oven-dry moisture content as determined using a separate subsample), and place in centrifuge tube.

Step 1. Add 33 ml of saturating solution, stopper the tube, and shake for 5 min. Unstopper, and centrifuge at relative centrifugal force of 1,000 until the supernatant liquid is clear (about 5 min). Decant the supernatant liquid and discard. Add fresh saturating solution, insert sonifier tip, and "sonify" for 10 to 30 sec to disperse sediment, then continue as above. Make four successive "equilibrations," discarding the supernatant liquid each time.

Step 2. Add 33 ml of extracting solution, shake for 5 min, centrifuge until the supernatant liquid is clear, and decant the extracted solution into a 100-ml volumetric flask. Repeat the extraction steps two more times with fresh extracting solution, and make to volume. Determine Na (Na_t) and Cl (Cl_t) in dilutions of this extracted solution using standards made up in the same batch of extracting solution (sections 10-3.4 and 10-3.5). Chloride is determined so that the soluble Na (Na_{sol}) carried over from the saturation step to the extraction step can be deducted from the total Na to obtain exchangeable Na (Na_{exch}):

$$CEC = (Na_t - Na_{sol}) = Na_t - (Cl_t)\,(Na/Cl)_{sat\ sol}.$$

8-3.4 Calculation

$$CEC \text{ in meq}/100\text{ g} = (10/\text{weight soil sample in g})$$

$$[(\text{Na concentration in meq/liter})\,(DF_{Na}) - (\text{Cl concentration in meq/liter})$$

$$(DF_{Cl})\,(NaCl)_{sat\ soil}]$$

where DF represents the dilution factor, i.e., (final analytical volume in milliliters)/(aliquot volume in milliliters).

8-3.5 Comments

If the soil is initially high in salts (EC $\geq$4 mmho/cm), wash the soil with one 33-ml increment of water before beginning saturation (avoid excessive washing to prevent loss of particles during decantation). The pH adjustment of the extracting solution has been eliminated from the procedure (Polemio & Rhoades, 1977) because it is not easy to adjust a relatively con-

centrated $Mg(NO_3)_2$ solution to pH 7 without adding considerable electrolyte. This added electrolyte may cause burner clogging and background interference problems during the atomic absorption spectrometric determination of Na (section 2-5.1) with some instruments. For the same reason, it is recommended that 0.5 rather than 1*N* $Mg(NO_3)_2$ be used as the extractant. This concentration of $Mg(NO_3)_2$ is sufficient to ensure complete desorption of Na during the replacement step. Quite satisfactory determinations of Na in 0.5*N* $Mg(NO_3)_2$ solution are obtained using an air-acetylene flame and a single-slot burner set at 45° to the optic path with the wavelength set at 295 nm. The concentration range of 0 to 1 meq/liter of Na may be covered linearly in this manner. Any dilutions should be made in such a way that the standards and unknowns have the same final $Mg(NO_3)_2$ concentration.

The amount of carryover of entrained saturating solution should be determined by direct analytical determination of Cl^- as described in section 10-3.5.3 and not by weight of solution retained. The latter is an inaccurate measure of Cl^- carryover because of the anion exclusion phenomenon. Indeed, this phenomenon can be used to estimate surface area of soil particles with the information collected during the CEC determination by using the method of Bower and Hatcher (1966).

The major advantages of this method are its simplicity, elimination of important sources of error inherent in other methods for certain soils, and its appropriateness for all kinds of soils, including saline and sodic soils and soils containing zeolites, feldspathoids, $CaCO_3$, and gypsum. The method should work equally well with acid soils for determining their permanent total negative charge.

Another method likely suited for determining the CEC of soils that are calcareous, gypsiferous, and relatively unweathered is that of Papanicolaou (1976). In the Papanicolaou CEC method, 1*N* $CaCl_2$ (pH 7.0) is used as the saturating solution; adsorbed Ca is replaced by extraction with 1*N* $NaNO_3$ (pH 7.0). Total (Ca^{2+} + Mg^{2+}), Cl^-, and (CO_3^{2-} + HCO_3^-) are determined in the combined extract. The CEC is calculated as (Ca^{2+} + Mg^{2+}) − (Cl^- + CO_3^{2-} + HCO_3^-). This method could probably be used for soils containing gypsum if SO_4^{2-} were determined. The method, however, requires numerous analyses.

8-4 CATION EXCHANGE CAPACITY OF ACID SOILS

The method described above for determining the CEC of arid-land soils should be applicable to acid soils for determining their permanent charge, but it will not yield a valid value of the capacity of highly weathered tropical soils, which consist primarily of variably charged matrices, to adsorb cations from an aqueous solution at a pH and ionic strength similar to that encountered in the field. Hence, a different method is needed for such soils. The method of Gillman (1979) is recommended because it is appropriate, simple, and inexpensive, and because it can be extended to determine the anion exchange capacity, which is often of equal interest for

such soils. Also, the exchangeable cations can be estimated with the method (Chapt. 9).

In this method the soil is saturated with Ba, subsequently equilibrated with $BaCl_2$ solution of a concentration about equivalent in ionic strength to the soil solution, and then reacted with $MgSO_4$ to replace Ba with Mg. The resultant $MgSO_4$ concentration is adjusted to achieve an ionic strength comparable with that of the soil solution. The use of unbuffered solutions throughout ensures that natural soil pH is not significantly altered. The loss of Mg from the reactant $MgSO_4$ solution is determined by analysis of the equilibrium solution and is equivalent to that adsorbed and hence to CEC. Thus, for 100 g of soil:

$$x\,\text{soil} - \text{Ba} + y\,BaCl_2\,(\text{entrained}) + z\,MgSO_4 \rightarrow$$

$$x\,\text{soil} - \text{Mg} + y\,MgCl_2 + (z - x - y)\,MgSO_4 + (x + y)\,BaSO_4 \downarrow$$

where x, y, and z are expressed in milliequivalents; hence:

$$\begin{aligned} \text{CEC} &= \text{Mg lost from reactant solution} \\ &= z - [y + (z - x - y)] \\ &= x\ \text{meq}/100\ \text{g}. \end{aligned}$$

8-4.1 Apparatus

1. Use the same as that described in section 8-3.1.

8-4.2 Reagents

1. Saturating solution, 0.1*M* barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$): Dissolve 24.4 g of $BaCl_2 \cdot 2H_2O$, and make to 1-liter volume with distilled water.
2. Equilibrating solution, 0.002*M* barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$): Dissolve 0.4889 g of $BaCl_2 \cdot 2H_2O$, and make to 1-liter volume with distilled water. Adjust the pH to 7.0 or other specified pH using $Ba(OH)_2$ or HCl.
3. Reactant solution, 0.005*M* magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$): Dissolve 1.232 g of $MgSO_4 \cdot 7H_2O$, and make to 1-liter volume with distilled water.
4. Ionic strength reference solution, 0.0015*M* magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$): Dissolve 0.3700 g of $MgSO_4 \cdot 7H_2O$, and make to 1 liter with distilled water.

8-4.3 Procedure

Place 2 g of air-dry (correct to oven-dry moisture content as determined using a separate subsample) plus 20 ml of 0.1*M* $BaCl_2$ saturating solution in a preweighed centrifuge tube; stopper and shake for 2 hours. Centrifuge and decant solution. Equilibrate soil with three successive 20-ml

PROCEDURE Cont. **VARIABLE Cation Exchange Capacity**

increments of 0.002*M* $BaCl_2$, equilibrating solution by "sonifying" for 10 to 30 sec to disperse sediment and shaking each for 1 hour between centrifugations, and discard the supernatants. Weigh the centrifuge tube plus soil and entrained 0.002*M* $BaCl_2$ of solution following the last decantation of supernatant. Add 10 ml of 0.005*M* $MgSO_4$ reactant solution, and shake gently for 1 hour. Adjust the EC of the reactant suspension to that of the 0.0015*M* $MgSO_4$ ionic strength reference solution at the ambient laboratory conditions by the addition of 0.005*M* $MgSO_4$ reactant solution or distilled water. Shake gently overnight, and again adjust the reactant suspension conductivity, if necessary. Weigh the centrifuge tubes plus contents to determine the volume of $MgSO_4$ or water added. Centrifuge and decant the supernatant, retaining it for analysis. Determine pH and Mg concentration of this supernatant solution (also its Cl^- concentration if anion exchange capacity is desired).

8-4.4 Calculation

1. If only distilled water was added:

$$\text{CEC in meq/100 g} = 100\,(0.1 - C_1V_3)/\text{oven-dry weight soil sample in g.}$$

2. If more $MgSO_4$ reactant solution had to be added:

$$\text{CEC in meq/100 g} = 100\,(0.01\,V_2 - C_1V_3)/$$

$$\text{(oven-dry weight soil sample in g)}$$

where V_2 and V_3 are volume (milliliters) of added $MgSO_4$ reactant solution and final supernatant solution, respectively, and C_1 is concentration of Mg in supernatant (milliequivalents/milliliter).

The CEC obtained is appropriate to the pH measured in the final $MgSO_4$ solution supernatant and is a close estimate of the soils CEC under natural field conditions of pH as measured in a 1:5 soil/water extract (Gilman, 1979).

8-4.5 Comments

If centrifuge tubes of 30-ml capacity are used, the use of 0.005*M* $MgSO_4$ is limited to soils with CEC values of < 10 meq/100 g. For higher CEC values, stronger $MgSO_4$ solution is required, e.g., 0.01*M* $MgSO_4$ for CEC values between 10 to 20 meq/100 g.

The volume of entrained 0.002*M* $BaCl_2$ equilibrating solution is determined (from the centrifuge tube plus contents weight after centrifugation and decantation) so that the dilution of the final $MgSO_4$ solution can be estimated.

The unbuffered 0.002*M* $BaCl_2$ and 0.0015*M* $MgSO_4$ solutions are used because their ionic strengths ($\mu = 0.006$) approximate that found in the soil solutions (at near field capacity) of highly weathered soils (Gillman & Bell,

PROCEDURE Cont. **VARIABLE** **Cation Exchange Capacity**

1978). This ionic strength can be altered at the discretion of the analyst for soils that differ in this respect.

Overnight equilibration is necessary for the complete replacement of Ba by Mg.

Variation in the ratio of soil to 0.002*M* $BaCl_2$ from 1:5 to 1:20 produces no change in CEC since the ionic strength and equilibrium pH are constant. Thus, flexibility in the choice of sample weights for soils with high and low CEC values is permitted.

The CEC values obtained for soils with this method are much lower than those obtained with conventional methods (such as the method in section 8-3.3) for the reasons discussed in section 8-1.

This method is suitable for routine determinations and for research needs where effects due to pH and ionic strength are to be studied. The desired pH can be attained during washings with 0.002*M* $BaCl_2$ using $Ba(OH)_2$ or HCl, and the reference $MgSO_4$ solution can be set at any desired ionic strength. Magnesium concentration in the supernatant is suitably determined by atomic absorption spectrometry (Chapt. 2 and section 10-3.4).

VARIABLE Exchangeable Hydrogen

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS**

METHOD

REFERENCE

INSTRUMENTATION

PROCEDURE

VARIABLE	Iron, Exchangeable
DATE	October 1990
VERSION	1
SAMPLE	Soil/Sediment
REPORTING UNITS	$mg \cdot kg^{-1}$
METHOD	Ammonium acetate extraction and atomic absorption
REFERENCE	Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Atomic absorption spectrophotometer.

PROCEDURE

9-3.1.1 PRINCIPLES

The soil sample is combined with an excess of 1*N* NH_4OAc such that maximum exchange occurs in a few minutes. The most doubtful cation extractions with this method are Ca^{2+}, in the presence of free $CaCO_3$ or gypsum and K^+ in soils dominated by mica or vermiculite. In the case of soils containing free $CaCO_3$ or gypsum ($CaSO_4 \cdot 2H_2O$), this method should not be used if exact results are required. (No other method is satisfactory either.) In the case of soils high in mica and vermiculite, NH_4AOc may give low (Barshad, 1954b) or excessively high (Rich & Black, 1964) results. There is no evidence at the present time that cations other than NH_4^+ give results that are less arbitary than those obtained using NH_4^+.

In the absence of excess $CaCO_3$, the cations Ca^{2+}, Mg^{2+}, and Na^+ appear to be completely exchangeable by NH_4OAc except in certain soils containing trioctahedral vermiculites (Barshad, 1954a). Such soils are known to occur on the west coast of the United States and probably the western parts of North and South America where granodiorite is the parent material and rainfall is < 60 cm/year.

PROCEDURE Cont. **VARIABLE Iron, Exchangeable**

9-3.1.2 METHOD

9-3.1.2.1 Reagent.

1. Ammonium acetate (NH_4OAc), 1*N*: Add 70 ml of reagent-grade NH_4OH, sp gr 0.90, to 57 ml of 99.5% acetic acid/liter of the final solution desired. Do the mixing in about half the final volume of distilled water desired, make up to volume with additional water, mix, thoroughly cool, and adjust exactly to pH 7.0 with either NH_4OH or acetic acid. (Only a small volume of acid or base will be required.)

9-3.1.2.2 **Büchner Funnel Procedure.** Place 10 g of <2-mm air-dried soil in a 125-ml Erlenmeyer flask, and add approximately 40 ml of 1*N* NH_4OAc to it. Swirl and let stand for 1 hour or more. Transfer the soil and solution to a 5.5-cm Büchner funnel fitted with no. 40 or 42 Whatman filter paper and connected to a 250-ml suction flask. Transfer the remaining traces of soil to the funnel by adding 10-ml portions of NH_4OAc to a final volume in the suction flask of 90 ml for soils with a CEC of 20 meq/100 g or less or 225 ml for soils with higher CEC's. Pour the extract into a 100- or 250-ml volumetric flask, and make up to volume by rinsing the suction flask with fresh 1*N* NH_4OAc. Determine the concentrations of Ca^{2+}, Mg^{2+}, K^+, and Na^+ as indicated in sections 13-3 and 13-4.

17-3 EXCHANGEABLE IRON

17-3.1 Introduction

The amount of Fe extracted from soils with neutral 1*N* NH_4OAc varies widely with soil conditions. In most well-aerated, neutral, or alkaline soils, little or no measurable Fe is extracted, probably because of hydrolysis of exchangeable Fe to $Fe(OH)_3$ and incomplete extraction of the latter. In highly acid or water-logged soils, large amounts may be obtained. The Fe extracted in this manner consists of both water-soluble and exchangeable Fe, and a correction for the water-soluble fraction must be applied for accurate estimates of exchangeable Fe. Also, NH_4OAc dissolves some organic matter that may contain Fe and cause considerable error in soils low in exchangeable Fe. Principles applying to the determination described below are discussed in section 17-2.2.

PROCEDURE Cont. **VARIABLE Iron, Exchangeable**

17-3.2 Method

17-3.2.1 EXTRACTION

17-3.2.1.1 Reagents.

1. Aqua regia: Mix 3 volumes of conc hydrochloric acid (HCl) with 1 volume of conc nitric acid (HNO_3) immediately before use.
2. Hydrochloric acid (HCl), 1*N*.

17-3.2.1.2 Procedure. For soils yielding large amounts of Fe upon extraction with neutral 1*N* NH_4OAc, use an aliquot of the extract described in section 9-3.1.2 for determination of exchangeable cations. This aliquot should contain between 0.01 and 0.10 mg of Fe. For most soils, however, the quantity of Fe extracted in this way is so small that the entire extract is needed for the determination.

Evaporate the NH_4OAc extract to dryness in a beaker on a steam hot plate. Treat the residue with 10 ml of aqua regia to oxidize organic matter, and again evaporate the solution to dryness. Add 3.5 ml of 1*N* HCl, and warm the beaker to dissolve the residue. Add a small amount of water to wash the sides of the beaker and complete the solution of the residue.

17-3.2.2 DETERMINATION BY ATOMIC ABSORPTION SPECTROMETRY

17-3.2.2.1 Procedure. Determination of exchangeable Fe by AAS is the method that many laboratories may prefer. The sample would be prepared by evaporating the NH_4OAc extract to dryness and treating the sample as described in the second paragraph of section 17-3.2.1.2. After treating the residue with 3.5 ml of 1*N* HCl, transfer the solution to a 50-ml volumetric flask and make to volume with deionized water. Use standard solutions in the range of 0 to 5 ppm of Fe, made up in 0.07*N* HCl, to prepare the calibration curve or standardize the instrument as described for the determination of total Fe in section 17-2.3.2.3. Calculate the parts per million exchangeable Fe in the soil as follows:

$$\text{ppm exchangeable Fe in soil} = \frac{50 \times (\text{ppm Fe in test solution} - \text{ppm Fe in blank})}{\text{g of soil represented by } NH_4OAc \text{ extract}}.$$

VARIABLE Lime requirement

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** kg $Ca\,CO_3 \cdot ha^{-1}$

METHOD Modified Adams-Evans procedure.

REFERENCE Boyd, C.E. 1979. Water quality in warm water fish ponds. Auburn University Agricultural Experiment Station, Auburn, Alabama, 359 pp.

INSTRUMENTATION

pH meter.

PROCEDURE

Reagents: Prepare a p-nitrophenol buffer of pH 8.0 ± 0.1 by dissolving 20 g of p-nitrophenol, 15 g of H_3BO_3, 74 g of KCl, and 10.5 g of KOH in distilled water and diluting to 1,000 mL in a volumetric flask.

Preparation of Sample: Spread the mud in thin layers on plastic sheets and air dry. Pulverize the dried mud gently and use for the test the material that passes through a 20 mesh screen (0.85 mm openings).

Determination of Mud-Water pH: Weigh 20.0 g of dry, screened mud into a 100 mL beaker, add 20 mL of distilled water, and stir intermitently for one hour. Measure the pH of the mud-water mixture with a glass electrode.

Determination of Buffer pH: Add 20.0 mL of the p-nitrophenol buffer to the sample used for the determination of mud-water pH. Stir intermittently for 20 minutes. Calibrate the pH meter at pH 8.0 with a 1:1 mixture of p-nitrophenol buffer and distilled water, and read the pH of the mud-water-buffer mixture while stirring vigorously.

Calculation: Select the appropriate liming rate from the table on the following page.

TABLE 12.2. LIME REQUIREMENT IN KG/HECTARE OF CALCIUM CARBONATE (NEUTRALIZING VALUE OF 100) TO INCREASE TOTAL HARDNESS AND TOTAL ALKALINITY OF POND WATER TO 20 MG/LITER OR ABOVE

Mud pH in water	Kg/hectare of calcium carbonate required according to mud pH in buffered solution									
	7.9	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.0
5.7	91	182	272	363	454	544	635	726	817	908
5.6	126	252	378	504	630	756	882	1,008	1,134	1,260
5.5	202	404	604	806	1,008	1,210	1,411	1,612	1,814	2,016
5.4	290	580	869	1,160	1,449	1,738	2,029	2,318	2,608	2,898
5.3	340	680	1,021	1,360	1,701	2,042	2,381	2,722	3,062	3,402
5.2	391	782	1,172	1,562	1,548	2,344	2,734	3,124	3,515	3,906
5.1	441	882	1,323	1,765	2,205	2,646	3,087	3,528	3,969	4,410
5.0	504	1,008	1,512	2,016	2,520	3,024	3,528	4,032	4,536	5,040
4.9	656	1,310	1,966	2,620	3,276	3,932	4,586	5,242	5,980	6,552
4.8	672	1,344	2,016	2,688	3,360	4,032	4,704	5,390	6,048	6,720
4.7	706	1,412	2,116	2,822	3,528	4,234	4,940	5,644	6,350	7,056

VARIABLE Nitrogen - Total

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** $mg \cdot kg^{-1}$

METHOD Kjeldahl (TKN).

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Kjeldahl digestion flasks.
Kjeldahl digestion stand and distillation apparatus.

PROCEDURE

Follow the detailed procedures as indicated in Methods of Soil Analysis (attached).

31-3 REGULAR KJELDAHL METHOD (Bremner, 1970)

31-3.1 Special Apparatus

1. Micro-Kjeldahl digestion flasks, 30 or 50 ml.
2. Micro-Kjeldahl digestion stand.
3. Hoskins (1944) steam distillation apparatus: This apparatus is available commercially (A. Gallenkamp & Co. Ltd., P.O. Box 290, Technico House, Christopher St., London, England EC2P 2ER) with interchange-

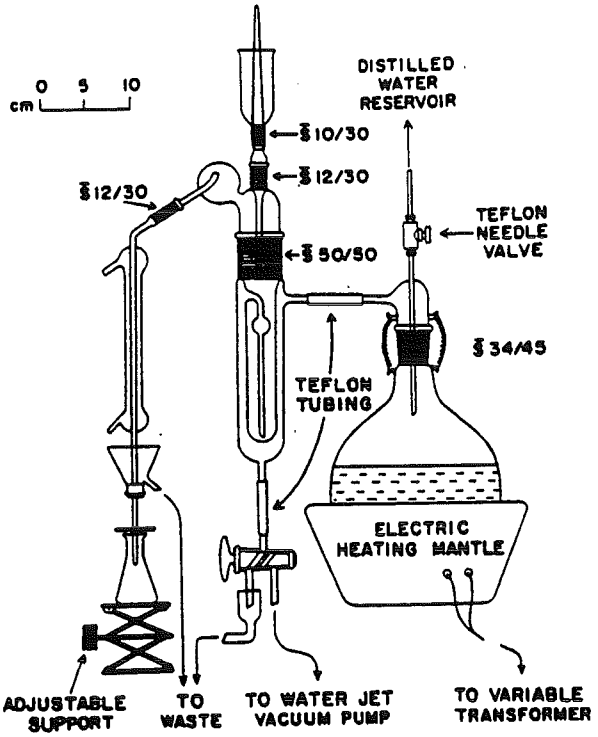


Fig. 31-1. Modified Hoskins steam distillation apparatus.

able microdistillation and macrodistillation chambers. The macrounit is required for the method described here. A modified macrounit specially designed in our laboratory (A. P. Edwards & J. M. Bremner, unpublished data) for distillation and determination of NH_4^+ in N isotope-ratio analysis is shown in Fig. 31-1. The main modifications of the Hoskins design in this apparatus are as follows: (i) the base of the funnel of the apparatus is fitted with a standard-taper (10/30) ground joint and funnel plug instead of a stopcock, (ii) the condenser and spray trap are connected by a standard-taper (12/30) ground joint, (iii) the base of the water jacket on the condenser is fitted with a condensate trap, (iv) the tube at the base of the steam jacket of the distillation chamber is connected by Teflon tubing to a three-way stopcock that is connected to a water-jet vacuum pump, (v) steam is generated by electrical heating of distilled water in a 5-liter flask that is connected to a reservoir of distilled water. Before use, the distillation apparatus should be steamed out to remove traces of NH_3, and the rate of steam generation should be adjusted so that about 6 ml of distillate are collected per minute. The flow of cold water through the condenser of the apparatus should be such that the temperature of the distillate obtained using this rate of distillation does not exceed 25°C.

31-3.2 Reagents

1. Potassium sulfate-catalyst mixture: Prepare an intimate mixture of 200 g of potassium sulfate (K_2SO_4), 20 g of cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), and 2 g of Se. Powder the reagents separately before mixing, and grind the mixture in a mortar to powder the cake that forms during mixing.
2. Sulfuric acid (H_2SO_4), concentrated.
3. Sodium hydroxide (NaOH) solution, approximately 10*N*: Place 3.2 kg of reagent-grade NaOH in a heavy-walled 10-liter Pyrex bottle marked to indicate a volume of 8 liters. Add 4 liters of CO_2-free water, and swirl the bottle until the alkali is dissolved. Cool the solution with a rubber stopper in the neck of the bottle, and make it to 8 liters by the addition of CO_2-free water. Then swirl the bottle vigorously to mix the contents, and fit the neck with some arrangement that permits the alkali to be stored and dispensed with protection from atmospheric CO_2 (Piper, 1947; Jackson, 1958).
4. Boric acid-indicator solution: Place 80 g of pure boric acid (H_3BO_3) in a 5-liter flask marked to indicate a volume of 4 liters, add about 3,800 ml of water, and heat and swirl the flask until the H_3BO_3 is dissolved. (Dissolution of the H_3BO_3 can also be effected by vigorous stirring with a high-speed laboratory stirrer.) Cool the solution, and add 80 ml of mixed indicator solution prepared by dissolving 0.099 g of bromocresol green and 0.066 g of methyl red in 100 ml of ethanol. Then add 0.1*N* sodium hydroxide (NaOH) cautiously until the solution assumes a reddish purple tint (pH ca. 5.0), and make the solution to 4 liters by addition of water. Mix the solution thoroughly before use.
5. Sulfuric acid (H_2SO_4) or hydrochloric acid (HCl), 0.01*N* standard.

31-3.3 Procedure

Place a sample of soil containing about 1 mg of N in a micro-Kjeldahl digestion flask, add 1.1 g of K_2SO_4-catalyst mixture and 3 ml of conc H_2SO_4, and heat the flask cautiously on the digestion stand. When frothing has ceased, increase the heat until the digest clears, and thereafter boil the mixture gently for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about one third of the way up the neck of the digestion flask.

After completion of digestion, allow the flask to cool, and add about 20 ml of water (slowly, and with shaking). Then swirl the flask to bring any insoluble material into suspension, and transfer the contents to the distillation chamber of the Hoskins apparatus via the funnel of the apparatus. Rinse the Kjeldahl flask three times with a total of about 9 ml of water to complete the transfer. Add enough water to the distillation chamber to bring the level of the solution to a mark made previously to indicate a

volume of 50 ml, and close the stopcock connecting the funnel and distillation chamber. Add 5 ml of H_3BO_3–indicator solution to a 50-ml Erlenmeyer flask marked to indicate a volume of 35 ml, and place the flask under the condenser of the distillation apparatus so that the end of the condenser is about 4 cm above the surface of the H_3BO_3. Then add 20 ml of 10*N* NaOH to the funnel of the apparatus, and run the alkali slowly into the distillation chamber by opening the funnel stopcock. When about 1 ml of alkali remains in the funnel, rinse the funnel rapidly with about 15 ml of water. Allow enough of this water to run into the distillation chamber to bring the level of the solution to a mark made previously to indicate a volume of 80 ml. Then close the funnel stopcock, and immediately commence distillation by closing the steam bypass tube at the base of the steam jacket of the distillation chamber. (It is not necessary to interrupt the flow of steam to the steam jacket ofthe distillation chamber before addition of the digest and alkali, but the bypass tube of the steam jacket must be kept open during these additions.) When the distillate reaches the 35-ml mark on the receiver flask, stop the distillation by opening the steam bypass tube, rinse the end of the condenser, and determine NH_4^+-N in the distillate by titration with 0.01*N* H_2SO_4 using a 10-ml burette graduated at 0.01-ml intervals (1 ml of 0.01*N* H_2SO_4 = 0.14 mg of NH_4^+-N). The color change at the endpoint is from green to pink.

31-3.4 Comments

Since a variety of gas-heated and electrically heated micro-Kjeldahl digestion stands are available commercially, comments on their suitability for soil analysis must be limited to the following general statements:

1) The gas or electric heaters should have individual controls that permit rapid adjustment of the heat, and they should provide sufficient heat to cause brisk boiling of a mixture of 3 ml of conc H_2SO_4 and 3 g of K_2SO_4. At full heat the heaters on a micro-Kjeldahl digestion stand found satisfactory for soil analysis brought 25 ml of water in a 50-ml Pyrex Kjeldahl flask to a rolling boil in approximately 2.5 min.[7]
2) The design should be such that the Kjeldahl flask is supported at an angle $<45°$ from the horizontal and is exposed to direct heat only in the region occupied by the liquid during digestion. Serious loss of N can occur during digestion if the flask is exposed to the heater above the level of the liquid.
3) The insulation and venting should be such that a mixture of 3 ml of H_2SO_4 and 1 g of K_2SO_4 can be boiled continuously for several hours, with the H_2SO_4 condensing about one third of the way up the neck of the digestion flask.

[7] The corresponding times for two digestion stands found unsatisfactory in our laboratory for soil analysis were 1.2 and 3 min, respectively.

PROCEDURE Cont. **VARIABLE Nitrogen - Total**

Whatever type of digestion stand is used, it is essential to have some arrangement for effective disposal of the acid fumes formed during digestion. Most of the micro-Kjeldahl digestion stands available commercially can be fitted with glass fume duct manifolds that effectively dispose of acid fumes when connected to a water-jet vacuum pump.

During the process of digestion, the Kjeldahl flasks should be swirled at intervals to dislodge any material adhering to the walls and to bring it into contact with the acid. This is particularly important with clay soils because clay promotes spattering, and the spattered material may adhere so tenaciously to the walls of the flask that it is not washed down into the digestion mixture by the condensing H_2SO_4.

Bumping is commonly encountered during Kjeldahl digestion of soils (particularly sandy soils), but this problem can usually be eliminated by addition of glass beads or other materials that promote smooth boiling. Kjeldahl methods involving short periods of digestion using a high concentration of K_2SO_4 may prove useful for analysis of sandy soils, because the severity of bumping usually increases with the period of digestion. The danger of loss of N due to spattering and bumping during digestion is considerably reduced if the Kjeldahl flask is supported at an angle $<45°$ from the horizontal as previously recommended.

The 5-hour period of digestion after clearing specified in the method described need not be adopted if it is not essential to obtain highly accurate results. The NH_4^+-N produced by digestion of soils for 2 hours is rarely $<98\%$ of the amount formed by digestion for 5 hours, and with most soils, the results of analyses using 2- and 5-hour periods of digestion are identical. A 1-hour period of digestion is adequate for routine analysis of soils and for other analytical work that does not require high accuracy.

Several types of steam distillation units for determination of NH_4^+ are available commercially, and most of these can be employed successfully to determine NH_4^+ in soil digests. The macroversion of the Hoskins unit is recommended because it has significant advantages over other commercial steam distillation units, and detailed information is available concerning its use for determination of NH_4^+ (Bremner, 1960). Its main advantages over the Markham (1942) apparatus are that it has a large distillation chamber (80 ml of liquid can be safely distilled) and a very efficient spray trap. The Parnas-Wagner (1921) micro-Kjeldahl steam distillation apparatus has similar advantages, and it is recommended that this apparatus be used if the Hoskins apparatus is not available. The steam distillation apparatus of Bremner and Edwards (1965) described in section 33–4.3.1 can also be employed if it is fitted with a 150- or 200-ml distillation flask.

Considerable time can be saved by employing two Hoskins units with their steam bypass tubes connected to a water-jet vacuum pump as shown in Fig. 31-1, because the chambers of the units can then be emptied and washed very rapidly by suction. With this arrangement it is not necessary to turn off the supply of steam between successive analyses to obtain the vacuum required to empty and wash the distillation chamber, and it is easily possible to perform 80 distillations in a normal working day.

PROCEDURE Cont. **VARIABLE Nitrogen - Total**

It is convenient to replace the 500-ml steam generator flask supplied with the Hoskins apparatus by a 5-liter flask and to use an electric heating mantle for generation of steam (Fig. 31-1). With this arrangement, the desired rate of distillation is readily obtained using a variable transformer to adjust the input to the heating mantle, and once this adjustment has been made, it is rarely necessary to alter it during a series of distillations to maintain the desired rate of distillation. A small amount of H_2SO_4 should be added to the steam generator flask to trap any NH_4^+-N in the distilled water used to generate steam. It is also advisable to add pumice or glass beads to eliminate bumping in the steam generator flask, because bumping can cause the liquid in the distillation chamber to siphon into the steam jacket.

Unsatisfactory results are sometimes obtained if water condensing on the external surface of the condenser of the steam distillation apparatus enters the NH_3 receiver flask, and a trap for this condensate (Fig. 31-1) or a collar of filter paper should be attached to the base of the water jacket on the condenser to prevent this. It is not necessary to distill with the end of the condenser under the surface of the H_3BO_3 (Bremner, 1960).

The sharpness of the endpoint obtained in the titration with standard acid to determine NH_4^+ in distillates collected in H_3BO_3 depends on the quality of the H_3BO_3, the strength of the H_3BO_3 solution, and the indicator. The better the quality of the H_3BO_3, and the more dilute the H_3BO_3 solution consistent with complete retention of NH_3, the sharper is the endpoint (Yuen & Pollard, 1953). Various indicators have been used for the titration (section 31-2.4), but most workers favor the bromocresol green–methyl red indicator mixture recommended by Ma and Zuazaga (1942). Our experience is that the sharpness of the endpoint obtained with bromocresol green–methyl red mixtures depends on the source of the indicators employed and that it can vary with different batches of indicator from the same source. It may be necessary, therefore, to alter the recommended proportions of the constituents of mixed indicators to obtain a satisfactory endpoint.

The 5 ml of 2% H_3BO_3 solution used in the method described should effectively absorb about 5 mg of NH_3-N (Scales & Harrison, 1920; Yuen & Pollard, 1953). This is about five times the amount that will be liberated if the recommendations concerning sample size in analysis are followed.

The H_2SO_4 (or HCl) solution employed for determination of NH_4^+ in the method described can be standardized satisfactorily using borax, anhydrous sodium carbonate, and other alkaline reagents (Vogel, 1951). The use of tris(hydroxymethyl)aminomethane [2-amino-2(hydroxymethyl)-1,3-propanediol] is recommended because it is available commercially (under the trade name of THAM)[4] as a highly purified reagent that can be used as a primary acidimetric standard. This reagent has a high equivalent weight, is not appreciably hygroscopic, and is readily soluble in water. Aqueous solutions of this substance absorb CO_2 at a slow rate and should be protected from atmospheric CO_2 (Bates & Hetzer, 1961).

[4] A technical data pamphlet describing the use of THAM as a primary acidimetric standard can be obtained from Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

PROCEDURE Cont. **VARIABLE Nitrogen - Total**

Controls should be performed in each series of analyses to allow for N in the reagents used.

It is recommended that acetanilide or THAM be used to check recovery of organic N by the method described. Acetanilide specially purified for use as a standard in N analysis can be obtained from the National Bureau of Standards, Washington, D.C. and highly purified THAM suitable for use as an N standard (Baker & Chesterfield, 1977) is readily available commercially.

Experience in our laboratory has shown that although the method described gives consistent results with most mineral soils when applied to samples ground to pass through a 60-mesh sieve, it is usually necessary to use more finely ground material to obtain highly reproducible results with organic soils or with mineral soils containing a high percentage of sand. To ensure precise results, it is recommended that soils containing $< 1\%$ N be ground to pass through a 100-mesh (0.14-mm) screen, that soils containing $> 1\%$ N be ground to pass through a 150-mesh (0.105-mm) screen, and that the sieved material be thoroughly mixed before analysis.

VARIABLE Organic Matter

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** %

METHOD Ashing and weighing, or Walkley-Black.

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Combustion apparatus for organic C determination.
Muffle furnace if using the Standard Methods procedure.

PROCEDURE

The Walkley-Black method is used to estimate organic C directly, but recovery of organic C from samples may be variable depending on the soil type.

The ignition method as described in Methods of Soil Analysis gives an indication of the actual organic matter (as opposed to organic C), but may result in overestimates due to loss of other components upon ignition at 950 °C.

Ignition of the sample at 550 °C in a muffle furnace as recommended in Standard Methods may be an alternate method of estimating organic matter (See Volatile Suspended Solids).
Methods of Soil Analysis recommends the direct determination of organic C (using Walkley-Black or other methods) over the ignition methods, and expressing the results in terms of Soil Organic C rather than Soil Organic Matter.

In all cases, follow the detailed procedures as indicated in the attached material.

WALKLEY-BLACK PROCEDURE (Walkley, 1946; Peech et al., 1947; Greweling & Peech, 1960)

29-3.5.2.1 Reagents.

1. Potassium dichromate ($K_2Cr_2O_7$), 1*N*: Dissolve 49.04 g of reagent-grade $K_2Cr_2O_7$ (dried at 105°C) in water, and dilute the solution to a volume of 1,000 ml.
2. Sulfuric acid (H_2SO_4), concentrated (not less than 96%): If Cl^- is present in soil, add Ag_2SO_4 to the acid at the rate of 15 g/liter.
3. Phosphoric acid (H_3PO_4), concentrated.
4. *o*-Phenanthroline–ferrous complex, 0.025*M*: Dissolve 14.85 g of *o*-phenanthroline monohydrate and 6.95 g of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in water. Dilute the solution to a volume of 1,000 ml. The *o*-phenanthroline–ferrous complex is available under the name of Ferroin from the G. Frederick Smith Chemical Co. (Columbus, Ohio).
5. Barium diphenylamine sulfonate: Prepare a 0.16% aqueous solution. This reagent is an optional substitute for no. 4.
6. Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) solution, 0.5*N*: Dissolve 140 g of reagent-grade $FeSO_4 \cdot 7H_2O$ in water, add 15 ml of conc sulfuric acid (H_2SO_4), cool the solution, and dilute it to a volume of 1,000 ml. Standardize this reagent daily by titrating it against 10 ml of 1*N* potassium dichromate ($K_2Cr_2O_7$), as described below.

29-3.5.2.2 Procedure. Grind the soil to pass through a 0.5-mm sieve, avoiding Fe or steel mortars. Transfer a weighed sample, containing 10 to 25 mg of organic C, but not in excess of 10 g of soil, into a 500-ml wide-mouth Erlenmeyer flask. Add 10 ml of 1*N* $K_2Cr_2O_7$, and swirl the flask gently to disperse the soil in the solution. Then rapidly add 20 ml of conc H_2SO_4, directing the stream into the suspension. Immediately swirl the flask gently until soil and reagents are mixed, then more vigorously for a total of 1 min. Allow the flask to stand on a sheet of asbestos for about 30 min. Then add 200 ml of water to the flask, and filter the suspension if experience shows that the endpoint of the titration cannot otherwise be clearly discerned. Add 3 to 4 drops of *o*-phenanthroline indicator, and titrate the solution with 0.5*N* $FeSO_4$. As the endpoint is approached, the solution takes on a greenish cast and then changes to a dark green: At this point, add the ferrous sulfate heptahydrate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background). Make a blank determination in the same manner, but without soil, to standardize the $Cr_2O_7^{2-}$. Repeat the determination with less soil if >75% of the dichromate is reduced.

Calculate the results according to the following formula, using a correction factor $f = 1.30$ or a more suitable value found experimentally:

PROCEDURE Cont. **VARIABLE Organic Matter**

$$\text{Organic C, \%} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq Fe } SO_4)(0.003)(100)}{\text{g water-free soil}} \times f. \quad [13]$$

29-3.5.2.3 Comments. Ferrous ammonium sulfate is also a suitable titrant for excess $Cr_2O_7^{2-}$ in conjunction with the Walkley-Black method. The Smith and Weldon (1941) modification involving complete reduction of $Cr_2O_7^{2-}$ with Fe^{2+}, and subsequent back-titration of excess Fe^{2+} with MnO_4^- solution may also be used to estimate unreacted $Cr_2O_7^{2-}$. Other oxidation-reduction indicators that have provided satisfactory results include barium diphenylamine sulfonate and *N*-phenylanthranilic acid. The amounts of $Cr_2O_7^{2-}$ reduced to Cr^{3+} by reaction with soil organic matter may also be estimated colorimetrically.

29-4 ORGANIC MATTER

29-4.1 Introduction

The organic matter content influences many soil properties, including (i) the capacity of a soil to supply N, P, S, and trace metals to plants; (ii) infiltration and retention of water; (iii) degree of aggregation and overall structure that affect air and water relationships; (iv) cation exchange capacity; (v) soil color, which in turn affects temperature relationships; and (vi) adsorption or deactivation (or both) of agricultural chemicals. Determination of organic matter content is a routine procedure carried out in soil analysis and testing laboratories throughout the United States because of the importance of organic matter in supplying plant available N and deactivating pesticides. However, no completely satisfactory method exists for determination of the organic matter content of soils. The direct method described below is that presented by Broadbent (1965) because few advances have been made in organic matter methodology in recent years.

29-4.2 Calculation of Organic Matter Content

The organic matter content of soil may be indirectly estimated through multiplication of the organic C concentration (as determined by procedures outlined in section 29-3) by the ratio of organic matter to organic C commonly found in soils. The organic matter content is normally arrived at by multiplying the organic C concentration by 1.724. However, a number of studies have suggested that this factor is too low for many soils, and consequently the organic matter content is underestimated. In summarizing much of the early work, Broadbent (1953) concluded that conversion factors of 1.9 and 2.5 would be appropriate for surface soils and subsoils, respectively. Other workers have found that a factor of 1.9 to 2.0 was satisfactory for surface layers of mineral soils (De Leenheer et al., 1957; Howard, 1965; Ponomareva & Platnikova, 1967). Loftus (1966) reported that the appropriate factors for mineral and organic soils in Pennsylvania were 2.2 and 1.8, respectively. More recently, Ranney (1969) found that the organic matter content of Pennsylvania surface soils may be accurately estimated by the equation

$$\% \text{ organic matter} = 0.35 + (1.80 \times \% \text{ organic C}). \qquad [15]$$

It is evident that estimation of organic matter content from organic C concentrations is not highly accurate, because the ratio of organic matter to organic C is variable from soil to soil and with depth in the profile. Accurate organic matter content estimates require a knowledge of the factor for the particular soil studied. However, if an estimate of organic matter content of surface soils must be made based on organic C data and no information on the exact factor is available, a factor of 2 appears to be most universally acceptable.

29-4.3 Direct Estimation of Organic Matter

29-4.3.1 PRINCIPLES

To achieve a direct determination of soil organic matter, one must separate it from the inorganic material, which in most soils makes up 90% or more of the weight of the soil. Extraction procedures that bring part of the organic matter into solution while leaving the inorganic material undissolved have only qualitative value, since no solvent has been found that will dissolve all or even a major portion of the organic fraction. The alternative is to destroy the organic matter, after which the loss in weight of the soil is taken as a measure of the organic content. The requirements of a suitable method are that the treatment used to destroy the organic matter should not destroy or alter the other soil constituents in such a way that their weight is changed and that the organic matter should be quantitatively removed.

The two most commonly used methods for achieving destruction of organic matter are (i) oxidation of the organic matter with H_2O_2 and (ii) ignition of the soil at high temperature. The H_2O_2 method (Robinson, 1927) has serious limitations in that the oxidation of organic matter by this reagent is incomplete, and the extent of oxidation varies from one soil to another. This method is therefore unsatisfactory as a means of determining total organic matter of soil, but it can be useful as a means of comparing the readily oxidizable material in different soils. The loss on ignition method gives quantitative oxidation of organic matter, but inorganic constituents of the soil, chiefly the hydrated aluminosilicates, lose structural water, and carbonate minerals are decomposed, upon heating, thus resulting in weight losses considerably in excess of the actual organic content. However, if the soil is pretreated with a mixture of HCl and HF to remove the hydrated mineral matter, loss on ignition then gives a valid estimate of organic content (Rather, 1917). This pretreatment dissolves part of the organic matter, so that a correction for the soluble material is necessary.

29-4.3.2 RATHER METHOD

29-4.3.2.1 Special Apparatus.

1. Polyethylene beakers, 600 ml.
2. Fritted-glass filter stick, coarse porosity.
3. Combustion apparatus for organic C (section 29-2.2.3).

29-4.3.2.2 Reagents.

1. Hydrochloric acid (HCl), 37% by weight.
2. Hydrofluoric acid (HF), 48% by weight.

29-4.3.2.3 Procedure.

Weigh 10 g of soil (40-mesh size or finer) into a polyethylene beaker, and add 440 ml of distilled water and 34 ml of 37% HCl. If the soil is calcareous, allow it to stand with occasional stirring until all the carbonates have dissolved. Filter off the soil on a Büchner funnel, wash it with water,

PROCEDURE Cont. **VARIABLE Organic Matter**

and return the sample to the polyethylene beaker. Resuspend the soil in HCl as above. (If the soil is not calcareous, omit the filtration after addition of HCl.) Add 6 ml of 48% HF using a polyethylene cylinder for measurement of the acid. Allow the soil suspension to stand, with occasional stirring for 24 hours. Let the soil settle out, and remove the supernatant solution using the fritted-glass filter stick. Repeat the treatment with the HCl-HF mixture for a second 24-hour period. Filter off the sample, and wash it several times with distilled water. Place the soil in an oven at 95°C, and dry it for 2 to 3 hours.

Transfer approximately 0.5 g of the dried soil to a previously ignited combustion boat, cover the soil with a thin layer of 90-mesh Alundum, previously ignited, and weigh the boat with its contents on an analytical balance. Burn the sample in a stream of O_2 in a combustion furnace at 950°C for 15 min, and determine the CO_2 evolved as described in section 29-2.2.3. Carefully remove the boat from the furnace, and place it in a desiccator to cool. When the boat is cool, weigh it again to determine ignition loss. Calculate the ratio of organic matter to organic C by dividing the loss on ignition by the C evolved as CO_2.

Determine the organic C content of a sample of the original untreated soil using the dry combustion procedure (section 29-2.2.3), and calculate organic matter using the factor determined by using the treated sample.

VARIABLE pH

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** pH units

METHOD Electrode.

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

pH meter and glass/calomel or gel-filled combination electrode.

PROCEDURE

Follow the detailed instructions as given in Methods of Soil Analysis (attached).

PROCEDURE Cont. **VARIABLE pH**

12-2.6.3 MAINTAINING ELECTRODES

12-2.6.3.1 Glass Electrode. Glass electrodes frequently become sluggish in their operation, as evidenced by slow changes or drift of the indicated pH when the measurement is made. Such drift or slow response may be caused by a dried layer of clay or precipitated carbonate on the glass bulb that cannot be removed by ordinary washing with water. Or, sluggish response may be caused by an aging of the glass surface of the bulb. Rejuvenation of the glass surface by immersion in a dilute solution of HF for 10 to 15 sec usually corrects the problem.

12-2.6.3.2 Calomel Electrode. The most frequent source of error with calomel electrodes is caused by stoppage of flow of electrolyte through the wick, fiber, porous ceramic, or sleeve. Although the instrument may calibrate satisfactorily in the solutions used for this purpose, it does not give correct values of pH in soil systems. The error tends to be in the direction of soil pH values that are too low and of high pH values in mixtures of soils and buffer solution used for measuring lime requirement. It is essential that the calomel electrode be operated with a functional liquid junction.

Another source of error in this respect occurs when the calomel electrode is forced into soil at the bottom of the cup in which the measurement is made, which stops the free movement of salt from the liquid junction. A stop in the electrode holder, which will prevent the tip of the electrode from penetrating to the bottom of the sample cup, will generally prevent this error.

12-2.6.4 EQUIPMENT AND REAGENTS

1. pH meter equipped with glass (indicating and reference electrodes).
2. Automatic pipette, portable stirrer (optional).
3. Paper cups, 28 g (1 oz), Solo souffle cup (Solo Cup Co. Urbana, Ill.) (optional).
4. Calcium chloride ($CaCl_2$) solution, 1 or 0.01*M* (optional).
5. Standard buffers, pH 7 and 4.
6. Distilled water.

12-2.6.5 pH[4]

1. Weigh or measure 5 g of air-dry soil into a 28-g paper cup. (Other containers, such as 50-ml beakers, may be used, but this may require adjustments in the shaking procedure called for below.)
2. Place cups in a 20- or 40-unit tray designed to go into the mechanical shaker used in the lime requirement determination (described below).
3. With automatic pipette, add 5 ml of distilled water to each cup.
4. Mix thoroughly for 5 sec, preferably with portable mechanical stirrer.

[4]The procedure described is designed to accommodate large numbers of samples as may be found in soil testing laboratory. However, it can readily be adapted to individual or small numbers of samples.

PROCEDURE Cont. **VARIABLE pH**

12-3 LIME REQUIREMENT

(A small stirrer motor, 1,550 rpm, mounted on a handle with a short, slightly bent plastic or glass rod agitator serves very well.)

5. Let stand for 10 min.
6. Insert the electrodes into the container, and stir the soil suspension by swirling the electrodes slightly. Protect the electrodes with a short glass rod attached to the electrode holder and extended just below the tips of the electrodes. If this method of agitation is used, it is not necessary to rinse the electrodes between successive samples.
7. Read the pH immediately on the standardized pH meter. Record as soil pH in water, or pH_w.
8. Optional test: To determine the soil pH in 0.01*M* $CaCl_2$, add 1 drop (0.05 ml) of 1*M* $CaCl_2$ solution to the soil water suspension.
9. Stir intermittently for 30 min.
10. Insert electrodes, and read the pH on the standardized pH meter. Record as soil pH in 0.01*M* $CaCl_2$, or pH_s. Alternatively, the soil pH in 0.01*M* $CaCl_2$ may be determined directly by substituting 0.01*M* $CaCl_2$ for water in no. 3 above.
11. If the lime requirement is to be determined on the samples, save them for this purpose after reading the pH in water or 0.01*M* $CaCl_2$.

VARIABLE Phosphorus

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** $mg \cdot kg^{-1}$

METHOD Ignition or HCL digestion.

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Spectrophotometer capable of reading from 840 to 880 nm with a 1 cm light path.
Muffle furnace capable of operating at 550 °C for the ignition method.

PROCEDURE

The ignition procedure is much simpler but it is also subject to a greater number of errors than the extraction method. In either case, follow the detailed instructions as given in Methods of Soil Analysis (attached).

24-3.2 Extraction Method (Mehta et al., 1954)

The extraction method involves sequential treatment of a soil with concentrated HCl, NaOH at room temperature, and NaOH at 90°C. Inorganic P is determined immediately after extraction, and total P is determined after oxidation of extracted organic matter with $HClO_4$.

24-3.2.1 APPARATUS

1. Aluminum block for tube digestion.

24-3.2.2 REAGENTS

1. Sulfuric acid (H_2SO_4), concentrated.
2. Hydrochloric acid (HCl), concentrated.
3. Sodium hydroxide (NaOH), 0.5*N*.
4. Sodium hydroxide (NaOH), 5*N*.
5. Perchloric acid ($HClO_4$), 72%.
6. *p*-Nitrophenol solution, 0.25% (wt/vol).
7. Reagents for P analysis listed in section 24-3.4.3.

24-3.2.3 PROCEDURE

Weigh 1 g of soil into a 100-ml polypropylene centrifuge tube, and add 10 ml of conc HCl. After mixing, place the tube on a steam bath for 10 min (final solution temperature about 70°C). Remove the tube from the steam bath, add 10 ml of conc HCl, mix, and allow to stand at room temperature for 1 hour. Add approximately 50 ml of H_2O, mix, and centrifuge the suspension ($\sim 1{,}500 \times g$). Decant the supernatant into a 250-ml volumetric flask containing 50 ml of distilled water. Treat the soil residue in the tube with 30 ml of 0.5*N* NaOH, mix, and allow to stand at room temperature for 1 hour. Centrifuge the soil suspension, and decant the supernatant into the 250-ml volumetric flask containing the concentrated HCl extract. Treat the soil in the tube with 60 ml of 0.5*N* NaOH, mix well, and place the tube in a 90°C oven for 8 hours. The tube should be loosely capped (e.g., 50-ml beaker, funnel, plastic cap) during heating. After cooling, centrifuge the suspension, and transfer the supernatant to the 250-ml volumetric flask containing the concentrated HCl and cold NaOH extracts. Dilute the flask to volume with distilled water, and mix thoroughly.

Thoroughly shake the flask to suspend the flocculated organic matter, pipette a 5-ml aliquot into a Folin-Wu (or comparable) digestion tube, add 1 drop of conc H_2SO_4 and 1 ml of 72% $HClO_4$, and place the tube in an Al digestion block. Slowly raise the temperature until white fumes of $HClO_4$ appear, and then place a 25-mm diam funnel atop the tube. Increase the temperature to 205°C and digest for 30 min. Remove the tube from the digestion block, allow to cool, and quantitatively transfer the digest to a 50-ml volumetric flask using <35 ml of water to rinse the digestion tube. (If the soil contains >1,000 μg of P/g, dilute the digest to 50 ml with water,

and transfer an aliquot containing 5 to 40 μg of P to a 50-ml volumetric flask for P analysis.) Treat the digest or aliquot contained in a 50-ml volumetric flask with 5 drops of 0.25% *p*-nitrophenol, then add 5*N* NaOH until a yellow color persists. Dilute the neutralized extract to ~40 ml, treat with 8 ml of reagent B, and dilute to 50 ml with water. After 10 min, read the absorbance at 840 to 880 nm using a 1-cm cuvette. Prepare a standard curve by analyzing aliquots of a KH_2PO_4 standard solution containing from 5 to 40 μg of P as described above. A blank containing all reagents used in extraction and digestion should be analyzed with each set of samples.

Allow the floculated organic matter in the extract to settle, and then remove an aliquot (usually 2 to 10 ml) for inorganic P determination. Transfer the aliquot to a 50-ml volumetric flask, add 5 drops of 0.25% *p*-nitrophenol, and treat with 5*N* NaOH until a yellow color persists. Dilute to ~40 ml with water, add 8 ml of reagent B, and determine P as described above. A blank containing all reagents used in the soil extraction should be analyzed, and the inorganic P analysis should be performed as soon as possible after soil extraction to minimize hydrolysis of organic P.

The organic P content of the soil is calculated from the difference between total and inorganic P in the extract.

24-3.2.4 COMMENTS

The oxidation of extracted organic matter can be performed in beakers as originally described by Mehta et al. (1954) if an Al block digestion apparatus is not available. For soils containing elevated levels of organic matter, the extract may be sufficiently colored to interfere with the inorganic P analysis. This interference can be overcome by treating a portion of the extract with activated charcoal, shaking, separating the charcoal from the extract by filtration or centrifugation, and analyzing an aliquot of the charcoal-treated extract for inorganic P. (Note: Many commercially available activated charcoals are contaminated with P. All batches of charcoal should be checked by treating the charcoal with the extracting solutions and analyzing for P before use on soil extracts. The P contaminants contained in activated charcoal can be removed by leaching with 6*N* HCl, followed by repeated washings with distilled water.) The ascorbic acid reduction procedure for P determination (Watanabe & Olsen, 1965) is less sensitive to interference by soluble organic matter than the $SnCl_2$ method used originally by Mehta et al. (1954).

The initial concentrated HCl treatment may result in hydrolysis of organic P if the soil contains appreciable amounts of P esters, such as glycerophosphate, RNA, DNA, and sugar phosphates (Anderson, 1960). To minimize hydrolysis, the above procedure can be altered as follows. Extract a 1-g soil sample with 70 ml of 0.3*N* NaOH for 16 hours before extraction with concentrated HCl and 0.5*N* NaOH as described in section 24-3.2.3. Neutralize an aliquot of the 0.3*N* NaOH extract with HCl, and analyze for P as described above. Combine aliquots of the 0.3*N* NaOH and concentrated HCl + 0.5*N* NaOH extracts, and digest with $HClO_4$ before determination of total P.

Unfortunately, a standard procedure is not obvious for determining organic P in soils. Any extraction procedure can underestimate the organic P content if incomplete extraction or hydrolysis occurs. An additional difficulty encountered is the random errors associated with any analytical procedure, especially a sequential extraction procedure, where the final result is obtained by difference. The lack of precision in organic P methods has been discussed in several reports (e.g., Kaila, 1962; Oniani et al., 1973; Anderson, 1960; Dormaar, 1964; Bornemisza & Igue, 1967). In general, little significance can be given to organic P values differing by $< 20\ \mu g\ P/g$.

24-3.3 Ignition Method (Saunders & Williams, 1955, as modified by Walker & Adams, 1958)

Organic P is estimated from the $1N$ H_2SO_4 extractable P in a soil sample ignited at 550°C and an unignited sample.

24-3.3.1 APPARATUS

1. Muffle furnace for igniting soils at 550°C.

24-3.3.2 REAGENTS

1. Sulfuric acid (H_2SO_4), $1N$.
2. Sodium hydroxide (NaOH), $5N$.
3. *p*-Nitrophenol, 0.25% (wt/vol).
4. Reagents for P analysis listed in section 24-3.4.3.

24-3.3.3 PROCEDURE

Place 1 g of soil in a procelain crucible, and place the crucible in a cool muffle furnace. Slowly raise the temperature of the muffle furnace to 550°C over a period of 1 to 2 hours. Maintain the temperature at 550°C for 1 hour, allow the crucible to cool, and transfer the ignited soil to a 100-ml polypropylene centrifuge tube.

In a separate 100-ml polypropylene centrifuge tube, place a 1-g sample of unignited soil. Add 50 ml of $1N$ H_2SO_4 to both samples, and place the tubes on a shaker for 16 hours. Centrifuge the samples at $\sim 1.500 \times g$ for 15 min (if the extract is not clear, filtration may be needed using acid-resistant filter paper). Pipette an aliquot of the $1N$ H_2SO_4 extract containing $< 40\ \mu g$ of P into a 50-ml volumetric flask, add 5 drops of 0.25% *p*-nitrophenol, and neutralize with $5N$ NaOH (yellow color). Dilute to ~40 ml with distilled water, and add the P reagent. After 10 min, measure the absorbance at 840 to 880 nm using a 1-cm cuvette. Prepare a calibration graph by analyzing aliquots of a KH_2PO_4 standard solution containing from 5 to 40 μg of P. Determine the P content of the $1N$ H_2SO_4 used to correct for any P contained in the reagents.

The organic P content of the soil is calculated by subtracting P in the unignited sample from P in the ignited sample.

VARIABLE Zinc

DATE October 1990 **VERSION** 1

SAMPLE Soil/Sediment **REPORTING UNITS** $mg \cdot kg^{-1}$

METHOD Atomic absorption spectrophotometry.

REFERENCE Page, A.L., Miller, R.H. and Keekey, D.R. editors. 1982. Methods of soil analysis, Part 2- Chemical and microbiological properties. Second Edition. American Society of Agronomy and Soil Science Society of America. Madison, Wisconsin.

INSTRUMENTATION

Atomic absorption spectrophotometer.

PROCEDURE

Follow the procedure as described in Methods of soil analysis and in the detailed operating guide for the instrument available. Use flame atomic absorption unless the concentration is too low and the samples must be analyzed by flameless atomic absorption.

19-2.1.3 PROCEDURE

Transfer a 2-g sample of soil (up to 10 g of soil may be needed where samples of lower horizons or sands are being analyzed), which is specified as air-dried or oven-dried at 105°C, to the polypropylene or Teflon digestion tube or beaker. If the sample is calcareous, it must be pretreated to remove carbonates. Add 25 ml of water and 2 ml of conc HNO_3, and take to dryness. For peats and mucks, long oxidation with H_2O_2 is required to destroy organic matter, particularly if montmorillonite is present. The samples may be transferred to porcelain or silica crucibles and heated for 2 hours at 450°C to destroy organic matter. The ash is then transferred to the polypropylene or Teflon tubes or beakers. To the residues from calcareous soils, the ash from peat or muck soils, or the original 2-g samples of noncalcareous soils, add 10 ml of H_2O_2, and evaporate the solution to near dryness at 90°C. Repeat this treatment until the sample no longer effervesces on addition of H_2O_2. Add 3 drops of conc H_2SO_4 and 10 ml of HF. Place the sample on the block digester or sand bath, slowly raise the temperature of the bath to 200°C, and evaporate the solution to dryness. Repeat the addition of H_2SO_4 and HF and the evaporation to dryness. Add 15 ml of conc HNO_3, 2 ml of conc H_2SO_4, and 5 ml of $HClO_4$, and continue heating until strong fumes of SO_3 are produced. Cool the polypropylene or Teflon container, and add 25 ml of water, rinsing down the sides of the container and policing with a polyethylene rod to complete the extraction of Ni, Cu, Zn, and Cd. Transfer the solution quantitatively to a 50-ml volumetric flask, and dilute to volume.

19-2.2 Determination of Nickel, Copper, Zinc, and Cadmium by Atomic Absorption Spectrometry

We recommend that those not familiar with atomic absorption spectrometry (AAS) read Chapter 2 before analyzing any samples. Samples can first be analyzed using flame AAS, and then any samples too low to be reliably determined by flame techniques can be run using flameless AAS. Also, the metals can be chelated and extracted into an organic liquid phase for analysis by flame AAS (USEPA, 1974). When using flame AAS, be sure that particulate material does not get aspirated with the solution, otherwise clogging may result. Samples can be filtered through fine, hard, acid-washed, acid-resistant filter paper to prevent this from happening.

To avoid interference from broad band absorption and scattering from other chemical species in the samples, background correction should be employed (Emmel et al., 1977). As indicated by Baker and Suhr (Chapt. 2), interferences may lead to errors of several orders of magnitude for samples containing low levels of the metals, especially Cd and Ni. Standards should contain the same amounts of acids that are in the samples to reduce matrix-type interferences.

19-2 TOTAL NICKEL, COPPER, ZINC, & CADMIUM

19-2.2.1 PREPARATION OF STANDARDS

Standards containing 1,000 μg/ml of the various metals are available from commercial sources. For those preferring to prepare their own standards, the following reagents are recommended:

1. Nickel, 1,000 μg/ml: Dissolve 1.000 g of Ni metal in 50 ml of 1 + 1 nitric acid (HNO_3). Dilute to 1 liter with deionized water.
2. Copper, 1,000 μg/ml: Dissolve 1.000 g of Cu metal in 50 ml of 1 + 1 nitric acid (HNO_3). Dilute to 1 liter with deionized water.
3. Zinc, 1,000 μg/ml: Dissolve 1.000 g of Zn metal in 50 ml of 1 + 1 hydrochloric acid (HCl). Dilute to 1 liter with deionized water.
4. Cadmium, 1,000 μg/ml: Dissolve 1.000 g of Cd metal in 50 ml of 1 + 1 hydrochloric acid (HCl). Dilute to 1 liter with deionized water.
5. Working standards: Working standards should be prepared by diluting the stock standards to include the optimum working ranges given in Table 19-2. The standards should be prepared with the same amounts of the acids or other reagents contained in the samples.

19-2.2.2 OPERATING PARAMETERS

It is recommended that the manufacturer's operating manual be consulted for specific operating conditions for the instrument on hand. A table of operating parameters is given below for flame AAS (Table 19-2). Samples with concentrations below the optimum range can be run by flameless AAS, or the metals can be chelated with pyrrolidine dithiocarbamic acid (PDCA) in $CHCl_3$, the $CHCl_3$ can be evaporated, and the ions can be dissolved in HNO_3 for analysis by flame AAS. Details of this standardized procedure are presented in the *Manual of Methods for Chemical Analysis of Water and Wastes* (USEPA, 1974). Other chelation-solvent extraction methods are available (Standard methods, 1980; Viets, 1978; Jenne, 1974). As pointed out by Baker and Suhr (section 2-3), the use of flameless AAS is preferred to solvent extraction. Where a solvent extraction method is used, it is important to determine that the blank prepared from the zero standard shows an acceptable low degree of contamination from the reagents.

Samples with concentrations above the optimum range can be diluted to bring them into range. The diluting solution should contain the same amounts of the acids or other reagents as in the samples and standards.

Table 19-2. Operating parameters for Ni, Cu, Zn, and Cd.†

	Ni	Cu	Zn	Cd
Optimum concentration range, μg/ml	0.3–10	0.2–10	0.05–2	0.05–2
Sensitivity, μg/ml‡	0.15	0.1	0.02	0.025
Detection limit, μg/ml‡	0.02	0.01	0.005	0.002
Wavelength, nm	232.0	324.7	213.9	228.8
Air-acetylene flame conditions	Oxidizing	Oxidizing	Oxidizing	Oxidizing

† Adapted from U.S. Environmental Protection Agency (1974).
‡ Sensitivity and detection limits vary among manufacturers and instruments.

Section Four

WATER

Water Sample Collection Procedure

Alkalinity
Chlorophyll a
Dark Bottle Respiration
Dissolved Oxygen
Nitrogen - Ammonia
Nitrogen - Total Kjeldahl
Nitrogen - Nitrate
Nitrogen - Nitrite
pH
Phosphorus - Total
Phytoplankton Composition
Pond Temperature
Primary Productivity
Salinity
Secchi Disk Depth
Solids - Total Suspended
Solids - Volatile Suspended
Zooplankton Composition

Water Sample Collection

Unless otherwise specified for a given analysis, water samples are to be collected as follows:

1. Use clean plastic bottle.

2. Fill bottle to the top and cap promptly.

3. Store samples refrigerated and in the dark while in the field. Some water quality parameters must be measured immediately after sample collection (e.g. dissolved oxygen and pH). Check the procedures for specific instructions on sample storage and preservation.

4. Collect sample at the depth indicated for the various analysis taking care not to disturb the water or sediments around the location where the sample is being collected. If samples from more than one depth are being collected at the same spot, collect the surface sample first, and follow with deeper samples, with the bottom sample being the last to be collected.

VARIABLE Alkalinity

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mgCaCO_3 \cdot L^{-1}$

METHOD Low or High Standard Total Alkalinity Method or Hach Test Kit.

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Hach Digital Titrator Test Kit/Alkalinity (optional).
pH meter (optional)

PROCEDURE

Near center of each pond, collect samples at 25 cm below the water surface midwater and 25 cm above the bottom.

The titration end point may be determined potentiometrically using a pH meter or using a color indicator (bromcresol green for an endpoint of 4.5). Use the pH endpoints suggested in Standard Methods for your particular water/alkalinity.

Use the low alkalinity method if alkalinity is less than 20 mg/L as calcium carbonate.

2320 ALKALINITY*

2320 A. Introduction

1. Discussion

Alkalinity of a water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constitutents. The measured values also may include contributions from borates, phosphates, silicates, or other bases if these are present. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of a water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than, or only slightly greater than, that of the water supply. Properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2000 to 4000 mg calcium carbonate ($CaCO_3$)/L.[1]

*Approved by Standard Methods Committee, 1985.

2. Reference

1. POHLAND, F.G. & D.E. BLOODGOOD. 1963. Laboratory studies on mesophilic and thermophilic anaerobic sludge digestion. *J. Water Pollut. Control Fed.* 35:11.

2320 B. Titration Method

1. General Discussion

a. Principle: Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Section 2310B.1*a*.

For samples of low alkalinity (less than 20 mg $CaCO_3$/L) use an extrapolation technique based on the near proportionality of concentration of hydrogen ions to excess of titrant beyond the equivalence point. The amount of standard acid re-

PROCEDURE Cont. **VARIABLE Alkalinity**

quired to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a simple extrapolation can be made to the equivalence point.[1,2]

b. End points: When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The following pH values are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams $CaCO_3$ per liter. "Phenolphthalein alkalinity" is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the colored indicator, if any, used in the determination. The sharp end-point color changes produced by metacresol purple (pH 8.3) and bromcresol green (pH 4.5) make these indicators suitable for the alkalinity titration.

	End Point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity, mg $CaCO_3$/L:		
30	4.9	8.3
150	4.6	8.3
500	4.3	8.3
Silicates, phosphates known or suspected	4.5	8.3
Routine or automated analyses	4.5	8.3
Industrial waste or complex system	4.5	8.3

c. Interferences: Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample.

d. Selection of procedure: Determine sample alkalinity from volume of standard acid required to titrate a portion to a designated pH taken from ¶ 1*b*. Titrate at room temperature with a properly calibrated pH meter or electrically operated titrator, or use color indicators.

Report alkalinity less than 20 mg $CaCO_3$/L only if it has been determined by the low-alkalinity method of ¶ 4*d*.

Construct a titration curve for standardization of reagents.

Color indicators may be used for routine and control titrations in the absence of interfering color and turbidity and for preliminary titrations to select sample size and strength of titrant (see below).

e. Sample size: See Section 2310B.1*e* for selection of size sample to be titrated and normality of titrant, substituting 0.02*N* or 0.1*N* sulfuric (H_2SO_4) or hydrochloric (HCl) acid for the standard alkali of that method. For the low-alkalinity method, titrate a 200-mL sample with 0.02*N* H_2SO_4 from a 10-mL buret.

f. Sampling and storage: See Section 2310B.1*f*.

2. Apparatus

See Section 2310B.2.

3. Reagents

a. Sodium carbonate solution, approximately 0.05*N:* Dry 3 to 5 g primary standard Na_2CO_3 at 250°C for 4 h and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, and dissolve and mix reagent. Do not keep longer than 1 week.

PROCEDURE Cont. **VARIABLE Alkalinity**

b. Standard sulfuric acid or hydrochloric acid, 0.1N: Prepare acid solution of approximate normality as indicated under Preparation of Desk Reagents (see inside front cover). Standardize against 40.00 mL 0.05*N* Na_2CO_3 solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to the pH inflection point. Calculate normality:

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

where:

A = g Na_2CO_3 weighed into 1-L flask,
B = mL Na_2CO_3 solution taken for titration, and
C = mL acid used.

Use measured normality in calculations or adjust to 0.1000*N*; 1 mL 0.1000*N* solution = 5.00 mg $CaCO_3$.

c. Standard sulfuric acid or hydrochloric acid, 0.02N: Dilute 200.00 mL 0.1000*N* standard acid to 1000 mL with distilled or deionized water. Standardize by potentiometric titration of 15.00 mL 0.05*N* Na_2CO_3 according to the procedure of ¶ 3*b*; 1 mL = 1.00 mg $CaCO_3$.

d. Bromcresol green indicator solution, pH 4.5 indicator: Dissolve 100 mg bromcresol green, sodium salt, in 100 mL distilled water.

e. Metacresol purple indicator solution, pH 8.3 indicator: Dissolve 100 mg metacresol purple in 100 mL water.

f. Phenolphthalein solution, alcoholic, pH 8.3 indicator.

g. Sodium thiosulfate, 0.1N: See Section 2310B.3*i*.

4. Procedure

a. Color change: See Section 2310B.4*b*.

b. Potentiometric titration curve: Follow the procedure for determining acidity (Section 2310B.4*c*), substituting the appropriate normality of standard acid solution for standard NaOH, and continue titration to pH 4.5 or lower. Do not filter, dilute, concentrate, or alter the sample.

c. Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to ¶ 1*b*. Prepare sample and titration assembly (Section 2310B.4*c*). Titrate to the end-point pH without recording intermediate pH values and without undue delay. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

d. Potentiometric titration of low alkalinity: For alkalinities less than 20 mg/L titrate 100 to 200 mL according to the procedure of ¶ 4*c*, above, using a 10-mL microburet and 0.02*N* standard acid solution. Stop the titration at a pH in the range 4.3 to 4.7 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume.

5. Calculations

a. Potentiometric titration to end-point pH:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50\,000}{\text{mL sample}}$$

where:

A = mL standard acid used and
N = normality of standard acid

or

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times t \times 1000}{\text{mL sample}}$$

where:

t = titer of standard acid, mg $CaCO_3$/mL.

PROCEDURE Cont. **VARIABLE Alkalinity**

Report pH of end point used as follows: "The alkalinity to pH _____ = _____ mg $CaCO_3$/L" and indicate clearly if this pH corresponds to an inflection point of the titration curve.

b. Potentiometric titration of low alkalinity:

Total alkalinity, mg $CaCO_3$/L

$$= \frac{(2B - C) \times N \times 50\,000}{\text{mL sample}}$$

where:

B = mL titrant to first recorded pH,
C = total mL titrant to reach pH 0.3 unit lower, and
N = normality of acid.

c. Calculation of alkalinity relationships: The results obtained from the phenolphthalein and total alkalinity determinations offer a means for stoichiometric classification of the three principal forms of alkalinity present in many waters. The classification ascribes the entire alkalinity to bicarbonate, carbonate, and hydroxide, and assumes the absence of other (weak) inorganic or organic acids, such as silicic, phosphoric, and boric acids. It further presupposes the incompatibility of hydroxide and bicarbonate alkalinities. Because the calculations are made on a stoichiometric basis, ion concentrations in the strictest sense are not represented in the results, which may differ significantly from actual concentrations especially at pH > 10. According to this scheme:

1) Carbonate (CO_3^{2-}) alkalinity is present when phenolphthalein alkalinity is not zero but is less than total alkalinity.

2) Hydroxide (OH^-) alkalinity is present if phenolphthalein alkalinity is more than half the total alkalinity.

3) Bicarbonate (HCO_3^-) alkalinity is present if phenolphthalein alkalinity is less than half the total alkalinity. These relationships may be calculated by the following scheme, where P is phenolphthalein alkalinity and T is total alkalinity (¶ 1*b*):

Select the smaller value of P or $(T-P)$. Then, carbonate alkalinity equals twice the smaller value. When the smaller value is P, the balance $(T-2P)$ is bicarbonate. When the smaller value is $(T-P)$, the balance $(2P-T)$ is hydroxide. All results are expressed as $CaCO_3$. The mathematical conversion of the results is shown in Table 2320:I. (A modification of Table 2320:I that is more accurate when $P \simeq \frac{1}{2}T$ has been proposed.[3])

Alkalinity relationships also may be computed nomographically (see Carbon Dioxide, Section 4500-CO_2). Accurately measure pH, calculate OH^- concentration as milligrams $CaCO_3$ per liter, and calculate concentrations of CO_3^{2-} and HCO_3^- as milligrams $CaCO_3$ per liter from the OH^- concentration, and the phenolphthalein and total alkalinities by the following equations:

$$CO_3^{2-} = 2P - 2[OH^-]$$

$$HCO_3^- = T - 2P + [OH^-]$$

Similarly, if difficulty is experienced with the phenolphthalein end point, or if a check on the phenolphthalein titration is desired, calculate phenolphthalein alkalinity as $CaCO_3$ from the results of the nomographic

TABLE 2320:I. ALKALINITY RELATIONSHIPS*

Result of Titration	Hydroxide Alkalinity as $CaCO_3$	Carbonate Alkalinity as $CaCO_3$	Bicarbonate Concentration as $CaCO_3$
$P = 0$	0	0	T
$P < \frac{1}{2}T$	0	$2P$	$T - 2P$
$P = \frac{1}{2}T$	0	$2P$	0
$P > \frac{1}{2}T$	$2P - T$	$2(T - P)$	0
$P = T$	T	0	0

*Key: P—phenolphthalein alkalinity; T—total alkalinity.

determinations of carbonate and hydroxide ion concentrations:

$$P = 1/2[CO_3^{2-}] + [OH^-]$$

6. Precision and Bias

No general statement can be made about precision because of the great variation in sample characteristics. The precision of the titration is likely to be much greater than the uncertainties involved in sampling and sample handling before the analysis.

In the range of 10 to 500 mg/L, when the alkalinity is due entirely to carbonates or bicarbonates, a standard deviation of 1 mg $CaCO_3$/L can be achieved. Forty analysts in 17 laboratories analyzed synthetic samples containing increments of bicarbonate equivalent to 120 mg $CaCO_3$/L. The titration procedure of ¶ 4*b* was used, with an end point pH of 4.5. The standard deviation was 5 mg/L and the average bias (lower than the true value) was 9 mg/L.[4]

Sodium carbonate solutions equivalent to 80 and 65 mg $CaCO_3$/L were analyzed by 12 laboratories according to the procedure of ¶ 4*c*.[5] The standard deviations were 8 and 5 mg/L, respectively, with negligible bias.[5] Four laboratories analyzed six samples having total alkalinities of about 1000 mg $CaCO_3$/L and containing various ratios of carbonate/bicarbonate by the procedures of both ¶ 4*a* and ¶ 4*c*. The pooled standard deviation was 40 mg/L, with negligible difference between the procedures.

7. References

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4. WINTER, J.A. & M.R. MIDGETT. 1969. FWPCA Method Study 1. Mineral and Physical Analyses. Federal Water Pollution Control Admin., Washington, D.C.
5. SMITH, R. 1980. Research Rep. No. 379, Council for Scientific and Industrial Research, South Africa.

8. Bibliography

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SKOUGSTAD M.W., M.J. FISHMAN, L.C. FRIEDMAN, D.E. ERDMAN, & S.S. DUNCAN. 1979. Methods for determination of inorganic substances in water and fluvial sediments. *In* Techniques of Water-Resources Investigation of the United States Geological Survey. U.S. Geological Survey, Book 5, Chapter A1, Washington, D.C.

VARIABLE Chlorophyll a (uncorrected)

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot m^{-3}$

METHOD Acetone Extraction and Spectrophotometric Determination.

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Spectrophotometer with a narrow band width (0.5 to 2.0 nm).
Clinical centrifuge.
Glass tube / teflon pestle tissue grinder

PROCEDURE

Collect one sample per pond by pooling three 90 cm column samples. Mix the samples thoroughly and collect a subsample for vacuum filtration through a 25 or 47 mm diameter glass fiber or membrane filter (0.45 to 1 µm nominal pore size).

Filter, grind, extract, and analyze the pigment as described in detail in Standard Methods (attached). Membrane filters are easier to use with (but considerably more expensive) than glass fiber filters since they dissolve almost completely in the acetone solution.

10200 H. Chlorophyll

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass.[1,2] All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of planktonic algae. 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.

The three methods for determining chlorophyll *a* in phytoplankton are the spectrophotometric,[3-5] the fluorometric,[6-8] and the high-performance liquid chromatographic (HPLC) techniques.[9] Fluorometry is more sensitive than spectrophotometry, requires less sample, and can be used for in-vivo measurements.[10] These optical methods can significantly under- or overestimate chlorophyll *a* concentrations,[11-18] in part because of the overlap of the absorption and fluorescence bands of cooccurring accessory pigments and chlorophyll degradation products.

Pheophorbide *a* and pheophytin *a*, two common degradation products of chlorophyll *a*, can interfere with the determination of chlorophyll *a* because they absorb light and fluoresce in the same region of the spectrum as does chlorophyll *a*. If these pheopigments are present, significant errors in chlorophyll *a* values will result. Pheopigments can be measured either by spectrophotometry or fluorometry, but in marine and freshwater environments the fluorometric method is unreliable when chlorophyll *b* co-occurs. Upon acidification of chlorophyll *b*, the resulting fluorescence emission of pheophytin *b* is coincident with that of pheophytin *a*, thus producing underestimation and overestimation of chlorophyll *a* and pheopigments, respectively.

HPLC is a useful method for quantifying photosynthetic pigments[9,13,15,16,19-21] including chlorophyll *a*, accessory pigments (e.g., chlorophylls *b* and *c*), and chlorophyll degradation products (chlorophyllides, pheophorbides, and pheophytins). Pigment distribution is useful for quantitative assessment of phytoplankton community composition and zooplankton grazing activity.[22]

1. Pigment Extraction

Conduct work with chlorophyll extracts in subdued light to avoid degradation. Use opaque containers or wrap with aluminum foil. The pigments are extracted from the plankton concentrate with aqueous acetone and the optical density (absorbance) of the

PROCEDURE Cont. **VARIABLE Chlorophyll a (uncorrected)**

extract is determined with a spectrophotometer. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve consistently the complete extraction of the pigments, disrupt the cells mechanically with a tissue grinder.

Glass fiber filters are preferred for removing algae from water. The glass fibers assist in breaking the cells during grinding, larger volumes of water can be filtered, and no precipitate forms after acidification. Inert membrane filters such as polyester filters may be used where these factors are irrelevant.

a. Equipment and reagents:

1) *Tissue grinder:** Successfully macerating glass fiber filters in tissue grinders with grinding tube and pestle of conical design may be difficult. Preferably use round-bottom grinding tubes with a matching pestle having grooves in the TFE tip.

2) *Clinical centrifuge.*

3) *Centrifuge tubes,* 15-mL graduated, screw-cap.

4) *Filtration equipment,* filters, glass fiber† or membrane (0.45-μm porosity, 47-mm diam); vacuum pump; solvent-resistant disposable filter assembly, 1.0-μm pore size;‡ 10-mL solvent-resistant syringe.

5) *Saturated magnesium carbonate solution:* Add 1.0 g finely powdered $MgCO_3$ to 100 mL distilled water.

6) *Aqueous acetone solution:* Mix 90 parts acetone (reagent grade BP 56°C) with 10 parts saturated magnesium carbonate solution.

b. Extraction procedure:

1) Concentrate sample by centrifuging or filtering as soon as possible after collection. If processing must be delayed, hold samples on ice or at 4°C and protect from exposure to light. Use opaque bottles because even brief exposure to light during storage will alter chlorophyll values. Samples on filters taken from water having pH 7 or higher may be placed in airtight plastic bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation. Use glassware and cuvettes that are clean and acid-free.

2) Place sample in a tissue grinder, cover with 2 to 3 mL 90% aqueous acetone solution, and macerate at 500 rpm for 1 min. Use TFE/glass grinder for a glass-fiber filter and glass/glass grinder for a membrane filter.

3) Transfer sample to a screw-cap centrifuge tube, rinse grinder with a few milliliters 90% aqueous acetone, and add the rinse to the extraction slurry. Adjust total volume to a constant level, 5 to 10 mL, with 90% aqueous acetone. Use solvent sparingly and avoid excessive dilution of pigments. Steep samples at least 2 h at 4°C in the dark.

4) Clarify by filtering through a solvent-resistant disposable filter (to minimize retention of extract in filter and filter holder, force 1 to 2 mL air through the filter after the extract), or by centrifuging in closed tubes for 20 min at 500 *g*. Decant clarified extract into a clean, calibrated, 15-mL, screw-cap centrifuge tube and measure total volume. Proceed as in 2, 3, or 4 below.

2. Spectrophotometric Determination of Chlorophyll

a. Equipment and reagents:

1) *Spectrophotometer,* with a narrow band (pass) width (0.5 to 2.0 nm) because the chlorophyll absorption peak is relatively narrow. At a spectral band width of 20 nm the chlorophyll *a* concentration may be underestimated by as much as 40%.

2) *Cuvettes,* with 1-, 4-, and 10-cm path lengths.

3) *Pipets,* 0.1- and 5.0-mL.

*Kontes Glass Co., Vineland, N.J. 08360: Glass/glass grinder, Model No. 8855: Glass/TFE grinder, Model 886000; or equivalent.

†Whatman GF/F (0.7 μm), GFB (1.0 μm), Gelman AE (1 μm),[11] or equivalent.

‡Gelman Acrodisc or equivalent.

PROCEDURE Cont. **VARIABLE Chlorophyll a (uncorrected)**

4) *Hydrochloric acid,* HCl, 0.1*N*.

b. Determination of chlorophyll a *in the presence of pheophytin* a: Chlorophyll *a* may be overestimated by including pheopigments that absorb near the same wavelength as chlorophyll *a*. Addition of acid to chlorophyll *a* results in loss of the magnesium atom, converting it to pheophytin *a*. Acidify carefully to a final molarity of not more than $3 \times 10^{-3}M$ to prevent certain accessory pigments from changing to absorb at the same wavelength as pheophytin *a*.[13] When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption-peak-ratio (OD664/OD665) of 1.70 is used in correcting the apparent chlorophyll *a* concentration for pheophytin *a*.

Samples with an OD664 before/OD665 after acidification ratio ($664_b/665_a$) of 1.70 are considered to contain no pheophytin *a* and to be in excellent physiological condition. Solutions of pure pheophytin show no reduction in OD665 upon acidification and have a $664_b/665_a$ ratio of 1.0. Thus, mixtures of chlorophyll *a* and pheophytin *a* have absorption peak ratios ranging between 1.0 and 1.7. These ratios are based on the use of 90% acetone as solvent. Using 100% acetone as solvent results in a chlorophyll *a* before-to-after acidification ratio of about 2.0.[3]

Spectrophotometric procedure—Transfer 3 mL clarified extract to a 1-cm cuvette and read optical density (OD) at 750 and 664 nm. Acidify extract in the cuvette with 0.1 mL 0.1*N* HCl. Gently agitate the acidified extract and read OD at 750 and at 665 nm, 90 s after acidification. The volumes of extract and acid and the time after acidification are critical for accurate, consistent results.

The OD664 before acidification should be between 0.1 and 1.0. For very dilute extracts use cuvettes having a longer path length. If a larger cell is used, add a proportionately larger volume of acid. Correct OD obtained with larger cuvettes to 1 cm before making calculations.

Subtract the 750-nm OD value from the readings before (OD 664 nm) and after acidification (OD 665 nm).

Using the corrected values calculate chlorophyll *a* and pheophytin *a* per cubic meter as follows:

$$\text{Chlorophyll } a,\ \text{mg/m}^3 = \frac{26.7\,(664_b - 665_a) \times V_1}{V_2 \times L}$$

$$\text{Pheophytin } a,\ \text{mg/m}^3 = \frac{26.7\,[1.7\,(665_a) - 664_b] \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, L,
V_2 = volume of sample, m^3,
L = light path length or width of cuvette, cm, and
664_b, 665_a = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.7 is the absorbance correction and equals $A \times K$

where:

A = absorbance coefficient for chlorophyll *a* at 664 nm = 11.0, and
K = ratio expressing correction for acidification,

$$= \frac{\left(\frac{664_b}{665_a}\right)_{\text{pure chlorophyll a}}}{\left(\frac{664_b}{665_a}\right)_{\text{pure chlorophyll a}} - \left(\frac{664_b}{665_a}\right)_{\text{pure pheophytin a}}}$$

$$= \frac{1.7}{1.7 - 1.0} = 2.43$$

c. Determination of chlorophyll a, b, *and* c *(trichromatic method):*

Spectrophotometric procedure—Transfer extract to a 1-cm cuvette and measure optical density (OD) at 750, 664, 647, and 630 nm. Choose a cell path length or di-

PROCEDURE Cont. **VARIABLE Chlorophyll a (uncorrected)**

lution to give an OD664 between 0.1 and 1.0.

Use the optical density readings at 664, 647, and 630 nm to determine chlorophyll *a*, *b*, and *c*, respectively. The OD reading at 750 nm is a correction for turbidity. Subtract this reading from each of the pigment OD values of the other wavelengths before using them in the equations below. Because the OD of the extract at 750 nm is very sensitive to changes in the acetone-to-water proportions, adhere closely to the 90 parts acetone:10 parts water (v/v) formula for pigment extraction. Turbidity can be removed easily by filtration through a disposable, solvent-resistant filter attached to a syringe or by centrifuging for 20 min at 500 *g*.

Calculate the concentrations of chlorophyll *a*, *b*, and *c* in the extract by inserting the corrected optical densities in following equations:[5]

a) $C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$

b) $C_b = 21.03(OD647) - 5.43(OD664) - 2.66(OD630)$

c) $C_c = 24.52(OD630) - 7.60(OD647) - 1.67(OD664)$

where:

C_a, C_b, and C_c = concentrations of chlorophyll *a*, *b*, and *c*, respectively, mg/L, and

OD664, OD647, and OD630 = corrected optical densities (with a 1-cm light path) at the respective wavelengths.

After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{C_a \times \text{extract volume, L}}{\text{Volume of sample, m}^3}$$

VARIABLE Dark Bottle Respiration

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg\ O_2 \cdot m^{-3} \cdot d^{-1}$

METHOD Oxygen method.

REFERENCE Adapted from:
American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Dissolved oxygen meter with BOD-type probe or reagents and equipment for Winkler titrations.

PROCEDURE

Collect one sample (by pooling three 90 cm column samples) from each pond. Make sure that initial dissolved oxygen concentrations are not above saturation. Incubate for four hours (or as appropriate to prevent oxygen depletion) in dark bottles suspended at mid-depth in the ponds.

PROCEDURE Cont. **VARIABLE Dark Bottle Respiration**

2. Productivity, Oxygen Method

Productivity is defined as the rate at which inorganic carbon is converted to an organic form. Chlorophyll-bearing organisms (phytoplankton, periphyton, macrophytes) serve as primary producers in the aquatic food chain. Photosynthesis ultimately results in the formation of a wide range of organic compounds, release of oxygen, and reduction of carbon dioxide (CO_2) in the surrounding waters. Primary productivity[6] can be determined by measuring the changes in oxygen and CO_2 concentrations.[7] In poorly buffered waters, pH can be a sensitive property for detecting variations in the system. As CO_2 is removed during photosynthesis, the pH rises. This shift can be used to estimate both photosynthesis and respiration.[8] The sea and many fresh waters are too highly buffered to make this useful, but it has been applied successfully to productivity studies in some lake waters.

Two methods of measuring the rate of carbon uptake and net photosynthesis in situ are the oxygen method[9] and the carbon 14 method.[10] In both methods, clear (light) and darkened (dark) bottles are filled with water samples and suspended at regular depth intervals for an incubation period of several hours or samples are incubated under controlled conditions in environmental growth chambers in the laboratory.

The basic reactions in algal photosynthesis involve uptake of inorganic carbon and release of oxygen, summarized by the relationship:

$$CO_2 + H_2O \rightarrow (CH_2O)_x + O_2$$

The chief advantages of the oxygen method are that it provides estimates of gross and net productivity and respiration and that analyses can be performed with inexpensive laboratory equipment and common reagents. The dissolved oxygen (DO) concentration is determined at the beginning and end of the incubation period. Productivity is calculated on the assumption that one atom of carbon is assimilated for each molecule of oxygen released.

a. Equipment:

1) *BOD bottles,* numbered, 300-mL, clear and opaque borosilicate glass, with ground-glass stopper and flared mouth, for sample incubation. Acid-clean the bottles, rinse thoroughly with distilled water, and just before use, rinse with water being tested. Do not use phosphorus-containing detergents.

If suitable opaque bottles are not available, make clear BOD bottles opaque by painting them black and wrapping with black waterproof tape. As a further precaution, wrap entire bottle in aluminum foil or place in a light-excluding container during incubation.

2) *Supporting line or rack* that does not shade suspended bottles.

3) *Nonmetallic opaque acrylic Van Dorn sampler* or equivalent, of 3- to 5-L capacity.

PROCEDURE Cont. **VARIABLE Dark Bottle Respiration**

4) *Equipment and reagents for dissolved oxygen determinations:* See Section 4500-O.

5) *Pyrheliometer.*

6) *Submarine photometer.*

7) *Thermometer.*

b. Procedure:

1) Obtain a profile of the input of solar radiation for the photoperiod with a pyrheliometer.

2) Determine depth of euphotic zone (the region that receives 1% or more of surface illumination) with a submarine photometer. Select depth intervals for bottle placement. The photosynthesis-depth curve will be approximated closely by placing samples at intervals equal to one-tenth the depth of the euphotic zone. Estimate productivity in relatively shallow water with fewer depth intervals.

3) Measure oxygen concentration with probe or by titration and temperature and salinity to determine whether water is supersaturated with respect to oxygen (see Table 4500-O:I). If water is supersaturated, bubble nitrogen gas through sample to lower initial oxygen concentration to less than 80% saturation.

4) Keep samples out of direct sunlight during handling. Introduce samples taken from each preselected depth into duplicate clear, darkened, and initial-analysis bottles. Insert delivery tube of sampler to bottom of sample bottle and fill so that three volumes of water are allowed to overflow. Remove tube slowly and close bottle. Use water from the same grab sample to fill a "set" (one light, one dark, and one initial bottle).

5) Immediately treat (fix) samples taken for the chemical determination of initial dissolved oxygen (see Section 4500-O) with manganous sulfate ($MnSO_4$), alkaline iodide, and sulfuric acid (H_2SO_4) or check with an oxygen probe. Analyses may be delayed several hours if necessary, if samples are fixed or iced and stored in the dark.

6) Suspend duplicate paired clear and darkened bottles at the depth from which the samples were taken and incubate for at least 2 h, but never longer than it takes for oxygen-gas bubbles to form in the clear bottles or DO to be depleted in the dark bottles.

7) At the end of the exposure period, immediately determine DO as described above.

c. Calculations: The increase in oxygen concentration in the light bottle during incubation is a measure of net production which, because of the concurrent use of oxygen in respiration, is somewhat less than the total (or gross) production. The loss of oxygen in the dark bottle is used as an estimate of total plankton respiration. Thus:

$$\text{Net photosynthesis} = \text{light bottle DO} - \text{initial DO}$$

$$\text{Respiration} = \text{initial DO} - \text{dark bottle DO}$$

$$\text{Gross photosynthesis} = \text{light bottle DO} - \text{dark bottle DO}$$

Average results from duplicates.

1) Calculate the gross or net production for each incubation depth and plot:

$$\text{mg carbon fixed/m}^3 = \text{mg oxygen released/L} \times 12/32 \times 1000 \times K$$

where K is the photosynthetic quotient (PQ), ranging from 1 to 2, depending on the nitrogen supply.[11,12]

Use the factor 12/32 to convert oxygen to carbon; under ideal conditions 1 mole of O_2 (32 g) is released for each mole of carbon (12 g) fixed.

2) Productivity is defined as the rate of production and generally is reported in grams carbon fixed per square meter per day. Determine the productivity of a vertical column of water 1 m square by plotting productivity for each exposure depth and graphically integrating the area under the curve.

3) Using the solar radiation profile and photosynthesis rate during incubation adjust the data to represent phytoplankton productivity for the entire photoperiod. Because photosynthetic rates vary widely during the daily cycle,[13,14] do not attempt to convert data to other test circumstances.

VARIABLE	Dissolved Oxygen
DATE	October 1990
VERSION	1
SAMPLE	Water
REPORTING UNITS	$mg \cdot L^{-1}$
METHOD	Azide modification of Iodometric (Winkler), or Membrane Electrode.
REFERENCE	American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Dissolved oxygen meter
(Yellow Springs Instrument Model 57 Dissolved Oxygen Meter, or equivalent)
Follow operating instructions carefully. Turn meter on at least 15 minutes prior to calibration, and leave meter on for the duration of the sampling. Maintain the membrane in good condition by periodic changing and by refilling the electrode.
The meter may be calibrated as indicated in the operating manual (air calibration or saturated water sample) or against the Winkler method.

PROCEDURE

Collect samples near the center of each pond at 25 cm below the water surface, mid-water, and 25 cm above the bottom. Analyze samples separately.

For the Winkler test, collect samples in BOD bottles, being careful not to agitate or aerate the sample as it is collected. Add Manganous sulfate, alkali-iodide-azide reagent, and sulfuric acid as soon as posible after sample collection. The samples may be stored for a few hours prior to titration if the water has no iodine demand. Titrate as soon as possible to prevent or minimize sample changes.

For the Membrane Electrode, keep the probe in motion while the sample is analyzed to ensure water flow past the membrane. If the dissolved oxygen is measured in situ, take care not to agitate the water to the extent that stratification or sediments are disturbed.

4500-O C. Azide Modification

1. General Discussion

Use the azide modification for most wastewater, effluent, and stream samples, especially if samples contain more than 50 μg NO_2^--N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF solution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. Manganous sulfate solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$, 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

b. Alkali-iodide-azide reagent:

1) For saturated or less-than-saturated samples—Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

2) For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide

(NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

c. Sulfuric acid, H_2SO_4, conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. Starch: Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. Standard sodium thiosulfate titrant: Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water. Add 1.5 mL 6*N* NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f. Standard potassium bi-iodate solution, 0.0021*M:* Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 mL.

Standardization—Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6*N* H_2SO_4 or a few drops of conc H_2SO_4 and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.025*M* $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025*M*.

g. Potassium fluoride solution: Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 mL.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc H_2SO_4. Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of $MnSO_4$ and alkali-iodide-azide reagents in a 300-mL bottle, titrate $200 \times 300/(300 - 2) = 201$ mL.

b. Titrate with 0.025*M* $Na_2S_2O_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0021*M* bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

a. For titration of 200 mL sample, 1 mL 0.025*M* $Na_2S_2O_3$ = 1 mg DO/L.

b. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 4500-O:I. Equations for correcting solubilities to barometric pressures other than mean sea level and for various chlorinities are given below the table.

5. Precision and Bias

DO can be determined with a precision, expressed as a standard deviation, of about 20 μg/L in distilled water and about 60 μg/L in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 μg/L. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

VARIABLE Nitrogen-Ammonia

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Nesslerization or Phenate Methods

REFERENCE Michigan State University Limnological Research Laboratory, 1984.
American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Spectrophotometer for use at 400 to 500 nm that provides a light path of 1 cm or longer.

PROCEDURE

Collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be filtered as soon as possible after collection, refrigerated and analyzed within 24 hours.

Follow the detailed procedures for sample preparation, distillation, and analysis as described in Standards Methods (attached).

4500-NH_3 C. Nesslerization Method (Direct and Following Distillation)

1. General Discussion

Use direct nesslerization only for purified drinking waters, natural water, and highly purified wastewater effluents, all of which should be low in color and have NH_3-N concentrations exceeding 20 μg/L. Apply the direct nesslerization method to domestic wastewaters only when errors of 1 to 2 mg/L are acceptable. Use this method only after it has been established that it yields results comparable to those obtained after distillation. Check validity of direct nesslerization measurements periodically.

Pretreatment before direct nesslerization with zinc sulfate and alkali precipitates calcium, iron, magnesium, and sulfide, which form turbidity when treated with nessler reagent. The floc also removes suspended matter and sometimes colored matter. Addition of EDTA or Rochelle salt solution inhibits precipitation of residual calcium and magnesium ions in the presence of the alkaline nessler reagent. However, use of EDTA demands an extra amount of nessler reagent to insure a sufficient nessler reagent excess for reaction with the ammonia.

The graduated yellow to brown colors produced by the nessler-ammonia reaction absorb strongly over a wide wavelength range. The yellow color characteristic of low ammonia nitrogen concentration (0.4 to 5 mg/L) can be measured with acceptable sensitivity in the wavelength region from 400 to 425 nm when a 1-cm light path is available. A light path of 5 cm extends measurements into the nitrogen concentration range of 5 to 60 μg/L. The reddish brown hues typical of ammonia nitrogen levels approaching 10 mg/L may be measured in the wavelength region of 450 to 500 nm. A judicious selection of light path and wavelength thus permits the photometric determination of ammonia nitrogen concentrations over a considerable range.

Departures from Beer's law may be evident when photometers equipped with broad-band color filters are used. For this reason, prepare the calibration curve under conditions identical with those adopted for the samples.

A carefully prepared nessler reagent may respond under optimum conditions to as little as 1 μg NH_3-N/50 mL. In direct nesslerization, this represents 20 μg/L. However, reproducibility below 100 μg/L may be erratic.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 400 to 500 nm and providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance at 400 to 425 nm. A blue filter can be used for higher NH_3-N concentrations.

3) *Nessler tubes,* matched, 50-mL, tall form.

b. pH meter, equipped with a high-pH electrode.

3. Reagents

Use ammonia-free water for preparing all reagents, rinsing, and making dilutions. All the reagents listed in Preliminary Distillation (4500-NH_3.B above), except the borate buffer and absorbent solution, are required, plus the following:

a. Zinc sulfate solution: Dissolve 100 g $ZnSO_4 \cdot 7H_2O$ and dilute to 1 L with water.

b. Stabilizer reagent: Use either EDTA or Rochelle salt to prevent calcium or magnesium precipitation in undistilled samples after addition of alkaline nessler reagent.

1) *EDTA reagent:* Dissolve 50 g disodium ethylenediamine tetraacetate dihydrate in 60 mL water containing 10 g NaOH. If necessary, apply gentle heat to complete dissolution. Cool to room temperature and dilute to 100 mL.

2) *Rochelle salt solution:* Dissolve 50 g potassium sodium tartrate tetrahydrate, $KNaC_4H_4O_6 \cdot 4H_2O$, in 100 mL water. Remove ammonia usually present in the salt by boiling off 30 mL of solution. After cooling, dilute to 100 mL.

c. Nessler reagent: Dissolve 100 g HgI_2 and 70 g KI in a small quantity of water and add this mixture slowly, with stirring, to a cool solution of 160 g NaOH dissolved in 500 mL water. Dilute to 1 L. Store in rubber-stoppered borosilicate glassware and out of sunlight to maintain reagent stability for up to a year under normal laboratory conditions. Check reagent to make sure that it yields the characteristic color with 0.1 mg NH_3-N/L within 10 min after addition and does not produce a precipitate with small amounts of ammonia within 2 h. (CAUTION: *Toxic—take care to avoid ingestion.*)

d. Stock ammonium solution: Dissolve 3.819 g anhydrous NH_4Cl, dried at 100°C, in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH_3.

e. Standard ammonium solution: Dilute 10.00 mL stock ammonium solution to 1000 mL with water; 1.00 mL = 10.00 μg N = 12.2 μg NH_3.

f. Permanent color solutions:

1) *Potassium chloroplatinate solution:* Dissolve 2.0 g K_2PtCl_4 in 300 to 400 mL water; add 100 mL conc HCl and dilute to 1 L.

2) *Cobaltous chloride solution:* Dissolve 12.0 g $CoCl_2 \cdot 6H_2O$ in 200 mL water. Add 100 mL conc HCl and dilute to 1 L.

4. Procedure

a. Treatment of undistilled samples: If necessary, remove residual chlorine from the freshly collected sample by adding an equivalent amount of dechlorinating agent. (Do not store chlorinated samples without prior dechlorination.) Add 1 mL $ZnSO_4$ solution to 100 mL sample and mix thoroughly. Add 0.4 to 0.5 mL 6*N* NaOH solution to obtain a pH of 10.5, as determined with a pH meter and a high-pH glass electrode, and mix gently. Let treated sample stand for a few minutes, whereupon a heavy flocculent precipitate should fall, leaving a clear and colorless supernate. Clarify by centrifuging or filtering. Pretest any filter paper used to be sure no ammonia is present as a contaminant. Do this by running water through the filter and testing the filtrate by nesslerization. Filter sample, discarding first 25 mL filtrate. (CAUTION: Samples containing more than about 10 mg NH_3-N/L may lose ammonia during this treatment of undistilled samples because of the high pH. Dilute such samples to the sensitive range for nesslerization before pretreatment.)

b. Color development:

1) Undistilled samples—Use 50.0 mL sample or a portion diluted to 50.0 mL with water. If the undistilled portion contains sufficient concentrations of calcium, magnesium, or other ions that produce turbidity or precipitate with nessler reagent, add 1 drop (0.05 mL) EDTA reagent or 1 to 2 drops (0.05 to 0.1 mL) Rochelle salt solution. Mix well. Add 2.0 mL nessler reagent if EDTA reagent is used or 1.0 mL nessler reagent if Rochelle salt is used.

2) Distilled samples—Neutralize the boric acid used for absorbing the ammonia distillate by adding either 2 mL nessler reagent, an excess that raises the pH to the desired high level, or alternatively, neutralizing the boric acid with NaOH before adding 1 mL nessler reagent.

3) Mix samples by capping nessler tubes with clean rubber stoppers (washed thoroughly with water) and then inverting tubes at least six times. Keep such conditions as temperature and reaction time the same in blank, samples, and standards. Let reaction proceed for at least 10 min after

adding nessler reagent. Measure color in sample and standards. If NH_3-N is very low, use a 30-min contact time for sample, blank, and standards. Measure color either photometrically or visually as directed in ¶s *c* or *d* below.

c. Photometric measurement: Measure absorbance or transmittance with a spectrophotometer or filter photometer. Prepare calibration curve at the same temperature and reaction time used for samples. Measure transmittance readings against a reagent blank and run parallel checks frequently against standards in the nitrogen range of the samples. Redetermine complete calibration curve for each new batch of nessler reagent.

For distilled samples, prepare standard curve under the same conditions as the samples. Distill reagent blank and appropriate standards, each diluted to 500 mL, in the same manner as the samples. Dilute 300 mL distillate plus 50 mL boric acid absorbent to 500 mL with water and take a 50-mL portion for nesslerization.

d. Visual comparison: Compare colors produced in sample against those of ammonia standards. Prepare temporary or permanent standards as follows:

1) Temporary standards—Prepare a series of visual standards in nessler tubes by adding the following volumes of standard NH_4Cl solution and diluting to 50 mL with water: 0, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0 mL. Nesslerize standards and portions of distillate by adding 1.0 mL nessler reagent to each tube and mixing well.

2) Permanent standards—Measure into 50-mL nessler tubes the volumes of K_2PtCl_6 and $CoCl_2$ solutions indicated in Table 4500-NH_3:III, dilute to mark, and mix thoroughly. The values given in the table are *approximate;* actual equivalents of the ammonium standards will differ with the quality of nessler reagent, the kind of illumination used, and the color sensitivity of the analyst's eye. Therefore, compare color standards with nesslerized temporary ammonia standards and modify the tints as necessary. Make such comparisons for each newly prepared nessler reagent and satisfy each analyst as to the aptness of the color match. Protect standards from dust to extend their usefulness for several months. Compare either 10 or 30 min after nesslerization, depending on reaction time used in preparing nesslerized ammonium standards against which they were matched.

TABLE 4500-NH_3:III. PREPARATION OF PERMANENT COLOR STANDARDS FOR VISUAL DETERMINATION OF AMMONIA NITROGEN

Value in Ammonia Nitrogen μg	Approximate Volume of Platinum Solution mL*	Approximate Volume of Cobalt Solution mL*
0	1.2	0.0
2	2.8	0.0
4	4.7	0.1
7	5.9	0.2
10	7.7	0.5
14	9.9	1.1
17	11.4	1.7
20	12.7	2.2
25	15.0	3.3
30	17.3	4.5
35	19.0	5.7
40	19.7	7.1
45	19.9	8.7
50	20.0	10.4
60	20.0	15.0

*In matched 50-mL nessler tubes.

5. Calculation

a. Deduct amount of NH_3-N in water used for diluting original sample before computing final nitrogen value.

b. Deduct also reagent blank for volume of borate buffer and 6*N* NaOH solutions used with sample.

c. Compute total NH_3-N by the following equation:

$$\text{mg } NH_3\text{-N/L (51 mL final volume)} = \frac{A}{\text{mL sample}} \times \frac{B}{C}$$

where:

A = μg NH_3-N (51 mL final volume),
B = total volume distillate collected, mL, including acid absorbent, and
C = volume distillate taken for nesslerization, mL.

The ratio B/C applies only to distilled samples; ignore in direct nesslerization.

6. Precision and Bias

See Section 4500-NH_3.A.4 and Table 4500-NH_3:I.

7. Bibliography

JACKSON, D.D. 1900. Permanent standards for use in the analysis of water. *Mass. Inst. Technol. Quart.* 13:314.

SAWYER, C.N. 1953. pH adjustment for determination of ammonia nitrogen. *Anal. Chem.* 25:816.

JENKINS, D. 1967. The differentiation, analysis and preservation of nitrogen and phosphorus forms in natural waters. *In* Trace Inorganics in Water. American Chemical Soc., Washington, D.C.

BOLTZ, D.F. & J.A. HOWELL, EDS. 1978. Colorimetric Determination of Nonmetals. Wiley Interscience, New York, N.Y.

4500-NH_3 D. Phenate Method

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by a manganous salt.

b. Interference: Alkalinity over 500 mg as $CaCO_3$/L, acidity over 100 mg as $CaCO_3$/L, and turbidity interfere. Remove these interferences by preliminary distillation.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 630 nm with a light path of approximately 1 cm.

2) *Filter photometer,* equipped with a red-orange filter having a maximum transmittance near 630 nm and providing a light path of approximately 1 cm.

b. Magnetic stirrer.

3. Reagents

a. Ammonia-free water: Prepare as directed in Section 4500-NH_3.B.3*a*. Use for making all reagents.

b. Hypochlorous acid reagent: To 40 mL water add 10 mL 5% NaOCl solution prepared from commercial bleach. Adjust pH to 6.5 to 7.0 with HCl. Prepare this unstable reagent weekly.

c. Manganous sulfate solution, 0.003 *M*: Dissolve 50 mg $MnSO_4 \cdot H_2O$ in 100 mL water.

d. Phenate reagent: Dissolve 2.5 g NaOH and 10 g phenol, C_6H_5OH, in 100 mL water. Because this reagent darkens on standing, prepare weekly. (CAUTION: *Handle phenol with care.*)

e. Stock ammonium solution: Dissolve 381.9 mg anhydrous NH_4Cl, dried at 100°C, in water, and dilute to 1000 mL; 1.00 mL = 100 μg N = 122 μg NH_3.

f. Standard ammonium solution: Dilute 5.00 mL stock ammonium solution to 1000 mL with water; 1.00 mL = 0.500 μg N = 0.607 μg NH_3.

4. Procedure

a. Treatment of sample: To a 10.0-mL sample in a 50-mL beaker, add 1 drop (0.05 mL) $MnSO_4$ solution. Place on a magnetic stirrer and add 0.5 mL hypochlorous acid reagent. Immediately add, a drop at a time, 0.6 mL phenate reagent. Add reagent with-

out delay using a bulb pipet or a buret for convenient delivery. Mark pipet for hypochlorous acid at the 0.5-mL level and deliver the phenate reagent from a pipet or a buret that has been calibrated by counting the number of drops previously found to be equivalent to 0.6 mL. Stir vigorously during addition of reagents. Because color intensity is affected by age of reagents, carry a blank and a standard through the procedure with each batch of samples. Measure absorbance using reagent blank to zero the spectrophotometer. Color formation is complete in 10 min and is stable for at least 24 h. Although the blue color has a maximum absorbance at 630 nm, satisfactory measurements can be made in the 600- to 660-nm region.

b. Preparation of standards: Prepare a calibration curve in the NH_3-N range of 0.1 to 5 μg, treating standards exactly as the sample. Beer's Law governs.

5. Calculation

Calculate ammonia concentration as follows:

$$\text{mg } NH_3\text{-N/L (11.1 mL final volume)} = \frac{A \times B}{C \times S} \times \frac{D}{E}$$

where:

A = absorbance of sample,
B = NH_3-N in standard, μg,
C = absorbance of standard,
S = volume of sample used, mL,
D = volume of total distillate collected, mL, including acid absorbent, neutralizing agent, and ammonia-free water added, and
E = volume of distillate used for color development, mL.

The ratio D/E applies only to distilled samples.

6. Precision and Bias

See Section 4500-NH_3.A.4 and Table 4500-NH_3:I.

7. Bibliography

ROSSUM, J.R. & P.A. VILLARRUZ. 1963. Determination of ammonia by the indophenol method. *J. Amer. Water Works Assoc.* 55:657.

WEATHERBURN, M.W. 1967. Phenolhypochlorite reaction for determination of ammonia. *Anal. Chem.* 39:971.

VARIABLE Nitrogen - Total Kjeldahl

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Semi-Micro-Kjeldahl Method.

REFERENCE Michigan State University Limnological Research Lab, 1984.
American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Kjeldahl digestion unit.
(Kontes Kjeldahl Nitrogen apparatus, or equivalent)
Instrumentation for ammonia measurement.

PROCEDURE

For each pond, pool three 90 cm column samples. Composite samples should be refrigerated and analyzed within 24 hours.

Follow detailed instructions for reagent preparation, sample digestion, and distillation given in Standard Methods (attached).

4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N_{org}.B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus* equipped with heating elements to accomodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 365 to 380°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater† (Figure 4500-N_{org}:1).

c. pH meter.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH_3.B.3) and Nitrogen (Organic) macro-kjeldahl (Section 4500-N_{org}.B.3) are required. Prepare all reagents and dilutions with ammonia-free water.

*Rotary kjeldahl digestion unit, Kontes, Model K551100, or equivalent.

†ASTM E-147 or equivalent.

PROCEDURE Cont. **VARIABLE Nitrogen - Total Kjeldahl**

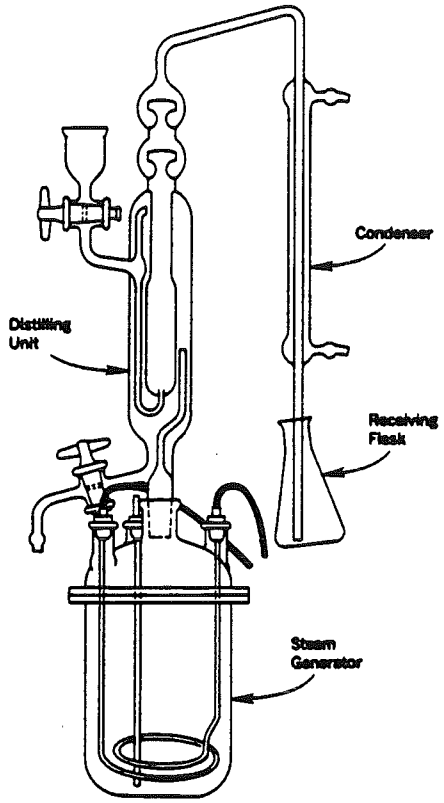

Figure 4500-N_{org}:1. Micro-kjeldahl distillation apparatus.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample *mg/L*	Sample Size *mL*
4–40	50
8–80	25
20–200	10
40–400	5

For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add 3 mL borate buffer and adjust to pH 9.5 with 6*N* NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl flask and boil off 30 mL.

Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ *c* below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add 5 or 6 glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO_3. Continue to boil briskly until solution clears (becomes colorless or a pale straw color) and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL boric acid solution contained in a 125-mL

PROCEDURE Cont. **VARIABLE Nitrogen - Total Kjeldahl**

erlenmeyer flask. Use plain boric acid solution when ammonia is to be determined by nesslerization and use indicating boric acid for a titrimetric finish. Use 10 mL 0.04*N* H_2SO_4 solution for collecting distillate for the phenate, nessler, or electrode methods. Extend tip of condenser well below level of boric acid solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with delivery tube and continue distillation during last 1 or 2 min to cleanse condenser.

e. Blank: Carry a reagent blank through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Determine ammonia by nesslerization, manual phenate, titration, or ammonia-selective electrode method.

5. Calculation

See Section 4500-NH_3.C.5, D.5, E.5, or F.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N_{org}.B.7.

VARIABLE Nitrogen - Nitrate

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Cadmium Reduction

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Spectrophotometer.

PROCEDURE

Samples should be filtered and analyzed as soon as possible after collection. If storage is necessary, store for up to 24 h at 4°C. Follow detailed instructions for reagent preparation and sample analysis given in Standard Methods (attached).

4500-NO_3^- E. Cadmium Reduction Method

1. General Discussion

a. Principle: NO_3^- is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate ($CuSO_4$) and packed in a glass column.

The NO_2^- produced thus is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically. A correction may be made for any NO_2^- present in the sample by analyzing without the reduction step. The applicable range of this method is 0.01 to 1.0 mg NO_3^--N/L. The method is recommended especially for NO_3^- levels below 0.1 mg N/L where other methods lack adequate sensitivity.

b. Interferences: Suspended matter in the column will restrict sample flow. For turbid samples, see ¶ A.1. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency. Add EDTA to samples to eliminate this interference. Oil and grease will coat the Cd surface. Remove by pre-extraction with an organic solvent (see Section 5520). Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Check samples for residual chlorine (see DPD methods in Section 4500-Cl). Remove residual chlorine by adding sodium thiosulfate ($Na_2S_2O_3$) solution [Section 4500-NH_3.B.3*d*4)]. Sample color that absorbs at about 540 nm interferes.

2. Apparatus

a. Reduction column: Purchase or construct the column* (Figure 4500-NO_3^-:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve[1] to control flow rate.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 543 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* with light path of 1 cm or longer and equipped with a filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrate-free water: See ¶ B.3*a*. The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilutions.

b. Copper-cadmium granules: Wash 25 g new or used 40- to 60-mesh Cd granules† with 6*N* HCl and rinse with water. Swirl Cd with 100 mL 2% $CuSO_4$ solution for 5 min or until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to de-

* Tudor Scientific Glass Co., 555 Edgefield Road, Belvedere, S.C. 29841, Cat. TP-1730, or equivalent.

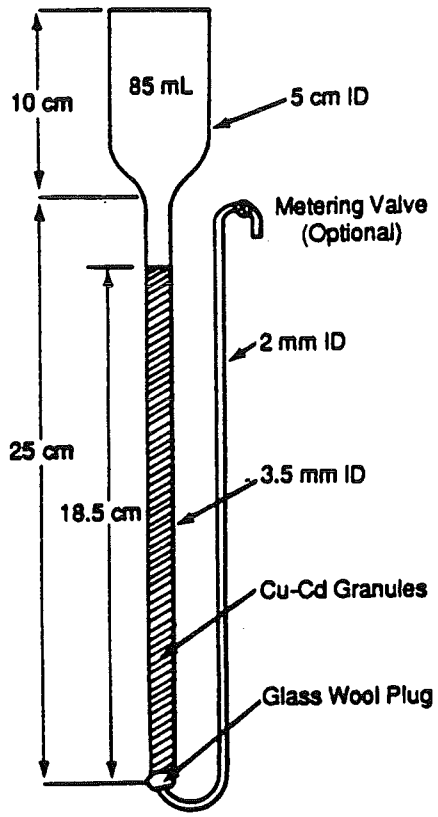

Figure 4500-NO_3^-:1. Reduction column.

velop. Gently flush with water to remove all precipitated Cu.

c. Color reagent: Prepare as directed in Section 4500-NO_2^-B.3*b*.

d. Ammonium chloride-EDTA solution: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with conc NH_4OH and dilute to 1 L.

† EM Laboratories, Inc., 500 Exec. Blvd., Elmsford, N.Y., Cat. 2001, or equivalent.

e. Dilute ammonium chloride-EDTA solution: Dilute 300 mL NH_4Cl-EDTA solution to 500 mL with water.

f. Hydrochloric acid, HCl, 6*N*.

g. Copper sulfate solution, 2%: Dissolve 20 g $CuSO_4.5H_2O$ in 500 mL water and dilute to 1 L.

h. Stock nitrate solution; Prepare as directed in ¶ B.3*b*.

i. Intermediate nitrate solution: Prepare as directed in ¶ B.3*c*.

j. Stock nitrite solution: See Section 4500-NO_2^-.B.3*e*.

k. Intermediate nitrite solution: See Section 4500-NO_2^-.B.3*f*.

l. Working nitrite solution: Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite-free water; 1.00 mL = 5 μg NO_2^--N.

4. Procedure

a. Preparation of reduction column: Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH_4Cl-EDTA solution. Activate column by passing through it, at 7 to 10 mL/min, at least 100 mL of a solution composed of 25% 1.0 mg NO_3^--N/L standard and 75% NH_4Cl-EDTA solution.

b. Treatment of sample:

1) Turbidity removal—For turbid samples, see ¶ A.1.

2) pH adjustment—Adjust pH to between 7 and 9, as necessary, using a pH meter and dilute HCl or NaOH. This insures a pH of 8.5 after adding NH_4Cl-EDTA solution.

3) Sample reduction—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 mL dilute NH_4Cl-EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

4) Color development and measurement—As soon as possible, and not more than 15 min after reduction, add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 h afterward, measure absorbance at 543 nm against a distilled water-reagent blank. NOTE: If NO_3^- concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

c. Standards: Using the intermediate NO_3^--N solution, prepare standards in the range 0.05 to 1.0 mg NO_3^--N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one NO_2^- standard to a reduced NO_3^- standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in ¶ 3*b* above when efficiency of reduction falls below about 75%.

5. Calculation

Obtain a standard curve by plotting absorbance of standards against NO_3^--N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized N per liter (the sum of NO_3^--N plus NO_2^--N) unless the concentration of NO_2^--N is separately determined and subtracted.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg NO_3^- + NO_2^--N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01, respectively.

PROCEDURE Cont. **VARIABLE Nitrogen - Nitrate**

In a single laboratory using wastewater with additions of 0.24, 0.55, and 1.05 mg NO_3^- + NO_2^--N/L, the recoveries were 100%, 102%, and 100%, respectively.[2]

7. References

1. WOOD, E.D., F.A.J. ARMSTRONG & F.A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *J. Mar. Biol. Assoc. U.K.* 47:23.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes, Method 353.3. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

STRICKLAND, J.D.H. & T.R. PARSONS. 1972. A Practical Handbook of Sea Water Analysis, 2nd ed. Bull. No. 167, Fisheries Research Board Canada, Ottawa, Ont.

NYDAHL, F. 1976. On the optimum conditions for the reduction of nitrate by cadmium. *Talanta* 23:349.

AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1987. Annual Book of ASTM Standards, Vol. 11.01. American Soc. Testing & Materials, Philadelphia, Pa.

VARIABLE Nitrogen - Nitrite

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Colorimetric.

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Spectrophotometer with a light path of 1 cm or longer for use at 543 nm.

PROCEDURE

Samples should be filtered and anlayzed as soon as possible after collection, frozen at -20°C or refrigerated at 4°C and analyzed within one to two days. Follow detailed instructions for reagent preparation and sample analysis given in Standard Methods (attached).

4500–NO_2^- B. Colorimetric Method

1. General Discussion

a. Principle: Nitrite (NO_2^-) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 μg NO_2^--N/L. Photometric measurements can be made in the range 5 to 50 μg N/L if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 μg N/ L with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

b. Interferences: Chemical incompatibility makes it unlikely that NO_2^-, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^+, chloroplatinate ($PtCl_6^{2-}$), and metavanadate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

c. Storage of sample: Never use acid preservation for samples to be analyzed for NO_2^-. Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. For short-term preservation for 1 to 2 d, freeze at −20°C or store at 4°C.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrite-free water: If it is not known that the distilled or demineralized water is free from NO_2^-, use either of the following procedures to prepare nitrite-free water:

1) Add to 1 L distilled water one small crystal each of $KMnO_4$ and either $Ba(OH)_2$ or $Ca(OH)_2$. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 mL of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent (Section 4500-Cl.F.2b), indicates the presence of permanganate.

2) Add 1 mL conc H_2SO_4 and 0.2 mL $MnSO_4$ solution (36.4 g $MnSO_4$ H_2O/100 mL distilled water) to each 1 L distilled water, and make pink with 1 to 3 mL KMnO4 solution (400 mg $KMnO_4$/L distilled water). Redistill as described in the preceding paragraph.

Use nitrite-free water in making all reagents and dilutions.

b. Color reagent: To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

c. Sodium oxalate, 0.025*M* (0.05*N*): Dissolve 3.350 g $Na_2C_2O_4$, primary standard grade, in water and dilute to 1000 mL.

d. Ferrous ammonium sulfate, 0.05*M* (0.05*N*): Dissolve 19.607 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize as in Section 5220B.3*d*.

e. Stock nitrite solution: Commercial reagent-grade $NaNO_2$ assays at less than 99%. Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine $NaNO_2$ content, add a known excess of standard 0.05*M* (0.05*N*) $KMnO_4$ solution prepared (with 8.0 g $KMnO_4$) and standardized as described in 3500-Ca.C.3*i*, discharge permanganate color with a known quantity of standard reductant such as 0.025*M* $Na_2C_2O_4$ or 0.05*M* $Fe(NH_4)_2(SO_4)_2$, and back-titrate with standard permanganate solution.

1) Preparation of stock solution—Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 μg N. Preserve with 1 mL $CHCl_3$.

2) Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.05*M* $KMnO_4$, 5 mL conc H_2SO_4, and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while adding stock NO_2^- solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient 10-mL portions of standard 0.025*M* $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.05*M* $KMnO_4$ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

If standard 0.05*M* ferrous ammonium sulfate solution is substituted for $Na_2C_2O_4$, omit heating and extend reaction period between $KMnO_4$ and Fe^{2+} to 5 min before making final $KMnO_4$ titration.

Calculate NO_2^--N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

A = mg NO_2^--N/mL in stock $NaNO_2$ solution,
B = total mL standard $KMnO_4$ used,
C = normality of standard $KMnO_4$,
D = total mL standard reductant added,
E = normality of standard reductant, and
F = mL stock $NaNO_2$ solution taken for titration.

Each 1.00 mL 0.05*M* $KMnO_4$ consumed by the $NaNO_2$ solution corresponds to 1750 g NO_2^--N.

f. Intermediate nitrite solution: Calculate the volume, G, of stock NO_2^- solution required for the intermediate NO_2^- solution from $G = 12.5/A$. Dilute the volume G (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 μg N. Prepare daily.

g. Standard nitrite solution: Dilute 10.00 mL intermediate NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500 μg N. Prepare daily.

4. Procedure

a. Removal of suspended solids: If sample contains suspended solids, filter through a 0.45-μm-pore-diam membrane filter.

b. Color development: If sample pH is not between 5 and 9, adjust to that range with 1*N* HCl or NH_4OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

c. Photometric measurement: Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO_2^--N concentrations:

Light Path Length *cm*	NO_2^--N *μg/L*
1	2–25
5	2–6
10	<2

5. Calculation

Prepare a standard curve by plotting absorbance of standards against NO_2^--N concentration. Compute sample concentration directly from curve.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg NO_3^- + NO_2^--N/L, the standard deviations were ±0.005, ± 0.004, ± 0.005, and ± 0.01, respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55, and 1.05 mg NO_3^- + NO_2^--N/L, the recoveries were 100%, 102%, and 100%, respectively.[1]

7. Reference

1. U.S. Environmental Protection Agency. 1979. Methods for Chemical Analysis of Water and Wastes. Method 353.3 U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

Boltz, D.F., ed. 1958. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.

Nydahl, F. 1976. On the optimum conditions for the reduction of nitrate by cadmium. *Talanta* 23:349.

VARIABLE pH

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** pH units

METHOD Electrometric pH measurement.

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

pH Meter with Combination Electrode.
(Orion 2000 Series with Ross Model 81-55 Electrode, or equivalent)

PROCEDURE

Near center of each pond, take sample readings 25 cm below the water surface, midwater, and 25 cm above the bottom. Stir sample gently during analysis with probe in a beaker of sample water. Rinse and blot dry the electrode between samples. Calibrate the pH meter following the manufacturer's recommendations. Adjust for temperature, or use a temperature compensating pH probe and meter. Store the probe according to manufacturer's recommendations.

4500-H^+ pH VALUE*

4500-H^+ A. Introduction

1. Principles

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams $CaCO_3$ per liter. Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1-L sample by 1 unit. pH as defined by Sorenson[1] is $-\log [H^+]$; it is the "intensity" factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$[H^+][OH^-] = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \quad (1)$$

and

$$[H^+] = [OH^-] = 1.005 \times 10^{-7}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
$[OH^-]$ = activity of hydroxyl ions, moles/L, and
K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the "activity" of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, a_{H+}, is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1 in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{H^+}) + (-\log_{10} a_{OH^-}) = 14 \quad (2)$$

or

$$pH + pOH = pK_w$$

where:

pH† $= -\log_{10} a_{H^+}$ and
$pOH = -\log_{10} a_{OH^-}$.

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because pK_w is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

2. Reference

1. SORENSON, S. 1909. Über die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21: 131.

*Approved by Standard Methods Committee, 1985.

†p designates $-\log_{10}$ of a number.

4500-H$^+$ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

Because single ion activities such as a_{H+} cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) buffers having assigned values so that:

$$pH_s = -\log_{10} a_{H^+}$$

where:

pH_s = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_s \pm \frac{F(E_x - E_s)}{2.303\,RT}$$

where:

pH_x = potentiometrically measured sample pH,
F = Faraday: 9.649×10^4 coulomb/mole,
E_x = sample emf, V,
E_s = buffer emf, V,
R = gas constant; 8.314 joule/(mole °K), and
T = absolute temperature, °K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the U.S. is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.[1,2]

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$pH\ (activity) = pH\ (Sorenson) + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes ($< 0.2M$). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH > 10. Reduce this error by using special "low sodium error" electrodes.

pH measurements are affected by temperature in two ways: mechanical effects

PROCEDURE Cont. **VARIABLE pH**

that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0.001 pH unit, but most instruments are not that precise.

For routine work use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagramatically in Figures 4500-H^+:1 and 2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the

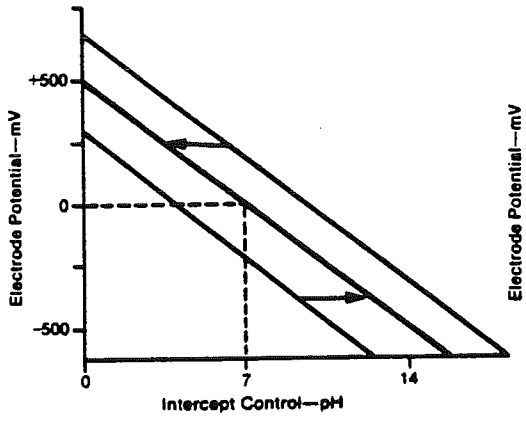

Figure 4500-H^+:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

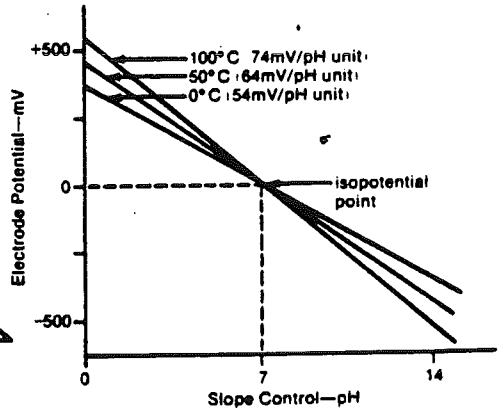

Figure 4500-H^+:2. Typical pH electrode response as a function of temperature.

electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference electrode. Upon immersion of a new electrode in a solution the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass-solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a "low sodium error" electrode that can operate at high temperatures for measuring pH over 10 because standard glass electrodes yield erroneously low values. For measuring pH below 1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE* beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

*Teflon or equivalent.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H^+:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 μmhos/cm. To 50 mL add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, insure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H^+:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500-H^+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 4 weeks.

PROCEDURE Cont. **VARIABLE pH**

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $KHC_4H_4O_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for 2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade $CaCO_3$ in a platinum dish by igniting for 1 h at 1000°C. Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid $Ca(OH)_2$ on a fritted glass filter of medium porosity. Dry at 110°C, cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1*N* NaOH, 0.1*N* HCl, 5*N* HCl (dilute five volumes 6*N* HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than 4000 μmhos/cm. Tap water is a better substitute than distilled water, but pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (¶ 2*a* above). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature, a fixed temperature such as 25°C, or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so that meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately 3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (see ¶s 5*a* and *b* below).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after clean-

ing by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Trouble Shooting

a. Potentiometer: To locate trouble source disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1*N* HCl and 0.1*N* NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used, i.e., silver: silver-chloride against calomel or vice versa, the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unit represents the limit of accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ±0.13 pH unit.

7. References

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2. LICHT, T.S. & A.J. DE BETHUNE. 1957. Recent developments concerning the signs of electrode potentials. *J. Chem. Educ.* 34:433.
3. DURST, R.A. 1975. Standard Reference Ma-

VARIABLE Phosphorus - Total

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Persulfate Digestion and Ascorbic Acid/Colorimetric Method.

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Spectrophotometer with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

PROCEDURE

Collect one sample (by pooling three 90 cm column samples) from each pond. Samples should be refrigerated and analyzed within 24 hours.

Follow the detailed procedure for sample preparation, digestion and analysis as indicated in Standard Methods (attached).

PROCEDURE Cont. **VARIABLE Phosphorus - Total**

5. Persulfate Digestion Method

a. Apparatus:

1) *Hot plate:* A 30- × 50-cm heating surface is adequate.

2) *Autoclave:* An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.

3) *Glass scoop,* to hold required amounts of persulfate crystals.

b. Reagents:

1) *Phenolphthalein indicator aqueous solution.*

2) *Sulfuric acid solution:* Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

3) *Ammonium persulfate,* $(NH_4)_2S_2O_8$, solid, or potassium persulfate, $K_2S_2O_8$, solid.

4) *Sodium hydroxide,* NaOH, 1*N*.

c. Procedure: Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(NH_4)_2S_2O_8$ or 0.5 g solid $K_2S_2O_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 hr for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

6. Bibliography

LEE, G.F., N.L. CLESCERI & G.P. FITZGERALD. 1965. Studies on the analysis of phosphates in algal cultures. *J. Air Water Pollut.* 9:715.

SHANNON, J.E. & G.F. LEE. 1966. Hydrolysis of condensed phosphates in natural waters. *J. Air Water Pollut.* 10:735.

GALES, M.E., JR., E.C. JULIAN & R.C. KRONER. 1966. Method for quantitative determination of total phosphorus in water. *J. Amer. Water Works Assoc.* 58:1363.

4500-P E. Ascorbic Acid Method

1. General Discussion

a. 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.

b. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. Minimum detectable concentration: Approximately 10 µg P/L. P ranges are as follows:

Approximate P Range *mg/L*	Light Path *cm*
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	5.0

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer,* equipped with a red color filter and a light path of 0.5 cm or longer.

b. Acid-washed glassware: See Section 4500-P.C.2*b*.

3. Reagents

a. Sulfuric acid, H_2SO_4, *5N:* Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.

b. Potassium antimonyl tartrate solution: Dissolve 1.3715 g $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle.

d. Ascorbic acid, 0.01*M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

e. Combined reagent: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL *5N* H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

f. Stock phosphate solution: See Section 4500-P.C.3*e*.

g. Standard phosphate solution: Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 µg P.

4. Procedure

a. Treatment of sample: Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add *5N* H_2SO_4 solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. Correction for turbidity or interfering color: Natural color of water generally does not interfere at the high wavelength used.

PROCEDURE Cont. **VARIABLE Phosphorus - Total**

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

Ascorbic Acid Method	Phosphorus Concentration, Dissolved Orthophosphate μg/L	No. of Laboratories	Relative Standard Deviation %		Relative Error %	
			Distilled Water	River Water	Distilled Water	River Water
13th Edition[1]	228	8	3.87	2.17	4.01	2.08
Current method[2]	228	8	3.03	1.75	2.38	1.39

For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimonyl potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

c. Preparation of calibration curve: Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1*c* above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P:I are for a single-solution procedure given in the 13th edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228-μg P/L level (Table 4500-P:II).

7. References

1. EDWARDS, G.P., A.H. MOLOF & R.W. SCHNEEMAN. 1965. Determination of orthophosphate in fresh and saline waters. *J. Amer. Water Works Assoc.* 57:917.
2. MURPHY, J. & J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31.

8. Bibliography

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VARIABLE Phytoplankton Composition

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $L \cdot L^{-1}$

METHOD CRSP.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

Van Dorn or Kemmerer bottle for phytoplankton collection.
Compound microscope.
Counting cell (Sedgwick-Rafter, or hemocytometer) or Whipple Square.

PROCEDURE

Collect samples using a Van Dorn or Kemmerer bottle. Use a compound microscope and appropriate references to identify major groups (green, blue-green, or diatom) and estimate biovolume for each group. This may be achieved by estimating the average cellular biovolume for each group and counting the number of individual cells per unit volume of water. The cellular biovolume in turn may be estimated by calculation from typical dimensions of length, width, depth, or radius (depending on the cell shape). If the cells are spherical, the biovolume would be 4/3 π (radius) ^3. If the cells are cylindrical, the biovolume would be π (radius)^2 * length.

VARIABLE Pond Temperature

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** °C

METHOD Thermometer.

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

Electronic thermometer accurate to 0.1 °C
(YSI Model 57 Dissolved Oxygen Meter with Temperature Indicator, or equivalent)
Establish the accuracy of the probe using a precision mercury thermometer.

PROCEDURE

Near center of each pond, take readings on samples from 25 cm below the water surface midwater and 25 cm above the bottom.

VARIABLE Primary Productivity

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS**

METHOD Modified Whole Pond.

REFERENCE CRSP Technical Committee

INSTRUMENTATION

Computer.

PROCEDURE

The purpose of this interim procedure is to produce standard calculations of gross primary production from standard CRSP data as they have been collected. The procedure is based on respiration and primary production estimates obtained from diel dissolved oxygen measurements. A spreadsheet version on Microsoft Excel ™ on Macintosh computers is available from the UC Davis DAST.

PROCEDURE Cont. **VARIABLE** **Primary Productivity**

Primary productivity is calculated from the CRSP database by using an oxygen balance based on the DO and temperature measurements taken at three depths during the 24 hour sampling cycles. The mass balance for dissolved oxygen in a pond can be written as:

DO RATE OF CHANGE = DO GROSS PRODUCTION - DO RESPIRATION + DO DIFFUSION

and

DO GROSS PRODUCTION = DO RATE OF CHANGE + DO RESPIRATION - DO DIFFUSION

The hourly rate of change of dissolved oxygen (DO) is estimated from the depth averaged measurements and is then adjusted for diffusion to yield hourly net productivity. Hourly net productivity is adjusted for estimated pond respiration to yield hourly gross primary productivity. The hourly productivity is then summed to yield total daily primary production.

The detailed calculations are described below, and have been implemented into an Excel spreadsheet which is available from the Davis DAST.

Data needed:

Wind Speed (m/s)	Template A
Temperature (°C)	Template D
Pond Depth (m)	Template B
O_2 Concentration (mg O_2/L)	Template D

Description of Calculations:

1) Dissolved oxygen (DO) and temperature data are depth averaged to yield single pond temperature and DO values for each of the sampling periods:

$$DO_I = \text{Average } (DO_{I,surface}, DO_{I,mid}, DO_{I,bottom})$$
$$TEMP_I = \text{Average } (TEMP_{I,surface}, TEMP_{I,mid}, TEMP_{I,bottom})$$

Where:

DO_I = Depth averaged dissolved oxygen concentration at time T_I (mg/L)
$TEMP_I$ = Depth averaged temperature at time T_I (°C)
I = Number of the five to seven sampling intervals (0 - 6)

2) The hourly rate of change of average pond temperature and DO are calculated for each sampling interval.

$$TRC_I = TEMP_{I+1} - TEMP_I / T_{I+1} - T_I$$
$$DORC_I = DO_{I+1} - DO_I / T_{I+1} - T_I$$

Where:

TRC_I = Temperature rate of change in the interval T_I (°C/h)
$DORC_I$ = Dissolved oxygen rate of change in the interval T_I (mg/L/h)

3) The duration of each sampling interval according to CRSP sampling procedures is approximately 4 hours, however because of variations in the times of the recorded data, the sampling interval is not always constant. To interpolate the data over 24 daily sub-intervals, the duration of each sampling interval is determined and is divided into 4 approximately hourly sub-intervals.

$$\Delta T_I = (T_{I+1} - T_I)$$
$$\Delta T_i = \Delta T_I / 4$$

Where:

ΔT_I = Duration of time interval between samplings (h)
ΔT_i = Duration of time sub-interval (h)
i = Number of time sub-interval (0-23)

This process is repeated for each time interval T_I.

4) The average pond DO concentration and temperature are interpolated for each sub-interval to complete the 24 hour cycle.

$$TEMP_i = TEMP_I + j \cdot (TRC_I \cdot \Delta T_i) \text{ , for j between 0 and 3.}$$
$$DO_i = DO_I + j \cdot (DORC_I \cdot \Delta T_i) \text{ , for j between 0 and 3.}$$

j = Number of time sub-interval within each interval (0-3)

This process is repeated for each time interval, T_I obtaining values for i between 0 and 23.

5) The upper layer DO concentration and temperature are interpolated for each sub-interval for use in determining the rate of diffusion across the boundary layer.

$$TEMPS_i = TEMPS_I + i \cdot (TEMPS_{I+1} - TEMPS_I) / 4, \text{ for i between 0 and 3}$$
$$DOS_i = DOS_I + i \cdot (DOS_{I+1} - DOS_I) / 4, \text{ for i between 0 and 3}$$

Where:

TEMPS = Temperature in the top or surface layer (°C)
DOS = Dissolved oxygen concentration in the top or surface layer (mg/L)

6) The O_2 saturation concentration is determined for each sub-interval in the upper layer from the empirical equation by Elmore and Hayes (1966).

$$SDO_i = 14.652 - 0.41022 \cdot TS_i + 0.007991 \cdot TS_i \text{ ^ } 2 - 0.000077774 \cdot TS_i \text{ ^ } 3$$

Where:

SDO = O_2 saturation concentration (mg/L)

7) The rate of oxygen diffusion into the upper pond layer is calculated for each sub-interval from the wind speed, and oxygen concentration gradient according to the empirical equation by Banks and Herrera (1977).

$$\text{Diff in}_i = (SDO_i - DOS_i)\ (1/Z)\ (\ 0.03 \cdot W \text{ ^ } 0.5 - 0.0132 \cdot W + 0.00157 \cdot W \text{ ^ } 2\ )$$

PROCEDURE Cont. **VARIABLE** **Primary Productivity**

Where:
Diff in_i = rate of oxygen diffusion into the pond (mg O_2/L/h).
Z = depth (m)
W = Wind speed (m/s)

8) The night time pond respiration for each sub-interval is calculated as the night time rate of change of average pond DO less diffusion in. This value is then averaged and used as a rough estimate of daytime pond respiration, in accordance with the procedures suggested by Hall and Moll (1975) and others. This pond respiration rate is not adjusted for temperature effects because of lack of data for this calculation in the CRSP database, and is therefore likely to be an underestimation of actual daytime pond respiration.

$NTPR_i$ = $DORC_i$ - Diff in_i, for all night time i's.
PR = Average ($NTPR_i$)

Where:
$NTPR_i$ = Night time pond respiration rate (mg O_2/L/h)
PR = Average pond respiration rate (mg O_2/L/h)

9) The phytoplankton production rate per hour is calculated for each sub-interval as the DO rate plus pond respiration less diffusion in. The daylight production rates (< 0) are then summed to yield daily primary production.

GPP_i = $DORC_i$ + PR - Diff in_i
GPP_{tot} = Σ GPP_i

Where:
GPP_i = Gross primary production (mg O_2/L/h)
GPP_{tot} = Gross primary production (mg O_2/L/day)

When using these calculations, it should be kept in mind that because night time pond respiration is used as an estimate of daytime respiration, this term is likely to be underestimated when there is a significant temperature differential between daytime and night time. This means that the daily gross primary production also is likely to be underestimated in this case.

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Elmore, H. L. and T. W. Hayes. 1960. Solubility of atmospheric oxygen in water. Jour. Sanit. Eng. Div., ASCE. 86(SA4):41.

Hall, C. A. S. and R. Moll. 1975. Methods of assessing aquatic primary production. pp 19-53 in H. Leith and R. H. Whittaker (eds) Primary Productivity of the Biosphere. Springer-Verlang, New York.

VARIABLE Salinity

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** ppt

METHOD Light refraction or conductivity.

REFERENCE

INSTRUMENTATION

Use a temperature-compensated refractometer or a salinity meter.
(Refractometer Argent Aquaculture model SS-1000A, or equivalent)
(Salinity meter, YSI model 33 S-C-T meter and probe, or equivalent)

PROCEDURE

Near the center of each pond, collect a 500 ml sample 25 cm below the water surface, midwater, and 25 cm above the bottom. Mix the samples and analyze.

VARIABLE Secchi Disk Depth

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** cm

METHOD Secchi Disk.

REFERENCE Lind, O.T. 1979. Handbook of common methods in Limnology. C.V. Mosby Co. Saint Louis.190 pp.

INSTRUMENTATION

Secchi Disk: 20 cm diameter disk with alternating black and white quarter circles. The disk is suspended from a calibrated line, or attached to a calibrated rod.

PROCEDURE

Measure secchi disk depth at two locations in each pond. To measure Secchi disk depth, lower the disk into the water and note the depth at which the disk dissapears from view. Lower the disk further and then bring it up slowly, noting the depth at which the disk becomes visible. Average the two depths. Repeat the procedure at a second location, average the values obtained for the two locations and report this average as the Secchi disk depth for the pond. Position of the sun with respect to the observer, time of day and light intensity affect the readings. For best results, measure secchi disk between 1000h and1400h in bright sunlight, make sure that the sun is behind the operator, and do not use sun glasses.

VARIABLE Solids - Total Suspended

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD Filtration

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Drying oven for operation at 103 to 105 °C.
Analytical balance capable of weighing to 0.1 mg.
Vacuum filtration apparatus.

PROCEDURE

Follow the detailed instructions as indicated in Standard Methods. Be particularly careful to mix the sample thoroughly before filtering, and in handling the filter paper through the filtration, drying, equalizing, and weighing processes. Use tweezers to handle the filter paper and the weighing boats or crucibles.

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

1. General Discussion

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

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

2. Apparatus

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

*Planchet,** aluminum or stainless steel, 65-mm diam.

3. Procedure

a. Preparation of glass-fiber filter disk: Insert disk with wrinkled side up in filtra-

*Available from New England Nuclear, Boston, Mass., or equivalent.

PROCEDURE Cont. **VARIABLE Solids - Total Suspended**

tion apparatus. Apply vacuum and wash disk with three successive 20-mL portions of distilled water. Continue suction to remove all traces of water, and discard washings. Remove filter from filtration apparatus and transfer to an aluminum or stainless steel planchet as a support. Alternatively remove crucible and filter combination if a Gooch crucible is used. Dry in an oven at 103 to 105°C for 1 h. If volatile solids are to be measured, ignite at 550 ± 50°C for 15 min in a muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 0.5 mg between successive weighings. Store in desiccator until needed. Weigh immediately before use.

b. Selection of filter and sample sizes: See Section 2540C.3*c*. For nonhomogeneous samples such as raw wastewater, use a large filter to permit filtering a representative sample.

c. Sample analysis: Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of distilled water to seat it. Filter a measured volume of well-mixed sample through the glass fiber filter. Wash with three successive 10-mL volumes of distilled water, allowing complete drainage between washings and continue suction for about 3 min after filtration is complete. Carefully remove filter from filtration apparatus and transfer to an aluminum or stainless steel planchet as a support. Alternatively, remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less.

4. Calculation

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

where:

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

5. Precision

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

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

6. Bibliography

DEGEN, J. & F.E. NUSSBERGER. 1956. Notes on the determination of suspended solids. *Sewage Ind. Wastes* 28:237.

CHANIN, G., E.H. CHOW, R.B. ALEXANDER & J. POWERS. 1958. Use of glass fiber filter medium in the suspended solids determination. *Sewage Ind. Wastes* 30:1062.

NUSBAUM, I. 1958. New method for determination of suspended solids. *Sewage Ind. Wastes* 30:1066.

SMITH, A.L. & A.E. GREENBERG. 1963. Evaluation of methods for determining suspended solids in wastewater. *J. Water Pollut. Control Fed.* 35:940.

WYCKOFF, B.M. 1964. Rapid solids determination using glass fiber filters. *Water Sewage Works* 111:277.

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VARIABLE Solids - Volatile Suspended

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** $mg \cdot L^{-1}$

METHOD

REFERENCE American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. APHA, Washington, D.C.

INSTRUMENTATION

Muffle furnace for operation at 550 ± 50 °C.
Analytical balance capable of weighing to 0.1 mg.
Vacuum filtration apparatus.

PROCEDURE

Follow the detailed instructions as indicated in Standard Methods.

2540 E. Fixed and Volatile Solids Ignited at 550°C

1. General Discussion

a. Principle: The residue from Method B, C, or D is ignited to constant weight at 550 ± 50°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 in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

b. 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 subject to considerable error. In such cases, measure for suspect volatile components by another test, for example, total organic carbon (Section 5310).

2. Apparatus

See Sections 2540B.2, 2540C.2, and 2540D.2.

3. Procedure

Ignite residue produced by Method B, C, or D to constant weight in a muffle furnace at a temperature of 550 ± 50°C. Have furnace up to temperature before inserting sample. Usually, 15 to 20 min ignition are required. Let dish or filter disk cool partially in air until most of the heat has been dissipated. Transfer to a desiccator for final cooling in a dry atmosphere. Do not overload desiccator. Weigh dish or disk as soon as it has cooled to balance temperature. Repeat cycle of igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 4% of previous weight.

4. Calculation

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

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

where:

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

5. Precision

The standard deviation was 11 mg/L at 170 mg/L volatile total solids in studies by three laboratories on four samples and 10 replicates. Bias data on actual samples cannot be obtained.

2540 F. Settleable Solids

1. General Discussion

Settleable solids in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (mL/L) or a weight (mg/L) basis.

2. Apparatus

The volumetric test requires only an Imhoff cone. The gravimetric test requires all the apparatus listed in Section 2540D.2 and a glass vessel with a minimum diameter of 9 cm.

3. Procedure

a. Volumetric: Fill an Imhoff cone to the 1-L mark with a well-mixed sample. Settle for 45 min, gently stir sides of cone with a rod or by spinning, settle 15 min longer, and record volume of settleable solids in the cone as milliliters per liter. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids. The practical lower limit of measurement depends on sample composition and generally is in the range of 0.1 to 1.0 mL/L. Where a separation of settleable and floating materials occurs, do not estimate the floating material as settleable matter.

b. Gravimetric:

1) Determine total suspended solids of well-mixed sample (Section 2540D).

2) Pour a well-mixed sample into a glass vessel of not less than 9 cm diam using not less than 1 L and sufficient to give a depth of 20 cm. Alternatively use a glass vessel of greater diameter and a larger volume of sample. Let stand quiescent for 1 h and, without disturbing the settled or floating material, siphon 250 mL from center of container at a point halfway between the surface of the settled material and the liquid surface. Determine total suspended solids (milligrams per liter) of this supernatant liquor (Section 2540D). These are the nonsettleable solids.

4. Calculation

$$\text{mg settleable solids/L} = \text{mg total suspended solids/L} - \text{mg nonsettleable solids/L}$$

5. Precision and Bias

Precision and bias data are not now available.

6. Bibliography

FISCHER, A.J. & G.E. SYMONS. 1944. The determination of settleable sewage solids by weight. *Water Sewage Works* 91:37.

2540 G. Total, Fixed, and Volatile Solids in Solid and Semisolid Samples

1. General Discussion

a. Applicability: This method is applicable to the determination of total solids and its fixed and volatile fractions in such solid and semisolid samples as river and lake sediments, sludges separated from water and wastewater treatment processes, and sludge cakes from vacuum filtration, centrifugation, or other sludge dewatering processes.

b. Interferences: The determination of both total and volatile solids in these materials is subject to negative error due to loss of ammonium carbonate and volatile organic matter during drying. Although

PROCEDURE Cont. **VARIABLE Solids - Volatile Suspended**

this is true also for wastewater, the effect tends to be more pronounced with sediments, and especially with sludges and sludge cakes. The mass of organic matter recovered from sludge and sediment requires a longer ignition time than that specified for wastewaters, effluents, or polluted waters. Carefully observe specified ignition time and temperature to control losses of volatile inorganic salts. Make all weighings quickly because wet samples tend to lose weight by evaporation. After drying or ignition, residues often are very hygroscopic and rapidly absorb moisture from the air.

2. Apparatus

All the apparatus listed in Section 2540B.2 is required except that a balance capable of weighing to 10 mg may be used.

3. Procedure

a. Total solids:

1) Preparation of evaporating dish—If volatile solids are to be measured, ignite a clean evaporating dish at 550 ± 50°C for 1 h in a muffle furnace. If only total solids are to be measured, heat dish at 103 to 105°C for 1 h in an oven. Cool in desiccator, weigh, and store in desiccator until ready for use.

2) Sample analysis

a) Fluid samples—If the sample contains enough moisture to flow more or less readily, stir to homogenize, place 25 to 50 g in a prepared evaporating dish, and weigh. Evaporate to dryness on a water bath, dry at 103 to 105°C for 1 h, cool to balance temperature in an individual desiccator containing fresh desiccant, and weigh.

b) Solid samples—If the sample consists of discrete pieces of solid material (dewatered sludge, for example), take cores from each piece with a No. 7 cork borer or pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves. Place 25 to 50 g in a prepared evaporating dish and weigh. Place in an oven at 103 to 105°C overnight. Cool to balance temperature in an individual desiccator containing fresh desiccant and weigh.

b. Fixed and volatile solids: Transfer to a cool muffle furnace, heat furnace to 550 ± 50°C, and ignite for 1 h. (If the residue from 2) above contains large amounts of organic matter, first ignite the residue over a gas burner and under an exhaust hood in the presence of adequate air to lessen losses due to reducing conditions and to avoid odors in the laboratory.) Cool in desiccator to balance temperature and weigh.

4. Calculation

$$\% \text{ total solids} = \frac{(A - B) \times 100}{C - B}$$

$$\% \text{ volatile solids} = \frac{(A - D) \times 100}{A - B}$$

$$\% \text{ fixed solids} = \frac{(D - B) \times 100}{A - B}$$

where:

A = weight of dried residue + dish, mg,
B = weight of dish,
C = weight of wet sample + dish, mg, and
D = weight of residue + dish after ignition, mg.

5. Precision and Bias

Precision and bias data are not now available.

6. Bibliography

GOODMAN, B.L. 1964. Processing thickened sludge with chemical conditioners. Pages 78 et seq. *in* Sludge Concentration, Filtration and Incineration. Univ. Michigan Continued Education Ser. No. 113, Ann Arbor.

GRATTEAU, J.C. & R.I. DICK. 1968. Activated sludge suspended solids determinations. *Water Sewage Works* 115:468.

VARIABLE Zooplankton Composition

DATE October 1990 **VERSION** 1

SAMPLE Water **REPORTING UNITS** No. $\cdot m^{-3}$

METHOD CRSP.

REFERENCE CRSP Technical Committee Meetings.

INSTRUMENTATION

Compound microscope for small zooplankton.
Dissecting microscope for larger zooplankton.

PROCEDURE

Collect at least three 90 cm column samples per pond or use trap or zooplankton net, as appropriate. Use a microscope to identify at the level of order and count the number of organisms per unit volume.

Section Five

WEATHER

Air Temperature

Rainfall

Solar Radiation

Wind Speed

VARIABLE Air Temperature

DATE October 1990 **VERSION** 1

SAMPLE Weather **REPORTING UNITS** Max: C; Min: C

METHOD Glass thermometer

REFERENCE

INSTRUMENTATION

Maximum-minimum thermometer.

PROCEDURE

Install three maximum-minimum thermometers in the shade near the ponds; read at 24-hour intervals and report the average maximum and average minimum temperatures.

VARIABLE Rainfall

DATE October 1990 **VERSION** 1

SAMPLE Weather **REPORTING UNITS** $cm \cdot d^{-1}$

METHOD Rain gauge

REFERENCE Pond Dynamics/Aquaculture CRSP Technical Committee

INSTRUMENTATION

No type specified.

PROCEDURE

Install three rain gauges at the study site; read and empty at 24-hour intervals or more frequently if needed to prevent gauge overflow. Report the average of the readings from the three gauges.

VARIABLE Solar Radiation

DATE October 1990 **VERSION** 1

SAMPLE Weather **REPORTING UNITS** $E \cdot m^{-2} \cdot d^{-1}$

METHOD Light Sensor.

REFERENCE

LI-COR
4421 Superior Street
P.O. Box 4425
Lincoln, Nebraska 68504

INSTRUMENTATION

Quantum sensor to measure photosynthetically active solar radiation (wavelength between 400 and 700 nm).

PROCEDURE

Install Solar Monitor and Quantum Sensor at study site and read at 24-hour intervals. During diel cycles, read the solar monitor and report radiation values whenever water is sampled.

The solar monitor records a running total of light intensity. Therefore, the time units reported depend on the frequency of reading of the solar monitor. For daily (every 24 hour) readings, the reading from the instrument corresponds to Einsteins per square meter per day. For diel sampling, the solar monitor readings should be divided by the duration of the time interval sampled (in hours), and the units become Einsteins per square meter per hour.

VARIABLE Wind Speed

DATE October 1990 **VERSION** 1

SAMPLE Weather **REPORTING UNITS** $km \cdot h^{-1}$

METHOD Anemometer

REFERENCE

INSTRUMENTATION

Totalizing anemometer. The instrument should be located in the pond complex 2 m above the level of the pond banks, away from trees, buildings, or other obstructions.

PROCEDURE

Read the totalizing anemometer at 24-hour intervals (in km), and calculate average hourly wind speed by dividing by 24 h (the duration of the time interval).

During the diel cycles, read the anemometer whenever water is sampled. Divide the readings (km) by the duration of the time period between readings (h).