MANUAL OF ANALYTICAL METHODS FOR SEAWATER AND SEDIMENT

Issued during the second Workshop on NATP Project "Impact of Dams on river run-off into Sea and Changes in the Nutrient and Productivity Profile of Coastal waters"

FISHERY ENVIRONMENT AND MANAGEMENT DIVISION

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE, PB NO. 1603, KOCHI - 682 014

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Preface

This "Manual of Analytical Methods for Seawater and Sediment" is a comprehensive laboratory manual prepared for the NATP project " Impact of Dams on river run-off into Sea and Changes in the Nutrient and Productivity Profile of Coastal waters" functioning at Kochi, Mangalore, Visakhapatnam and Veraval Centres with an intention to obtain a relatively comparable results by employing uniform methodology in collection, processing and analyses of samples.

The help and assistance rendered by Shri Rajkumar, M.S. and Ms. Dhanya Lenin, K.L., Senior Research Fellows in the preparation of this manual is sincerely acknowledged.

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Part A WATER

Part B SEDIMENT

SUMMARY OF REQUIREMENTS FOR SAMPLING & HANDLING WATER SAMPLES

Determination	Container†	Minimum Sample Size mL	Sample Type‡	Preservation§	Maximum Storage Recommended/ Regulatory‖
Acidity	P, G(B)	100	g	Refrigerate	24 h/14 d
Alkalinity	P, G	200	g	Refrigerate	24 h/14 d
BOD	P, G	1000	g	Refrigerate	6 h/48 h
Boron	P	100	g, c	None required	28 d/6 months
Bromide	P, G	100	g, c	None required	28 d/28 d
Carbon, organic, total	G	100	g, c	Analyze immediately; or refrigerate and add H_3PO_4 or H_2SO_4 to pH<2	7 d/28 d
Carbon dioxide	P, G	100	g	Analyze immediately	stat/N.S.
COD	P, G	100	g, c	Analyze as soon as possible, or add H_2SO_4 to pH<2; refrigerate	7 d/28 d
Chloride	P, G	50	g, c	None required	28 d
Chlorine, residual	P, G	500	g	Analyze immediately	0.5 h/stat
Chlorine dioxide	P, G	500	g	Analyze immediately	0.5 h/N.S.
Chlorophyll	P, G	500	g, c	30 d in dark	30 d/N.S.
Color	P, G	500	g, c	Refrigerate	48 h/48 h
Conductivity	P, G	500	g, c	Refrigerate	28 d/28 d
Cyanide:					
Total	P, G	500	g, c	Add NaOH to pH>12, refrigerate in dark#	24 h/14 d; 24 h if sulfide present
Amenable to chlorination	P, G	500	g, c	Add 100 mg $Na_2S_2O_3$/L	stat/14 d; 24 h if sulfide present
Fluoride	P	300	g, c	None required	28 d/28 d
Hardness	P, G	100	g, c	Add HNO_3 to pH<2	6 months/6 months
Iodine	P, G	500	g, c	Analyze immediately	0.5 h/N.S.
Metals, general	P(A), G(A)	500	g	For dissolved metals filter immediately, add HNO_3 to pH<2	6 months/6 months
Chromium VI	P(A), G(A)	300	g	Refrigerate	24 h/24 h
Copper by colorimetry*					
Mercury	P(A), G(A)	500	g, c	Add HNO_3 to pH<2, 4°C, refrigerate	28 d/28 d
Nitrogen:					
Ammonia	P, G	500	g, c	Analyze as soon as possible or add H_2SO_4 to pH<2, refrigerate	7 d/28 d
Nitrate	P, G	100	g, c	Analyze as soon as possible or refrigerate	48 h/48 h (28 d for chlorinated samples)
Nitrate + nitrite	P, G	200	g, c	Add H_2SO_4 to pH<2, refrigerate	none/28 d
Nitrite	P, G	100	g, c	Analyze as soon as possible or refrigerate	none/48 h
Organic, Kjeldahl*	P, G	500	g, c	Refrigerate; add H_2SO_4 to pH<2	7 d/28 d
Odor	G	500	g	Analyze as soon as possible; refrigerate	6 h/N.S.
Oil and grease	G, wide-mouth calibrated	1000	g, c	Add HCl to pH<2, refrigerate	28 d/28 d
Organic compounds:					
MBAS	P, G	250	g, c	Refrigerate	48 h
Pesticides*	G(S), TFE-lined cap	1000	g, c	Refrigerate; add 1000 mg ascorbic acid/L if residual chlorine present	7 d/7 d until extraction; 40 d after extraction
Phenols	P, G	500	g, c	Refrigerate, add H_2SO_4 to pH<2	*/28 d
Purgeables* by purge and trap	G, TFE-lined cap	2 × 40	g	Refrigerate; add HCl to pH <2; add 1000 mg ascorbic acid/L if residual chlorine present	7 d/14 d
Oxygen, dissolved:	G, BOD bottle	300	g		
Electrode				Analyze immediately	0.5 h/stat
Winkler				Titration may be delayed after acidification	8 h/8 h
Ozone	G	1000	g	Analyze immediately	0.5 h/N.S.
pH	P, G	50	g	Analyze immediately	2 h/stat
Phosphate	G(A)	100	g	For dissolved phosphate filter immediately; refrigerate	48 h/N.S.
Salinity	G, wax seal	240	g	Analyze immediately or use wax seal	6 months/N.S.
Silica	P	200	g, c	Refrigerate, do not freeze	28 d/28 d
Sludge digester gas	G, gas bottle	—	g	—	N.S.

Ref: American Public Health Association (1995)

SUMMARY OF TECHNIQUES FOR PRESERVING WATER SAMPLES

Acidity and alkalinity	Cool to 4°C	24 hours
Biochemical oxygen demand ...	Cool to 4°C	6 hours
Carbon dioxide	Cool to 4°C	2 hours
Chemical oxygen demand	1 ml of concentrated H_2SO_4 per liter	7 days
Dissolved oxygen by Winkler technique ....:....	Fix DO immediately on site in BOD bottle[1]	6 hours
Total or calcium hardness ...	(1) 1 ml HNO_3 per liter	7 days
	(2) Cool to 4°C	7 days
Ammonia and nitrate	(1) Cool to 4°C	24 hours
	(2) 1 ml H_2SO_4 per liter	7 days
	(3) 40 mg/liter $HgCl_2$	7 days
Soluble orthophosphate	(1) Cool to 4°C	2 hours
	(2) Storage in I_2 treated bottles[1]	7 days
Total phosphorus	Cool to 4°C	7 days
Settleable matter	Not required	24 hours
Total, dissolved, and volatile solids	Cool to 4°C	7 days
Particulate organic matter ...	Cool to 4°C	12 hours
Chlorophyll a	Cool to 4°C	12 hours

Ref: United States Environmental Protection Agency (1976)

PART A WATER

3. STREAM PARAMETERS

Length:

Total length of stream or length of segment under study.

Breadth:

Width at either actual water level or flood level. Mean breadth is average width of segment under study.

Depth:

Either actual depth or depth at flood level. Mean depth, d =A/W, where A = area of cross section, W = width.

Area:

Either the surface area of water segment under study or area of a cross section of the stream. For the latter, plot cross section of stream from depth measurements, obtain area with polar planimeter (or graph paper) and calculate cross section in ft^2 or m^2.

Velocity:

Water velocity is measured either by

a) Dutchman's log by timing a floating object over a measured distance (e.g. 100m) or

b) With a current meter, in which case check flow rates in channel or streams, amid vegetation and along shore.

The Dutchman's log or surface float method is subject to many errors caused by wind, current variations etc. Weighing down the float reduces wind errors. Mean velocity is estimated at 0.8 times the float velocity. With a current meter, average velocity can be measured by taking readings at intervals across stream and at intervals of depth.

With the Gurley current meter, the velocity of flow in ft/sec
$$= \frac{No:\ of\ clicks\ /\ min.}{60}$$

Volume of flow or discharge: -

When using the Dutchman's log, it can be calculated from Embody's formula as given by Welch (1948)

$$V = \frac{wdal}{t} \quad or \quad V = \frac{Aal}{t}$$

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Where

V = discharge in ft^3/sec.

w = width of channel in ft. (average)

d = average depth (ft.)

a = a constant depending on smoothness of bottom (0.8 if rough, 0.9 if smooth) and depth (1.33 if less than 2 ft; 1.05 if more than 10 ft and interpolate between 2 and 10 ft.)

l = length of channel measured (ft.)

t = time (average of three tests) to cover the length.

A = cross section area (replaces w and d when measured directly).

Note: $1ft^3$/sec. = 450 gal/min.

1 ft^3 = 7.481 US gal.

4. HYDROGEN ION CONCENTRATION (pH)

Principle:

pH of a solution is measured with a pH meter. pH is the negative log of the hydrogen ion concentration. Hydrogen ion concentration and pH are not the same. The former can be averaged; but pH being a log function should not be averaged. When the electrodes are dipped in two solutions of different pH levels and connected, a potential difference is set up between the two electrodes, which is measured by the potentiometer. This is directly related to the pH of the solution.

Procedure:

1) Warm up the instrument for 15-20 minutes before use.
2) Calibrate the instrument with the standard buffer solutions, (pH 4, 7 or 9). Calibration is done by a buffer solution whose pH is close to that of the sample.
3) Clean the electrode with double distilled water/ deionised water.
4) Immerse the electrode in the unknown sample and stir for 3 minutes and note the pH.

Note:
Bring the sample to room temperature before measuring the pH.

N A ⌐

Introduction:

Salinity is usually estimated by either titrimetric method or using a salinometer. The method detailed below is the titrimetric method. Knudson, who modified Mohr's method, developed the basic procedure.

Principle:

In this method the halogen ions in seawater are titrated with silver nitrate using potassium chromate as indicator. The halogen ions (except fluoride) readily react with silver to give insoluble silver halides. In this method silver will react with chromate only after all the halide ions, other than fluoride, are precipitated and as soon as a slight excess of silver ion is present, red silver chromate is formed. A faint red colour of the solution indicates the end point of the titration. The total quantity of silver required to react with chloride, bromide and iodide is a measure of the chlorinity of seawater.

Reagents required:

1) Silver nitrate (24.5 gm/ltr.)
2) Potassium chromate (10%)- 10 gm in 100 ml.
3) Standard seawater

Procedure:

Pipette out 10 ml of Standard seawater into a 250 ml conical flask. Add four drops of potassium chromate solution and using a mechanical stirrer titrate against silver nitrate solution. Repeat to concordance. Pipette out 10 ml of the seawater sample into the conical flask and proceed as above.

Calculation:

$$\text{Salinity of sample} = \frac{V_2 \times S}{V_1}$$

Where

V_1 = Volume of silver nitrate for 10 ml standard seawater

V_2 = Volume of silver nitrate for 10 ml sample

S = Salinity of Standard seawater


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6. DISSOLVED OXYGEN
## (Winkler method)

Principle:

This method, popularly known as Winkler method, depends upon the oxidation of manganous dioxide (bivalent manganese) by the oxygen dissolved in the sample resulting in the formation of a tetravalent compound. When the water containing the tetravalent compound is acidified free iodine is liberated from the oxidation of potassium iodide. The free iodine is chemically equivalent to the amount of dissolved oxygen present in the sample and is determined by titration with a standard solution of sodium thiosulphate.

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$$
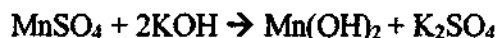
If the precipitate is white there is very little dissolved oxygen in the sample. A brown precipitate indicates that oxygen was dissolved in it and reacted with the manganous hydroxide to form manganic oxide.

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$
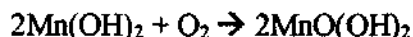
On addition of acid the precipitate is dissolved forming manganic sulphate.

$$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$$
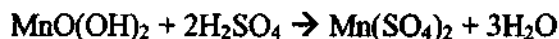
Due to an immediate reaction between this compound and the potassium iodide added previously, iodine is liberated resulting in the typical iodine colouration of the sample.

$$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2$$

The number of molecules of iodine liberated by the reaction is equivalent to the number of molecules of oxygen dissolved in the sample and this can be determined by titrating against standard solution of sodium thiosulphate using starch as indicator.

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$
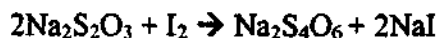
Reagents:

1) Sodium thiosulphate solution (1.25 gms in 1 ltr.)
2) Starch solution – 1gm starch made into a paste with distilled water and diluted to 100 ml, boiled and kept with 1 ml formalin as preservative.
3) Winkler solution A – 20 gms of manganous sulphate in 100 ml water.
4) Winkler solution B – 41 gm of sodium hydroxide + 25 gm of potassium iodide in 100 ml water.
5) Concentrated sulphuric acid.

6) Standard potassium iodate – Accurately weigh out 0.1784 gm of potassium iodate into a 1 ltr. volumetric flask and dissolve and make up to the volume - (this is 0.005N)
7) Potassium iodide.

Procedure:

Collect the water sample in a 125 ml glass stoppered bottle with out entangling any air bubbles. Take out the stopper and add 1ml each of Winkler A and Winkler B solution. Close the bottle. Shake the bottle gently till the precipitation formed is evenly distributed. Allow settling. Then add 2 ml con. H_2SO_4 close bottle and gently shake till the precipitate is completely dissolved.

Pipette out 10 ml of potassium iodate solution into a conical flask. Add 1 gm of potassium iodide and 2 ml of conc. sulphuric acid. Dilute to 100 ml and titrate against sodium thiosulphate solution till the colour becomes pale yellow. Add 1 ml of starch solution, shake well and continue the titration till the blue colour disappears. Repeat to concordance.

Pipette out 100 ml of the preserved sample and titrate against standard sodium thiosulphate as above.

Calculation:

Normality of potassium iodate $= \dfrac{\text{Weight/litre}}{35.67} = N_1$

Normality of thiosulphate $= \dfrac{N_1 \times 10}{\text{Titrated volume of thiosulphate for 10 ml of potassium iodate}} = N_2$

Hence amount of dissolved oxygen in ml/ltr. $= \dfrac{\text{ml. Thiosulphate} \times N_2 \times 8 \times 1000 \times R}{100 \times 1.429}$

Where
R is the correction factor =1.01 i.e. 125/125-2
1.429 is the conversion factor from ppm to ml/lit.

Ref: - Winkler, L.W., 1888. *Ber. Dtsch. Chem. Ges.*, **21**, 2843.

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7. DISSOLVED ORTHOPHOSPHATE
(Ascorbic acid method)

Introduction:

Phosphorous present in seawater in the form of dissolved orthophosphate can be easily determined quantitatively based on the method given by Murphey and Riley, (1962).

Principle:

Ammonium molybdate and potassium antimony tartrate react in an acid medium with dilute solutions of orthophosphate to form phosphomolybdic acid that is reduced to the intensely coloured molybdenum blue by ascorbic acid. The intensity of the blue colour increases in proportion to the amount of phosphorous present and can be measured photometrically.

Reagents:

1) Sulphuric acid solution 5N: Dilute 70 ml concentrated H_2SO_4 with 500 ml distilled water.
2) Potassium antimony tartrate solution: dissolve 1.3715 g K (SbO) $C_4H_4O_6$. $\frac{1}{2}H_2O$ in 400 ml distilled water in a 500 ml volumetric flask and dilute to volume. Store in a glass stopper bottle.
3) Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6$ Mo_7 O_{24}. $4H_2O$ in 500 ml distilled water. Store in a plastic bottle at 4° C.
4) Dissolve 1.76 g of ascorbic acid in 100 ml distilled water. This solution is stable.
5) Mixed reagent: Mix the above reagents in the following proportions. For 100 ml combined reagent, 50 ml 5N H_2SO_4, 5 ml potassium antimony tartrate solution, 15 ml ammonium molybdate solution and 30 ml ascorbic acid solution. Mix after addition of each reagent. The reagent is stable for 4 hrs.
6) Standard stock phosphate solution: Dissolve accurately 0.816 gm of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water. Store in dark bottle with 1 ml of chloroform.1 ml of this solution contains 6µg at PO_4-P.

Procedure:

To 100 ml of the sample at laboratory temperature add 8.0 ml of mixed reagent. After 5 minutes and preferably within the first 30 minutes measure the extinction of the solution, in a 1 cm cell against distilled water at a wavelength of 885nm.

Warm another portion of the sample to laboratory temperature and measure the extinction to obtain turbidity correction. Correct the measured extinction of the sample by subtracting both the turbidity and reagent blank.

Preparation of calibration graph:

Dilute the standard stock solution to get working standards of 1.2, 2.4, 4.8, 7.2, 9.6 and 12.0 µg at PO_4-P/ ltr. concentrations. Follow the above procedure and measure the absorbance of the standards at 885nm. Draw a calibration graph.

Calculation:

Obtain the concentration of PO_4-P in the sample from the calibration graph.

Note:

1) Samples are to be collected in polythene bottles and analysis is to be carried out within an hour of collection. If the analysis is delayed the samples must be frozen.
2) All the reagents must reach the room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the mixed reagent shake and let it stand for a few minutes until the turbidity disappears before proceeding.
3) If the samples are high in phosphate, dilute them with distilled water before the reagents are added.

Ref: - Murphy, J. & Riley, J.P., 1962. *Anal. Chem. Acta.*, **27**, 31-36.

8. REACTIVE SILICATE

Introduction:

Silicon present in seawater in the dissolved form mainly as the alkali salts of orthosilicic acid $Si(OH)_4$, is estimated by the method described by Mullin and Riley (1955) and as modified by Strickland and Parsons (1968).

Principle:

Determination of Silicate in natural waters is based on the principle that yellow silicomolybdic acid is produced when silicomolybdates react with acids. But all forms of silica in solution will not react to give the silicomolybdate complex. Depending on the pH, the silicomolybdate complex exists in two isomeric forms (the alpha and beta silicomolybdic acids). The beta form is very unstable. The alpha form, termed 'reactive silicate' is the most available form, turns into a blue complex on reduction with ascorbic acid, which can be measured photometrically.

Reagents:

1. Acid ammonium molybdate: Shake 2 g of ammonium molybdate with approximately 70 ml of water, add 6 ml of conc. HCl to dissolve the salt completely. Dilute to 100 ml and if necessary filter. Since the reagent takes up silica from glass it should be stored in polythene bottles.
2. Oxalic acid: Dissolve 10 g of oxalic acid dihydrate in water, dilute to 100 ml and filter.
3. Sulphuric acid 25 % v/v
4. Metol-sulphite solution: Dissolve by shaking, 5 g of metol in about 240 ml of water, containing 3 g of anhydrous sodium sulphite and dilute to 250 ml. The solution, after filtration through a Whatman No.1 filter paper, should be stored in a dark glass bottle.
5. Reducing agent:
   Mix 100 ml of the metol sulphite solution with 60 ml of 10 % oxalic acid and add, while cooling, 120 ml of 25 % H_2SO_4, dilute to 300 ml. The fresh reducing agent should be prepared fortnightly.
6. Standard silicate solution: 0.960 g of sodium silico fluoride is dissolved in distilled water and make up to 1000 ml. 1 ml of this solution contains 5µg-at Si.

Treatment of apparatus:

Graduated flasks should be allowed to stand overnight with a mixture of concentrated nitric acid and sulphuric acids (1:1) to render them insoluble. After this

treatment they should be well washed with tap water and distilled water. The flasks may be drained, but should not be allowed to become completely dry, as this appears to render them more soluble.

Method:

Pipette 20 ml of the sample (up to 2 µg-at Si) – (If the sample contains more than 2 µg-at Si take 15 ml of seawater and add about 5 ml of distilled water) - in to a 50 ml graduated flask containing 3 ml of the acid molybdate reagent and mix thoroughly. After 10 minutes add 15 ml of reducing agent and make up to 50 ml with distilled water. Allow to stand for 3 hours. Measure the optical density of the solution at 812 nm in a spectrophotometer. Use a reagent blank and set the instrument at 0.0 absorbance.

Preparation of calibration graph:

From the stock solution a series of working standards of known concentrations of silicates are prepared by suitably diluting with distilled water. The diluted working solutions of 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 µg-at Si/ lit. are prepared and treated with reagents and OD values are measured at 812 nm. Draw a calibration graph.

Calculation:

Concentration of the reactive silicate in the given sample is obtained from the graph.

Note:

1) Glass bottles must be avoided for sampling or storage; plastic containers are suitable. Because of the possible presence of siliceous organisms, storage in the dark is advised but analysis should in any case not be delayed for more than 24 hours. If this were unavoidable, freezing of the sample would probably help to minimize changes.
2) For samples of salinity below 27‰, overnight standing after thawing is essential to allow silicon polymerized by freezing to depolymerize.

Ref: -1) Mullin, J.B. and Riley, J.P. 1955: *Analyt. Chem. Acta.*, **12**, 162-76.
       2) Strickland, J.D.H. and Parson, T.R., 1968: *Fish. Res. Bd. Can.*, Bull. No. **167**, 311pp

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9. NITRATE

Introduction:

The estimation of Nitrate in seawater is based on a method by Morris and Riley (1963) with some modifications suggested by Grasshoff (1964) and Wood et. al. (1967).

Principle:

Nitrate in seawater is reduced almost quantitatively to nitrite when a sample is run through a column containing cadmium filings coated with metallic copper. The nitrite produced is determined by diazotizing with sulphanilamide and coupling with N- (1-naphthyl)-ethylenediamine to form a highly coloured azodye, which can be measured spectrophotometrically. Any nitrite initially present in the sample should be corrected for.

Special apparatus:
A reduction column may be conveniently made.

Reagents:

1) Conc. Ammonium chloride solution
   Dissolve 125 gm of AR grade ammonium chloride in 500 ml of distilled water and store in a glass or plastic bottle.

2) Dil. Ammonium chloride solution
   Dilute 50 ml of conc. ammonium chloride solution to 2000 ml with distilled water. Store the solution in a glass or plastic bottle.

3) Cadmium-copper filings

   Cadmium filings of a specific size range are required for the columns. They may be brought or made from cadmium metal by filing the metal with a coarse wood file. Filings should pass through 2 mm mesh size and be retained by 0.5 mm mesh size. Stir about 100 g of filings (sufficient for 2 columns with 500 ml of 2% w/v solution of $CuSO_4$ ($CuSo_4.5H_2O$) until the blue colour has left the solution. Place a small plug of copper wool (turnings) in the bottom of the reduction column and fill the column with dilute ammonium chloride solution.

   Pour in slurry of the cadmium-copper filings and gently pack the column to the required height. Do not allow the filings to become dried out during the procedure. They should continue to be covered with dil. ammonium chloride or the seawater samples at all times. Wash the column thoroughly with dil. ammonium chloride and adjust the flow rate by tapping the side of the column so that about 100 ml is

ml is collected in 8-12 minutes. If the flow rate is slower than this, the column has to be re-packed. Add a small plug of copper wool to the top of the column.
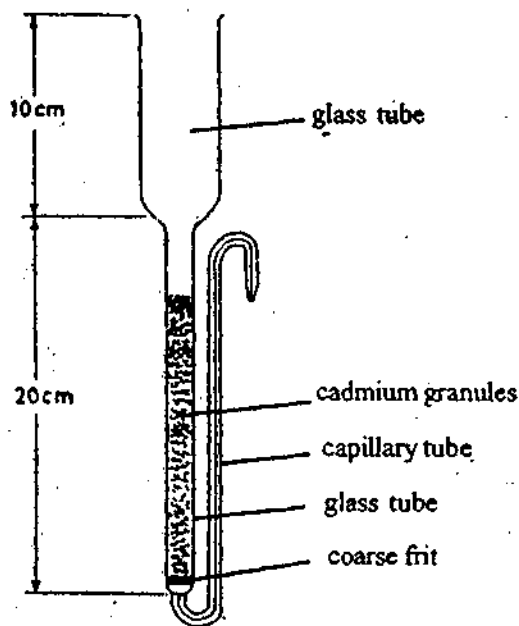
Cadmium column

The cadmium-copper filings may be reactivated after continued use (as judged by the F-value obtained for the standard). Filings are removed from the column, washed with 5% v/v HCl and then washed with distilled water until the pH of the decanted solution is ~5. The filings can then be reactivated with copper sulphate using the procedure given below.

4) Sulphanilamide solution

Dissolve 5 g of sulphanilamide in a mixture of 50 ml of con. HCl (sp.gr.1.18) and about 300 ml of distilled water. The solution is stable for many months.

5) N- (1-naphthyl)- ethylene diamine dihydrochloride solution
Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or directly a strong brown colouration develops.

6) Synthetic seawater
Dissolve 310 g of AR quality sodium chloride (NaCl), 100 g of AR quality magnesium sulphate ($MgSO_4.7H_2O$) and 0.5 gm of sodium bicarbonate ($NaHCO_3.H_2O$) in 10 lit. of distilled water.

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7) Standard nitrate solution

Dissolve 1.02 g of AR quality potassium nitrate (KNO₃) in 1000 ml of distilled water. The solution is generally stable in the absence of evaporation. Dilute 2 ml of this solution to 1000 ml with synthetic seawater. This solution should be stored in a dark bottle and prepared fresh before use.

Concentration = 20µ g –at N/l.

Procedure:

Add 2 ml of conc. ammonium chloride to the sample. Transfer100+2 ml of the sample into an Erlenmeyer flask. Mix the solution and pour about 5 ml on to the top of the column and allow it to pass through.

Add the remainder of the sample to the column and place the drained Erlenmeyer flask under the collection tube. Collect about 40 ml and discard. Collect about 50 ml in a graduated cylinder and dispense this into the Erlenmeyer flask, which contained the original sample. Allow the column to drain before adding the next 5 ml sample (as above).

To the 50 ml sample add 1 ml of sulphanilamide solution from an automatic pipette. Mix and allow the reagent to react for a period greater than 2 minute but not exceeding 8 minutes. Add 1 ml of naphthyl ethylene diamine (NNED) solution and mix immediately. After 10 minutes and not later than 1/2 hr., measure the extinction of the solution in a 1 cm cuvette against distilled water at a wavelength of 545 nm in a spectrophotometer. Correct the observed extinction by that of the reagent blank(B).

Carry out the procedure given above, using 100 ml of dil. Ammonium chloride instead of the seawater sample. Measures the extinction using the same cuvette as is used for the samples and subtract the blank value from the sample values for each column.

Also carry out the procedure with 100 ml of the dilute standard nitrate solution. Measure the extinction for each individual column; then the factor F is

$$F= 20/E_S$$

Where
E_S is the extinction of the standard corrected for the blank.

Calculation:

µg-at N/l = (Corrected extinction (B)x F) – 0.95 C

Where 'C' is the concentration of nitrite in the sample in µ g-at N/l.

Estimation of Nitrite © in the sample:

Prepare a standard stock nitrite solution by dissolving 0.345 g of sodium nitrite in 1000 ml of distilled water (I ml = 5μg-at). 1 ml of stock solution is diluted to 100 ml with distilled water. Prepare dilutions of 0.05, 0.2, 0.5, 2.0, and 4.0μg-at/ltr. Measure the absorbance at 545 nm and prepare the calibration graph to obtain the nitrite concentration.

To 50 ml of sample, add 1 ml of sulphanilamide followed by 1 ml NNED solution. Mix well and after 10 minutes measure the absorbance of the solution at 545 nm against a reagent blank. Obtain the nitrite concentration of the sample (c) from the calibration graph.

Notes:

1) If the samples are stored they should be frozen at -20° C. In the presence of high concentrations of phytoplankton, samples should be filtered before analysis.

2) Because of the small salt effect, standard nitrate solutions should be made up in synthetic seawater or a low nitrate seawater sample should be 'spiked' with a standard amount of nitrate.

3) Column dimensions can be scaled down proportionally and smaller seawater samples can be used as required by users.

4) For extinction values of >1.0 or <1.0 use an appropriate cuvette cell length (i.e. 0.5 cm or 10 cm respectively) and adjust the factor where appropriate.

5) In most samples of seawater, the level of nitrite will be insignificant and the correction can be largely ignored. However in some cases, particularly with respect to depth profiles where a nitrite maximum is expected, a correction should be employed. The factor of 0.95 allows for an approximate 5% loss of nitrite on the column compared with the direct determination.

6) For the blank and standard values, the extinctions obtained should be applied to individual cadmium columns and not averaged. Each column may have small consistent differences that are allowed for only if the blank and standards are applied on an individual basis.

Ref:- 1) Grasshoff, 1964. *Kiel. Meeresforsch.*, **20:** 5
2) Morris and Riley, 1963. *Anal.Chem. Acta.* **29:** 272
3) Wood et. al., 1967. *J. Mar. Biol. Assoc. U.K.*, **47:** 23, 1967

Introduction:

For the determination of ammonia in the seawater the method involving indophenol blue reaction is well known and the one followed here is that of Zolarzano (1969).

Principle:

In this method phenol and hypochlorite react in an alkaline solution to form phenyl quinone-monoimine, which in turn, react with ammonia to form indophenol. Indophenol gives the solution a blue colour, the intensity of which is propotional to the concentration of ammonia present. Sodium nitroprusside is added to intensify the blue colour. Both ammonia and ammonium are measured, because in a strong alkaline solution all the ammonium is converted to ammonia. This procedure gives an estimate of total ammonia nitrogen.

Reagents required:

1) Phenol-alcohol solution: Dissolve 10 g of reagent grade phenol in 100 ml of 95% v/v ethyl alcohol. U.S.P.
2) Sodium nitroprusside 0.5%: Dissolve 1 g of sodium nitroprusside in 200 ml of water.
3) Alkaline solution: Dissolve 100 g of trisodium citrate and 5 g of sodium hydroxide in 500 ml of water.
4) Sodium hypochlorite solution: Use a solution of commercial hypochlorite, which should be atleast 1.5 N.
5) Oxidising solution: Mix 100 ml of sodium citrate solution (alkaline solution) and 25 ml of hypochlorite solution and use the same day (1:4 ratio- sodium hypo chlorite: alkaline solution).
6) Stock standard solution: 0.100 g of ammonium sulphate (A.R Grade) in 1000 ml of distilled water (1 ml = 1.5 μg at N).

Procedure:

The procedure consists of the successive addition of 2 ml of phenol solution, 2 ml of nitroprusside solution and 5 ml of oxidizing solution to 50 ml of sample mixing thoroughly after each addition. The colour is allowed to develop at room temperature (22-27° C) for 1 hr and the absorbance recorded at 640 nm in a spectrophotometer. Correct the absorbance with that of the reagent blank.

Preparation of calibration graph:

Dilute the standard stock solution to get working standards of 1.5, 3.0, 6.0, 9.0, 12, 15 µg at NH_3-N/ltr. concentrations. Follow the above procedure and measure the absorbance at a wavelength at 640 nm in a spectrophotometer and draw a calibration graph.

Calculation:

Obtain the concentration of NH_3-N in the sample from the calibration graph.

Note:

1) All the reagents are prepared using ammonia free distilled water.
2) All the glassware used must be cleaned by washing initially with warm dilute hydrochloric acid and rinsing thoroughly with distilled water.
3) Filter the water sample prior to analysis through Whatman No: 42, or equivalent filter paper.
4) If the strength of hypochlorite is not satisfactory, a fresh reagent should be used for analysis.

Ref: - Zolarzano, L., 1969. *Limnol. Oceanogr.*14: 799-801.

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11. BIOCHEMICAL OXYGEN DEMAND

Introduction:

Heterotrophic bacteria decompose biodegradable organic matter by respiring oxygen present in water. Biochemical Oxygen Demand (BOD) is explained as a measure of dissolved oxygen consumed by the heterotrophic bacteria to convert available biodegradable organic matter into inorganic plant nutrients and carbon dioxide.

Principle:

Aerated water sample of known oxygen content is taken in BOD bottles, which is stoppered and incubated at $20°$ C for 5 days. Oxygen consumed for decomposing the organic matter is estimated by measuring the initial oxygen level and final oxygen level after consumption for 5 days.

The procedure used to determine BOD depends upon the nature and extent of pollution. Three methods are commonly used.
1. The direct method
2. The unseeded dilution method and
3. The seeded dilution method.

Seeding:

The purpose of seeding is to introduce into the sample a biological population capable of oxidizing the organic matter in the wastewater. Where such microorganisms are already present, as in domestic waste or unchlorinated effluents and surface waters, seeding is unnecessary and should not be used.

Unseeded dilution method:

Reagents:

1) All the reagents used for Dissolved Oxygen estimation by Winkler method.

2) Buffer solution: Dissolve potassium dihydrogen phosphate (KH_2PO_4. 8.5 g), Potassium hydrogen phosphate (K_2HPO_4, 21.75 g), disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$), 33.4 g), and ammonium chloride (NH_4Cl, 1.7 g) in distilled water and dilute to one litre.

3) Magnesium sulphate solution: Dissolve 22.5 g of $MgSO_4.7H_2O$ in distilled water and dilute to one litre.

4) Calcium chloride solution: Dissolve 27.5 g of anhydrous calcium chloride in distilled water and dilute to one litre.

5) Ferric chloride solution: Dissolve 0.25 g of $FeCl_3.6H_2O$ in one litre of distilled water.

6) Dilution water: Prepare the dilution water by adding 1 ml of each of the above four reagents (2-5) to a litre of distilled water. Store the dilution water at 20° C.

TABLE 1
Data for estimation of Dilution

Type of waste	Five-day BOD range	Dilution scale	Milliliters of sample in a 300 ml BOD bottle
Strong industrial waste	500-5000	1:100-1:1000	3-0.3
Normal and settled sewage	100-500	1:20-1:100	15-3
Treated effluent	20-100	1:5-1:20	60-15
Moderately polluted water	10-20	1:1-1:5	300-60

A range of dilutions is desirable, and a dilution in the range of 40-90% of the original dissolved oxygen content will give the best results. At least three dilutions should be prepared, and these should be run in duplicate.

Procedure:

Accurately measure the required amount of the well-aerated sample into the BOD bottles. Fill the bottles completely with dilution water.

Use the appropriate Winkler modification and determine the dissolved oxygen content of the diluted sample. Calculate the initial dissolved oxygen.

Incubate the samples at 20° C for five days in complete darkness in a BOD incubator.

After five days, determine the dissolved oxygen of the samples. Average the dissolved oxygen concentrations of the duplicate samples and record as final dissolved oxygen.

Calculation:

Calculate the BOD$_5$ (mg/ltr) of the sample using the following formula:

$$BOD_5 \text{ (mg/ltr)} = \frac{D_1 - D_2}{P}$$

Where

D_1 = Dissolved oxygen of diluted sample 15 minutes after preparation
D_2 = Dissolved oxygen of diluted sample after incubation
P = Decimal fraction of the sample used,

i.e. $\frac{\text{ml of waste water}}{\text{volume of the BOD bottle}}$

Note:

To convert the values of dissolved oxygen from ml/l to mg/l ,the ml/l
values are divided by 0.7

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12. CHEMICAL OXYGEN DEMAND (COD)

Introduction:

Chemical oxygen demand is the measure of the total amount of oxygen that is required to completely oxidize all of the organic matter in a sample to carbon dioxide and water. Therefore COD of the water samples increases with increasing organic matter concentrations.

Principle:

In this method, potassium dichromate is used as the oxidizing agent. A known quantity of potassium dichromate is added to a water sample, which is acidified with sulphuric acid. The sample is then heated and the organic matter is oxidized to carbon dioxide and water, while the dichromate gets reduced. The excess dichromate can be measured by back titration with ferrous ammonium sulphate using ferroin indicator (an oxidation-reduction indicator) to detect the end point.

The amount of dichromate consumed in the oxidation of organic matter may be calculated from the milliequivalents of ferrous ammonium sulphate used in the back titration to reduce the excess dichromate. The amount of potassium dichromate consumed may easily be converted to oxygen equivalent since 1meq. of $K_2Cr_2O_7$ is equal in oxidizing power to 1 meq. or 8 mg of oxygen.

Special apparatus:

A reflux apparatus may be prepared with a hot plate, a West condenser with ground glass joints, and a 125 ml Erlenmeyer flask with a ground glass neck.

Reagents:

1) Potassium dichromate solution, 1.000N: Dry primary standard grade $K_2Cr_2O_7$ at 103° C for 2 hours and cool in a desiccator. Dissolve 49.036 g of $K_2Cr_2O_7$ in distilled water and dilute to 1000 ml.
2) Potassium dichromate solution, 0.0250N: Dilute 25 ml of 1.000N $K_2Cr_2O_7$ and 100 mg of sulphamic acid to 1000 ml with distilled water.
3) Ferrous ammonium sulphate solution: Dissolve 9.8 gm of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water and add 20 ml of con. H_2SO_4. Cool and then dilute to 1000 ml and store in the dark. This reagent must be standardized daily.
4) Ferroin indicator: Dissolve 1.8877 gm of 1,10-phenanthroline monohydrate and 0.70 g of $FeSO_4.7H_2O$ in 100 ml of distilled water.
5) Other reagents: Silver sulphate, mercuric sulphate and concentrated sulphuric acid.

I) Digestion method:

Procedure:

Transfer 20 ml of sample into a 125 ml Erlenmeyer flask. Using a clean pipette transfer 10 ml of 0.0250 N potassium dichromate to the flask. Add 0.4 gm of silver sulphate crystals, 0.4 gm of mercuric sulphate crystals and several glass beads to the flask. The silver sulphate and mercuric sulphate may be added with a calibrated scoop or spoon. Carefully add 30 ml of concentrated sulphuric acid, swirl the flask thoroughly, attach to the condenser and heat. Failure to mix well will result in the solution being blown out of the condenser upon application of heat.

Reflux for 2 hours, cool, wash the condensate present inside the condenser into the flask with 20 ml distilled water. Remove the flask from the condenser, and dilute the contents to about 80 ml with distilled water. One or more reagent blanks prepared with distilled water must be carried through the digestion procedure with each set of determinations.

Add 2 or 3 drops of ferroin indicator to the digested sample and to the reagent blank and titrate with ferrous ammonium sulphate. The initial colour of solutions will vary from yellowish-orange to blue-green. However just before reaching the end point the colour of the solutions will be blue-green. At the end point, the addition of a single drop of titrant causes the colour to change from blue-green to red-brown.

Calculation:

Equation for the calculation of COD:

$$COD \ (mg/ltr.) = \frac{(B-S) \ (N) \ (8) \ (1000)}{Sample \ volume \ in \ ml}$$

Where
B = Milliliters of ferrous ammonium sulphate (FAS) used in titration of the reagent blank.
S = Milliliters of FAS used in the titration of the sample
N = Normality of the FAS

Standardization of ferrous ammonium sulphate:

Dilute 10 ml of 0.0250 N potassium dichromate to about 45 ml with distilled water in an Erlenmeyer flask and add 30 ml of con. H_2SO_4. Do not carry this solution through the digestion. When cool add 2 or 3 drops of ferroin indicator and titrate with ferrous ammonium sulphate. Standardization should be made at the same time the samples and blanks are titrated. Calculate the normality of ferrous ammonium sulphate from the equation:

$$V_1N_1 = V_2N_2$$

$$10 \times 0.0250 = \text{Titrated volume} \times N_2$$

$$N_2 = \frac{10 \times 0.025}{\text{Titrated volume}}$$

Note:

1) The procedure outlined above is suitable for waters that have a COD of less than 70 mg/ltr. If the samples contain greater quantities of organic matter, the normality of the potassium dichromate solution must be increased. The strength of ferrous ammonium sulphate should also be increased proportionally to keep the titration volume at a reasonable size.

2) The importance of clean glassware and high quality distilled water cannot be overemphasized. Traces of organic matter cause serious error. Deionised water cannot be used for preparing reagents or washing glassware since it contains organic matter.

3) Chloride seriously interferes with the COD procedure because it is oxidized to chlorine by dichromate causing high results. Mercuric sulphate is added to complex chloride as mercuric chloride.

4) Nitrites also cause high results since they are oxidized to nitrate by dichromate. Sulphamic acid is used to prevent interference by nitrite.

5) Silver sulphate is added to the digestion solution to catalyze the oxidation of aromatic hydrocarbons and pyridine.

II) Heat of dilution COD procedure for brackish waters:

Principle:

In this modification of COD procedure (Ruttanagosright and Boyd, 1989) no heat other than that is produced by the dilution of concentrated sulphuric acid is applied to the sample and hence eliminate the need for a reflux apparatus. The heat of dilution COD value for any particular sample will invariably be lower than the standard COD value because the oxidation of organic matter is not complete. However a close correlation between heat of dilution and standard COD values have being observed by the authors with an 'r' value of 0.97.

Procedure:

Using a volumetric pipette transfer 20 ml of water sample into a clean 250 ml Erlenmeyer flask. Pipette 10 ml of 0.025 N potassium dichromate to the flask. Carefully add 30 ml of con. H_2SO_4 and swirl. Cover the flask with a clean cover glass and let stand for 30 minutes. Dilute the contents of the flask to 75 ml and titrate with ferrous

ammonium sulphate by adding 2 or 3 drops of ferroin indicator as described above in the standard COD procedure. A reagent blank prepared with 20 ml of distilled water is also carried through the procedure.

Calculate the COD value using the equation as given above.

Note:

In this procedure, a ratio of 10:1 of mercuric sulphate: chloride is maintained to prevent interference by chloride. 200 mg of $HgSO_4$ is to be added for each 1000 mg/ltr. of chloride present in the sample. If salinity is known, a sufficiently accurate estimate of chloride concentration may be obtained by dividing salinity in mg/ltr. by 1.8.

Ref: - 1) Ruttanagosrigit and Boyd, C.E., 1989. *J. World Aquaculture Soc.*, **20**: 170-172.

SOLIDS
(Total dissolved solids and Total suspended solids)

Introduction:

Solids represent that portion of the water sample that is not lost upon evaporation. Solids include dissolved organic matter, particulate organic matter, dissolved inorganic matter, dissolved inorganic substances except gases, the carbon dioxide contained in bicarbonate and particulate inorganic substances.

13. TOTAL DISSOLVED SOLIDS (TDS)

Principle:

To measure the total dissolved solids (TDS) concentration, a sample is filtered to remove the particulate matter, the filtrate is evaporated, and the residue weighed. The TDS concentration indicates the milligram per litre of dissolved organic and inorganic matter in a sample.

Special Apparatus:

Depending upon the solid analysis conducted, one or more of the following items are required: Glass fibre filtration apparatus, Gelman type A/E glass fibre filters or equivalent, Imhoff cones, 100 ml evaporating dishes, muffle furnace, large desiccators and semi micro analytical balance.

Procedure:

Prepare glass fibre filters by soaking them in distilled water for 24 hrs. and then drying. Ignite a clean evaporating dish in a muffle furnace at 550° C for 30 minutes, cool the dish in a desiccator and weigh it. Position a filter holder in a suction flask, place a glass fibre filter on the holder, attach the funnel to the holder and attach the apparatus to a vacuum source. Mix the sample and filter 125-150 ml of it through the glass fibre filter.

Measure 100 ml of the filtrate into the tared evaporating dish with a graduated cylinder. Evaporate the contents of the dish in an oven at 95° C. Increase the oven temperature to 103° C for 1 hr. Cool the dish and residue in a desiccator and weigh.

Calculation:

$$TDS\ (mg/ltr) = \frac{(F\text{-}T)\ 1000}{V}$$

Where

        F= Final weight of Evaporating dish and residue in milligrams
        T= Tare weight of evaporating dish in milligrams
        V= Sample volume in milliliters

14. TOTAL SUSPENDED SOLIDS (TSS)

Principle:

        The Total Suspended solids (TSS) can be estimated by weighing the residue retained on the glass fibre filter used in the TDS analysis. The TSS in milligrams per litre is a measure of the particulate matter in suspension.

Procedure:

        Prepare a glass fibre filter by soaking them in distilled water for 24 hours and then drying. Dry filters in oven at 80-90° C for 24 hrs. and tare. Pass a 100 ml (or larger) sample through the tared glass fibre filter. Remove the filter with small tongs (do not touch) and dry for 24 hrs. at 80-90° C. Cool the filter in a desiccator and weigh to five decimal places.

Calculation:

$$TSS\ (mg/ltr) = \frac{(F-T)\ 1000}{V}$$

        Where F= Final weight of Filter and residue in milligrams
                  T= Tare weight of Filter in milligrams
                  V= Sample volume in milliliters

Note:

        The TSS analysis can easily be conducted in conjunction with the TDS analysis. One can simply tare the filter used in the TDS analysis, determine the quantity of the residue resulting from the filtration of the TDS sample, and calculate TSS.

Where

F= Final weight of Evaporating dish and residue in milligrams
T= Tare weight of evaporating dish in milligrams
V= Sample volume in milliliters

14. TOTAL SUSPENDED SOLIDS (TSS)

Principle:

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Calculation:

$$TSS \ (mg/ltr) = \frac{(F-T) \ 1000}{V}$$

Where F= Final weight of Filter and residue in milligrams
T= Tare weight of Filter in milligrams
V= Sample volume in milliliters

Note:

The TSS analysis can easily be conducted in conjunction with the TDS analysis. One can simply tare the filter used in the TDS analysis, determine the quantity of the residue resulting from the filtration of the TDS sample, and calculate TSS.

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15. PRIMARY PRODUCTIVITY
(Light and dark bottle method)

Principle:

Two sets of bottles are incubated with comparable plankton samples, one exposed to light and other kept in darkness for a suitable period of time at controlled light and temperature. The changes in dissolved oxygen levels in these bottles are measured and in turn the productivity is expressed in g C fixed/ unit volume/ hr.

Glassware:
1) 125 ml dissolved oxygen bottles (4 numbers clear)
2) 125 ml dissolved oxygen bottles (2 numbers dark)
3) 2 dark cloth bags.

Reagents:
1. Winkler A
2. Winkler B
3. Sodium thiosulphate
4. Con. H_2SO_4
5. Starch solution

Procedure:

Collect water sample in 4 clear BOD bottles and 2 dark BOD bottles as usual. Fix two of the clear bottles immediately with Winkler A and B. Incubate the other 2 clear bottles and 2 dark bottles (keep inside dark cloth bags), for 3 hours at the place where collection was made. (Alternatively all the bottles may be incubated in a glass trough with periodic change of water). After 3 hours of incubation fix the clear and dark bottles with equal volumes (1 ml each) of Winkler A and B.

Titrate against sodium thiosulphate and calculate the O_2 in ml/l.

Calculation:

Let O_2 of light bottle after incubation = x
Let O_2 of dark bottle after incubation = y
Let O_2 of light bottle initially fixed = z

Then gross production $= \dfrac{(x-y) \times 0.536}{PQ \times t}$ mgC/ l/ hr

net production $= \dfrac{(x-z) \times 0.536}{PQ \times t}$ mgC/ l/ hr

Where
PQ is the photosynthetic quotient = 1.25
't' is the number of hours of incubation = (3 hours)

Note:

For expressing the productivity for m^3/day, multiply the above by 10000;
assuming 10 hrs being the sunshine hours affecting photosynthesis in a day.

Ref: - Gaarder, T and Gran, H.H., 1927. *Rapp. Et. Proc. Verb. Cons. Internat. Explor. Mer.,* **No: 42,** 48 pp.

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16. CHLOROPHYLL PIGMENTS
(Spectrophotometric method)

Principle:

Chlorophyll bearing organisms present in known volume of water sample is filtered and dissolved in a solvent (Acetone 90% v/v). The pigment content dissolved in unit volume of acetone is measured spectrophotometrically. Since on an average, primary production in the ocean bears a fairly constant relation to the chlorophyll content, measurement of these pigments is also used as an index of productivity.

Requirements:

Glass fibre filter papers, vaccum-filtering unit, measuring jar, centrifuge and centrifuge tubes with cap.

Method:

Water samples collected for chlorophyll pigments must be passed through a coarse filter 0.2 mm mesh to remove zooplankters. Thoroughly mix the sample. A known volume (500 ml) of the sample was filtered through a 47 mm GF/C filter paper. The pigment is extracted by adding 10 ml of 90 % v/v acetone to each filter in a centrifuge tube. Tightly stopper the tube with aluminium foil or plastic cap. The extraction was carried out at low temperature for 20 hours in dark. The extract is centrifuged (6000 rpm for 8 minutes) and the final volume is adjusted to 10 ml with the same solvent. Decant the supernatant into a cuvette and measure the extinction at the following wavelengths (750, 664, 647 & 630 nm). The amount of pigments in the sample is calculated using the revised formula of Jeffery and Humphrey (1975).

Calculation:

(Chl a) Chlorophyll a = 11.85 x E 664 – 1.54 x E 647 – 0.08 x E 630
(Chl b) Chlorophyll b = 21.03 x E 647 – 5.43 x E 664 – 2.66 x E 630
(Chl c) Chlorophyll c = 24.52 x E 630 – 1.67 x E 664 – 7.6 x E 647

Where
E stands for the absorbance at different wavelengths obtained above and corrected by the 750 nm reading; Chlorophyll a, b and c are the amounts of chlorophyll.

Then mg Chlorophyll/ m^3 = $\dfrac{C \times v}{V \times 10}$

Where

v = volume of acetone used.

V = volume of sample in litres.

C = Amount of Chlorophylls a, b & c

Note:

Water sample must be frozen if filtration could not be done immediately. While filtration the sample should be mixed thoroughly.

Ref: - Parsons, T.R., Yoshiaki Maita and Carol Lalli, 1984. *A manual of chemical and biological methods for seawater analysis,* 101-104.

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1. SEDIMENT SAMPLING AND PROCESSING

Sediment sampling:

Sediment sample is collected from the sea / river using a grab. Sample should be representative of the area sampled. For this, collect samples from at least four sites in an area. Pool these samples in a wide basin. The pooled sediment is mixed thoroughly. Then quartering is done, removing the opposite quarters as shown below.
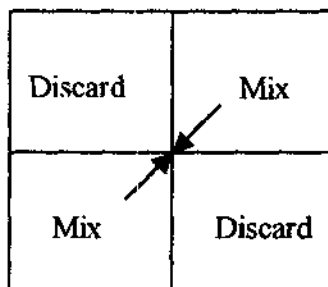
Quartering is done till a sizeable quantity i.e., around 500 g sediment is obtained. Collect in heavy-duty plastic bags.

Sediment processing:

Do not store the sediment wet for more than one day. Wet samples should be kept under refrigeration if storage is needed in the wet stage for more than one day. Then air-dry the sediment in shade in well-ventilated places. To reduce drying time, oven drying can be done at 50-60°C. (Temperature should not be increased above 60°C since it will cause loss of nutrients). Then pulverize the sediment gently by breaking clods using a pestle and mortar. (Do not over grind the sediment so as to break the sand particles, which will cause errors in textural analysis). Then sediment samples can be stored in well-labeled, capped plastic bottles or heavy-duty plastic bags. At this stage, it is ready for sending for analysis. While storing, store in a dry place.

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2. HYDROGEN ION CONCENTRATION (pH)

Principle:

The degree of acidity or alkalinity in soils also known as soil reaction is determined by hydrogen ion (H^+) conc. in soil solution. An acid soil has more H^+ than OH^- ions, whereas basic or alkaline solution contains more OH^- ions than H^+ ions. To categorize these conditions, the term soil pH is used.

Determination of dry pH of sediment

Procedure:

Calibrate the pH meter with buffer solution according to the maker's instructions and wash the electrode well.

To a 10 g sample add 25 ml water. The suspension is stirred at regular intervals for 20-30 min. Then pH is measured with glass electrode. The suspension is stirred just before electrode is immersed.

Determination of wet pH of sediment

Procedure:

Wet pH is to be determined before air-drying the sediment. After calibrating the pH meter, insert the electrode of the pH meter directly in to a wet lot of sediment sample and the pH recorded is the wet pH of the sediment.

Principle:

When standard silver nitrate solution is added to the soil extract, the chlorides will be precipitated as silver chloride. When all the chlorides being precipitated, next drop of silver nitrate will react with potassium chromate giving red colour of silver chromate.

Reagents:

(1) Silver nitrate (0.144 N): Dissolve 24.5 g of silver nitrate in 1 liter water.
(2) Indicator: 10 % potassium chromate.

Procedure:

Weigh 5 g of sediment and transfer it into a conical flask. Add 25 ml distilled water and shake it for 30 minutes .The sample is then filtered through No.42 filter paper. To 10 ml of the filtrate is added 4 drops of 10 % potassium chromate indicator. This is then titrated against silver nitrate. The end point is the colour change from pale yellow to pale pinkish red.

Calculations:

1 ml of $1N$ Ag NO_3 = 35.46/ 1000 g of Cl

$$Salinity = \frac{x * 0.144 * 35.46 * 25 * 1000}{10 * 5 * 1000} \quad g/kg$$

$$= \frac{x * 0.144 * 35.46 * 25}{10 * 5} \quad mg/g \quad or \quad ppt$$

where
x	=	volume of $AgNO_3$ used
0.144	=	normality of $AgNO_3$
35.46	=	eq.wt of Cl
25	=	distilled water used for extraction
10	=	volume of extractant used for titration
5	=	quantity of sediment taken.
1000	=	for converting 35.46mg to g
1000	=	for converting g to kg

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4. SOIL TEXTURE
(Mechanical Analysis by International Pipette Method)

Principle:

Mechanical analysis is defined as the analytical procedure by which individual particles are separated and to determine the size distribution of the soil. According to the relative proportion of various sizes of the individual particle, they are separated in to various groups and is known as textural classification of soil. According to Attenburg system, the following classification of mechanical separates was suggested.

Soil separate	Diameter limit (mm)
Coarse sand	2 to 0.2
Fine sand	0.2 to 0.02
Silt	0.02 to 0.002
Clay	< 0.002

Mechanical analysis consists essentially of two distinct operations:

1) Dispersion: The most important cementing agents are organic matter, colloidal clay and dehydrated colloidal oxides of Fe, Al.

   The important physical techniques that have been used to effect the mechanical disruption of aggregates into completely dispersed particles are shaking, stirring and boiling etc.

The chemical aid in soil dispersion are based on

(a) Oxidation of organic matter by H_2O_2; (b) Removal of flocculating ions by introducing single cations; (c) Peptization of colloidal particles through the introduction of ions that increase negative potential.

2) Fractionation of the sample in to various separates: The relation between the particle size and its rate of fall through a fluid is expressed by Stokes law as follows:

$$V = 2gr^2(dp-dw)/9n$$

Where, V = velocity of fall of particles, r = radius of particles, dp = density of particles, dw = density of medium, n = coeff. of viscosity of liquid and g = acceleration due to gravity.

Pipette method:

According to this method, instead of completely separating the fractions of the mechanical compositions, suspension samples are taken from the different depths subsequent to shaking, after the lapse of certain pre- determined period of settling which are dependent on the dimensions of the mechanical elements and on the temperature of water.

Requirements:

Sieves (2mm, 60 mesh), beaker, sedimentation cylinder (1 litre), meter scale, Buchner funnel and suction filtration (vacuum) assembly, aluminium box, $2N$ HCl, $1N$ NaOH.

Procedure:

1) Dispersion:
(a) Weigh 20 g soil in a beaker. Moisten with water.
(b) Add 30 ml of 6% H_2O_2, heat intermittently for 1 hour. When the effervescence and evolution of CO_2 will cease add 30 ml H_2O_2 and digest on hot plate for ½ hour. Repeat this process for 3-4 times.
(c) Cool and dilute with 100 ml water. Add 25 ml of $2N$ HCl and stir vigorously. Filter through No.1 filter paper. Wash with hot distilled water several times until free of Cl ion.
(d) Transfer the soil quantitatively in to a 500 ml beaker and add 10 ml $1N$ NaOH and stir for 10 minutes using a mechanical stirrer.
(e) Transfer the soil quantitatively in to a sedimentation cylinder by washing with distilled water and fill up to the volume with water. Shake the suspension vigorously for 10 minutes.

2) Fractionation:

(a) Separation and determination of clay + silt: Note the room temp. and time of settling from table of sedimentation time at different temperature in International System (Table 1). Then draw 10 ml of suspension from a pipette at 10 cm depth in a weighed crucible and dry it at 105°C to a constant weight. Difference in weight will give the quantity of clay + silt present in 10 ml suspension. Express on percent dry weight basis.
(b) Separation and determination of clay: The whole suspension is again shaken and kept undisturbed according to time and temperature chart. Draw 10 ml suspension in a similar manner as described earlier in a weighed crucible and then dry at 105°C until the constant weight attains. Express the result in percent of dry weight basis.
(c) Separation and determination of fine sand + coarse sand: Transfer the whole quantity of suspension in a large beaker quantitatively. Fill water carefully in the beaker. Wait 4 min., decant the supernatant liquid. Repeat this process several times till the clay + silt fraction completely removed. Care must be taken during decantation of the supernatant liquid as no fine sand is removed from the

suspension. Evaporate the beaker containing fine + coarse sand to reduce the volume and finally transfer quantitatively in to a weighed crucible and dry in an oven at 105^0 C to constant weight. Express the percent fine sand + coarse sand on dry weight basis.

(d) Separation and distribution of coarse sand: Transfer the fine + coarse sand present in the crucible on a 60 mesh (0.2mm) sieve. Then take the weight of the particles remain on the sieve. This is coarse sand fraction. Express on percent dry weight basis.

(e) Determination of loss in solution: Determine clay, silt, fine sand and coarse sand separately as percent on dry weight basis. Then add all the constituents and subtract from 100, which yield the percent loss in solution.

Determination of sediment moisture:

Take 10 g of wet sediment in a previously weighed petridish. Let the weight of petridish be Xg. Oven-dry the 10g sediment at 105°C for 24 hours. Note the dry weight (dry weight of sediment + weight of petridish). Let it be Y g. Find out the dry weight of sediment alone by deducting the weight of petridish X from (Y). Then, the percentage moisture in the sediment will be (10 – dry weight of the sediment)/10 *100.

Weight of petridish alone   = Xg
Weight of wet sediment   = 10g
Dry weight of sediment + petridish = Yg
Dry weight of sediment alone   = (Y – X) g
% Moisture in the sediment   = [10 – (Y- X)] /10 * 100

This sediment moisture percentage is needed for the calculation in soil textural analysis.

Calculation:

(a) <u>Silt + clay fraction</u>:
    Let, wt. of soil   = w g
    % moisture   = x
    Total volume of suspension = V
    Volume of suspension taken = v
    Wt. of silt + clay   = m g
    ∴ % silt + clay   = [m/v x V(100-x) / w * 100] *100

(b) <u>Clay Fraction</u>:
    Let, Weight of clay = n g
   ∴ % Clay = [n/v x V (100-x) /w * 100] * 100

(c) <u>Fine sand + coarse sand fraction</u>:
    Let weight of crucible = A g
    Weight of crucible + fine sand + coarse sand = M g
   ∴ % Fine sand + coarse sand = [(M-A) (100-x) / W * 100] * 100

(d) <u>Coarse sand fraction</u>:
      Let, Wt. of crucible + Coarse sand = N g
      $\therefore$ % Coarse sand = [(N-A) (100-X) / w * 100] *100

(e) <u>Loss in solution</u>:
      Let, a = % of clay
          b = % of silt
          c = % of coarse sand
      &   d = % of fine sand
      $\therefore$ % Loss in solution = 100 − (a+b+c+d)

Now, soil texture composition = % clay + % silt + % fine sand + % coarse sand + % loss in solution = 100.

TABLE 1
SEPARATION OF CLAY AND CLAY + SILT AT DIFFERENT TEMPERATURE - TIME

Temperature (°C)	Clay Decantation		Silt Decantation	
	Hrs	Min.	Min.	Sec.
11	10	10	6	10
12	9	50	6	0
13	9	35	5	50
14	9	20	5	40
15	9	5	5	30
16	8	50	5	20
17	8	35	5	10
18	8	25	5	0
19	8	10	5	10
20	8	0	4	48
21	7	50	4	40
22	7	40	4	30
23	7	25	4	30
24	7	15	4	20
25	7	5	4	15
26	6	55	4	10
30	6	20	3	50
31	6	15	3	45
33	5	55	3	35

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5. ORGANIC CARBON
(Walkley and Black's titration method)

Principle:

The soil is digested with potassium dichromate solution and sulphuric acid making use of the heat of dilution of sulphuric acid. The excess of potassium dichromate not reduced by the organic matter of the soil, is determined by titration with standard ferrous sulphate or ferrous ammonium sulphate solution.

Reagents:

1) Potassium dichromate 1N: Dissolve 49 gram of potassium dichromate in water and make it to 1 liter.

2) Sulphuric acid + silver sulphate: Dissolve 25 gm of silver sulphate in 1 liter of conc. sulphuric acid.

3) Phosphoric acid (85%)

4) Diphenylamine indicator solution: Dissolve 0.5 gm of diphenylamine in a mixture of 100 ml sulphuric acid and 20 ml water and store in a colored bottle.

5) Ferrous ammonium sulphate ($N/2$): Dissolve 196 gm of A.R grade $FeSO_4$ $(NH_4)_2SO_4$. $6H_2O$ in water. Add 20 ml sulphuric acid and dilute to 1 liter.

Procedure:

Take 0.5g of soil (0.5 mm sieved) in 500 ml conical flask. Add 10ml of 1N potassium dichromate and 20 ml conc. H_2SO_4. Shake well for a minute or two and allow it to stand for about 30 minutes. Add 200 ml water, 10 ml phosphoric acid and 1 ml diphenylamine indicator solution. A deep violet color will appear. Titrate with $N/2$ ferrous ammonium sulphate solution, till the violet color changes to blue and finally to green. In the same way carry out a blank determination also and calculate the results as follows.

Calculation:

Weight of soil taken = W g

Volume of 0.5 N ferrous ammonium
sulphate required for reducing 10 ml
$K_2Cr_2O_7$ solution (Blank Reading) = X ml

Volume of 0.5 N ferrous ammonium
Sulphate required for reducing the
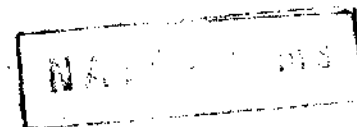excess of dichromate (experimental reading) = Y ml

Difference = (X - Y) ml

1 ml of $1N$ $K_2Cr_2O_7$ = 0.003 g carbon

% of carbon in soil $= \dfrac{(X-Y) \times N \times 0.003 \times 100}{W}$

Where N = normality of ferrous ammonium sulphate

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6. TOTAL NITROGEN
(Modified Kjeldahl method)

Principle:

The total N in the sample is converted to ammonium by digestion with conc. H_2SO_4 in the presence of salicylic acid and a catalyst mixture. NH_3 is determined after steam distillation by capture in an excess boric acid on titration with standard acid (H_2SO_4 or HCl).

Reagents:

1. H_2SO_4 -Salicylic acid mixture: Dissolve 50 g salicylic acid in 1litre H_2SO_4.
2. 40% NaOH: 40 g NaOH in 100 ml distilled water.
3. Indicator: Prepare a mixture of equal volume of methyl red (0.66%) and bromocresol green (0.99%) in 95 % ethanol.
4. Boric acid indicator mixture: 20 g boric acid dissolved in 600 ml distilled water are mixed with 10 ml indicator and diluted to 1 liter with distilled water.
5. Catalyst mixture: Prepare a mixture of $CuSO_4$ and K_2SO_4 (ratio 1:10).

Procedure:

Treat exactly 1 g air-dry soil in Kjeldahl digestion flask or digestion tube with 10 ml sulphuric-salicylic acid mixture. After 30 min., add 0.5 g $Na_2S_2O_3$ and shake. Wait 15 min. and add 3 ml H_2SO_4 and one teaspoon of catalyst mixture. Heat the flask till the solution becomes clear. After cooling, dilute by addition of water and make up the volume to 100 ml in 100 ml vol. flask. Draw an aliquot of 10 ml, alkalize with 40 % NaOH (20 ml) in micro Kjeldahl distillation assembly and start steam distillation immediately. Collect the distillate in to 250 ml flask containing 10 ml boric acid indicator mixture. After distillation of all ammonia, titrate the boric acid solution with 0.1 N standard acid. At the end point indicator turns from green to red.

Calculation:

1 g sample diluted to 100 ml, hence dilution factor = 100
1 ml of $N/10$ H_2SO_4 = 0.0014 g of N

$$\% \text{ total N} = \text{vol. of } H_2SO_4 \times \frac{0.0014 \times 100 \times 100}{10 \text{ ml (aliquot)} \times \text{wt. of soil.}}$$

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7. AVAILABLE PHOSPHORUS
(Olsen's method)
(0.5 M sodium bicarbonate extraction)
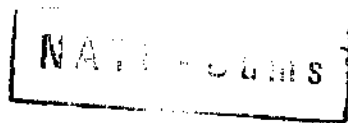
Principle:

All soils contain insoluble phosphates mainly di- and tri-calcium phosphates in neutral and alkaline solution and aluminium and ferric phosphates in acid soils. Phosphate ions are present in small concentration in soil solution according to relative amounts of calcium, aluminium and ferric ions. If the concentrations of metallic ions are reduced, concentration of phosphate ions increases in order to maintain various solubility products at their constant values.

An alkaline (pH 8.5) bicarbonate solution can repress the concentration of Ca ions by precipitation as $CaCO_3$ and Al and ferric ions by precipitation as hydroxides. Thus phosphate ion concentrations are increased and available phosphate can be extracted from soil by shaking with alkaline sodium bicarbonate and filtering. Activated carbon(phosphate free) must be used with most soils to absorb soluble organic matter and it is necessary to allow time for CO_2 bubbles to escape.

Reagents:

1. Extracting solution: 0.5 M sodium bicarbonate (pH 8.5).
   Dissolve 420 gm sodium bicarbonate to 10 liters, incorporating about 45 ml 5N sodium hydroxide to adjust the pH to 8.5 + or – 0.1.

2. Activated carbon, purified:
   Test the carbon for phosphorus by shaking with extractant, filtering and developing molybdenum blue. If measurable amount of phosphorus is obtained, shake the main stock of carbon with extracting solution, filter and wash the carbon well with water, dry in an oven and pulverize to powder. Retest to establish the absence of phosphorus.

3. Stannous chloride (approx 0.5 M) (Stock solution):
   (a) Dissolve 10 g stannous chloride in 25 ml Conc. HCl.
   (b) Dilute Solution – Add 1 ml conc. stannous chloride to 66 ml water.

4. Ammonium molybdate – HCl solution: Dissolve 15 g of ammonim molybdate in 400 ml warm water and filter , add 400 ml of 10 N HCl slowly with mixing and make it to one litre.

Procedure:

Air dry soil should be ground to pass a 0.5 mm sieve. A 2.5 g soil sample is suspended in 50 ml of $NaHCO_3$ solution of pH 8.5 along with 1 teaspoon of carbon black. Fine suspension is shaken for a period of 30 minutes. The solution is filtered through a Whatman No.42 or other suitable filter paper. A 5 ml aliquot of clear filtrate is pipetted in to 25 ml vol. flask. A volume of 5ml acid molybdate is added and the flask is allowed to

stand for the evolution of CO_2. After 20 minutes add 10 ml distilled water, then 1 ml stannous chloride (working solution), by immediate shaking and make up the volume to 25 ml and mix thoroughly. Prepare a blank as above and read the intensity of color developed, at 660 nm, after 10 minutes and within 20 minutes.

Preparation of standard curve:

Dissolve 0.2195 g KH_2PO_4 in 1 liter $NaHCO_3$ solution. This stock solution contains 50µg P/ml. Pipette out different quantities of solution from the standard in 25 ml vol. flask, add 5 ml molybdate reagent, add 1 ml dilute stannous chloride. Read the intensity of colour developed after 10 min (660 nm), within 20 minutes.

Calculation:

Plot the absorbance values obtained with standard phosphorus solutions against the amount of phosphorus present. From the graph record the number of micrograms of phosphorus corresponding to the absorbance values given by test solution.

ppm P in soil = ppm P in solution × 25/5 × 50/2.5 .

Bray 1 method for acid soils.

Reagents:
1. Extracting solution (Bray 1): 1.11g of solid NH_4F and 4.16 ml of 6N HCl per litre .
2. Stannous chloride (approx 0.5 M) (Stock solution): Dissolve 10 g stannous chloride in 25 ml Conc. HCl. Dilute Solution – Add 1 ml conc. stannous chloride to 66 ml water.
3. Ammonium molybdate – HCl solution: Dissolve 15 g of ammonium molybdate in 400 ml warm water and filter , add 400 ml of 10 N HCl slowly with mixing and make it to 1litre.

Procedure:

Weigh 5 g of soil, add 50 ml of extracting solution mixture and shake for 10 min. and filter through Whatman No. 42 . Take 5 ml aliquot, add 5 ml distilled water and add 5 ml ammonium molybdate and solution is mixed. Finally add 1 ml freshly diluted stannous chloride reagent with immediate mixing and make up the volume to 25 ml. After 10 min read the intensity of the colour within 15-20 min at 660 nm. The P standards are made up in the range of 0.1 to 1 ppm of P through the same steps including 5 ml of extraction solution in each 25 ml final volume.

Calculation:

ppm P in soil = ppm P in solution × 25/5 × 50/5
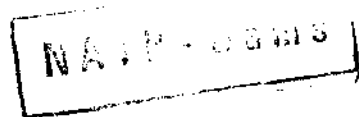
Principle:

For most soils the potassium removed is largely that associated with the clay and humus complex as exchangeable ions but in some saline extracts there may be a fair amount of water-soluble potassium. In the assessment of availability, the exchangeable and water-soluble potassium ions are not differentiated, the sum of the two being measured in the soil extract, usually by flame photometry.

It is accurate enough to shake soil with ammonium acetate solution (1:20 ratio) a procedure that removes 90-95% exchangeable potassium and all water-soluble potassium.

Reagents:

1.Extracting solution- ammonium acetate, $1N$ (pH 7.0): Dissolve 77 g of ammonium acetate in 1 liter of distilled water.
2.Standard Potassium-1000 ppm: Dissolve 1.907 g dry potassium chloride in 1 liter distilled water.

Procedure:

Transfer a weight of air-dry soil containing 5 g of oven dry soil to 250 ml flask and add 100 ml ammonium acetate. Shake for 30 min. and filter. Measure the concentration of extracts by flame photometry, calibrating photometer with standards containing 0-25 ppm K in $1N$ ammonium acetate.

Calculation:

From graph, let the concentration of soil extract be 'A' ppm K.
Then concentration of potassium in oven dry soil = A x 100/5 ppm

9. TOTAL SILICA
(Molybdenum – Blue method)

Principle:

Small quantities of dissolved silicic acid react with a solution of ammonium molybdate in an acid medium to form an intense yellow coloration due to the formation of complex silicomolybdate acid. This will again be reduced to molybdenum - blue by reducing against a mixture of 1-amino 2 – napthol 4 – salphonic acid, sodium sulphite, and sodium bisulphite.

Preparation of solution A:

1. 0.1 g of the powdered sample is weighed accurately in a covered, nickel crucible.

2. Add 1.5 g of sodium hydroxide (NaOH), which has been carefully stored in a firmly stoppered polyethylene bottle. About 17 pellets of NaOH is approximately the right weight and, as this is not critical, it is common practice to add this number of pellets to avoid contamination of the alkali.

3. The covered crucible and contents are placed in a muffle furnace at a temperature between 800 and 850^0 C. At this temperature, complete fusion is attained in about 5 minutes. Experience at the efficiency of the furnace employed will determine the precise duration for the complete fusion, but this rarely take more than 10 minutes, even when using 0.1g of powdered sample.

4. The crucible is removed to safe cooling place and allowed to cool. Some workers swirl the NaOH melt around the sides of the crucible during the transfer as this tends to assist eventual removal of the melt in to solution.

5. The covered crucible and contents are warmed on a water bath after adding 20 ml of distilled water to the solidified melt.

6. A solution of 20 ml of $2.5N$ sulphuric acid in about 200 ml of distilled water is made up in a 1-liter graduated flask fitted with a stopper. A solution of 20 ml 1:1 HCl is used by many workers in place of H_2SO_4 as this reduces the number of acid radicals in the eventual solution and facilitates the use of Solution A for the determination of Al.

7. Great care must be taken to remove all traces of melt in to the solution. Consequently, the crucible and lid are carefully washed through the polyethylene funnel in to the flask. The scouring of the funnel and the crucible is done with a rubber policeman.

8. The washings and solution of the hydroxide melt are made up to 1 liter in the flask by washing distilled water through the funnel to ensure that no traces are allowed to remain upon it. Before finally making up the solution to the graduation mark, a small crystal of ferrous sulphate should be added if the solution has pink coloration. This is usually due to the presence of excessive quantities of manganese. The ferrous sulphate reduces the permanganates to a colorless solution, which is more suitable for calorimetric determination. Solution A should be visibly free from suspended matter and virtually colorless. This completes the formation of Solution A

Reagents:

1) Ammonium molybdate: strength 7.5%
   Dissolve 7.5g of G.R ammonium molybdate in 75 ml of water, warming gently if necessary. Cool and add 10 ml 1:1 H_2SO_4, dilute to 100 ml and mix thoroughly. Store in a plastic bottle. Discard the solution when it becomes cloudy.

2) Tartaric acid – 8%
   Dissolve 20g of G.R tartaric in water and dilute to 250 ml with distilled water. Store in a plastic bottle. Prepare a fresh solution when sediment forms.

3) Sodium bisulphate
4) Sodium metasilicate
5) Dilute H_2SO_4
6) Anhydrous sodium sulphite
7) 1-amino 2-napthol 4-salphonic acid
8) Reducing Agent:
   Dissolve 0.5 g G.R anhydrous sodium sulphite in 10 ml water. Add 0.15g 1-amino 2-napthol 4-salphonic acid and stir well to aid in the dissolving process. Dissolve 9 g G.R sodium bisulphite in 90 ml of water and add thi solution to the first solution. Mix thoroughly and store in a plastic bottle in a dark place. Do not store this solution for more than 3 days.

Procedure:

Preparation of standard solutions of silica:

Dissolve 10.1189g of sodium metasilicate in de-ionized water and make it up to 1000 ml. This standard solution contains 1g of silica in 1 liter H_2O giving a 1000 ppm solution. Pipette out 10 ml of the above solution and dilute to 100 ml (making a 100 ppm solution). Again pipette out 1 ml of this solution and make up to volume of 100 ml, which gives a 1 ppm solution. In the same way, prepare 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, standard silica solutions.

Pipette out standard solutions (1 ml, 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and make up to 100 ml) and the unknown sample solution(10 ml) in a 100 ml volumetric flasks(these

flasks should be cleaned with 1 : 1 HCl and then rinsed with water). All solutions should be at the temperature of $20 - 25^0$ C.

Add by pipette 1 ml of ammonium molybdate solution to each flask, swirling the flasks during the additions. Mix and allow to stand for at least 10 minutes to form a slightly yellow coloration.

Add by pipette 5 ml of tartaric acid, swirling the flasks during the additions and immediately add 1 ml of the reducing agent to it. Mix thoroughly and allow to stand for 30 minutes. By this time the solution will form an intense blue coloration. Dilute the solution up to 100 ml.

Determine the absorbance of each solution at 650 nm in a previously set spectrophotometer using the blank solution A as a reference solution.

Calculations:

Optical density obtained plotted against the concentrations for standard solutions will display a linear relationship. By this principle, we can calculate the concentrations of the unknown sample by measuring its optical density and comparing to the standard graph.

Calculate the % silica by using the equation

$$\% \text{ silica } = \text{ppm} * \frac{1000 * 100}{10 * 0.1}$$

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10. HEAVY METALS

Principle:

The sample is treated with NaCl and acid solution. The free chlorine, which is developed, loosens the chemical bonds in the easiest volatilized organic mercury compounds. Gentle heating for 12 hours destroys the organic material and transfers the metal in to solution. Hydroxylamonium chloride is added to eliminate the free chlorine and other oxidizing substances.

Reagents:

1. Sodium chloride: Dissolve 30 g NaCl in metal free distilled water and dilute to 100ml.
2. Acid solution: Mix carefully 10 vol. of con. Perchloric acid ($HClO_4$ sp gr 1.67) and 2 vol. of con. HNO_3(sp gr 1.40)
3. Hydroxylamonium chloride: Dissolve 50 g of hydroxylamonium chloride in 100 ml distilled water.

Procedure:

Weigh 0.5 –1 g material (dry weight) or 1-5 g (wet wt.) .Add 3 drops of NaCl and 8 ml acid solution, heat at 70^0 C for 12 hours in water bath or other heating equipment. Add 3 drops of hydroxylamonium chloride solution. Mix and dilute to 50 ml with metal free distilled water, filter (through No. 42 filter paper) and take readings using Atomic Absorption Spectrophotometer.

Calculation:

$$ppm(\mu g /g) = AAS(ppm) \times 50/1$$

where

    Vol made up = 50 ml
    Sediment taken = 1 g

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11. WET-ASHING OF BIOLOGICAL MATERIAL FOR DETERMINATION OF TRACE ELEMENTS AND RECOVERY OF ORGANIC MERCURY FOR AAS

1. Remove the sub-samples from the deep-freezer and allow time for them to thaw before opening the plastic tubes.
2. Open each tube and transfer the whole sample of about 10 g that was weighed previously, into a clean 100 ml beaker.
3. Add 10 ml of freshly prepared 1:1 v/v hydrogen peroxide/nitric acid, prepared in a small measuring cylinder as required, and cover the beaker with a watch glass.
4. Set the beaker aside, for about an hour, until the first reaction subsides.
5. Place the beaker on the hot plate and carefully allow the temperature to rise to 160° C.
6. Continue to boil gently for about two hours and reduce the volume to between 2-5 ml. Any reaction should have ceased but do not allow the sample to go dry or it must be discarded.
7. Allow the solution to cool, transfer to a volumetric flask (25 ml) and dilute to the mark with distilled water (i.e. the test solution will be about 4-10% v/v nitric acid and not more.)

Blanks and known amounts of the working standard solutions should be taken through this wet-ashing procedure to test for methodic and operative errors. A great advantage here is that this sample wet-ashing procedure is sufficient to solubilize all of the trace elements and destroy most of the organic matter, which then do not interfere with the determination. Earlier methods required the complete destruction of the organic matter and it was necessary to use perchloric acid in the final stages. This can be extremely dangerous and has been the cause of serious explosions even when used correctly.

The total mercury will be determined after wet-ashing the organic matter with hydrogen peroxide and nitric acid as described above. For organic mercury follow this procedure:

1. Take an aliquot of the aqueous cysteine hydrochloride solution, which has been extracted from the samples used to determine methyl mercury by GLC.
2. Dilute to about 10 ml with water.
3. Add 1.0 ml sulphuric acid and 2 ml of 10% v/v ammonium persulphate.

4. Heat slowly to boiling and continue for a minute or two in order to remove most of the excess persulphate.
5. Cool, transfer to a volumetric flask (25 ml) and dilute to the mark.
6. Proceed with the determination of mercury by flameless AAS in exactly the same way as for the total mercury.

Suggested reading:

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