CWC/2022/76

सत्यमेव जयते

Government of India
Ministry of Jal Shakti
Department of Water Resources, River Development
& Ganga Rejuvenation

STANDARD OPERATING PROCEDURES

for Level-II Water Quality Laboratories of CWC

River Data Compilation-II Directorate,
Planning & Development Organisation
Central Water Commission, New Delhi
October, 2022

सत्यमेव जयते

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PREFACE

Surface water resources have a significant role in the existence of human beings, flora and fauna in any region, especially in India; considering the main uses of surface water include utilisation as drinking water, for irrigation, in industries, for cattle, for various indoor and outdoor activities etc. Hence, it is important to assess and monitor the quality of surface water, mainly rivers, as they are the major source of surface water in our country. Being the apex technical body for development of water resources in the country, CWC is responsible for assessment and management of surface water resources of the country in general.

Keeping in view quality management, uniformity and optimum utilisation of resources of CWC laboratories, the need of a compiled handy SOP document was being felt keenly for long time. This document contains the standard procedure of analysis followed by the CWC Level - II laboratories.

I would like to put on record my appreciation of the initiative taken by Shri P M Scott, Member(RM) and works carried out by Shri Devendra Pratap Mathuria, Chief Engineer (P&DO) and Shri Pankaj Kumar Sharma, Director, RDC-II Directorate as well as the dedicated efforts put in by each officer of RDC-II Directorate and the scientific officers of all 23 laboratories of CWC in compilation and preparation of this report. I hope this document would be found useful by all the offices of CWC, Central / State agencies and other stakeholders in the field of Water Quality.

Rk Gupta

(Dr. Rakesh Kumar Gupta)
Chairman, CWC

सत्यमेव जयते

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FOREWORD

CWC is monitoring the water quality of rivers since 1963. Its water quality network consists of 764 water quality sites (as on January, 2021) and a 3-tier laboratory system of 378 Level-I, 19 Level-II and 5 Level-III laboratories across the country.

The preparation of a compiled, handy document containing details related to water quality activities and laboratories of CWC, which can be used as a ready-reference was felt as a need-of-the hour. This document has been prepared by compiling the information regarding various activities performed by the WQ laboratories and laboratory personnel. It contains the details of all activities going on in Level-II laboratories of CWC on routine basis. This SOP document may be read along with "Field Water Analysis Manual, January 2020" and "Water Quality Activities of Central Water Commission" published by CWC (available in CWC website).

The methodologies for sample analysis are adopted from the 23rd Edition of Standard Methods for the Examination of Water and Waste Water, published by American Public Health Association, American Water Works association and Water Environment Federation in 2017. Efforts have been made to tabulate the time required for the analysis of each parameter, the routine analysis activities and other activities (in addition to analysis) in a CWC WQ laboratory. A list of Instruments/Equipment/Chemicals required for the analysis of each parameter and a note on validation of water quality data are also included.

I appreciate the hard work and efforts put in by Shri Devendra Pratap Mathuria, Chief Engineer (P&DO), CWC; Shri Pankaj Kumar Sharma, Director, RDC-II, CWC, Sh. Rakesh Kumar Gupta, Dy. Director, RDC-II, CWC, Sh. Devendra Patel, Asst. Director, RDC-II and Smt.Geethu Krishna, SRA, RDC-II in the preparation of this unique document.

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Member (River Management), CWC

सत्यमेव जयते

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PREAMBLE

Central Water Commission (CWC) over the years has developed expertise and capabilities in areas of water resources management in the country through its extensive monitoring network covering major river basins and observing river gauge / discharge / sediment / quality parameters. Being the apex national body for development of water resources in the country, one of its mandates is assessment of water resources in general.

At present, CWC follows a three-tier water quality laboratory system which consists of Level I, II and III types of laboratories. As on October, 2022, out of 23 Level II/III laboratories of CWC, 19 got accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) in accordance with Standard ISO/IEC 17025:2017.

The Standard Operating Procedure (SOP) for Level-II Laboratories has been prepared by compiling the information received from the labs regarding various activities performed by the Water Quality laboratories and laboratory personnel. It has been finalised after several rounds of discussions and communication with the laboratories of CWC. This is a compiled, handy document containing details and routine activities related to water quality laboratories of CWC.

In the Standard Operating Procedure (SOP), the following can be found at one place:

- The day-to-day activities of lab
- The time needed for analysis of each parameter
- The manpower requirement of the laboratory to complete the analysis works of a set of 50 samples in 8 days.
- The requirement of manpower/time/chemicals/instruments/equipment for labs presuming that the lab is NABL accredited.
- Other activities of the laboratory staff in addition to analysis

This work has been attempted for the first time in CWC and can be used as a ready-reference in respect of Water Quality activities of CWC.

(Devendra Pratap Mathuria)
Chief Engineer, P&DO, CWC

Table of Contents

Content

Table of Contents

Content

CHAPTER: 1

Introduction

Water Quality Activities of CWC

Central Water Commission (CWC) is playing an important role in the field of water quality monitoring of river water. CWC is monitoring the water quality of river since 1963. In the beginning, water quality observation work was started for observing the river water for its suitability for irrigation purpose. By year 2000, water quality parameters were observed in CWC at 353 locations as a value addition to water quantity. As on 01.01.2021, CWC is observing water quality at 764 key locations (652 on Hydrological Observation network and 112 Water Quality Sampling Sites).

At present, CWC follows a three-tier laboratory system which consists of Level I, II and III types of laboratories for providing analytical facilities for the analysis of river water samples collected from water quality monitoring stations covering all the important river basins of India.

1. **Level-I Laboratories:** These are the field laboratories located at field water quality monitoring stations on various rivers of India, where analysis of 6 in-situ parameters: Temperature, Colour, Odour, pH, Electrical Conductivity (EC) and Dissolved Oxygen (DO)of river water are being monitored. As on January 2021, there are 378 Level-I laboratories located at field water quality monitoring stations situated on various rivers of India.

2. **Level-II Laboratories:** There are 18 level-II laboratories located at division offices of CWC to analyse 25 physico-chemical and biological parameters of river water.

3. **Level-III Laboratories:** There are 5 regional Level-III laboratories for analysis of 41 parameters including trace & toxic metals and pesticides. Level-III labs are located at New Delhi, Varanasi, Guwahati, Hyderabad and Coimbatore.

As on September, 2022, 19 out of 23 laboratories in CWC, functioning under different divisional offices, got accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL) in the field of Testing in accordance with Standard ISO/IEC 17025:2017. All Level-III labs are accredited.

The details of parameters being analysed in each level laboratories can be seen at Table: 1.

Table 1: List of Water Quality Parameters

Sl. No.	Level-I	Level-II	Level-III
1	Temperature	Temperature	Temperature
2	Colour	pH	pH
3	Odour	Electrical Conductivity	Electrical Conductivity
4	pH	Dissolved Oxygen (DO)	Dissolved Oxygen (DO)
5	Electrical Conductivity	Turbidity	Turbidity
6	Dissolved Oxygen (DO)	Biochemical Oxygen Demand (BOD)	Biochemical Oxygen Demand (BOD)
7		Chemical Oxygen Demand (COD)	Chemical Oxygen Demand (COD)
8		Total Dissolved Solids (TDS)	Total Dissolved Solids (TDS)
9		Sodium	Sodium
10		Calcium	Calcium
11		Magnesium	Magnesium
12		Potassium	Potassium
13		Carbonate	Carbonate
14		Bicarbonate	Bicarbonate
15		Chloride	Chloride
16		Sulphate	Sulphate
17		Fluoride	Fluoride
18		Boron	Boron
19		Ammoniacal Nitrogen	Ammoniacal Nitrogen
20		Nitrate	Nitrate
21		Nitrite	Nitrite
22		Phosphate	Phosphate
23		Silicate	Silicate
24		Total Coliform	Total Coliform
25		Fecal Coliform	Fecal Coliform
26			Arsenic
27			Cadmium
28			Chromium
29			Copper
30			Iron
31			Lead
32			Nickel
33			Mercury
34			Zinc
35			Alpha Benzenehexachloride(BHC), Beta BHC, Gama BHC (Lindane)
36			OP-Dichlorodiphenyltrichloroethane (OP DDT), PP-DDT
37			Alpha Endosulphan, Beta Endosulphan
38			Aldrin, Dieldrin
39			Carbaryl (Carbamate)
40			Malathion, Methyl Parathion
41			Anilophos, Chloropyriphos

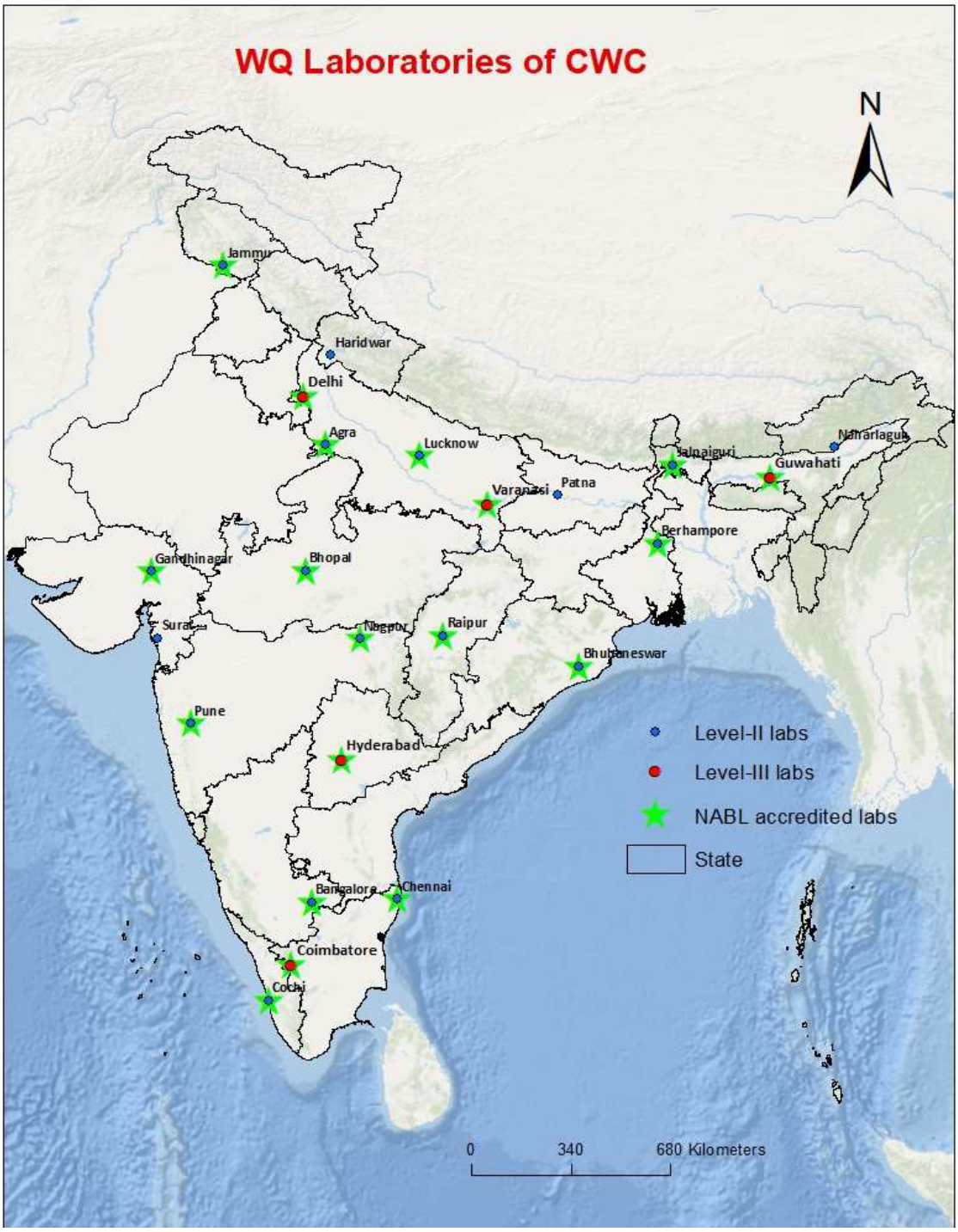

Figure 1: Laboratories of CWC

The first draft was prepared by compiling the information received from regional offices regarding various activities performed by the water quality laboratories and laboratory personnel. Thereafter, the draft was further improvised by incorporating the inputs & suggestions of 23 laboratories of CWC received during several rounds of communication & discussions with them. In-depth technical discussion on the document was also done during two online workshops organised by RDC-II Directorate in the month of October, 2021. The SOP contains the details of all activities going on in Level-II laboratories on routine basis. This document is a compiled, handy document containing all details related to water quality laboratories of CWC, which can be used as a ready-reference.

For sample collection and related details like preservation, transportation, analysis of Level-I parameters etc. the "Field Water Analysis Manual, January 2020" (http://cwc.gov.in/sites/default/files/field-water-analysis-manual.pdf) may be referred, which is already published and hosted on CWC website. Generic information about CWC water quality activities, safety measures to be followed in laboratories etc. can be seen on web-page "Water Quality Activities of Central Water Commission" (http://cwc.gov.in/sites/default/files/water-quality-activities-cwc.pdf) of CWC.

Note:

- This SOP should be followed by both Level-II & Level-III labs for analysis of Level-II parameters.
- Manpower required and time required (Appendix: I & Appendix: II) are estimated for laboratory activities only. For other activities listed under Appendix: III, additional manpower should be planned.
- The digestion of metals and extraction & concentration of pesticides from river water samples shall be carried out at Level-II laboratories. But right now, the methods for the same have not been included in the SOP. For these activities, additional manpower / equipment / chemicals / apparatus is needed.
- SOP for Level-III labs shall be prepared and submitted separately.

CHAPTER: 2

Standard Procedure of Analysis for each Water Quality Parameter

(Based on Standard Methods for the Examination of Water and Waste Water, APHA, AWWA, WEF, 23rd Edition, 2017)

1. TEMPERATURE

(2550 B. Laboratory and Field Methods)

Temperature is an important parameter which affects many other parameters like alkalinity, pH, EC etc.

Instrument/apparatus/equipment:

A mercury-filled Celsius thermometer with a scale marked for every 0.1°C, with markings etched on the capillary glass. The thermometer should have a minimal thermal capacity to permit rapid equilibration. Periodically check the thermometer against a precision thermometer certified by the National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) that is used with its certificate and correction chart. For field operations use a thermometer having a metal case to prevent breakage.

Procedure

Immerse thermometer in the sample up-to the mark specified by the manufacturer and read temperature after equilibration.

Report

Report results for temperature in units of degrees Celsius, with 1 digit after the decimal point, e.g., 21.5 °C.

2. TURBIDITY

(APHA 2130 B. Nephelometric Method)

This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. Higher the intensity of scattered light, higher the turbidity. Formazin polymer is used as the primary standard reference suspension. The turbidity of a specified concentration of formazin suspension is defined as 4000 NTU.

Interference

Turbidity can be determined for any water sample that is free of debris and rapidly settling coarse sediment. Dirty glassware and the presence of air bubbles give false results. ‘‘True colour,’’ i.e., water colour due to dissolved substances that absorb light, causes measured turbidity to be low. This effect usually is not significant in treated water.

Instrument/apparatus/equipment:

Nephelometric turbidity meter with sample cells

Reagents

Solution I:

Dissolve 1.000 g hydrazine sulphate, $(NH_2)_2.H_2SO_4$ in distilled water and dilute to 100 mL in a volumetric flask. **Caution: Hydrazine sulphate is a carcinogen, avoid inhalation, ingestion and skin contact.**

Solution II:

Dissolve 10.00 g hexamethylenetetramine, $(CH_2)_6N_4$, in distilled water and dilute to 100 mL in a volumetric flask.

4000 NTU suspension:

In a flask, mix 5.0 mL of Solution I and 5.0 mL of Solution II. Let stand for 24 h at (25 ± 3) °C. This results in a 4000 NTU suspension. Store in an amber glass bottle. The suspension is stable for up to 1 year. Dilute 4000 NTU stock solution with distilled water to prepare dilute standards just before use and discard after use.

Procedure

i. Calibrate Nephelometric turbidity meter according to manufacturer’s operating instructions. Run at least one standard in each instrument range to be used.

ii. Gently agitate sample. Wait until air bubbles disappear and pour sample into cell. Read turbidity directly from instrument display.

Report– Report turbidity, in NTU as follows:

Turbidity range in NTU unit	**Record to the Nearest**
0-1	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
Greater than 1000	100

3. pH

(APHA 4500 – H^+ B. Electrometric Method)

Principle

The pH value is determined by measurement of the electromotive force of a cell consisting of an indicator electrode immersed in the test solution and a reference electrode. Contact between the test solution and the reference electrode is usually achieved by means of a liquid junction, which forms part of the reference electrode. The electromotive force is measured with a pH meter, that is, a high impedance voltmeter calibrated in terms of pH.

Interference

1. Above pH 10, high sodium concentrations interfere with the measurement. Correction for the sodium error may be made by consulting the chart supplied by the manufacturer of electrodes being used. It can be reduced or eliminated by using a low sodium error electrode.
2. Oil and grease may interfere by coating the pH electrode and causing a sluggish response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid (1%) may be necessary to remove any remaining film.
3. Temperature affects the pH values in two ways; the first is covered by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample- dependent and cannot be controlled. Therefore, the temperature at the time of analysis should be reported.

Instrument/apparatus/equipment

a) pH meter with temperature compensating device, accurate and reproducible to 0.1 pH unit with a range of 0 to 14.

b) Reference electrode: Consisting of a half-cell that provides a standard electrode potential. Generally, calomel, silver-silver chloride electrodes are used as reference electrode.

c) Sensor (glass) electrode: Several types of glass electrodes are available. The glass electrode consists essentially of a very thick-walled glass bulb, made of low melting point glass of high electrical conductivity, blown at the end of a glass tube. This bulb contains an electrode, which has a constant potential, e.g., a platinum wire inserted in a solution of hydrochloric acid saturated with quinhydrone. The bulb is placed in the liquid where pH is to be determined.

d) Beakers: Preferably use polyethylene or TFE beakers.

e) Stirrer: Use a magnetic TFE coated stirring bar.

Reagents

Use ready-made standard buffer solutions available in market or prepare as following:

pH 4 buffer solution:

Dissolve 10.12 g potassium hydrogen phthalate, $KHC_8H_4O_9$ in distilled water. Dilute to 1 L.

pH 7 buffer solution:

Dissolve 1.361 g anhydrous potassium dihydrogen phosphate, KH_2PO_4, and 1.42 g anhydrous disodium hydrogen phosphate, Na_2HPO_4, which have been dried at 110°C to 130°C. Use distilled water which has been boiled and cooled. Dilute to 1L.

pH 9.2 buffer solution:

Dissolve 3.81 g borax, $Na_2B_4O_7.10H_2O$ in distilled water, which has been previously boiled and cooled. Dilute to 1000 mL. Store buffer solutions in polyethylene bottles. Replace buffer solutions every 4 weeks.

Calibration and standardisation

Before use, remove the electrodes from the water and rinse with distilled water. Dry the electrodes by gentle wiping with a soft tissue. Calibrate the electrode system against standard buffer solution of known pH. Buffer solutions should be freshly made or use readily available pH buffers. Boil and cool distilled water having a conductivity of less than 2 μS. 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. Prepare and calibrate the electrode system with buffer solutions with pH approximating that of the sample, to minimize error resulting from non-linear response of the electrode.

Procedure

i. Before use, remove electrodes from storage solutions (recommended by manufacturer) and rinse with distilled water.

ii. Dry electrodes by gently blotting with a soft tissue paper, standardize instrument with electrodes immersed in a buffer solution within 2 pH units of sample pH.

iii. Remove electrodes from buffer, rinse thoroughly with distilled water and blot dry.

iv. Immerse in a second buffer below pH 10, approximately 3 pH units different from the first, the reading should be within 0.1 unit for the pH of the second buffer. (If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or pH meter)

v. For sample analysis, establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity and measure pH.

vi. For buffered samples (or those with high ionic strength), condition the electrodes after cleaning by dipping them into the sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

vii. With poorly buffered solutions (dilute), equilibrate electrodes by immersing in three or four successive portions of samples. Take a fresh sample and record the pH.

Reporting

Report results for pH with 1 digit after the decimal point, e.g., 6.5.

4. ELECTRICLAL CONDUCTIVITY

(APHA 2510 B. Laboratory Method)

Principle

This method is used to measure the conductance generated by various ions in the solution/water. Rough estimation of dissolved ionic contents of water sample can be made by multiplying specific conductance (in µS/cm) by an empirical factor which may vary from 0.55 to 0.90 depending on the soluble components of water and on the temperature of measurement. Conductivity measurement gives rapid and practical estimate of the variations in the dissolved mineral contents of a water body.

Instrument/apparatus/equipment

Conductivity meter capable of measuring conductivity with an error not exceeding 1% or 0.1 mS/m whichever is greater.

Reagents and standards

Conductivity Water:

Reagent grade water can be used. The conductivity should be small compared to the value being measured.

Standard potassium chloride: 0.01M:

Dissolve 745.6 mg anhydrous KCl in conductivity water and make up to 1,000 mL at 25°C. This standard reference solution has a specific conductance of 1413 µmhos/cm at 25°C. It is satisfactory for most types of water when using a cell with a constant between 1 and 2. Store the solutions in glass stoppered Pyrex bottles.

Procedure

Conductivity can be measured as per the instruction manual supplied with the instrument and the results may be expressed as µmhos/cm or µS/cm. Note the temperature at which measurement is made. Conductivity meter needs very little maintenance and gives accurate results. However, a few important points in this respect are:

i. Adherent coating formation of the sample substances on the electrodes should be avoided which requires thorough washing of cell with distilled water at the end of each measurement.
ii. Keep the electrode immersed in distilled water.
iii. Organic material coating can be removed with alcohol or acetone followed by washing with distilled water.

Reporting

Report results for EC in µmhos/cm or µS/cm, with no decimal points, e.g., 450 µmhos/cm.

5. TOTAL HARDNESS

(APHA 2340 C. EDTA Titrimetric Method)

Principle

Hardness is determined by the EDTA method in alkaline condition; EDTA and its sodium salts form a soluble chelated complex with certain metal ions. Calcium and Magnesium ions develop wine red colour with Eriochrome black T in aqueous solution at pH 10.0 ± 0.1. When EDTA is added as a titrant, Calcium and Magnesium divalent ions get complexed resulting in a sharp change from wine red to blue which indicates endpoint of the titration. Magnesium ion must be present to yield satisfactory end point. Hence, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer. The sharpness of the end point increases with increasing pH. However, the specified pH of 10.0 ± 0.1 is a satisfactory compromise. At a higher pH i.e., at about 12.0, Mg^{++} ions precipitate and only Ca^{++} ions remain in solution. At this pH murexide (ammonium purpurate) indicator forms a pink colour with Ca^{++}. When EDTA is added, Ca^{++} gets complexed resulting in a change from pink to purple which indicates end point of the reaction. To minimize the tendency towards $CaCO_3$ – precipitation limit the duration of titration period to 5 minutes.

Instrument/apparatus/equipment

Conical flasks 100 mL, Burette, Pipette, Spatula

Reagents and Standards

Buffer solution*:*

Dissolve 16.9 gm NH_4Cl in 143 mL conc. NH_4OH. Add 1.25 gm magnesium salt of ethylenediaminetetraacetate (EDTA) and dilute to 250 mL with distilled water. Store in a plastic / borosilicate bottle stoppered tightly for no longer than one month.

Complexing agent:

Magnesium salt of 1, 2 cyclohexanediaminetetraacetic acid. Add 250 mg per 100 mL sample only if interfering ions are present and sharp end point is not obtained.

Indicator: Eriochrome Black T sodium salt*:*

The salt can also be used in dry powder form by grinding 0.5 g dye with 100 g NaCl.

Standard EDTA titrant, 0.01M:

Weigh 3.723 g di-sodium salt of EDTA dihydrate, dissolve in distilled water and dilute to 1000mL Store in polyethylene /borosilicate glass bottle.

Standard Calcium Solution*:*

Weigh 1.000 g anhydrous $CaCO_3$ in a 500 mL flask. Add 1 + 1 HCl slowly through a funnel till all $CaCO_3$ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2.

Cool and add a few drops of methyl red indicator and adjust to the intermediate orange colour by adding 3N NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 1 mg $CaCO_3$.

Calibration and standardization

The EDTA solution needs to be standardized against standard calcium solution such that the strength of EDTA will be 1 mL = 1 mg as $CaCO_3$.

Procedure

i. Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel.

ii. Add 1 to 2 mL buffer solution. Usually, 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point colour change in the titration usually means that an inhibitor must be added at this point or that the indicator has deteriorated.

iii. Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation.

iv. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears. Add the last few drops at 3- to 5-s intervals.

v. At the end point the solution normally is blue.

vi. Standardise the EDTA titrant against standard calcium solution using the above procedure.

Calculation

Total Hardness as mg $CaCO_3$/L= $\frac{A \times B \times 1000}{mL\ sample}$

Where: A = mL EDTA titrated for sample

B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant

Reporting

Report results for TH as mg $CaCO_3$/L with 1 digit after decimal point, e.g., 45.0 mg $CaCO_3$/L.

6. CALCIUM

(APHA 3500-Ca B. EDTA Titrimetric Method)

Principle:

When EDTA (ethylenediaminetetraacetic acid or its salts) is added to water containing both calcium and magnesium, it combines first with the calcium. Calcium can be determined directly, with EDTA, when the pH is made sufficiently high that the magnesium is largely precipitated as the hydroxide and an indicator is used that combines with calcium only. At a higher pH i.e., at about 12.0 Mg^{++} ions precipitate and only Ca^{++} ions remain in solution. At this pH murexide (ammonium purpurate) indicator forms a pink colour with Ca^{++}. When EDTA is added, Ca^{++} gets complexed resulting in a change from pink to purple which indicates end point of the reaction.

Interference:

Under conditions of this test, the following concentrations of ions cause no interference with the calcium hardness determination: Cu^{2+}, 2 mg/L; Fe^{2+}, 20 mg/L; Fe^{3+}, 20 mg/L; Mn^{2+}, 10 mg/L; Zn^{2+}, 5 mg/L; Pb^{2+}, 5 mg/L; Al^{3+}, 5 mg/L; and Sn^{4+}, 5 mg/L. Orthophosphate precipitates calcium at the pH of the test. Strontium and barium give a positive interference and alkalinity in excess of 300 mg/L may cause an indistinct end point in hard waters.

Instrument/apparatus/equipment: Conical flasks, Burette, Pipette, Spatula

Reagents and Standards

Sodium hydroxide, NaOH, 1N

Dissolve 40 g of NaOH in 1000 mL of distilled water

Murexide (ammonium purpurate) indicator:

Mix 200 mg dye with100 g solid NaCl. Grind to 40 to 50 mesh size.

Standard EDTA titrant, 0.01M:

Weigh 3.723 g di-sodium salt of EDTA, EDTA dihydrate, dissolve in distilled water and dilute to 1000mL. Store in polyethylene bottle, 1 mL = 400.8 µg Ca. Standardise EDTA against standard calcium solution periodically following the method described below.

Standard calcium solution:

Weigh 1.000 g anhydrous $CaCO_3$ in 500 mL flask (primary standard). Add 1 + 1 HCl in small amounts through a small funnel till all $CaCO_3$ is dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool and add a few drops of methyl red indicator and adjust to intermediate orange colour by adding 3N NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water, 1 mL = 400.8 µg Ca.

Procedure

i. Take 50 mL sample or an aliquot diluted to 50 mL such that the calcium content is not more than 10 mg. Samples which contain alkalinity greater than 300 mg/L should be neutralised with acid, boiled for 1 min and cooled before titration.

ii. Add 2 mL NaOH solution or a volume sufficient to produce a pH of 12 to 13.

iii. Start titration immediately after addition of the alkali. Add 0.1 to 0.2 g indicator mixture. Titrate with EDTA solution, with continuous mixing, till the colour changes from pink to purple.

iv. Check end point by adding 1 to 2 drops excess titrant to make certain that no further colour change occurs.

Calculation

$$\textbf{mg Ca/L} = \frac{\mathbf{A \times B \times 400.8}}{\mathbf{mL Sample}}$$

where: A= mL titrant used for sample

$$B = \frac{\text{mL of standard calcium solution taken for titration}}{\text{mL EDTA titrant}}$$

$$\textbf{Calcium hardness as mg } CaCO_3\textbf{/L} = \frac{\mathbf{A \times B \times 1000}}{\mathbf{mL\ Sample}}$$

Reporting

Report results for Ca as mg Ca/L with 1 digit after decimal point, e.g., 45.0 mg Ca/L.

7. MAGNESIUM

(APHA 3500-Mg B. Calculation Method)

Principle

Magnesium may be estimated as the difference between hardness and calcium as $CaCO_3$ if interfering metals are present in non-interfering concentrations in the calcium titration and suitable inhibitors are used in the total hardness titration.

Procedure

Get the values for Total Hardness and Calcium Hardness determined by EDTA and calculate magnesium.

Calculation

mg Mg/L= (total hardness as mg $CaCO_3$/L –calcium hardness as mg $CaCO_3$/L) ×0.243

Reporting

Report results for Mg as mg Mg /L with 1 digit after decimal point, e.g., 45.0 mg Mg /L

8. SULPHATE

(APHA 4500-SO_4^{-2} E. Turbidimetric Method)

Principle

Sulphate ion (SO_4^{2-}) is precipitated in an acetic acid medium with barium chloride ($BaCl_2$) so as to form barium sulphate ($BaSO_4$) crystals of uniform size. Light absorbance of the $BaSO_4$ suspension is measured by a photometer and the SO_4^{2-} concentration is determined by comparison of the reading with a standard curve.

Interference

Colour or suspended matter in large amounts will interfere. Some suspended matter may be removed by filtration. Silica in excess of 500 mg/L will interfere, and in waters containing large quantities of organic material it may not be possible to precipitate $BaSO_4$ satisfactorily.

Instrument/apparatus/equipment

a) Nephelometric turbidity meter with sample cells. Alternatively, a spectrophotometer at 420 nm wavelength with a light path of 2.5 to 10 cm may be used.

b) Magnetic stirrer, magnetic bar

c) Stopwatch (Timer with indication of seconds)

Reagents

Standard Solution:

Dissolve 0.1479 anhydrous Na_2SO_4 in distilled water, make up to 1000 mL.1.00 mL=100µg SO_4^{2-}

Buffer solution A

Dissolve 30 g magnesium chloride ($MgCl_2.6H_2O$), 5 g sodium acetate ($CH_3COONa.3H_2O$), 1 g potassium nitrate (KNO_3) and 20 mL acetic acid CH_3COOH (99%) in 500 mL distilled water and make up to 1000 mL.

Buffer solution B

Only required if sample SO_4 concentration is less than 10 mg/L. Prepare as buffer solution A and add 0.111 g sodium sulphate, Na_2SO_4.

Barium chloride $BaCl_2$: crystals, 20 to 30 mesh

Procedure

i. Standardise nephelometer following manufacturer's instructions.

ii. Formation of barium sulphate turbidity: Measure 100 mL sample or a suitable portion made up to 100 mL into a 250-mL Erlenmeyer flask. Add 20 mL buffer solution and mix in stirring apparatus. While stirring, add a spoonful of $BaCl_2$ crystals and begin timing immediately. Stir for (60 ± 2) seconds at constant speed.

iii. Measurement of barium sulfate turbidity: After stirring period has ended, pour solution into absorption cell of photometer and measure turbidity at 5 ± 0.5 min.

iv. Preparation of calibration curve: Estimate SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO_4^{2-} standards through the entire procedure. Take standards in 0-40 mg/L range. Above 40 mg/L, $BaSO_4$ suspensions lose stability.

v. Correct for sample color and turbidity by running blanks to which $BaCl_2$ is not added.

Calculation

$$\text{mg } SO_4^{2-}/\text{L} = \frac{\text{mg } SO_4^{2-} \times 1000}{\text{mL sample}}$$

Reporting

Report results for SO_4^{2-} as mg SO_4^{2-}/L with 1 digit after decimal point, e.g., 45.0 mg SO_4^{2-}/L

9. ALKALINITY

(APHA 2320 B. Titration Method)

Principle

Alkalinity is the acid-neutralising capacity of the sample. It can be estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator. Titration to decolourisation of phenolphthalein indicator will indicate complete neutralisation of OH^- and 1/2 of CO_3^{-2}, while sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH^-, CO_3^{-2}, HCO_3^-).

End Point:

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.

''Phenolphthalein alkalinity'' is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the coloured indicator, if any, used in the determination. Phenolphthalein or metacresol purple may be used for alkalinity titration to pH 8.3. Bromocresol green or a mixed bromocresol green-methyl red indicator may be used for pH 4.5.

Instrument/apparatus/equipment

Burettes, Beakers, Pipettes, conical flasks, volumetric flasks etc.

Reagents and standards

Standard sodium carbonate, approximately 0.05N:

Dry 3 to 5 g sodium carbonate, Na_2CO_3, at 250°C for 4 h and cool in a desiccator. Accurately weigh 2.65±0.2 g to the nearest mg, dissolve in distilled water and make to 1 L.

Standard H_2SO_4, approximately 0.1N:

Dilute 2.8 mL conc. sulphuric acid to 1 L. Standardise against 40.00 mL 0.05N Na_2CO_3 with about 60 mL distilled water, in a beaker by titrating potentiometrically to pH 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 titration to pH 4.3. Calculate normality of sulphuric acid:

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

where:

A = g Na_2CO_3 weighed into the 1 L-flask for the Na_2CO_3 standard

B = mL Na_2CO_3 solution taken for standardisation titration

C = mL acid used in standardisation titration

Standard sulphuric acid, 0.02N:

Dilute the approximate 0.1N solution to 1 L. Calculate volume to be diluted as:

$$\text{mL volume} = \frac{20}{N}$$

Where: N = exact normality of the approximate 0.1N solution

Phenolphthalein indicator:

Dissolve 0.5 g in 500 mL 95% ethyl alcohol. Add 500 mL distilled water. Add drop wise 0.02N NaOH till faint pink colour appears (pH 8.3).

Methyl orange indicator:

Dissolve 0.5 g and dilute to 1000 mL with CO_2 free distilled water (pH 4.3-4.5).

OR

Bromo-cresol green indicator:

Dissolve 0.1 g bromocresol green, sodium salt, in 100 mL distilled water (pH 4.5 indicator).

Procedure

i. Take 25- or 50-mL sample in a conical flask and add 2-3 drops of phenolphthalein indicator.

ii. If pink colour develops titrate with 0.02N H_2SO_4 till it disappears or pH is 8.3. Note the volume of H_2SO_4 required.

iii. Add 2-3 drops methyl orange to the same flask and continue titration till yellow colour changes to orange. Note the volumes of H_2SO_4 required.

Calculation

Phenolphthalein alkalinity, mg $CaCO_3/L = \frac{A \times N \times 50000}{\text{mL sample}}$

where:

A = mL titrant used to phenolphthalein end point

N = normality of titrant

Total alkalinity, mg $CaCO_3/L = \frac{B \times N \times 50000}{\text{mL sample}}$

where:

B = total mL of titrant used to methyl orange / bromocresol green end point

N = normality of titrant.

Result	Type of alkalinity as $CaCO_3$		
	Hydroxide	**Carbonate**	**Bicarbonate**
P=0	0	0	T
$P<{}^{1}/_{2}$ T	0	2P	T-2P
$P={}^{1}/_{2}$ T	0	2P	0
$P>{}^{1}/_{2}$T	2P-T	2(T-P)	0
P=T	T	0	0

Reporting

Report results for alkalinity as mg $CaCO_3$/L with 1 digit after decimal point, e.g. 45.0 mg $CaCO_3$/L.

10. CHLORIDE

(APHA 4500-Cl^- B. Argentometric Method)

Principle

In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

Interference

Substances in amounts normally found in potable waters will not interfere. Bromide, iodide, and cyanide register as equivalent chloride concentrations. Sulphide, thiosulfate, and sulphite ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphate in excess of 25 mg/L interferes by precipitating as silver phosphate. Iron in excess of 10 mg/L interferes by masking the end point.

Instrument/apparatus/equipment

Erlenmeyer flask, Digital Burette, volumetric flask, pipette

Reagents

Potassium chromate indicator solution:

Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

Standard silver nitrate titrant, 0.0141M (0.0141N):

Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000mL. Standardize against NaCl by the procedure described below; 1.00 mL = 500 µg Cl^-. Store in amber coloured bottle.

Standard sodium chloride, 0.0141M (0.0141N):

Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL = 500 µg Cl^-.

Special reagents for removal of interference:

- Aluminum hydroxide suspension: Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$, in 1 L distilled water. Warm to 60°C and add 55 mL conc. ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.
- Phenolphthalein indicator solution.
- Sodium hydroxide, NaOH, 1N.

- Sulfuric acid, H_2SO_4, 1N.
- Hydrogen peroxide, H_2O_2, 30%.

Procedure

i. Sample preparation:

Use a 100-mL sample or a suitable portion diluted to 100mL. If the sample is highly coloured, add 3 mL Al $(OH)_3$ suspension, mix, let settle, and filter. If sulphide, sulphite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

ii. **Titration:**

Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode. (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.) Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition.

Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

Calculation

$$\text{mg } Cl^- / L = \frac{(A-B)\times N\times 35450}{mL\ sample}$$

Where:

A = mL titration for sample,

B = mL titration for blank, and

N = normality of $AgNO_3$.

Reporting

Chloride should be reported in units of mg/L and should include 1 digit after the decimal place, e.g.,32.1 mg/L.

11. BORON

(APHA 4500-B B. Curcumin Method)

Principle:

When a water sample containing boron is acidified and evaporated in the presence of curcumin, a red-coloured product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent and the red colour is compared with standards, visually or photometrically.

Instrument/apparatus/equipment

a) Spectrophotometer or photometer with a green filter, for use at 540 nm.
b) High-silica glass or porcelain evaporating dishes, (100 – 150) mL
c) Water-bath, set at 55±2 °C
d) Glass-stoppered volumetric flasks, (25 – 50) mL capacity.
e) Ion- exchange column, 1.3 cm diameter, 50 cm length.
f) Containers, boron free or polyethylene.

Reagents

Store all reagents in polyethylene or boron-free containers.

Stock boron solution:

Dissolve 571.6 mg anhydrous boric acid, H_3BO_3, in distilled water and dilute to 1 L, 1 mL = 100 µg B.

Standard boron solution

Dilute 10 mL stock boron solution to 1 L with distilled water; 1.00 mL = 1.00 µg B.

Curcumin reagent

Dissolve 40 mg finely ground curcumin and 5 g oxalic acid in 80 mL 95% ethyl alcohol, add 4.2 mL conc. HCl, make up to 100 mL with ethyl alcohol and filter if reagent is turbid - store in refrigerator (stable for several days). 95% isopropyl alcohol may be used in place of ethyl alcohol.

Ethyl or isopropyl alcohol, 95%.

Hydrochloric acid, HCl, 1:5

Procedure

Preparation of calibration curve:

Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 µg boron into evaporating dishes of the same type, shape, and size.

i. Add distilled water to each standard to bring total volume to 1.0 mL.
ii. Add 4.0 mL curcumin reagent to each and swirl gently to mix contents thoroughly. Float dishes on a water bath set at (55 ± 2) °C and let them remain for 80 min, which is usually

sufficient for complete drying and removal of HCl. Keep drying time constant for standards and samples. After dishes cool to room temperature, add 10 mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to insure complete dissolution of the red-colored product.

iii. Wash contents of dish into a 25-mL volumetric flask, using 95% ethyl alcohol. Make up to mark with 95% ethyl alcohol and mix thoroughly by inverting. Repeat the same steps for samples.

iv. Read absorbance of standards and samples at a wavelength of 540 nm after setting reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 µg boron. Make photometric readings within 1 h of drying samples.

Sample treatment:

For water expected to have 0.1 – 1 mg B/L, use 1 mL sample. For higher concentrations, take appropriate sample to make dilutions to 1 mL with distilled water. Run the sample with the standard and blank.

Removal of hardness and cation interference:

For samples containing more than 100 mg /L hardness as $CaCO_3$. use a column with strongly acidic cation-exchange resin, backwash with distilled water, pass 50 mL 1 + 5 HCl at a rate of 0.2 mL acid/mL resin in column/min. Wash column free of acid with distilled water. Add 25 mL sample to resin column, adjust flow to 2 drops/s and collect in 50 mL volumetric flask and wash column with distilled water to make up the volume. Alternatively, filter the final solution in step ‘b’ above if any turbidity appears due to hardness of the sample.

Calculation

Plot calibration curve giving absorbance versus mg B. Read weight of Boron in mg in the sample from the curve. Calculate mg B/L by dividing the weight by the volume of the sample in mL.

Reporting

Boron should be reported in units of mg/L and should include 2 digits after the decimal point, e.g.,1.62 mg/L.

12. PHOSPHATE

(APHA 4500-P E. Ascorbic Acid Method)

Principle:

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

Instrument/apparatus/equipment

a) Spectrophotometer with infrared phototube for use at 880 nm or filter photometer, equipped with a red filter.

b) Acid washed glassware, use dilute HCl and rinse with distilled water.

Interference:

Arsenates react with the molybdate reagent to produce a blue colour 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. Sulphide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

Reagents

Sulfuric acid, H_2SO_4, 5N:

Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.

Potassium antimonyl tartrate solution:

Dissolve 1.3715 g $K(SbO)C_4H_4O_6$. $½H_2O$ in 400 mL distilled water in a 500-ml volumetric flask and dilute to 1000 mL. Store in a glass-stoppered bottle.

Ammonium molybdate solution:

Dissolve 20 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle.

Ascorbic acid, 0.1M:

Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

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.

Standard phosphate solution:

Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 µg PO_4^{3-}-P.

Procedure

Treatment of sample:

Take 50 mL sample into a 125 mL conical flask, add 1 drop of phenolphthalein indicator. Discharge any red colour by adding 5N H_2SO_4. Add 8 mL combined reagent and mix.

i. Wait for 10 minutes, but not more than 30 minutes and measure absorbance of each sample at 880 nm. Use reagent blank as reference.

ii. Correction for turbid or coloured samples. Prepare a sample blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from sample absorbance reading.

iii. Preparation of calibration curve: Prepare calibration from a series of standards between 0.15-1.30 mg P/L range (for a 1 cm light path). Use distilled water blank with the combined reagent.

iv. Plot a graph with absorbance versus phosphate concentration to give a straight line. Test at least one phosphate standard with each set of samples.

Calculation

$$\text{mg/L P} = \frac{\text{mg P (in approximately 58 mL final volume) X 1000}}{\text{Volume of Sample}}$$

Reporting

Phosphate should be reported in units of mg/L and should include 3 digits after the decimal point, e.g.,1.632 mg/L

13. FLUORIDE

13.1 (APHA 4500-F^- C. Ion-Selective Electrode Method)

Principle:

The fluoride electrode is an ion-selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other. The cell may be represented by:

$$Ag|AgCl, Cl^-(0.3M), F^-(0.001M)\ |LaF_3|\ \text{test solution}|\text{reference electrode}$$

The fluoride electrode can be used with a standard calomel reference electrode and almost any modern pH meter having an expanded millivolt scale. Calomel electrodes contain both metallic and dissolved mercury; therefore, dispose of them only in approved sites or recycle. For this reason, the Ag/AgCl reference electrode is preferred.

The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. Adding an appropriate buffer provides a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration.

Interference:

Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride, and complexing species. However, CDTA (cyclohexylenediaminetetraaceticacid), a component of the buffer, preferentially will complex interfering cations and release free fluoride ions. Concentrations of aluminum, the most common interference, up to 3.0 mg/L can be complexed preferentially. In acid solution, F^- forms a poorly ionized HF·HF complex but the buffer maintains a pH above 5 to minimize hydrogen fluoride complex formation. In alkaline solution hydroxide ion also can interfere with electrode response to fluoride ion whenever the hydroxide ion concentration is greater than one-tenth the concentration of fluoride ion. At the pH maintained by the buffer, no hydroxide interference occurs. Distil the sample if the dissolved solids concentration exceeds 10000 mg/L.

Instrument/apparatus/equipment

a) ion meter.
b) Sleeve-type reference electrode: Do not use fibre-tip reference electrodes because they exhibit erratic behaviour in very dilute solutions.
c) Fluoride electrode.

d) Magnetic stirrer, with TFE-coated stirring bar, retriever, micropipette

e) Timer.

Reagents

Stock fluoride solution:

Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1.00 mL = 100 μg F^-.

Standard fluoride solution:

Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1.00 mL = 10.0 μg F^-.

Fluoride buffer:

Place approximately 500 mL distilled water in a 1-L beaker and add 57 mL glacial acetic acid, 58 g NaCl, and 4.0 g 1,2 cyclohexylenediaminetetraacetic acid. Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to a 1-L volumetric flask and add distilled water to the mark. This buffer, as well as a more concentrated version, is available commercially. In using the concentrated buffer follow the manufacturer's directions.

Procedure

Prepare a series of working standards by diluting 5.0, 10.0 and 20.0 mL of standard solution to 100 mL, corresponding to 0.5, 1.0 and 2 mg F^- /L, respectively.

i. Take between 10 to 25 mL standards and sample in 100 mL beakers. Bring the samples and the standards to the room temperature and add an equal volume of buffer to each beaker. The total volume should be sufficient to immerse the electrode and permit the use of the stirrer.

ii. Follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range. Standards already diluted with the buffer may have been supplied with the meter. Avoid stirring before immersing electrodes so as not to entrap air bubbles.

iii. If a direct reading instrument is not used, plot on a semi logarithmic graph paper potential measurement of fluoride standards on arithmetic scale vs. fluoride concentration on logarithmic scale.

iv. **Important:** Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read fluoride concentration in the sample from the calibration curve or directly from the meter.

Reporting

Fluoride should be reported in units of mg/L and should include 3 digits after the decimal point, e.g.,1.63 mg/L

13.2 (APHA 4500-F^- D. SPADNS Method)

Principle:

The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colourless complex anion (ZrF_6^{2-}); and the dye. As the amount of fluoride increases, the colour produced becomes progressively lighter.

Interference:

Whenever any one substance is present in sufficient quantity to produce an error of 0.1 mg/L or whenever the total interfering effect is in doubt, distil the sample. Also distil coloured or turbid samples. In some instances, sample dilution or adding appropriate amounts of interfering substances to the standards may be used to compensate for the interference effect. If alkalinity is the only significant interference, neutralize it with either hydrochloric or nitric acid. Chlorine interferes and provision for its removal is made. Volumetric measurement of sample and reagent is extremely important to analytical accuracy. Use samples and standards at the same temperature or at least within 2°C. Maintain constant temperature throughout the colour development period. Prepare different calibration curves for different temperature ranges.

Instrument/apparatus/equipment

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 570 nm, providing a light path of at least 1 cm.

b. Filter photometer, providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.

Reagents

Stock fluoride solution:

Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 ml; 1.00 ml = 100 µg F^-.

Standard fluoride solution:

Dilute 100 ml stock fluoride solution to 1000 ml with distilled water; 1.00 ml = 10.0 µg F^-.

SPADNS solution:

Dissolve 958 mg SPADNS, sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6naphthalene disulfonate, also called 4,5-dihydroxy-3-(parasulfophenylazo)-2,7-naphthalenedisulfonic acid trisodium salt, in distilled water and dilute to 500 mL. This solution is stable for at least 1 year if protected from direct sunlight.

Zirconyl-acid reagent:

Dissolve 133 mg zirconyl chloride octahydrate, $ZrOCl_2 .8H_2O$, in about 25 mL distilled water. Add 350 mL conc HCl and dilute to 500 mL with distilled water.

Acid zirconyl-SPADNS reagent:

Mix equal volumes of SPADNS solution and zirconyl-acid reagent. The combined reagent is stable for at least 2 years.

Reference solution:

Add 10 mL SPADNS solution to 100 mL distilled water. Dilute 7 mL conc HCl to 10 mL and add to the diluted SPADNS solution. The resulting solution, used for setting the instrument reference point (zero), is stable for at least 1 year. Alternatively, use a prepared standard of 0 mg F^-/L as a reference.

Procedure

a. Preparation of standard curve*:*

Prepare fluoride standards in the range of 0 to 1.0 mg F^-/L by diluting appropriate quantities of standard fluoride solution to 50 mL with distilled water. Pipet 5.00 mL each of SPADNS solution and zirconyl acid reagent, or 10.00 mL mixed acid-zirconyl-SPADNS reagent, to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards. Plot a curve of the milligram fluoride-absorbance relationship. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired

b. Colour development*:*

Use a 50.0-mL sample or a portion diluted to 50 mL with distilled water. Adjust sample temperature to that used for the standard curve. Add 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL acid zirconyl-SPADNS reagent; mix well and read absorbance, first setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

Calculation

Read fluoride concentration in the sample from the calibration curve.

When the prepared 0 mg F^-/L standard is used to set the photometer, alternatively calculate fluoride concentration as follows:

$$\text{mg } F^-/L = \frac{A0 - Ax}{A0 - A1}$$

where:

$A0$ = absorbance of the prepared 0 mg F^-/L standard,

A_x =absorbance of the prepared sample, and

A_1 =absorbance of a prepared 1.0 mg F^-/L standard.

14. NITROGEN - NITRITE

(APHA 4500–NO_2^- B. Colorimetric Method)

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 sulphanilamide 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 colour filter are used. The colour 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.

Interferences:

Chemical incompatibility makes it unlikely that NO_2^-, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red colour when colour 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 catalysing decomposition of the diazonium salt. Coloured ions that alter the colour system also should be absent. Remove suspended solids by filtration.

Instrument/apparatus/equipment

Spectrophotometer for use at 543 nm or filter photometer with green filter, maximum transmittance near 540 nm, providing 1 cm light path or longer.

Reagents

Colour reagent*:*

To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulphanilamide. After dissolving add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for one month when stored in dark bottle in refrigerator.

Sodium oxalate, 0.025M (0.05N):

Dissolve 3.350 g $Na_2C_2O_4$ primary standard grade, in water and dilute to 1000mL.

Ferrous ammonium sulphate, 0.05M (0.05N):

Dissolve 19.607 g $Fe(NH_4)_2$ $(SO_4)_2.6H_2O$ plus 20 mL conc H_2SO_4 in water and dilute to 1000mL.

Stock nitrite*:*

Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 µgN. Preserve with 1 mL $CHCl_3$. Standardise by pipetting, in order, 50 mL 0.01M $KMnO_4$, 5 mL conc H_2SO_4 and 50.00 mL stock NO_2^- solution in to a glass stoppered flask. Shake gently and warm to 70-80°C.

Discharge permanganate colour by adding 10 mL portions of 0.025M sodium oxalate. Titrate excess oxalate with 0.01M (0.05N) $KMnO_4$ to faint pink end point. Calculate nitrite content of stock solution:

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

where: A = mg NO_2^- - N/mL in stock solution

B = mL total $KMnO_4$ used

C = normality of $KMnO_4$

D = total mL oxalate added

E = normality of oxalate

F = mL stock nitrite taken for titration

Intermediate nitrite solution:

Calculate the volume, G, of stock nitrite solution required for the intermediate nitrite solution from G = 12.5/A. Dilute the volume G to 250 mL with water; 1 mL =50.0 µg NO_2^--N. Prepare daily.

Standard nitrite solution:

Dilute 10 mL intermediate NO_2^- solution to 1000 mL with water; 1.00mL = 0.500 µg NO_2^--N. Prepare daily.

Standard potassium permanganate titrant, 0.01M (0.05N):

Dissolve 1.6 g $KMnO_4$ in 1 L distilled water. Allow ageing for 1 week then decant supernatant. Standardise this solution frequently as follows:

Weigh to nearest 0.1 mg several 100 to 200 mg samples of anhydrous sodium oxalate in beakers. To each beaker add 100 mL distilled water, 10 mL 1 + 1 H_2SO_4 and heat rapidly to 90 to 95 °C. Titrate with permanganate solution to a slight pink end point that persists to at least 1 min. Do not allow temperature to fall below 85°C. Run a blank on distilled water + H_2SO_4.

$$\text{Normality of } KMnO_4 = \frac{\text{g Na2C2O4}}{(A-B)} \text{ X } 0.067$$

Where- A = mL titrant for sample

B= mL titrant for blank

Average the result of several titrations.

Procedure

i. Add 2 mL colour reagent to 50 mL sample, or to a portion diluted to 50 mL, and mix.

ii. Measure absorbance at 543 nm. Wait between 10 min and 2 h after addition of colour reagent before measurement

iii. Prepare standard curve by diluting 1, 2, 3, 4 and 5 mL of standard nitrite solution to 100 mL to give 5, 10, 15, 20 and 25 µg/L concentration, respectively.

Reporting

Report results as mg N/L with 1 digit after the decimal point, e.g., 0.9 mg N/L.

15. NITROGEN - AMMONIA

15.1 (APHA 4500–NH_3 D. Ammonia Selective Electrode method)

Principle

The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia (NH_3(aq) and NH_4^+) is converted to NH_3 (aq) by raising pH to above 11 with a strong base. NH_3 (aq) diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded mill volt scale or with a specific ion meter.

Interference

Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution is used.

Instrument/apparatus/equipment

a) Ion meter
b) Ammonia and reference electrodes
c) Magnetic stirrer with TFE coated stirring bar

Reagents

Ammonia free water:

Add 0.1 mL conc. H_2SO_4 to 1 L distilled water and redistill. Alternatively, prepare de-ionised water from distilled water using a mixed cation and anion exchange resin bed. In case the anion resin releases traces of ammonia, use only a cation exchange resin. Use ammonia free water to prepare all reagents.

Sodium hydroxide, 10N

NaOH/EDTA solution, 10N:

Dissolve 400g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetra sodium salt, tetrahydrate ($Na_4EDTA.4H_2O$) and stir to dissolve. Cool and dilute to 1000mL.

Stock ammonium chloride solution:

Dissolve 3.819 g anhydrous NH_4Cl (dried at 100°C) in water, dilute to 1000 mL; 1 mL = 1 mg N.

Procedure

i. Transfer 25 mL of the ammonium chloride stock solution to a 250 mL flask and make up to the mark. Using this diluted standard, transfer 25 mL to another 250 mL flask and make upto

the mark. Similarly prepare two more successive dilutions to give serial decimal dilutions of 100, 10, 1.0, and 0.1 mg NH_3-N /L.

ii. Place 100 mL of each standard in 150 mL beaker. Immerse electrodes in standard of lowest concentration and mix with magnetic stirrer. Stir at a very low speed to minimise the loss of ammonia under alkaline condition. Add sufficient volume of 10N NaOH to raise the pH to 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution. Wait for the reading to stabilise (at least 2 to 3 min) before recording.

iii. Repeat b) above with each of the standards and the samples. Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedure.

iv. Record the volume of the alkali used if more than 1 mL is used to adjust the pH of any of the samples.

v. Plot on a semi logarithmic graph paper potential measurement, in mV, of the standards in mill volts, on arithmetic scale, vs. mg NH_3-N/L concentration on logarithmic scale. The calibration curve should be a straight line with a slope of about 59/decade at 25°C. Recalibrate the probes and the instruments several times every day.

vi. In case of direct reading ion meters, follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range.

Important: Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read ammonia nitrogen concentration in the sample from the calibration curve or directly from the meter.

Reporting

Report results as mg N/L with 1 digit after the decimal point, e.g., 0.9 mg N/L.

15.2 (APHA 4500-NH_3 F. Phenate Method)

Principle

The method involves addition of phenol solution together with hypochlorite and nitroprusside catalyst to the sample. Ammonia reacts to form indophenol, which has an intense blue colour. The developed blue colour absorbs light of 640 nm wavelength.

Instrument/apparatus/equipment

Digital balance, Hot Air Oven, UV-Visible Spectrophotometer

Reagents

Phenol, Sodium nitroprusside, Trisodium Citrate, Sodium Hydroxide, Sodium Hypochlorite, NH_4Cl

Phenol solution: Mix 11.1 mL liquified phenol (89%) with 95% v/v ethyl alcohol to a final volume of 100 mL. Prepare weekly. **CAUTION: Wear gloves and eye protection when handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile substance.**

Sodium nitroprusside, 0.5% w/v: Dissolve 0.5 g sodium nitroprusside in 100 mL deionized water. Store in amber bottle for up to 1 month.

Alkaline citrate: Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized water. Dilute to 1000 mL.

Sodium hypochlorite, commercial solution, about 5%. This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.

Oxidizing solution: Mix 100 mL alkaline citrate solution with 25 mL sodium hypochlorite. Prepare fresh daily.

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

Standard ammonium solution: Use stock ammonium solution and water to prepare a calibration curve in a range appropriate for the concentrations of the samples.

Procedure

i. To a 25-mL sample in a 50-mL Erlenmeyer flask, add, with thorough mixing after each addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution and 2.5 mL oxidizing solution.

ii. Cover samples with plastic wrap or paraffin wrapper film. Let colour develop at room temperature (22 to 27°C) in subdued light for at least 1 h. Colour is stable for 24 h.

iii. Measure absorbance at 640 nm. Prepare a blank and at least two other standards by diluting stock ammonia solution into the sample concentration range. Treat standards the same as samples.

Calculation

Prepare a standard curve by plotting absorbance readings of standards against ammonia concentrations of standards. Compute sample concentration by comparing sample absorbance with the standard curve.

16. NITROGEN-NITRATE

16.1 (APHA 4500–NO_3^- D. Nitrate Selective Electrode method)

Principle

The nitrate ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to nitrate ion activity between about 10^{-5} and 10^{-1} M (0.14 to 1400 mg NO_3^- N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

Interferences

Chloride and bicarbonate ions interfere when their weight ratios to NO_3^- N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in potable waters are NO_2^-, CN^-, S^{2-}, Br^-, I^-, ClO_3^-, and ClO_4^-. Although the electrodes function satisfactorily in buffers over the range pH 3 to 9, erratic responses have been noted where pH is not held constant. Because the electrode responds to NO_3^- activity rather than concentration, ionic strength must be constant in all samples and standards. Minimize these problems by using a buffer solution containing Ag_2SO_4 to remove Cl^-, Br^-, I^-, S^{2-}, and CN^-, sulfamic acid to remove NO_2^-, a buffer at pH 3 to eliminate HCO_3^- and to maintain a constant pH and ionic strength, and $Al_2(SO_4)_3$ to complex organic acids.

Instrument/apparatus/equipment

a) Ion meter
b) Nitrate and reference electrodes
c) Magnetic stirrer with TFE coated stirring bar

Reagents

Nitrate free water:

Use double distilled or de-ionised water to prepare all solutions.

Stock nitrate solution:

Dissolve 0.7218 g, previously dried and cooled potassium nitrate (KNO_3) in water and dilute to 1000 mL; 1 mL = 100 µg NO_3^- - N.

Standard nitrate solutions:

Dilute 1.0, 10 and 50 mL stock nitrate solution to 100 mL to obtain standards of 1.0, 10 and 50 mg NO_3^- - N/L, respectively.

Buffer solution*:*

Dissolve 17.32 g $Al_2(SO_4)_3.18H_2O$, 3.43 g Ag_2SO_4, 1.28 g H_3BO_3, and 2.52 g sulfamic acid (H_2NSO_3H), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10 N NaOH. Dilute to 1000 mL and store in a dark glass bottle.

Procedure

i. Transfer 10 mL of 1.0 mg NO_3^- - N/L standard to a 50 mL beaker, add 10 mL buffer and stir with magnetic stirrer. Stop stirring after mixing and immerse electrodes. Start stirring again.

ii. Take mV reading when stable (after about 1 min). Repeat with 10 and 50 mg NO_3^-N/L standards.

iii. Plot on a semi logarithmic graph paper potential measurement of the standards in mV, on arithmetic scale, vs. NO_3^- - N concentration on logarithmic scale. The calibration curve should be a straight line with a slope of + 57 ± 3/ decade at 25 °C. Recalibrate the probes and the instruments several times every day using the 10 mg NO_3^--N/L standard.

iv. Transfer 10 mL sample to a 50 mL beaker, add 10 mL buffer and stir with magnetic stirrer. Stop stirring after mixing and immerse electrodes. Start stirring again. Take millivolt reading when stable (after about 1 min).

v. In case of direct reading ion meters, follow manufacturer's instructions to set up and calibrate the ion meter using standards in the prescribed range. Standards already diluted with the buffer may have been supplied with the meter.

Important: Wash and blot dry electrodes and stirring bar when used for different solutions and samples. Samples and standards should be maintained at nearly the same temperature throughout calibration and testing procedure.

Calculation

Read nitrate nitrogen concentration in the sample from the calibration curve or directly from the meter.

Reporting

Nitrate Nitrogen should be reported in units of mg N/L and should have 2 digits after the decimal point, e.g., 6.71 mg N/L.

16.2 4500-NO_3^- B. Ultraviolet Spectrophotometric Screening Method

Principle

Used for screening samples containing low organic matter (i.e., uncontaminated natural waters and potable water supplies). The NO_3^- calibration curve follows Beer's law up to 11 mg N/L. Measuring UV absorption at 220 nm enables analysts to determine NO_3^- rapidly. Be aware that dissolved organic matter also may absorb at 220 nm but NO_3^- does not absorb at 275 nm, so a second measurement can be made at 275 nm and used to correct the NO_3^- value, if needed. The extent of this empirical correction is related to the nature and concentration of the organic matter and may vary from one water to another, so this method is not recommended if a significant correction is required.

Interferences

Potential interferences include dissolved organic matter, surfactants, NO_2^-, hexavalent chromium and various inorganic ions not normally found in natural water, such as chlorite and chlorate. Sample filtration eliminates interference by suspended particles. Acidification with 1M HCl to pH = 2 prevents interference from hydroxide or carbonate concentrations up to 1000 mg calcium carbonate ($CaCO_3$)/L. Chloride does not affect the determination. Inorganic substances can be compensated for by independently analysing their concentrations and preparing individual correction curves. Filter turbid samples. Test filters for NO_3-contamination.

Instrument/apparatus/equipment

Spectrophotometer, for use at 220 and 275 nm with matched silica cells of 1cm or longer light path.

Reagents

Nitrate free water:

Use double distilled or de-ionised water to prepare all solutions.

Stock nitrate solution:

Dissolve 0.7218 g, previously dried (at 103-105°C for 24 hour) and cooled potassium nitrate (KNO_3) in water and dilute to 1000 mL; 1 mL = 100 µg NO_3^-- N. Preserve with 2 mL chloroform ($CHCl_3$)/L. Solution is stable for at least 6 months.

Standard nitrate solutions:

Dilute 100 mL stock NO_3^--N solution to 1000 mL with water; 1.00 mL = 10.0 µg NO_3^-- N. Preserve with 2 mL $CHCl_3$/L. Solution is stable for 6 months.

Hydrochloric acid solution(~1M):

Dilute 83 mL concentrated HCl to 1L with reagent water. Store in a glass or high-density polyethylene (HDPE) bottle. Stable for 1 year if kept closed.

Procedure

a. Treatment of sample: To 50 mL clear sample (filtered if necessary), add 1 mL 1M HCl solution and mix thoroughly.

b. Preparation of Standard Curve: Prepare calibration standards in the range 0 to 7 mg NO_3^--N/L by diluting to 50 mL the required volumes of standard solution. Add 1 mL of HCl and mix. Treat standards in same manner as samples.

c. Spectrophotometric measurement: Read absorbance or transmittance against reagent water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain NO_3^--N reading and a wavelength of 275 nm to determine any interference due to dissolved organic matter.

Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^--N. If correction value is >10% of reading at 220 nm for a particular sample, then the NO_3^--N concentration is considered a rough estimate. Prepare a standard curve by plotting absorbance due to $NO3^-$ against $NO3^-$-N concentration of standards. Obtain sample concentrations directly from the calibration curve by using corrected sample absorbances.

17. SODIUM

(APHA 3500-Na B. Flame Emission Photometric Method)

Principle

Trace amounts of sodium can be determined by flame emission photometry at the wavelength of 589 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at 589 nm is approximately proportional to the concentration of the element.

Interference

Flame photometers operating on the internal standard principle may require adding a standard lithium solution to each working standard and sample. The optimum lithium concentration may vary among individual instruments; therefore, ascertain it for the instrument used. Minimise interference by the following:

- Operate in the lowest practical sodium concentration range.
- Add radiation buffers to suppress ionisation and anion interference. Among common anions capable of causing radiation interference are Cl^-, SO_4^{-2} and HCO_3^- in relatively large amounts.
- Introduce identical amounts of the same interfering substances present in the sample into the calibration standards.
- Apply an experimentally determined correction in those instances where the sample contains a single important interference.
- Remove interfering ions.
- Remove burner-clogging particulate matter from the sample by filtering through a quantitative filter paper of medium retentiveness.
- Incorporate a non-ionic detergent in the standard lithium solution to assure proper aspirator function.
- Use the standard addition technique as described in the flame photometric method for strontium. Its use involves adding an identical portion of sample to each standard and determining the sample concentration by mathematical or graphical evaluation of the calibration data.

- Use the internal standard technique. Potassium and calcium interfere with sodium determination by the internal standard method, if the potassium to sodium ratio is > 5:1 and the calcium to sodium ratio is > 10:1. When these ratios are exceeded, measure calcium and potassium first so that the approximate concentration of interfering ions may be added to the sodium calibration standards. Magnesium interference does not appear until the magnesium to sodium ratio exceeds 100, a rare occurrence.

Instrument/apparatus/equipment

a) Flame photometer, direct reading type.
b) Glassware, rinsed with 1 + 15 HNO_3, followed by de-ionised distilled water.
c) Plastic bottles, to store all solutions.

Reagents

Stock sodium solution

weigh 2.542 g NaCl, dried at 140°C and cooled in desiccators, transfer to1 L volumetric flask and make to 1 L with water; 1 mL = 1.00 mg Na.

Intermediate sodium solution

Dilute 10 mL stock sodium solution with water to 100 mL; 1 mL =0.1 mg Na, prepare calibration curve in the range of 1 to 10 mg/L

Standard sodium solution*:*

Dilute 10 mL intermediate solution with water to 100 mL, 1 mL = 10µg Na, prepare calibration curve in the range of 0.1 to 1 mg/L.

Procedure

Follow instructions of flame photometer manufacturer for selecting proper photocell, wavelength, slit width adjustments, fuel gas and air pressure, steps for warm up, correcting for interference and flame background, rinsing of burner, sample ignition and emission intensity measurements.

Reporting

Report results as mg/L, with 1 digit after the decimal point, e.g., 32.9 mg/L.

18. POTASSIUM

(APHA 3500-K B. Flame Photometric Method)

Principle

Trace amounts of potassium can be determined in either a direct-reading or internal-standard type of flame photometer at a wavelength of 766.5 nm. Because much of the information pertaining to sodium applies equally to the potassium determination, carefully study the entire discussion dealing with the flame photometric determination of sodium before making a potassium determination.

Interference

Interference in the internal-standard method may occur at sodium-to-potassium ratios of 5:1 or greater. Calcium may interfere if the calcium-to-potassium ratio is 10:1 or more. Magnesium begins to interfere when the magnesium-to-potassium ratio exceeds 100:1.

Instrument/apparatus/equipment

a) Flame photometer, direct reading type.
b) Glassware, rinse with 1 + 15 HNO_3, followed by de-ionised distilled water.
c) Plastic bottles, to store all solutions

Reagents

Stock potassium solution:

Weigh 1.907 g KCl, dried at 110°C and cooled in desiccators, transfer to 1 L volumetric flask and make to 1 L with water; 1 mL = 1.00 mg K.

Intermediate potassium solution

Dilute 10ml stock potassium solution with water to 100 mL;1ml = 0.1 mg K, prepare calibration curve in the range of 1 to 10 mg/L

Standard potassium solution*:*

Dilute 10ml intermediate solution with water to 100 mL, 1 mL =10 µg K, prepare calibration curve in the range of 0.1 to 1 mg/L.

Procedure

Follow instructions of flame photometer manufacturer for selecting proper photocell, wavelength, slit width adjustments, fuel gas and air pressure, steps for warm up, correcting for interference and flame background, rinsing of burner, sample ignition and emission intensity measurements.

Reporting

Report results for Potassium as mg/L, with 1 digit after the decimal point, e.g., 13.9 mg/L.

19. SILICATE

(APHA 4500-SiO_2 D. Heteropoly Blue Method)

Instrument/apparatus/equipment

Spectrophotometer, for use at 815 nm, having 1 cm light path.

Reagents

Store all samples and standards in plastic containers.

a) **Sulphuric acid**, H_2SO_4, 1N.

b) **Hydrochloric acid,** HCl (1 + 1)

c) **Ammonium molybdate reagent**: Dissolve 10 g (NH) $Mo_7O_{24}.4H_20$ in distilled water, with stirring and gentle warming, and dilute to 100mL. Filter if necessary. Adjust pH between 7 and 8 with silica free NH_4OH or NaOH and store in polyethylene bottle to stabilise.

d) **Oxalic acid solution**: Dissolve 7.5 g Oxalic acid in distilled water and dilute to 100mL.

e) **Stock silica solution**: Dissolve 313.0 mg sodium hexafluorosilicate $Na_2[SiF_6]$ in 1000 mL distilled water. 1 mL = 0.1 mg SiO_2.

f) **Silica standard working solution**: Dilute 100 mL stock solution to 1000ml, 1mL = 10 ug SiO_2.

g) **Reducing agent**: Dissolve 500 mg 1-amino-2-naphthol-4-sulphonic acid and 1 g Na_2SO_3 in 50 mL distilled water, with gentle warming, if necessary, add this to a solution of 30 g $NaHSO_3$, in 150 mL distilled water. Filter into a plastic bottle. Discard when the solution becomes dark. Prolong reagent life by storing a refrigerator away from light.

Procedure

i. To 50 mL sample, containing between 20 and 100 ug silica, add in rapid succession 1.0 mL (1 + 1) HCI and 2 mL ammonium molybdate reagent. Mix thoroughly and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix. Measuring time from the moment of adding oxalic acid, wait at least 2 min but not more than 15 min, add 2 mL reducing agent and mix thoroughly.

ii. Read absorbance at 815 nm after 5 min, adjusting the instrument to zero absorbance using distilled water blank.

iii. Dilute 2.0, 4.0, 6.0, 8.0 and 10.0 mL silica working standard solution to 50 mL volumes and proceed as above to prepare a calibration curve.

Calculation

Read silica content of sample from the calibration curve.

Note: Standard Methods recommends use of sodium metasilicate nonahydrate, $Na_2SiO_3.9H_2O$, for preparation of standards and its standardisation by gravimetric method. The method

measures molybdate reactive silica. To determine molybdate unreactive silica a digestion step is necessary.

20. CHEMICAL OXYGEN DEMAND

20.1 (APHA 5220 B. Open Reflux Method)

Principle:

Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulphate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent.

Keep ratios of reagent weights, volumes, and strengths constant when sample volume other than 50 mL are used. The standard 2-hour reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analysed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

Instrument/apparatus/equipment

a) Reflux apparatus
b) Blender
c) Pipets, burette

Reagents

Standard potassium dichromate solution:

Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000mL. The equivalent concentration is 0.2500N.

Sulfuric acid reagent

Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc. H_2SO_4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix well.

Ferroin indicator solution

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100mL. This indicator solution may be purchased already prepared.

Standard ferrous ammonium sulphate (FAS) titrant:

Dissolve 98 g of Fe $(NH_4)_2(SO_4)_2$.$6H_2O$ in distilled water. Add 20 mL conc.H_2SO_4, cool, and dilute to 1000mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:

Dilute 25.00 mL standard $K_2Cr_2O_7$ to about 100mL. Add 30 mLconc.H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

$$\text{Molarity of FAS solution} = \frac{\text{Volume of K2Cr2O7 solution titrated} \times 0.2500}{\text{Volume of FAS used in titration}}$$

*Volumes in mL

Mercuric sulphate, $HgSO_4$, crystals or powder*:*

Procedure

i. Blend sample if necessary and pipet 50 mL into 500 mL refluxing flask.

ii. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$.

iii. Cool while mixing to avoid possible loss of volatile materials.

iv. Add 20 mL of $K_2Cr_2O_7$ solution and mix.

v. Attach flask to condenser and turn on cooling water.

vi. Add remaining sulfuric acid reagent (25 mL) through open end of condenser.

vii. Continue swirling and mixing while adding sulfuric acid reagent.

viii. Reflux for 2 hours.

ix. Cool and wash down condenser with distilled water.

x. Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

xi. Take the first sharp colour change from blue-green to reddish brown that persists for 1 minute or longer as the end point of the titration.

xii. Carry a blank reagent through the same procedure.

Calculation

$$\text{COD as mg } O_2/\text{L} = \frac{(A-B) \times M \times 8000}{mL\ sample}$$

where:

A = mL FAS used for blank,

B = mL FAS used for sample,

M = molarity of FAS, and

8000 = milli equivalent weight of oxygen × 1000 mL/L

20.2. (APHA 5220 D. Closed Reflux, Colorimetric Method)

Instrument/apparatus/equipment

Digestion vessels with premixed reagents, COD Digester with holes to accommodate digestion vessels, Colorimeter, Micro pipette

Reagents

Digestion vessels with premixed reagents, potassium hydrogen phthalate, standard

Procedure

Digestion and colour measurement

1. Measure suitable volume of the sample/blank/standards into each vial as prescribed.
2. Digest for 2 hours at 150°C in a preheated digester.
3. Let cool down to room temperature, vent if necessary to release any pressure and mix contents well.
4. Measure reading using colorimeter

Reporting

COD should be reported in units of mg/L and should include 1 digit after the decimal point, e.g., 9.7 mg/L.

21. DISSLOVED OXYGEN

(APHA 4500-O C. Azide Modification Method)

Principle

The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate. The titration end point can be detected visually, with a starch indicator. Experienced analysts can maintain a precision of ±50 µg/L with visual end-point detection.

Reagents

Manganous sulphate solution:

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

Alkali-iodide-azide reagent:

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 colour with starch solution when diluted and acidified.

Sulfuric acid, conc:

One millilitre is equivalent to about 3 mL alkali-iodide-azide reagent.

Starch:

Dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

Standard sodium thiosulfate titrant, 0.025 M:

Dissolve 6.205 g $Na_2S_2O_3$. $5H_2O$ in distilled water. Add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000mL. Standardize with bi-iodate solution.

Standard potassium bi-iodate solution:

Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000mL. Standardize with bi-iodate solution.

Standardisation

Dissolve approximately 2 g KI, free from iodate, in an Erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6N 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 colour is reached. When the solutions are of equal strength, 20.00 mL 0.025M $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025M.

Procedure

i. To the sample collected in a 300-mL bottle, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent.
ii. If pipets are dipped into sample, rinse them before returning them to reagent bottles.
iii. Alternatively, hold pipet tips just above liquid surface when adding reagents.
iv. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times.
v. 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 H2SO4.
vi. Restopper and mix by inverting several times until dissolution is complete.
vii. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents.
viii. 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.
ix. Titrate with 0.025M $Na_2S_2O_3$ solution to a pale straw colour.
x. Add a few drops of starch solution and continue titration to first disappearance of blue colour.
xi. If end point is overrun, back-titrate with 0.0021M bi-iodate solution added drop wise, or by adding a measured volume of treated sample.
xii. Correct for amount of bi-iodated solution or sample.

Calculation

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

Reporting

Dissolved oxygen should be reported in units of mg/L and should include 1 digit after the decimal point, e.g., 8.2 mg/L.

22. BIOCHEMICAL OXYGEN DEMAND

(APHA 5210 B. 5-Day BOD Test)

Principle

This is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of water. This method measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand). The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

The method consists of filling an airtight BOD bottle of 300 mL, to overflowing, and incubating it at the specified temperature (20ºC) for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement.

Sampling and storage

Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values. Minimize reduction of BOD by analysing sample promptly or by cooling it to near-freezing temperature during storage. However, even at low temperature, keep holding time to a minimum. Warm chilled samples to 20 ± 3°C before analysis.

Instrument/apparatus/equipment

a) BOD bottle, 300 mL capacity
b) Air incubator - thermostatically controlled at 20 ±1°C. Exclude all light prevent possibility of photosynthetic production of DO.
c) Digital burette

Reagents

Prepare reagents in advance but discard if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable and different stock concentrations may be used if doses are adjusted proportionally. Use reagent grade or better for all chemicals and use distilled or equivalent water, preferably sterilized, for making all solutions.

Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$, and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 and 1.7 g NH_4Cl in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

Magnesium sulphate solution: Dissolve 22.5 g $MgSO_4 7H_2O$ in distilled water and dilute to 1 L.

Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

Ferric chloride solution: Dissolve 0.25 g $FeCl_3.6H_2O$ in distilled water and dilute to 1 L.

Acid and alkali solutions, 1N, for neutralization of caustic or acidic waste samples.

1) Acid—slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

2) Alkali—Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

Sodium sulphite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water. This solution is not stable; prepare daily.

Nitrification inhibitor: 1) 2-chloro-6-(trichloromethyl) pyridine—Use pure TCMP or commercial preparations*. 2) Allyl thiourea (ATU) solution—Dissolve 2.0 g allyl thiourea ($C_4H_8N_2S$) in about 500 mL water and dilute to 1 L. Store at 4°C. The solution is stable for not more than 2 weeks.

Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile condition. Store all glucose-glutamic acid mixtures at 4°C or lower. Commercial preparations may be used but concentrations may vary.

Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

Source water for preparing BOD dilution water: Use demineralized, distilled, tap, or natural water for making sample dilutions.

Standardisation

Dissolve approximately 2 g KI, free from iodate, in an Erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6N 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 colour is reached. When the solutions are of equal strength, 20.00 mL 0.025M $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025M.

Procedure

Grab or composite samples are collected. Keep composite samples at or below 4°C during compositing. Samples for BOD may degrade significantly during storage. Minimise reduction of BOD by analysing samples promptly or by cooling it to near freezing temperature during

storage. The maximum holding time recommended between collection and analysis is 48 hours. Warm chilled samples to 20-27°C ± 3°C before analysis. State storage time and condition as part of results.

Procedure

Preparation of dilution water:

Store the distilled water/tap/ receiving stream water free of biodegradable organics and bio inhibitory substances such as chlorine or heavy metals in a BOD incubator at 20°C. Aerate the required quantity of this water at 20°C with clean compressed air. Add 1 mL each of $CaCl_2$, $MgSO_4$, $FeCl_3$ and phosphate buffer solutions per litre of the above aerated distilled water and mix thoroughly. This standard dilution water should be prepared just before use.

Seeding with micro-organisms

The addition of small measured volume of water containing a good bacterial population to the dilution water is called 'seeding'. Seeding is not required for sewage and sewage effluents because they contain the bacterial flora. Seeding is necessary for industrial effluents, which generally do not contain any bacteria. The seeding material that is generally used is fresh and settled raw sewage or final effluent of a good quality from a n aerobic biological process. The seed should be kept for 1 to 5 days at 20°C before use. The seed concentration recommended is 1 to 2 mL per litre of dilution water.

1. Prepare aerated water in a glass container by bubbling the compressed air in distilled water for about 30 min.
2. Add 1 mL each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride for each litre of dilution water and mix properly.
3. Neutralize the sample to pH=7 by using 1N NaOH or 1N HCl depending on the initial medium and pH.
4. If the DO in the sample is likely to be exhausted it is therefore necessary to prepare suitable dilution of the sample according to the expected BOD range.
5. Make dilution in large container, mix the contents thoroughly and fill the contents in 2 BOD bottles.
6. Keep one BOD bottle in BOD incubator at 20°C for 5 days and determine the DO level in other set immediately.
7. Determine DO in the sample and in the blank on initial day and end of incubation period by Winkler method as described for DO measurement.

Calculation

$$\text{BOD, mg/L} = (\text{Initial DO} - \text{Final DO}) \times \text{Dilution factor}$$

Reporting

Dissolved oxygen should be reported in units of mg/L and should include 1 digit after the decimal point, e.g., 8.2 mg/L.

23. TOTAL DISSOLVED SOLIDS

(APHA 2540 C. TDS Dried at 180^0C)

Principle:

A well-mixed sample is filtered through a standard glass fibre filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

Interferences:

Highly mineralized waters with a considerable calcium, magnesium, chloride, and/or sulphate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Samples high in bicarbonate require careful and possibly prolonged drying at 180°C to insure complete conversion of bicarbonate to carbonate. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue.

Instrument/apparatus/equipment

a. Glass-fibre filter disks without organic binder.

b. **Filtration apparatus:** One of the following, suitable for the filter disk selected:

i. Membrane filter funnel.

ii. Gooch crucible, 25-mL to 40-mL capacity, with Gooch crucible adapter.

iii. Filtration apparatus with reservoir and coarse (40- to 60-µm) fritted disk as filter

c. Suction flask, of sufficient capacity for sample size selected.

d. Drying oven, for operation at 180 ± 2°C.

Procedure

i. Take an evaporating dish of suitable size make it clean, dry at 103-105^0C, cool in desiccator. Weigh immediately before use, this is the initial weight (A)

ii. Filter about 100 mL or 250 mL of sample through filter paper (Whatman no. 41) and take the filtrate in the evaporating dish. (**Note:** if more than 10 min are required for complete filtration, decrease the sample volume.)

iii. Evaporate the sample in a hot air oven (or electric oven) at 180^0C $\pm$ 2^0C and after the whole water is evaporated, cool the dish in a desiccator and take the final weight (B).

Calculation

Total Dissolved Solid (TDS) = $\frac{(B-A)\ X1000}{V}$

V = Volume of Sample taken (mL)

A = Weight of dish, mg

B = Weight of dried residue + dish, mg

Reporting

TDS should be reported in units of mg/L and should include 0 digit after the decimal point, e.g., 82 mg/L.

24. TOTAL COLIFORM

(APHA 9221 B. Standard Total Coliform Fermentation Technique)

Principle

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. In terms of fermentation, Enterobacteriaceae group can be defined as facultative anaerobic gram negative, non-spore forming, rod shaped bacteria that ferment lactose with gas and acid fermentation within 48 hours at 35º C. The results will be reported in terms of Most Probable Number (MPN). This number is an estimate of mean density of coliforms in the sample.

Glassware & Equipment

- Fermentation tubes
- Petri plates
- Pipettes
- Glass slides
- Conical flasks
- Autoclave
- Hot air oven
- Water bath
- Incubator
- Laminar air flow
- Microscope

Procedure

This technique will be performed in 3 phases:

a) Presumptive phase

b) Confirmed phase

c) Completed phase

a) Presumptive phase

Use lauryl tryptose broth in presumptive phase of multiple tube test. If the medium has been refrigerated after sterilization, incubate overnight at room temperature (20^0 C) before use. Discard tubes showing growth.

Reagents and culture medium:

Lauryl tryptose broth composition:

Tryptose	20.0 g
Lactose	5.0 g
K_2HPO_4	2.75 g
KH_2PO_4	2.75 g
Sodium chloride	5.0 g
Sodium lauryl sulphate	0.1 g
Distilled water	1 L

Add dehydrated reagents to water mix thoroughly and heat to dissolve. Adjust pH to 6.8 $\pm$ 0.2 after sterilization. Before sterilization dispense 10 mL medium into the fermentation tubes with an inverted Durham's tube. Close tubes with metal or heat resistant plastic caps.

Procedure

I. Three sets of 5 fermentation tubes each containing (2 sets containing single strength and one set containing double strength) lauryl tryptose broth of known concentration (10 mL) should be inoculated with known amount of water sample (0.1ml, 1 mL and 10 mL respectively)

II. Shake all the water samples vigorously before inoculating into the 3 sets of test tubes.

III. Add water samples to the fermentation tubes using sterilized pipettes and mix thoroughly.

IV. Use separate pipettes for different samples as well as for dilutions.

V. While withdrawing sample portions, the tip of the pipette should never be dipped more than one inch below the surface of the water sample.

VI. This procedure minimizes the accumulative drainage from exterior of pipette into the media.

VII. Place all the inoculated fermentation tubes in a water bath at 35 $\pm$ 0.5^0C for 24 h. After 24 $\pm$ 2 h swirl each tube and examine carefully for growth, gas and acid reaction. If no growth

or acid reaction is observed reincubate and re-examine after 48 ± 3 h. record presence or absence of growth, gas and acid production

Interpretation

Production of gas or acid in the tubes within 48 ± 3 h constitutes positive presumptive reaction. Submit positive presumptive reaction tubes to confirmed phase.

b) Confirmed phase

Culture medium

Use Brilliant green lactose bile broth fermentation tubes for confirmed phase.

- ***Medium Composition:***

Peptone	10 g
Lactose	10 g
Oxgall	20 g
Brilliant green	0.0133 g
Distilled water	1 L

Add dehydrated reagents to water, mix thoroughly and heat to dissolve. Adjust pH to 7.2 ± 0.2 after sterilization. Before sterilization dispense 10 mL medium into the fermentation tubes with an inverted Durham's tube. Close tubes with metal or heat resistant plastic caps.

Procedure

i. Submit all positive presumptive reaction tubes within 24 ± 2 h to confirmed phase.
ii. Gently shake all positive presumptive reaction tubes and with a sterile loop 3.0 to 3.5 mm diameter, transfer culture to brilliant green lactose bile broth tubes.
iii. Incubate inoculated tubes at 35 ± 0.5^0C. Formation of gas in the durhams tube at any time within 48 ± 3 h constitutes a positive confirmed phase. Calculate MPN value from table.

c) Completed phase

To establish the presence of coliform bacteria and to provide quality control data, use the completed test on at least 10% of positive confirmed tubes.

1. Culture media and reagents

1.1 LES Endo agar

Medium composition:

Yeast extract	1.2 g
Trypticase	3.7 g
Thiopeptone	3.7 g
Tryptose	7.5 g

Lactose	9.4 g
K_2HPO_4	3.3 g
KH_2PO_4	1 g
NaCl	3 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite	1.6 g
Basic fuchsin	0.8 g
Agar	15 g
Distilled water	1 L

Rehydrate product in 1 L water containing 20ml 95% ethanol. Boil to dissolve agar. Remove from heat and cool to 45-50^0 C. Do not sterilize by autoclave. Adjust pH to 7.2 $\pm$ 0.2. Pour Petri plates and do not expose to sunlight. Store the plates by refrigeration, in dark, for not more than 2 weeks.

1.2 MacConkey agar

Medium composition:

Peptone	17 g
Proteose peptone	3 g
Lactose	10 g
Bile salts	1.5 g
Sodium chloride	5 g
Agar	13.5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Distilled water	1 L

Add ingredients to water, mix thoroughly, and heat to dissolve. Sterilize in autoclave at 121^0 C for 15 min. Temper agar after sterilization and pour into sterile Petri dishes. pH should be 7.1 $\pm$ 0.2 after sterilization.

1.3 Nutrient agar

Medium composition:

Peptone	5 g
Beef extract	3 g

Agar	15 g
Distilled water	1 L

Add ingredients to water, mix thoroughly, and heat to dissolve. Adjust pH to 6.8 ± 0.2 after sterilization. Before sterilization, dispense in screw – caped tubes. After sterilization, immediately place tubes in an inclined position so that agar will solidify with a sloped surface.

Procedure

i. Using aseptic technique, streak one LES Endo agar or MacConkey agar plate from each tube of brilliant green lactose bile broth showing gas. Incubate inoculated plates at 35 ± 0.5^0C for 24 ± 2 h.
ii. The colonies developing on LES Endo agar are defined as typical (pink to dark red with green metallic surface sheen), atypical (pink, red, white or colour less colonies without sheen) after 24 h incubation.
iii. Typical lactose fermenting colonies developing on MacConkey agar are red and may be surrounded by an opaque zone of precipitated bile.
iv. From each plate pick one isolated colony and transfer into single strength lauryl tryptose broth fermentation tube and onto nutrient agar slant.
v. Incubate secondary broth tubes at 35 ± 0.5^0C for 24 ± 2 h. If gas is not produced within 24 ± 2 h, reincubate and re-examine after 48 ± 3 h.
vi. Microscopically examine gram-stained nutrient agar cultures corresponding to secondary tubes that show growth.

Gram stain reagents

Ammonium oxalate-crystal violet:

Dissolve 2 g crystal violet in 20ml 95% ethyl alcohol. Dissolve 0.8 g $(NH_4)_2C_2O_4.H_20$ in 80ml reagent grade water. Mix the 2 solutions and age for 24 h before use. Filter through paper into staining bottle.

Lugol's solution:

Grind 1 g iodine crystals and 2 g KI in a mortar. Add reagent grade water, a few millilitres at a time and thoroughly after each addition until solution is complete. Rinse solution an amber colour bottle with the remaining water (using a total of 300 mL).

Counter stain:

Dissolve 2.5 g of safranin dye in 100 95% ethyl alcohol. Add 10ml to 100ml reagent-grade water.

Acetone alcohol:

Mix equal volumes of ethyl alcohol (95%) with acetone.

Interpretation

Formation of gas in the secondary tube of lauryl tryptose broth with in48 + 3 h and demonstration of gram negative, non-spore forming, rod shaped bacteria from agar culture constitute a positive result for the completed phase, demonstrating the presence of a member of the coli form group.

Calculation of most probable number

The calculation of Most Probable Number of coli forms is done by combination of positive and negative results in multiple tube tests. The values can be calculated for any of the combinations given in Table 3. Refer Table 2 for estimation of MPN, if the tubes of only one sample portion (10 mL) have been used, as is usually done for potable water samples.

The important thing to remember is that the positive and negative combinations can be used of any one test e.g., if a test has been carried out only up to the presumptive test stage, then the positive and negative combinations of this test can be used to calculate the MPN. If all the three tests have been carried out, the MPN can be calculated on the basis of either presumptive or confirmatory or completed tests.

For example, if 5 tubes each of 10ml, 1ml, and 0.1ml sample inoculum portions have been used and results are as follows:

10mL portion-2 tubes positive, 3 tubes negative

1mL portion-2 tubes positive, 3 tubes negative

0.1mL portion-all tubes negative

Combination can be written as 2-2-0 and MPN index according to table will be 9-cells/100mL. If combinations other than those given table appear, then perhaps the test has not been carried out according to instructions.

The table is given for a starting solution 10 mL and 1/10th and 1/100th of it (10, 1 and 0.1 mL of 10mL), the same table can be used, but here the 100, 10 will be supposed as 10 mL 10, 1, and 0.1. This table value will be put in the given formula to get MPN/100 mL

$$\text{MPN/100ml} = \frac{\text{MPN table value X10}}{\text{Starting dilution}}$$

For example, if the results are same, as previously given 2-2-0 but the used are 100mL, 10mL and 1mL, then according to the formula, 100mL will be:

$$\text{MPN/100mL} = \frac{9\text{X}10}{100} = 0.9$$

Table 2: MPN/100mL values when the 5 tubes of only 10mL are used

No. of tubes giving positive results out of 5	MPN/100 mL
0	<2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	>16.0

Table 3: MPN/100mL for various combinations of positive results when 5 tubes each of 10, 1, and 0.1ml sample fractions are used.

Combinations	MPN/100mL	Combinations	MPN/100mL
0-0-0	<1.8	4-1-1	21
0-0-1	1.8	4-1-2	26
0-1-0	1.8	4-1-3	31
0-1-1	3.6	4-2-0	22
0-2-0	3.7	4-2-1	26
1-0-0	2.0	4-2-2	32
0-2-1	5.5	4-2-3	38
0-3-0	5.6	4-3-0	27
1-0-0	2.0	4-3-1	33
1-0-1	4.0	4-3-2	39
1-0-2	6.0	4-4-0	34
1-1-0	4.0	4-4-1	40
1-1-1	6.1	4-4-2	47
1-1-2	8.1	4-5-0	41
1-2-0	6.1	4-5-1	48
1-2-1	8.2	5-0-0	23
1-3-0	8.3	5-0-1	31
1-3-1	10.0	5-0-2	43
1-4-0	10.0	5-0-3	58
2-0-0	4.5	5-1-0	33
2-0-1	6.8	5-1-1	46
2-0-2	9.1	5-1-2	63

2-1-0	6.8	5-1-3	84
2-1-1	9.2	5-2-0	49
2-1-2	12.0	5-2-1	70
2-2-0	9.3	5-2-2	94
2-2-1	12	5-2-3	120
2-2-2	14	5-2-4	150
2-3-0	12	5-3-0	79
2-3-1	14	5-3-1	110
2-4-0	15	5-3-2	140
3-0-0	7.8	5-3-3	170
3-0-1	11	5-3-4	210
3-0-2	13	5-4-0	130
3-1-0	11	5-4-1	170
3-1-1	14	5-4-2	220
3-1-2	17	5-4-3	280
3-2-0	14	5-4-4	350
3-2-1	17	5-4-5	430
3-2-2	20	5-5-0	240
3-3-0	17	5-5-1	350
3-3-1	21	5-5-2	540
3-3-2	24	5-5-3	920
3-4-0	21	5-5-4	1600
3-4-1	24	5-5-5	>1600
3-5-0	25		
4-0-0	13		
4-0-1	17		
4-0-2	21		
4-1-0	17		
4-0-2	21		
4-0-3	25		
4-1-0	17		

Reporting

Report in units of MPN/100 mL and should include 0 digit after the decimal point, e.g., 800 MPN/100 mL.

25. FECAL COLIFORM

(APHA 9221 E. Thermotolerant (Fecal Coliform) Procedure

Principle

Elevated temperature tests for distinguishing organisms of the total Coliforms that also belong to the Fecal Coliform group are described in this procedure. This test differentiates between Coliforms of fecal origin and Coliforms of other origin. The Fecal Coliforms test is applicable to drinking water, stream pollution, raw water sources, and wastewater treatment systems.

Glassware and Equipment

a. Fermentation tubes (test tubes)- 10 mL, 25 mL

b. Durham's tubes

e. Pipettes

c. Incubator with a stable temperature 44.5 $\pm$ 0.2^0C

d. Autoclave or sterilizer

Medium

EC Medium broth pH 6.9$\pm$2.0

Chemical composition of the medium

Tryptose	20.0 g
Lactose	5.0 g
Potassium dihydrogen phosphate	1.5 g
Dipotassium hydrogen phosphate	4.0 g
Sodium chloride	5 g
Bile salts mixture	1.5 g
Distilled water	1 L
pH	6.9$\pm$0.2

Procedure

i. Three sets of 5 fermentation tubes each containing 2 sets containing single strength and one set containing double strength EC broth may be prepared.

ii. Select all positive presumptive fermentation tubes (Total Coliforms) showing any amount of acid or growth or acidity within 48 h of incubation to fecal coliform test.

iii. Gently shake or rotate all the positive presumptive fermentation tubes (Total coliforms). Using a sterile 3 or 3.5 mm diameter metal loop or sterile wooden applicator stick transfer growth from presumptive fermentation tubes to EC broth tubes.

iv. Within 30 minutes of inoculation, incubate inoculated EC broth tubes in water bath maintained at 44.5 $\pm$ 0.2^0 C for 24 $\pm$ 2 h. Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of medium.

v. Submit all positive presumptive fermentation tubes showing any amount of acid or growth or acidity within 48 h of incubation to E. coli test.

Interpretation

Production of gas in the tubes within 24 $\pm$ 2 h constitutes a positive Fecal Coliform reaction**.** Failure to produce gas constitutes a negative reaction. Calculate MPN value.

Report

Total no. of fecal coliforms= MPN/100 mL

Reporting

Report in units of MPN/100mL and should include 0 digit after the decimal point, e.g., 800MPN/100mL.

Note:

- In order to ensure comparability of water quality data across all laboratories of CWC, uniform analysis methods should be adopted. For achieving this, the methods circulated vide Letter no. No. T-100014/4/2019-RD-II DTE, Dt:25.10.2021 to the field offices, consequent upon a technical discussion held on 11.10.2021 on the topic "Uniformity in Water Quality Methodology", may be followed. The circulated table of preferred methods to be adopted for the analysis of each parameter has been given in Table 4.

Table 4: Uniform methods to be followed

Sl No.	PARAMETER	METHOD OF ANALYSIS	
	COLOUR	Visual observation(in-situ) method	Physical parametrs
	ODOUR	Qualitative human receptor method	
1	TEMPERATURE	APHA 2550 B (Laboratory and field Methods)	
2	pH	APHA 4500-H+ B (Electrometric Method)	
3	EC	APHA 2510 B (Laboratory Method)	
4	Total Dissolved Solids	APHA 2540 C (TDS dried at 180ºC)	
5	Turbidity	APHA 2130 B Nephelometric Method	
6	Ammonia	APHA 4500 APHA NH3 - D (Ammonia- Selective Electrode Method)	Chemical parametrs
7	Sodium	APHA 3500 Na-B (Flame Emission Photometric Method)	
8	Calcium	APHA 3500 Ca-B (EDTA Titrimetric Method)	
9	Magnesium	APHA 3500 Mg-B (Calculation Method)	
10	Potassium	APHA 3500 K-D (Flame Emission Photometric Method)	
11	Boron	APHA 4500 B (Curcumin Method)	
12	Carbonate	APHA 2320 B (Titration Method)	
13	Bicarbonate	APHA 2320 B (Titration Method)	
14	Fluoride	APHA 4500 F-C (ISE Method), APHA 4500-D (SPADNS Method)	
15	Chloride	APHA 4500 Cl^- B (Argentometric Method)	
16	Sulphate	APHA 4500- SO_4^{2-} E (Turbidimetric Method)	
17	Nitrate	APHA 4500 NO_3^- D (Nitrate Electrode Method)	
18	Nitrite	APHA 4500 NO2- B (Colorimetric Method)	
19	Silicate	APHA 4500 -SiO_2 C (Molybdosilicate Method), APHA 4500 – SiO_2- D. (Heteropoly Blue Method)	
20	Phosphate	APHA 4500- P E(Ascorbic Acid Method) (as total phosphate)	
21	Dissolved Oxygen (DO)	APHA 4500, O-C (Azide Modification Method)	
22	Biochemical Oxygen Demand	APHA 5210 B (5 day BOD Test)	
23	Chemical Oxygen Demand	APHA 5220 D (Closed Reflux Colorimetric Method), APHA 5220 B (Open Reflux Method-when observed less than 4 mg/L)	
24	Total Coliform	MPN method	Biological parametrs
25	Fecal Coliform	MPN method	
26	Arsenic	By AAS/ICP-MS(Discussion proposed after six months of operation of ICP-MS)	Heavy metals
27	Cadmium		
28	Chromium		
29	Copper		
30	Lead		
31	Iron		
32	Mercury		
33	Nickel		
34	Zinc		
35	Alpha, Beta or Gamma BHC	By GC-MS(Discussion proposed after six months of operation of GC-MS)	Pesticides
36	O,P' and P,P' DDT		
37	Aldrin, Dieldrin		
38	Alpha and Beta Endosulfan		
39	Carbaryl(Carbamate)		
40	Malathion, 2-4 D, Methyl Parathion		
41	Anilophos, Chlorpyrifos		

Time Required for Analysis

*Estimated for a batch of 50 river water samples

Analysis time in mins(for a batch of 50 samples)							
S.No.	**Parameter**	**Sample preparation+ standardisation +calibration**	**Sample Analysis**	**Record& Calculation**	**Total time (minutes)**	**Manpower**	**Remarks**
1	pH	90	120	30	240	2	
2	Electrical Conductivity	90	120	30	240	2	
3	Dissolved Oxygen	200	200	60	460	2	
4	Turbidity	90	120	40	250	2	
5	Biochemical Oxygen Demand	300	240	60	600	2	*Incubation time:5 days
6	Chemical Oxygen Demand(closed reflux method)	90	90	60	240	2	*Preheating:30 min+Heating:120 min+cooling:30 min, for a digestor of 25 holes: need to be digested in 3 batches
	Chemical Oxygen Demand(open reflux method)	1060	250	60	1370	2	*Preheating:30 min+Heating:120 min+cooling:30 min for a digestor of 6 holes, need to be digested in 9 batches
7	Total Dissolved Solids	480		60	540	2	*include pre-heating of dishes, filtration, heating, cooling,weighing
8	Total hardness	120	120	60	300	2	
9	Calcium	120	120	60	300	2	
10	Magnesium	0	0	30	30	1	*calculaion method
11	Sodium	120	120	60	300	2	
12	Potassium	120	120	60	300	2	
13	Carbonate	120	120	60	300	2	
14	Bicarbonate						
15	Alkalinity						
16	Chloride	120	120	60	300	2	
17	Sulphate	120	150	60	330	2	*Wait 5 min after stirring
18	Fluoride(ISE)	120	120	60	300	2	*ISE:Ion Selective electrode method
	Fluoride(UV-VIS)	180	180	60	420	2	*UV-VIS:using uv-visible spectrophotometer
19	Boron	540		60	600	2	*heating time:80-90 min for each sample, 90 min for heating×5 batches in a water bath of 12 holes, 5 min for preparation, 3 min for analysis of each sample
20	Ammoniacal Nitrogen(ISE)	120	120	60	300	2	*ISE:Ion Selective electrode method
	Ammoniacal Nitrogen(UV-VIS)	180	180	60	420	2	*time for color development:minimum 1 hour, UV-VIS:using uv-visible spectrophotometer
21	Nitrate-N (ISE)	120	120	60	300	2	*ISE:Ion Selective electrode method
	Nitrate-N (UV-VIS)	180	180	90	450	2	UV-VIS:using uv-visible spectrophotometer, absorbance to be measured at 220 nm & 275 nm both
22	Nitrite-N	180	180	60	420	2	*Wait 10-120 min after addition of color reagent to sample
23	Phosphate	240	180	60	480	2	*Wait 10-30 min after addition of reagents to sample
24	Silicate	180	180	60	420	2	*Wait 5 min after addition of reagents to sample
25	Total Coliform	6 working days				2	*Involves preparation, sterilization, innoculation, incubation, presumptive, confirmation& completed phases, sterilisation& disposal after testing
26	Fecal Coliform						

****Given above is the estimated time for analysis only; actual time taken may vary according to the expertise/efficiency/proficiency of analysts and equipment/instruments used.**

Appendix: II

Activities in Level II laboratory for a cycle of 8 days

- Assumed that samples are being received in the lab thrice in a month, in interval of 10 days and being analysed in a cycle of 8 days (24 working days, considering 6 holidays in a month including Sundays, second Saturday and one general holiday).
- Assumed that laboratory is NABL accredited for all parameters.
- **Manpower needed for analysing 50 samples in 8 days:**

Level II lab	
Research Officer, RO	01
Assistant Research Officer, ARO	02
Senior Research Assistant, SRA	04
Research Assistant, RA*	03
Lab Assistant, LA* / Skilled Work Assistant	01

*Contractual staff

- Assumed that all 50 samples are being received in the lab on 1st and 2nd day of the cycle. If any sample is arriving in the lab other than the first 2 working days, the given cycle will not be followed, they will be analysed as per convenience and time sensitivity of parameters.
- Time sensitive parameters:
  - pH, DO, BOD, Total Coliform/Faecal Coliform, Alkalinity, Turbidity (done as soon as possible after receipt in the lab)
  - Nitrate, Nitrite, NH_3-N, Phosphate (within 48 hours)
  - COD, TDS (within 7 days of collection)

- The activities listed here are related to the laboratory analysis work and data entry in WIMS only. The additional works are listed at Appendix: III.
- Manpower required is estimated for laboratory activities only. For other activities listed under Appendix: III, additional manpower and time are required.
- Analysis involves preparation of reagents, sample preparation, standardisation of solutions, calibration of instruments, analysis of samples, replicate tests, filling of instrument log registers, record of CRMs/standards, filling of consumption records, record in a single register etc.

Activities for Level II laboratory

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 1&2	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_2+ RA_2 (30 minutes twice in a day)
	Receiving of WQ samples from sample messenger (from field offices) after proper checking w.r.to sample quantity, sample condition and container of different type samples (like general analysis sample, BOD sample, microbiological sample, sample for pesticide/heavy metal analysis etc.), verification of sample collection details form, filling of analytical request form, Coding & Decoding of Samples, issue of job card, filling of sample receipt record, files etc.	RO+ARO_1+LA (Whole day)
	Calibration of field equipment received from sample messengers, preparing & providing containers / chemicals / reagents to messengers, training sample messengers regarding sampling/sample transportation as needed.	SRA_1+ ARO_2 (3 hours a day)
	Analysis of pH	SRA_1+ RA_1 (2 hours a day)
	Analysis of DO and BOD (2 batches for BOD: -**Batch I**: incubated on 1st and **Batch II**: incubated on 2nd)	SRA_2+ RA_2 (6 hours a day)
	Analysis of TC/FC	SRA_3+ RA_3 (Whole day)
	Analysis of EC	SRA_4+ ARO_2 (2 hours a day)
	Analysis of Alkalinity (total alkalinity, carbonate, bicarbonate)	SRA_4+ RA_1 (2.5 hours a day)
	Analysis of Turbidity	SRA_4+ RA_1 (2.1 hours a day)
	Preparation/filling of various registers/templates, intermediate checks of instruments/standards.	SRA_1+ ARO_2 (2 hours a day)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 3	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_3+ RA_3 (30 minutes twice in a day)
	Analysis of nitrate (assumed that analysis done by Ion-Selective Electrode Method).	ARO_2+SRA_4 (5 hours)
	Analysis of nitrite.	SRA_1+RA_1 (7 hours)
	Analysis of TC/FC	SRA_3+RA_3 (whole day)
	Intermediate checks of instruments/standards.	ARO_1+SRA_4 (2 hours)
	Necessary checking, maintenance& filling of NABL documents/ records.	RO +ARO_1 (Whole day)
	Entry of analysis results of WQ in WIMS/SWDES.	SRA_2+ RA_2 (4 hours)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_2+ RA_2 (3 hours)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	ARO_2+LA (2 hours)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 4	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_2+RA_1 (30 minutes twice in a day)
	Analysis of TC/FC	SRA_3+RA_3 (whole day)
	Analysis of Total Dissolved Solids.	SRA_4+ RA_1 (5 hours)
	Analysis of Phosphate.	SRA_2+ RA_2 (6 hours)
	Analysis of COD, 2 batches (assumed that analysis done by closed reflux method), entry of analysis results in excel sheets, calculation, entry in data registers, etc.	ARO_1 + SRA_1 (6 hours)
	Checking, preparation& maintenance of NABL documents	RO+ARO_2 (Whole day)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_4+ RA_1 (2 hours)
	Intermediate checks of instruments/standards.	SRA_1+ ARO_2 (3 hours)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 5	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_3+RA_2 (30 minutes twice in a day)
	Analysis of TC/FC	SRA_3+RA_3 (whole day)
	Analysis of Total Dissolved Solids.	SRA_4+ RA1 (4 hours)
	Analysis of COD, 3rd batch (assumed that analysis done by closed reflux method).	ARO_1+ SRA_4 (4 hours)
	Analysis of Ammoniacal nitrogen (assumed that analysis done by Ion-Selective Electrode Method).	SRA_2+ ARO_2 (5 hours)
	Analysis of phosphate.	SRA_2+ RA_2 (2 hours)
	Analysis of sodium & potassium.	SRA_1+ RA_2 (3hours)
	Supervision of laboratory activities, maintenance of NABL documents, checking of registers/records related to analysis, preparation of various reports.	ARO_1+RO (Whole day)
	Entry of analysis results of WQ in WIMS/SWDES.	SRA_1+ RA_1 (3 hours)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	ARO_1+ RA_2 (1 hour)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_1 (1 hour)
	Intermediate checks of instruments/standards.	ARO_2 +LA (2 hours)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 6	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_2+LA (30 minutes twice in a day)
	Analysis of sodium & potassium, entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_1+ RA_2 (7 hours)
	Analysis of boron.	ARO_1+ RA_1 (1 hour)
	Analysis of fluoride (assumed that analysis done by Ion-Selective Electrode Method)	SRA_4+ ARO_2 (5 hours)
	Analysis of TC/FC, corresponding entry of analysis results in excel sheets, calculation, entry in data registers, etc., disposal of remnants.	SRA_3+RA_3 (whole day)
	Analysis of BOD (Batch I; after incubation:).	SRA_2+ RA_1 (3 hours)
	Preparation& maintenance of NABL documents/records, checking of registers/records, supervision of and guidance to overall lab activities.	ARO_1+ RO (Whole day)
	Entry of analysis results of WQ in WIMS/SWDES.	ARO_2+LA (2 hours)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_2+ RA_1 (3 hours)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_4 (2 hours)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 7	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_3+LA (30 minutes twice in a day)
	Analysis of total hardness, calcium.	SRA_2+ RA_1 (4 hours)
	Analysis of boron and corresponding entry of analysis results in excel sheets, calculation, entry in data registers, etc.	ARO_1+ SRA_3 (7 hours)
	Analysis of silicate.	SRA_1+ RA_3 (7 hours)
	Analysis of BOD (Batch II; after incubation).	SRA_2+ RA_1 (3 hours)
	Intermediate checks of instruments/standards.	SRA_4+RA_2 (3 hours)
	Preparation& maintenance of NABL documents, supervision of overall lab activities, checking of data calculation sheets etc.	RO+ARO_2 (Whole day)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	ARO_2+RA_2 (4 hours)
	Entry of analysis results of WQ in WIMS/SWDES.	SRA_4+ LA (3 hours)
	Cleaning of glassware/utensils and assistance in various activities.	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Day	Activities in Level II lab for a cycle of 8 days	Manpower/Time
Day 8	Checking of instruments and equipment, supervision of cleaning & sanitization of laboratory premises, filling of necessary documents as per NABL clause no.6.3 (Facilities & Environmental condition) for each and every room/section of laboratory etc.	SRA_3+ RA_3 (30 minutes twice in a day)
	Analysis of boron.	ARO_1+RA_3 (2 hours)
	Analysis of total hardness, calcium.	SRA_2+ RA_2 (6 hours)
	Analysis of sulphate.	SRA_1+ RA_1 (6 hours)
	Analysis of chloride.	SRA_3+ LA (5 hours)
	Intermediate checks of instruments/standards.	ARO_1+RA_3 (3 hours)
	Checking of registers and results, allotment of samples for spike test, retest, blind test etc., preparation& maintenance of NABL documents, supervision of overall lab activities.	ARO_1+ RO (Whole day)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_2+ RA_2 (1 hour)
	Entry of analysis results in excel sheets, calculation, entry in data registers, etc.	SRA_1+ RA_1 (1 hour)
	Retest, spike test, blind test etc. needed for the quality control.	ARO_2+SRA_4 (Whole day)
	Cleaning of glassware/utensils and assistance in various activities	LA (Whole day)

*Whole day: working hours (9:30-17:00 hrs)

Note: The digestion of metals and extraction & concentration of pesticides from river water samples shall be carried out at Level-III laboratories. But right now, the methods for the same have not been included in the SOP. For these activities, additional time/manpower/equipment/chemicals/apparatus are needed.

Appendix: III

Other Activities Involved (in addition to analysis)

Sl. No.	Work	Person involved	Remarks
1	Data entry, data validation and preparation of WQ bulletins & Test Reports	SRA/ARO/RO	The analysis results are entered, primary validation is carried out and monthly water quality bulletins and test reports in the prescribed format are being prepared.
2	Handling of correspondence related to Laboratory	RO/ARO/SRA	Any correspondence regarding activities to be handled or any other information related to laboratory has to be shared as and when required by the higher authority.
3	Maintenance of instruments in the laboratory	RO/ARO/SRA	Each and every instrument/equipment including sophisticated instruments need to be maintained properly. Explicit time is required for the processes such as external/internal calibration, repairing works, replacement of parts/accessories, preventive maintenance etc. of them.
4	Purchasing of Chemicals, Glassware, equipment, instruments etc.	RO/ARO/SRA	Stock checking, record keeping and purchasing of a large number of Chemicals/Glassware/instrument/equipment /other laboratory items for smooth functioning of the lab are being carried out regularly. It involves preparation of specifications/quotation and checking of incoming materials etc.
5	Record keeping & management of data of Sediment analysis	RO/ARO/SRA/RA	The analysis results received from the field offices are maintained by the laboratory. The results are entered in the software WIMS and SWDES.
6	Bed material sample analysis.	RO/ARO/SRA/RA	The bed material samples are analysed by 13 laboratories, samples being received thrice in a year: during pre-monsoon, monsoon and post- monsoon. The complete analysis includes gradation/separation using Siltometer& Sieve shaker, further heating, weighing etc and calculation.

7	Regular updation and handling of NABL documents for Internal Audit, Management Review Meeting and preparation for NABL audit.	RO/ARO/SRA/RA	Regular updating of NABL documents, conduction of internal audit (at least once in a year), management review meeting, surveillance audit etc.
8	Participation in/guidance to NABL activities of other laboratories.	RO/ARO	The senior officers give guidance to other laboratories for NABL accreditation and get deputed for internal auditing/assessment.
9	Participation in AQC exercises such as Proficiency Testing and Inter laboratory Comparison (ILC) exercise	RO/ARO/SRA/RA	According to clause 7.7 of NABL participation in Inter Laboratory Comparison (ILC) program or Proficiency testing program is mandatory requirement. The labs are participating in PT programme and ILC exercises by NABL accredited PT providers/CWC laboratories. Level III labs organise AQC exercise.
10	Publication of Water quality year book, Suspended Sediment Year book and Bed material year book	RO/ARO/SRA/RA	Water Quality Year Book: It is prepared for every basin and contains validated data, analysis thereof for a water year (June-May) and comparisons with past water years. The analysis results of a water year are compiled basin-wise, computed, analysed, report is prepared in the prescribed format and sent to the higher authority/circle office each year. Sediment/bed material year book: Data is compiled and sent to the higher authority/circle office.
11	Preparation of Reports/Papers/Presentation	RO/ARO/SRA	Various reports on WQ of rivers are being prepared as and when needed, with the direction of higher authority/HQ.
12	WQ Site visit for inspection and Training, WQ sampling	RO/ARO/SRA	The sampling sites are visited for giving proper training & instructions to the site staff regarding sampling/preservation/transport of samples as well as analysis of in-situ parameters.
13	Presentation in Training, Workshop etc.	RO/ARO	Senior officers attend and give lectures/training conducted by various agencies.

14	Participation in meetings/workshops/ training programs.	RO/ARO/SRA	The scientific officers participate in trainings, workshops etc. related to WQ analysis/NABL accreditation time to time.
15	Data required by external agencies related to WQ/sediment.	RO/ARO/SRA	Various agencies request historical data related to WQ/Sediment. data has to be provided as per the direction of higher authority.
16	Proper preservation, packaging and dispatch of samples for heavy metal and pesticide analysis to Level III labs.	RA/SRA/ARO/RO	Properly preserved samples for heavy metal and pesticide analysis are being sent to designated Level III labs.
17	Special studies and sample analysis, preparation of reports/study reports thereof.	RA/SRA/ARO/RO	Water samples from particular sites/rivers are being collected, analysed and reports submitted in emergency situations as directed by the higher authority.
18	Handling of PI, T&P, Chemical, Glassware and Stationary charges	SRA/ARO	The charges of T&P items, chemicals/glassware/other stationary items related to lab activities.
19	CMIS sample analysis for particle size and specific gravity	RO/ARO/SRA/RA	Coastal Management Information System (CMIS) samples are being analysed by 2 laboratories: Kochi and Chennai for Particle size & specific gravity, 6 samples from 3 sites each are being analysed since June, 2021.

Appendix: IV

Chemicals / Instruments / Equipment Required for Analysis

Sl. No.	Name of water quality Parameter	Instruments/equipment used in parameter analysis	Chemical/Reagents/Consumables required
1	Temperature	Thermometer	--
2	Turbidity	Turbidity meter	Formazin, 4000 NTU(CRM)
			hydrazine sulphate, $(NH2)_2.H_2SO_4$
			hexamethylenetetramine, $(CH_2)_6N_4$
3	pH	pH meter	Buffer Solution pH= 4, 7, 10(CRMs)
4	Electrical Conductivity	Conductivity meter	KCl (CRM)
5	Total Dissolved Solids (TDS)	Electric oven, digital balance, filtration apparatus	Whatman no. 41 filter paper
6	Total Hardness	Digital burette	EDTA disodium salt
			Calcium carbonate (CRM)
			Magnesium sulfate ($MgSO_4.7H_2O$)
			Ammonium chloride
			Ammonium hydroxide
			Sodium chloride
			Eriochrome Black T
			Methyl red indicator
7	Calcium Hardness	Digital burette	EDTA disodium salt
			Calcium carbonate (CRM)
			Sodium hydroxide
			Sodium chloride
			Murexide (Ammonium purpurate)
8	Magnesium Hardness	By Calculation	--
9	Total Alkalinity, Carbonate, Bicarbonate	Digital burette	Sulphuric Acid
			Sodium Carbonate (CRM)
			Phenolphthalein
			Methyl Orange/bromocresol green
			Ethanol
10	Chloride	Digital burette	Silver Nitrate
			Potassium Chromate
			Sodium Chloride (CRM)
11	Chemical Oxygen Demand (COD)	1. Closed Reflux Method: COD Digester, colorimeter	COD Vials with pre-mixed reagents, Potassium hydrogen phthalate (CRM)
			Potassium dichromate
			conc.sulphuric acid

		2. Open Reflux Method: Reflux apparatus, condenser, burette	Ferroin indicator
			Ferrous ammonium sulphate
			Mercuric sulphate
			Silver sulphate
12	Dissolved Oxygen (DO)	Digital burette	Potassium Iodide
			Starch
			Potassium bi-iodate (CRM)
			Sodium Azide
			Sodium Hydroxide
			Mangnous Sulphate ($MnSO_4.4H_2O$)
			Sulphuric Acid
13	Biochemical Oxygen Demand (BOD)	BOD incubator, Digital burette	Glucose
			Glutamic Acid
			Potassium Iodide
			Starch
			1000 mg/L BOD solution (CRM)
			Potassium bi-iodate (CRM)
			Sodium Azide
			Sodium Hydroxide
			Manganous Sulphate ($MnSO_4.4H_2O$)
			Ferric Chloride
			Calcium Chloride(anhydrous)
			Disodium hydrogen phosphate
			Sulphuric Acid
			Monopotassium phosphate (KH_2PO_4)
			Dipotassium phosphate (K_2HPO_4)
			Ammonium chloride
			$MgSO_4.7H_2O$
			Sodium thiosulphate (Hypo)
14	Nitrite	uv-visible spectrophotometer, micropipette	NEDA (N-(1-naphthyl)-ethylenediamine dihydrochloride)
			Sulfanilamide
			O-Phosphoric acid 85%
			Sodium oxalate
			Ferrous ammonium sulfate
			Sodium nitrite, $NaNO_2$
			Potassium permanganate, $KMnO_4$
			1+1 H_2SO_4

15	Boron	uv-visible spectrophotometer, micropipette, water bath	Hydrochloric acid
			Curcumin
			Oxalic acid
			Ethanol
			Boric acid, H_3BO_3, anhydrous
16	Phosphate	uv-visible spectrophotometer, micropipette	Ascorbic acid
			Potassium Antimonyl Tartrate
			Ammonium Molybdate
			Sulfuric acid
			anhydrous KH_2PO_4
17	Sulphate	Nephelometer, Magnetic stirrer, Stop Watch, Magnetic Bar, retriever, Micro pipette	Magnesium Chloride
			Sodium acetate
			Acetic Acid
			Potassium Nitrate
			anhydrous Na_2SO4
			$BaCl_2$
			Sulphate CRM (1000 mg/L)
18	Ammonia (UV-VIS)	uv-visible spectrophotometer, micropipette	liquified phenol (≥89%)
			Sodium nitroprusside
			Trisodium citrate
			Sodium hydroxide
			Sodium hypochlorite, commercial solution, about 5%
			anhydrous NH_4Cl (dried at 100°C)
	Ammonia (ISE)	Ion meter, ammonia Electrode, Micro pipette, Magnetic stirrer, Magnetic Bar, retriever	Ammonia free water
			Sodium hydroxide, EDTA
			Ammonium chloride
19	Fluoride (ISE)	Ion meter, Fluoride Electrode, Micro pipette, Magnetic stirrer, Magnetic Bar, retriever	Fluoride CRM 100 ppm
			Fluoride REFERENCE filling solution
			CDTA
			sodium chloride
			sodium hydroxide, 6N
			Acetic acid 100%
			anhydrous sodium fluoride
	Fluoride (UV-VIS)	uv-visible spectrophotometer, micropipette	Sodium fluoride
			SPADNS reagent, zirconyl chloride octahydrate, conc.HCl

20	Nitrate (ISE)	Ion meter, Nitrate Electrode, Micro pipette, Magnetic stirrer, Magnetic Bar, retriever	Nitrate CRM 1000 ppm AS NO_3-
			Nitrate filling solution
			Aluminium sulfate. 18 H_2O
			Boric acid
			Silver sulfate
			Sulfamic acid
			Sodium hydroxide, 0.1 N
			Ammonium sulfate
	Nitrate (UV-VIS)	uv-visible spectrophotometer, micropipette	Potassium nitrate, Chloroform, hydrochloric acid
21	Sodium	Flame Photometer, LPG cylinder, Micro pipette	Sodium chloride CRM
22	Potassium		Potassium chloride CRM
23	Silicate	uv-visible spectrophotometer, micropipette	Ammonium Molybdate
			Sulfuric acid
			HCl
			Na_2SO_3
			$NaHSO_3$
			Oxalic acid
			1-amino-2-naphthol-4-sulfonic acid
			Sodium hexafluorosilicate
24	Total Coliform & Fecal Coliform	Bio safety Cabinet., Hot Air Oven, Microscope, Water Bath, Microbiology Incubator, pH Meter, Fumigation Machine, Micro pipettes	Lauryal Tryptose(M080-500Gram)
			Brilliant Green Bile Broth 2%
			Nutrient Agar pH 7.0 Granulated
			S. D. Agar
			Tryptic Soya Broth
			Nutrient Broth M002
			Swab transport MS684A with peptone water
			Ampules Biological Indicator
			LES m-endo Agar
			Steriswift Cleaning wipes, Mask, Petridish, Virosil / peroxide Silver, Gram Stain Kit K001, pH buffer solutions: pH- 4, 7, 10, Alcohol
			Durham tubes
			Petriseal, Cotton Roll, Aluminium Foil, Head Disposal cap, Shoe Cover, Disposal Apron, Micro Tips, Petri Plate, Glass slides, Poly bags, Hand Sanitizer, Gloves
			Hi flexi loop
			Bacillus Steriothermophillus(autoclave)
			Reference Cultures
			Germitol
			Hi Staph Identification Kit KB004, KB001

Other than these, equipment/instruments for various general purposes like air conditioner, hygrometer, double distillation apparatus, water bath, digital balance, UPS/generator for uninterrupted power supply, electric oven, fume cupboard, hot plate, fire extinguishers & eye/body shower for safety, deep freezer/refrigerator for sample storage, desiccator, analytical weight box, trolly for sample movement, vacuum pumps and filtration assembly, bottle top dispensers, centrifuge etc. are also needed.

Note:

- The digestion of metals and extraction & concentration of pesticides from river water samples shall be carried out at Level-II laboratories. But right now, the methods for the same have not been included in the SOP. For these activities, additional manpower/equipment/chemicals/apparatus are needed.

Appendix: V

Validation of Water Quality Data

Validation of Water Quality Data in Laboratories

Primary validation of water quality data is done by the laboratory where the water samples are analysed and analysis results entered.

Prior to primary validation, results and entries should be again inspected for complete information and errors. Make sure that data entry and typing mistakes are not there, check for the significant figures and fractional digits. Check whether result is within the detection limits of the method of analysis and within the expected range of a parameter.

1. Primary Validation

- Ion balance between major cations and major anions should not exceed 5 %.

  $$\text{Ionic Balance} = \frac{[\text{Total Cations} - \text{Total Anions}]}{[\text{Total Cations} + \text{Total Anions}]} * 100$$

  all concentrations in milli equivalents/litre (m.eq/L)
- Sodium and Chlorine ratio – the ratio should be between 0.8 and 1.2(concentrations in milli equivalents/L).
- Total Dissolved Solids (TDS) and Conductivity (EC) ratio – the ratio should be between 0.55 and 0.9
- COD and BOD ratio – COD values should always be higher than BOD values
- Carbonate and pH relationship – carbonate should be zero for pH values below 8.3
- Total coliform and fecal coliform ratio – TC values should be higher than FC values
- Total hardness = Ca hardness + Mg hardness
- The value of measured EC should be close to the value of calculated EC.
- TDS calculated and TDS measured ratio – the ratio should be between 1.0 and 1.2
- Presence of nitrate in the absence of dissolved oxygen may indicate an error since nitrate is rapidly reduced in the absence of oxygen.

Primary validation of analysis results is required to be completed immediately, preferably within one week. This time schedule ensures that any obvious problems (e.g., indicating an instrument malfunction, observer error etc.) are spotted at the earliest opportunity and resolved. Other problems may not become apparent until more data have been collected, and data can be viewed in a longer-term context during secondary validation.

2. Secondary validation

Secondary validation is largely concerned with detecting outliers. An outlier is an observation that does not match the pattern of earlier observations. They may be identified by using the historical dataset for a particular parameter for a particular station using control

charts/graphical analysis (Refer Chapter 5: Water Quality Handbook: Sediment and Water Quality, HP II, Indian Hydrology Project, May 2014).

Outliers in water quality datasets may occur due to practical mistakes or instrumental failure in all aspects of water quality sampling and analysis: from sample collection, transport, storage, analysis or data entry. They may also result from transcription or data entry errors, or can be the result of instrument breakdowns, calibration problems or power failures.

However, outliers may be an indication of a true change in the system under study, such as an accidental spill on a river stretch. The presence of one or more outliers within a data set may greatly influence any calculated statistics and yield biased results, so outliers should be identified, flagged and, if truly erroneous, possibly removed from a dataset.

If outliers are identified,

i. The value may be confirmed by rechecking/recalculation. If an obvious mistake is detected, the correct data value may be entered, if available. Remove from the dataset, if they are truly erroneous and retesting of sample is not possible.
ii. If retesting is possible, outliers may be removed by reanalysis of the sample in the laboratory itself.
iii. If outlier is not removed by step ii, the sample may be sent to the nearest laboratory of CWC.
iv. If the value is confirmed; outlier is not removed by step iv also, the values may be entered in the WQ bulletin; flagged. The probable reason of the unusual value may be indicated in remarks.

Source:

Water Quality Handbook: Sediment and Water Quality, HP II, Indian Hydrology Project, may 2014

Reference

1. Standard Methods for the Examination of Water and Waste Water, APHA, AWWA, WEF, 23rd Edition, 2017
2. Uniform Protocol on Water Quality Monitoring Order, 2005, MoEF &CC
3. Draft Guidelines on Water Quality Monitoring, 2017, CPCB
4. Field Water Analysis Manual, January 2020, CWC
5. Water Quality Activities of Central Water Commission, CWC
6. Manual on Analysis of Water and Waste Water, 2nd Edition, CWC, December 2000
7. Water Quality Handbook: Sediment and Water Quality, HP II, Indian Hydrology Project, May 2014
8. Gauging the Ganga: Guidelines for Sampling & Monitoring WQ, Centre for Science & Environment
9. Manual of Methods of Analysis of Foods: Water, FSSAI, 2016
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11. Course material of Induction Training Programme for newly appointed SRAs of CWC by NWA, CWC, 2018
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TOTAL ALKALINITY


TABLE OF THE ELEMENTS-LONG FORM


HOT PLATE