Laboratory Manual

on

Poor Quality Water Analysis

ICAR-Central Soil Salinity Research Institute

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PREFACE

Annual demand for food, fiber, fodder and bio-energy in African-Asian Rural Development Organization (AARDO) member developing countries is growing at the rate of 3.7%. Land and water degradation are the two principal constraints in achieving the desired growth rate. Asia suffers from the maximum extent of salinisation covering 52.7 mha (69%) followed by 14.8 mha (19%) in Africa as compared to the minimum 3.8 mha (5%) in Europe. Asia exploits 67% of the total groundwater used for agriculture in the world and Africa has only modest reserves of groundwater. Gradual decline in surface water resources due to contamination, climatic change and other hazards causes additional stress on the available groundwater resources of AARDO nations in semi-arid and arid regions. On the other hand, over-exploitation of groundwater resources has led to continuous deterioration in quality. Further, expected rise in temperature with changes in climate is likely to increase salinisation of land and water resources. Contamination of water resources from point and non-point sources has also aggravated with urbanization and industrialization. These factors and fast emerging competition from other sectors of economy challenge the availability and accessibility of fresh water resources to agriculture. Thus the farmers are forced to use poor quality groundwater and wastewater for irrigation posing serious implications to agricultural production, sustainability of natural eco-systems and increased health problems.

The indiscriminate and unscientific use of such water although pose grave threat to natural resources, crop production and human health; but their rational use by adopting suitable time tested agro-practices can supplement shortage of fresh water and conserve their nutrient potential. However, characterization of such poor quality water is the first and foremost step for making their optimal use. For this purpose, the standard procedures of detecting a range of water quality parameters used to judge its suitability for irrigation, nutrients and toxic substances and public health have been compiled in this laboratory manual. We hope that this laboratory manual will help in building the capabilities and capacity of water resources managers and implementers to optimize nutrient and irrigation use potential of poor quality water in safe manner, augmenting the fresh water supplies to agriculture and thus food production in all developing AARDO nations.

Since this laboratory manual is specifically compiled for capacity building of AARDO member countries; we are highly grateful to AARDO for sponsoring and Ministry of Rural Development, Govt. of India for financing the international training programmes at Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal. We are obliged to Director, CSSRI, Karnal who has been a source of inspiration, guidance and encouragement in conducting the international capacity building programmes and compilation of this laboratory manual.

– Authors

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1. Collection of Samples

Irrigation is an important activity in crop production. Long term application of this water affects the soil property depending upon the quality of water used for irrigation. Concentration of soluble salt, alkalinity or excess of any particular element leads to the accumulation and related problem in crop production. Hence, quality should be determine and its suitability should be established before planning for long term use of any water resource.

Water sampling is a vital part in analysis of the water for its quality appraisal for agricultural and environmental applications. Therefore, water sampling program starts with collections of samples which accurately represent the characteristics of the bulk material and handled conveniently in the laboratory while still providing test results. The major source of error in the whole process of obtaining water quality information often occurs during sampling. Over 50 % of the faulty data that occur in laboratory test results are due to sampling error, rather than during laboratory analysis. Adoption of the standard recommended practices helps in minimization of the sampling error.

Selection of the sampling containers

- Containers should be examined for cleanliness, ensuring it is strong and durable, so that it will not break in transit and that the cap does not leak once it is secured.
- Containers must not contain any of the compounds that samples are to be analyzed for.
- Container must be of the appropriate size.
- Containers must be high density polyethylene or glass containers with Teflon® lid liners for most analyses.
- Rubber and cork stoppers must not be used so as to avoid the risk of contaminating the sample.

Water sampling techniques

For collection of water sample following method may be adopted:

- Glass or plastic bottles thoroughly cleaned and rinsed 3-4 times with the water to be tested should be used for collection of samples. The bottle should not be washed with detergents or soaps.
- Take about 500 mL of sample after runing the tube well or hand pump for about 15-20 min. to drain out the water retained in the pipe.
- If a new tube well boring is in progress, collect water samples at different depths at intervals of about 3-4 m. Since the water in such a situation is always turbid, with suspended impurities, it should be collected as such without caring for turbidity, as it does not affect the test results.
- From a tank or pond collect the sample from at least 5 to 10 m away from the boundaries after displacing surface water, which might contain organic material floating over it.
- From a well, the sample can be drawn either during irrigation, just before the water falls in the channel or by drawing it with the help of a bucket or any other clean container using a rope. The water surface should be disturbed a little to remove any floating material before collection of the sample.
- Place the cap on the bottle tightly.
- Write the name, address, sample no., identification mark etc. on the bottle.
- Separately provide following information on the sheet of paper:
    - Name of the crop to be grown
    - Texture of soil to be irrigated,
    - Previous experience about the effect of the water on soil surface,
    - Previous test results if any,
    - Other sources available for irrigation, and
    - Crop performance so far.
- Sample should be analysed preferably within 2-3 days of collection of the sample.
- It is desirable to send both soil and water samples together for testing, as it helps in making the test reports and recommendations more fruitful.

2. pH

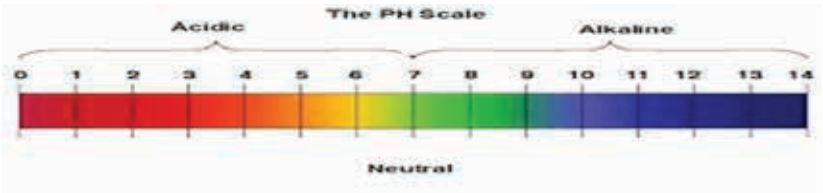
pH of saline irrigation water, is not very much important since presence of neutral salts keeps it around 7.0. However the pH of bicarbonate waters is usually more than 7.5, its determination is very important because it may reflects the degree of sodicity in the water samples.

Principle

pH is the negative logarithm of hydrogen ions concentration. It is expressed as: $pH = -\log_{10} (H^+)$

If pH below 7, the H^+ ion concentration is exceeds to OH^- and range is acidic. When the OH- concentration is more than H^+, pH lies between 7 to 14 (alkaline range).

The scale of measurement is from 1 to 14 on a pH scale:



Equipment and Apparatus

pH meter and 100 mL beakers

Reagents

- **Buffer solutions (pH 4 and 9.2)**

pH 4.0: Prepare stock solution of 0.3 *M* potassium hydrogen phthalate by dissolving 15.3 g of the analytical grade salt in about 225 mL of hot water, cool the solution and dilut to 250 mL. Add a drop of toluene to discourage growth of micro-organisms. For the standard buffer pH 4.0 mix 100 mL of the stock solution with 500 mL water. Prepare a fresh solution every week.

pH 9.2: Dissolve 3.81 g sodium tetraborate (AR) in water and dilute to 1000 mL.

Procedure

1. Calibrate the pH meter
2. Take a 50 mL water sample in 100 mL flask, Put the combined electrode in the water sample and take the reading after 30 seconds.
3. Remove the combined electrode from the sample and rinse thoroughly with distilled (DI) water in a separate beaker and carefully dry excess water with a tissue.

Practical suggestions

- Make sure that the combined electrode contains saturated KCl solution and some solid KCl.
- Allow the pH meter to warm for 10 minutes before recording the pH.
- Never allow the lower portion of glass electrode to touch the bottom of the beaker.

3. Electrical Conductivity (EC)

Electrical Conductivity (EC) is the measure of the ability of a solution to carry an electric current or the concentration of soluble salts in the sample at any particular temperature. The EC measurement is affected by dissolved CO_2, turbidity, temperature and the nature of various ions and their relative concentration.

Equipment and apparatus

Electrical conductivity meter, beakers

Reagent

- **Potassium chloride (KCl) 0.01 *M*:** Dissolve 0.7456 g of KCl in distilled water and make up the volume to 1 L at 25 ^{0}C. This is standard reference solution. At 25 ^{0}C it has an electrical conductivity of 1.412 dS m^{-1}.

Procedure

1. Take about 75 mL water sample in a 100 mL beaker, and then put the clean and dried conductivity cell in beaker.
2. Take the reading the display will also need some time to stabilize before the reading.
3. Remove the conductivity cell from the glass beaker, rinse thoroughly with distilled water, and carefully dry excess water with tissue paper.

Calculation

The EC value can either be used as such for categorizing the water on salinity basis or may be used to get the concentration of as given below;

Total soluble salt content (mg L^{-1}) =EC (dS m^{-1}) at 25 ^{o}C × 640
Total salt content me L^{-1} (approximately) =EC (dS m^{-1}) at 25 ^{o}C × 10

Practical suggestions

- Cleaning of the conductivity cell is needed if contaminated.
- The reference temperature should be 25 °C, and the result expressed in dS m^{-1}. If the measurement is carried out at a different temperature, the result should be corrected to 25 °C. Check accuracy of the EC meter using a 0.01 *M* KCl solution, which should give a reading of 1.413 dSm^{-1} at 25 °C.
- The use of the unit deci-Siemens per meter is preferred over the unit milli-mhos. Both units are equal, that is, 1 dSm^{-1} = 1 mmhocm^{-1}.

4. Total Dissolved Solids (TDS)

TDS is defined as the substances remaining after evaporation and drying of a water samples. The remaining fraction is approximately equivalent to the total content of the dissolved and suspended matter in the water sample. Non-filterable residue corresponds to the total suspended solids (TSS) and the filterable residue is the TDS. This is accomplished by comparing the value of calculated TDS with the measured value. Ion concentration, in mgL^{-1} of constituents, required to calculate the TDS are as follow:

Calculated TDS ($g\ L^{-1}$) = $Na+K+Ca+Mg+Cl+SO_4+SiO_3+ (NO_3-N)+F$

Principle

A well mixed, measured portion of a sample is filtered through a standard glass-fiber 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.

Apparatus

- Evaporating dishes made porcelain
- Platinum or high-silica glass
- Steam bath
- Desiccators provided with a desiccant containing a colour indicator for moisture concentration
- Glass-fiber filters
- Suction flask
- Analytical balance

Procedure

1. Take an aliquot of sample to yield between 2.5 and 200 mg dried residue.
2. Filter measured volume of well mixed water sample through the glass-fiber filter;
3. Wash with 3 successive 10 mL volumes of distilled water, allowing complete draining between washing.
4. Continue suction for about 3 minutes after filtration is complete.
5. Transfer filtrate to a weighed evaporating dish (*Wtd*) and evaporate to dryness on a steam bath.
6. Dry for at least 1 hour in an oven at 180 °C, cool in desiccators, and weigh (*Wtd+s*).
7. Repeat the cycle of drying, desiccating and weighing until a constant weight is obtained or until weight loss between successive weighing is less than 4 % or 0.5 mg which is less.

Calculation

$$\text{TDS (mg L}^{-1}) = \frac{(Wt_{d+s} - Wt_d) \times 1000}{V}$$

Where:

Wt_{d+s}= Weight of dish plus solids (mg)

Wtd= Weight of dish before use (mg)

V= Volume of water sample used for measurement (mL)

5. Total Suspended Solids

Total Suspended Solids (TSS) applies to dry weight of the material that is removed from a measured volume of water sample by filtration with a standard filter. The test is basically empirical and is not subject to usual criteria of accuracy. To achieve reproducibility and comparability of results requires close attention to procedural details, especially filter characteristics and time and temperature of drying.

Apparatus

- Desiccators provided with a desiccant containing a colour indicator for moisture concentration
- Analytical balance capable of weighing to 0.001 g
- Glass-fiber filter disc, Whatman GF/C or equivalent
- Drying oven
- Buchner funnels
- Vacuum pump

Pre-treatment of filter disc and crucible

1. Place a filter disc on the filter holder. Assemble filter holder in suction flask apparatus, connect to vacuum source and apply vacuum.
2. Wash the filter disc with 3 successive 20 mL portions of distilled water. Continue to apply vacuum for 2-3 minutes after the water has passed through the filter. Discard the filtrate.
3. Remove the filter paper from the membrane filter funnel or the Buchner funnel and place it on a supporting surface in drying oven.
4. Place the crucible (s) in the drying oven. The oven should be maintained at 105 ^{o}C and drying should be continued for at least 1 hour.
5. Cool the filter (s) and crucible (s) in desiccators and weigh it on an analytical balance.
6. Repeat the cycle of drying, desiccating and weighing until the weight loss between two successive series operations is less than 0.5 mg.
7. Store filter (s) and crucible (s) in desiccators until required.

Procedure

1. Remove the filter disc and crucible from the desiccators, and weigh ($\boldsymbol{Wt_f+c}$).
2. Place the filter in the filter holder and assemble the filter holder in the suction flask apparatus. Connect to the vacuum source and apply vacuum.
3. Wet the filter with a few drops of distilled water to seat the filter.
4. Shake the sample vigorously and measure out 100 mL in a 100 mL graduated cylinder or volume flask. Pour this portion of the sample into the filter funnel (be careful not to disturb the placing of the filter disc).

5. Rinse out the measuring flask or cylinder with a small quantity of distilled water. If the sample is very low in suspended material, a large volume of sample may be used.
6. When filtration is complete, carefully remove the filter disc from the filter holder with tweezers (or remove the crucible from its supporting socket with a pair of tongs), and place it in the drying oven.
7. Dry for at least 1 hour at 105 °C. Cool in desiccators, and weigh (***Wt_f+c+s***).
8. Repeat the drying, desiccating and weighing cycle until the weight loss between 2 successive weighing is less than 0.5 mg.
9. Record the final weight obtained

Calculation

$$\text{TSS (mg L}^{-1}) = \frac{(Wt_{fc+s} - Wt_{f+c}) \times 1000}{V}$$

Where:

Wt_f+c+s= Weight of filter and crucible plus solids (mg)
Wt_f+c= Weight of filter and crucible before use (mg)
V = Volume of water sample used for measurement (mL)

6. Biological Oxygen Demand

Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter is discharged into a watercourse it serves as a food source for the bacteria present there. If organic matter such as dead plants, leaves, grass clippings, manure, sewage, or even food waste is present in a water supply, the bacteria will begin the process of breaking down this waste. Biological Oxygen Demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. BOD refers to the amount of oxygen that would be consumed if all the organics in one litre of water were oxidized by bacteria and protozoa. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste.

Principle

This test is based on Winkler's Method. Oxygen combines with Mangnous hydroxide to form higher hydroxides, which on acidification liberate iodine equivalent to that of oxygen fixed. This iodine is titrated by standard Sodium thiosulfate solution using starch as an indicator.

Test Procedure

The BOD test takes 5 days to completeand is performed using a dissolved oxygen (DO) test procedure. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

Reagents:

- **0.0250 *M* Sodium thiosulfate:** 3.1 g sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) in reagent water. Add 0.2 g sodium hydroxide (NaOH, 1 to 2 pellets) and dilute to 500 mL. Alternate: Dissolve exactly 6.206 grams sodium thiosulfate crystals in freshly boiled and cooled reagent water and make up water to a volume of 1 liter. For preservation, add 0.4 gm or 1 pellet of sodium hydroxide. Solutions of "thio" should be used within two weeks to avoid loss of accuracy because of decomposition of the solution. Alternate: Phenylarsine Oxide solution (PAO) may be used instead of "thio" [0.025 *N* PAO available and standardized from commercial sources.]
- **Alkaline Iodine Azide (AIA) solution:** 50 g sodium hydroxide (NaOH) and 15 g potassium iodide (KI) are dissolved in reagent water and diluted to 100 mL. Add 1 g sodium azide (NaN_3) dissolved in 4 mL distilled water.
- **Starch solution:** 2 g starch and 0.2 g salicylic acid dissolved in 100 mL hot water.
- **Manganous Sulfate solution:** Dissolve 40 g Manganoussulfatedihydrate ($MnSO_4 \cdot 2H_2O$) in reagent water, filter and dilute to 100 mL.
- **Sulfuric acid:** Use concentrated reagent grade acid (H_2SO_4). Handle carefully, since this material will burn skin and clothes.
- **Potassium Iodide (KI) crystals**

Standardization (Procedure):

1. Dissolve 2 g potassium iodide in approximately 100 mL Erlenmeyer flask.
2. Add 1.0 mL concentrated Sulfuric acid.
3. Add a magnetic stir bar.
4. Fill burette with the sodium thiosulfate solution and lower level to 0.0 marks. Be sure that the tip of the buret is filled with solution and not air.
5. Begin stirring and add sodium thiosulfate solution to the flask until a light yellow colordeveloped.
6. Add about 2 mL of starch solution. The color will change to deep blue or purple.
7. Continue addition of sodium thiosulfate until the solution clears permanently. Stop the titration and record the volume of of sodium thiosulfate used.
8. If exactly 20 mL of sodium thiosulfate solution is used it can be recorded as standardized at 0.0250 *M*. If less than 20 mL are needed, add some reagent water to the sodium thiosulfate solution (5 mL reagent water to 100 mL titrant for each 1 mL that the titrant is low). If more than 20 mL are required, add solid sodium thiosulfate to the titrant solution (310 mg per 1000 mL for every 1.00 mL the titrant is high). Recheck the standardization.
9. Repeat the standardization and record both determinations and the average in the notebook.

Procedure

1. Collect samples in 300 mL BOD bottle taking special care to avoid aeration of the liquid being collected. Fill bottle completely (no air under cap). Samples should be taken in triplicate. Keep one bottle of each set in an incubator at 20°C for 5 days.
2. Insert stopper in bottle prepared for DO, then remove.
3. Add, under the surface of the sample, 1-2 mL Manganous Sulfate solution.
4. Immediately add 1-2 mL Alkaline Iodide Azide solution below the surface of the liquid.
5. Insert stopper in bottle, avoid trapping air bubbles, and invert it until it mix well. Repeat this shaking after the flock has settled halfway.
6. Allow flock to settle to half the volume of the bottle a second time, then open and add 1-2 mL concentrated Sulfuric acid by allowing the acid to run down the neck of the bottle above the surface of the liquid.
7. Insert stopper and invert to mix until the flock dissolves.
8. Transfer the solution to an Erlenmeyer flask with magnetic stir bar for the titration procedure.
9. Titrate with the 0.0250 M sodium thiosulfate solution until the solution is pale yellow.
10. Add 2 mL starch and continue titration until the color is permanently discharged. Record the volume of sodium thiosulfate used.Each 1.00 mL of 0.025 M sodium thiosulfate used is equivalent to 1.00 mg L^{-1} DO.
11. Repeat same steps with other samples earlier kept in incubator and record DO.

Calculation:

$$\text{DO mg equivalent} = \frac{\text{Vol. of } Na_2S_2O_3 \text{ sol. Used x } N \text{ of } Na_2S_2O_3 \text{ x } 8000}{\text{Vol. of taken}}$$

$$\text{BOD (mg L}^{-1}) = \frac{DO_i - DO_f}{P}$$

Where,

DO_i and DO_f are initial and final values of DO.

P is the decimal fraction of sample diluted in the 300 mL BOD bottle.

Ranges for typical BOD levels in water bodies are

- < 5 mg L^{-1} in natural, unpolluted waterways
- 20–30 mg $L^{-1}$for well-treated sewage
- 150–300 mg $L^{-1}$for raw sewage
- 100–500 mg $L^{-1}$for urban storm water runoff.

Precautions

- Test should be done as early as possible after sample collection.
- If test is not started within two hours of collection, it should be kept at 4 °C up to 6 hours.
- Do not open BOD bottles before analysis.
- The pH of samples should be adjusted between 6.5 - 7.5 using sulfuric acid or sodium hydroxide.

7. Chemical Oxygen Demand

The chemical oxygen demand (COD) is a measure of water and wastewater quality. The COD test is often used to monitor water treatment plant efficiency. The COD method determines the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of oxidizing agent, temperature, and time. The chemical oxygen demand test procedure is based on the chemical decomposition of organic and inorganic contaminants.

Principle

The COD is often measured using a strong oxidant (e.g. potassium dichromate, potassium iodate, potassium permanganate) under acidic conditions. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$).The organic matter present in sample gets oxidized completely by potassium dichromate ($K_2Cr_2O_7$) in the presence of sulphuric acid (H_2SO_4), silver sulphate ($AgSO_4$) and mercury sulphate($HgSO_4$) to produce CO_2 and H_2O.After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfateusing ferroin as an indicator to determine the amount of $K_2Cr_2O_7$ remaining in the solution. The dichromate consumed by the sample is equivalent to the amount of O_2 required to oxidize the organic matter.

Test Procedure

Sample should be collected in glass bottles, if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers. Biologically active samples should be tested as soon as possible. Samples containing settle able material should be well mixed, preferably homogenized, to permit removal of representative aliquots.

Reagents

- **Potassium dichromate ($K_2Cr_2O_7$) 0.25N**: Dissolve 12.259 g of oven-dried (primary standard grade dried at 103 °C to a constant weight) potassium dichromate in distilled water and dilute to 1 litre volume in a volumetric flask.
- **Sulphuric acid (H_2SO_4) and silver sulphate (Ag_2SO_4) solution:** Add 5.5 g of silver sulphate to a 1kg bottle of concentrated sulphuric acid (1kg H_2SO_4 = 550 mL H_2SO_4) and mix until the silver sulphate goes into solution.
- **Ferrous ammonium sulphate (FAS) [$Fe(NH_4)_2(SO_4)_2$] (0.1N):** Dissolve 39 g reagent grade ferrous ammonium sulphate hexahydrate in distilled water. Add 20 mL of concentrated sulphuric acid (H_2SO_4). Cool and dilute to exactly 1 litre in a volumetric flask using distilled water. The ferrous ammonium sulfate (FAS) titrant must be standardized daily by the following procedure:
  - Dilute 10mL of standard potassium dichromate ($K_2Cr_2O_7$) solution to 100 mL with distilled water.
  - Slowly add 30 mL of concentrated sulphuric acid and cool to room temperature.
  - Titrate with ferrous ammonium sulphate titrant, using 2 to 3 drops (0.10 to 0.15 mL) of Ferroin indicator.
    Normality of FAS = (mL $K_2Cr_2O_7$)(0.25) mL FAS required
    The deterioration of FAS can be decreased if it is stored in a dark bottle.
- **Ferroin indicator (1, 10-phenanthroline and ferrous ammonium sulphate):** Dissolve 1.485 g of 1,10-phenanthroline monohydrate and 695 mg of ferrous ammonium sulphate heptahydrate in distilled water and dilute to approximately 100 mL. (Alternatively, this indicator may be purchased as Ferroin Indicator from most scientific suppliers.)
- **Mercuric Sulphate ($HgSO_4$).**

Procedure

1. Place 20 mL sample in a 500 mL refluxing flask. The blank is prepared using 20 mL of distilled water and add 5 to 7 glass boiling beads.
2. Add 0.4 g of mercuric sulphate ($HgSO_4$), Place reflux flask in an ice bath and slowly add, with swirling, 30 mL of concentrated sulphuric acid / silver sulphate solution, and mix until the $HgSO_4$ is in solution. The function of the mercuric sulphate is to bind or complex chlorides. (Caution: Care must be taken to assure that the contents of the flask are well mixed. If not, superheating may result, and the mixture may be blown out of the open end of the condenser.)
3. Again using slow addition with swirling motion, accuratelyadd 10 mL of 0.25 *N* potassium dichromate ($K_2Cr_2O_7$) and mix.
4. After thorough mixing, attach the flask to the reflux condenser, apply heat, and reflux for 2 hours. The standard 2 h reflux time may be reduced if it has been shown that a shorter period yields the same results. This may be determined by refluxing for periods from 15 minutes to 2 hours and comparing the results.
5. A reagent blank containing distilled water should be refluxed with samples.
6. Cool the apparatus to room temperature after the refluxing period.
7. Add 4 to 5 drops of Ferroin indicator and a magnetic stirring bar.
8. Place flask on a magnetic stirrer and rapidly titrate with 0.1 *N* ferrous ammonium sulphate to the first. The colour change will be sharp, changing from a blue-green to red-brown endpoint.
9. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data.

Calculation:

$$\text{COD (mg L}^{-1}) = \frac{(V_1 - V_2) \times N \times 800}{V_0}$$

Where,

V_1 = vol. of FAS required for titration against the blank, in mL.
V_2 = vol. of FAS required for titration against the different samples, in mL.
N = normality of FAS.
V_0 = vol. of sample taken for testing, in mL.

Precautions

- Samples should be preserved with sulfuric acid to a pH < 2 and maintained at 4 °C until analysis.
- Traces of organic material either from the glassware or atmosphere may cause a gross, positive error. Extreme care should be exercised to avoid inclusion of organic materials in the distilled water used for reagent preparation or sample dilution.
- Glassware used in the test should be conditioned by running blank procedures to eliminate traces of organic material.
- Volatile materials may be lost when the sample temperature rises during the sulfuric acid addition step. To minimize this loss the flask should be cooled during addition of the sulfuric acid solution.

8. Coliform Count

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery.

*Escherichia coli*is a normal inhabitant of the intestinal tract and is not normally found in fresh water. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect *E. coli*. In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms.

The standard test for the coliform group is carried out qualitatively by the multiple-tube fermentation technique (through the presumptive-confirmed phases or completed test) or quantitatively by the membrane filters (MF) technique. When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample.

Principle

The Presumptive Test

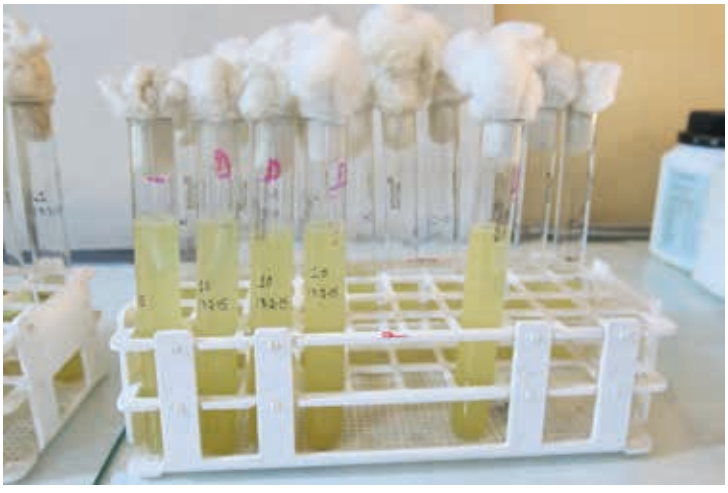
In the presumptive test, a series of lactose broth tubes containing an inverted Durham tube are inoculated with measured amounts of the water sample to be tested. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms because these bacteria are capable of using lactose as a carbon source. The most probable number (MPN) of coliforms can be estimated by the number of positive tubes. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination of the water.

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms. In order to confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. *E. coli* colonies are small and have a green metallic sheen, whereas *E. aerogenes* forms large pinkish colonies.

The Completed Test

The completed test is made using the organisms which grew on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37 °C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.



Procedure:

A. Presumptive Test:

1. Add dehydrated lactose broth (the other enteric organisms are not able to use lactose as a carbon source) to water, mix thoroughly, and heat to dissolve. pH should be 6.8 ± 0.2 after sterilization. Before sterilization, dispense sufficient medium, in fermentation tubes with an inverted durham tube for gas collection in each. Close tubes with cotton plugs.
2. Tubes of this lactose medium are inoculated with 10 mL, 1mL and 0.1mL of the water sample each. Five with 10mL, five with 1mL and five with 0.1mL and incubate them for 24 hrs at 37 ^{0}C.
3. The reading is recorded as positive gas producer and the MPN is determined by using MPN index (Table 1).

B. Confirmed Test:

1. EMB Agar (Eosin methylene blue agar) media plates prepared. Lactose fermentation produces acids, which lower the pH. This encourages dye absorption by the colonies, which show purple-green colonies on EMB Agar.
2. Inoculate 5 plates from 10mL tubes each and mark it, inoculate similarly from different tubes showing positive result and incubate all plates in inverted position at 37 ^{0}C for 24 hrs.

C. Completed Test:

1. The EMB agar plates showing positive result with dark centers and green metallic sheen colonies are taken and a loop full of the colonies is inoculated in the lactose broth tubes containing inverted Durham tubes after numbering them according to the plates.
2. Inoculate nutrient agar slant from same isolate.
3. Incubate these tubes and plates at 37^0 C for 24 hrs and the results should record with the source of its water sample.
4. After microscopic examination of culture from positive colonies confirms the presence of E. coli in water sample.

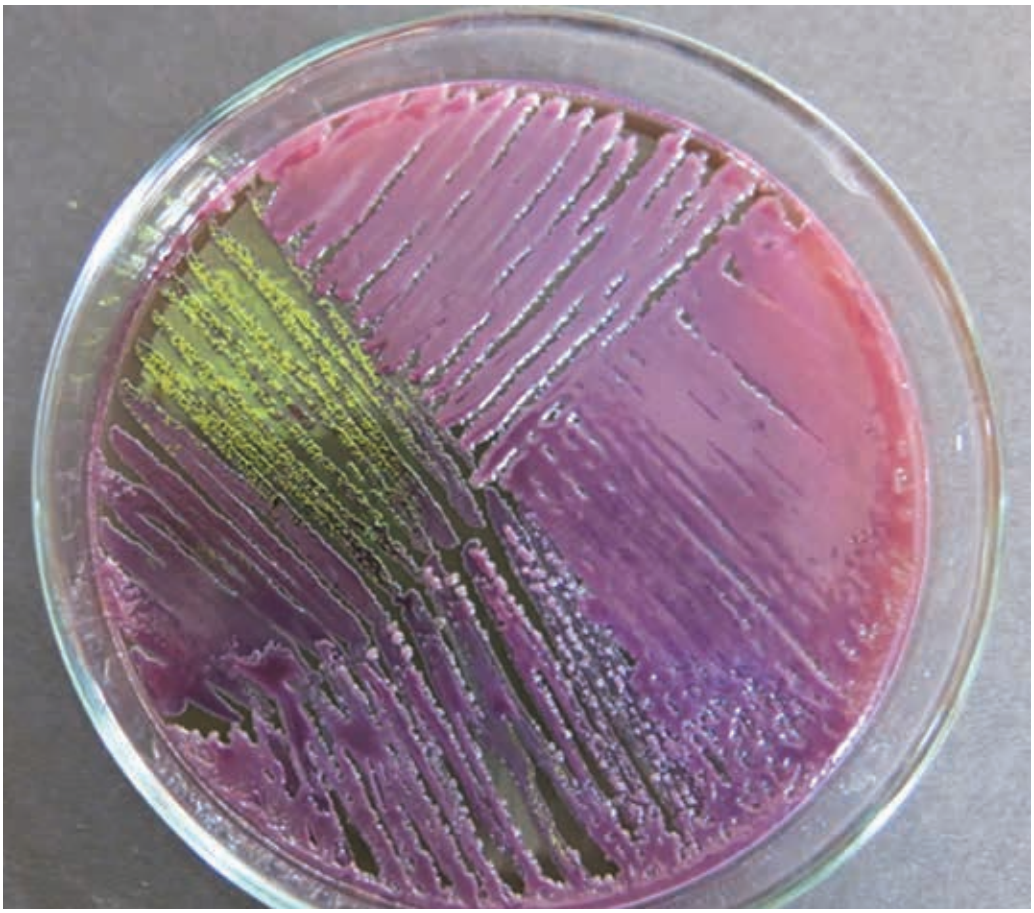


Table 1: The MPN Index per 100 mL for combination of positive results with five tubes of each portion of sample (10 mL, 1 mL and 0.1mL)

Combination of positives	MPN index per 100 mL	95% confidence limit		Combination of positives	MPN index per 100 mL	95% confidence limit	
		Upper	Lower			Upper	Lower
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1 4-4-0	33 34	15 16	77 80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1 5-1-2	50 60	20 30	150 180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1,300
4-1-0	17	7.0	46	5-5-2	500	200	2,000
4-1-1	21	9.0	55	5-5-3	900	300	2,900
4-1-2	26	12.0	63	5-5-4 5-5-5	1600 >1,600	600 -	5,300 -

9. Nitrogen

Nitrogen generally occurs in trace amount in surface (river, canal) water but can be present in higher concentration in some ground waters (tubewells). Beneficial effect of nitrogen on crop production has been widely reported. The presence of K^+ and NO_3^- ions in appreciable amounts in irrigation water has been found to partially counteract the adverse effect of salinity and sodicity on plant growth. In drinking waters, nitrates content higher than 10 mg L^{-1} it causes illness called methaeglobinaemia or blue baby syndrome in infants. Water may contain nitrogen in different forms such as nitrate, nitrite, ammonia and organic nitrogen. Total nitrogen includes all forms of nitrogen. The concentration of nitrogen in water samples can be used to assess nutrient status of the water resources or as indicator water pollution due to enrichment from various sources such as fertilization of crop lands, animal wastes, sewage and growth of nitrogen fixing plants. Depending upon the objective, the water samples can be analysed for total as well as water soluble inorganic nitrogen.

A. Water soluble inorganic nitrogen

Principle

Ammonium nitrogen and NO_3^- N plus NO_2^- N are determined by steam distillation, using heavy MgO for NH_4 and Devarda's Alloy for NO_3 .The distillate is collected in saturated H_3BO_3 and titrated to pH 5.0 with dilute H_2SO_4.

Apparatus

- Distillation unit
- Automatic titrator connected to a pH-meter
- Stirrer

Reagents

- **Magnesium Oxide (MgO), powder**: Heat heavy magnesium oxidein a muffle furnace at 600-700 °C for 2 hours, and cool in a desiccators containing KOH pellets, and store in a tightly stoppered bottle.
- **Devarda's Alloy (50 Cu: 45 Al: 5 Zn):**Ball-mill reagent-grade Devarda's Alloyuntil the product will pass a 100-mesh sieve (0.150 mm) and at least 75% will pass a 300-mesh sieve (0.05 mm).
- **Boric Acid Solution (H_3BO_3), saturated:** Add 500 g H_3BO_3into a 5-L volume. Add 3 L distilled water (DI), and swirl vigorously and leave overnight.
- **Tris Solution (hydroxymethylaminomethane) ($C_4H_{11}NO_3$), 0.01 *N:*** Dry reagent-grade Trisin an oven at 80 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle.
- **Sulfuric Acid Solution (H_2SO_4), 0.01 *N* :** Add 28 mL concentrated H_2SO_4, to about 600-800 mL distilled water in a 1L flask, mix well, let it cool, and bring to 1 L volume. This solution contains 1 *N* H_2SO_4 solution (Stock Solution). Pipette 10 mL Stock Solutionto 1 L flask, and bring to volume with distilled water. This solution contains 0.01 *N* H_2SO_4.
- **Standard Stock Solution**
  - Dry reagent-grade ammonium sulfate $(NH_4)_2SO_4$, and potassium nitrate (KNO_3) in an oven at 100 °C for 2 h, cool in a desiccators, and store in a tightly stoppered bottle.

- Dissolve 5.6605 g $(NH_4)_2 SO_4$ and 8.6624 g KNO_3 in distilled water, and bring to 1 L volume. This solution contains (1.2 g NH_4-N, and 1.2 g NO_3^-N) L^{-1} (Stock Solution).
- Prepare a Standard Solution from the Stock Solution as follows: Dilute 50 mL Stock Solution to 1 L volume by adding 2 *M*KCl solution (Diluted Stock Solution).
- A 20 mL aliquot of Diluted Stock Solution contains 1.2 mg NH_4 N and 1.2 mg NO_3 N.

Procedure

Pre-treatment of the distillation unit

1. The distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate/minute.
2. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.

Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 – 8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the 0.01 *N* H_2SO_4 in the Auto-Titrator by titrating three separate 10 mL aliquots of the primary standard, 0.01 *N* Tris solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found. The H_2SO_4 normality is:

$$N\ H_2SO_4 = \frac{10 \times N_{Tris}}{V\ H_2SO_4}$$

Carry out distillations as follows

To determine NH_4-N

1. Pipette 20 mL water or wastewater sample into a 100 mL distillation flask.
2. Pipette 1 mL saturated H_3BO_3 solution and 1 mL distilled water into a 50 mL beaker (duplicate beakers).
3. Place the first beaker underneath the condenser tip, with the tip touching the solution surface.
4. Add about 0.2 g heavy MgO, with a calibrated spoon, to the distillation flask.
5. Immediately, attach the distillation flask to the distillation unit with a clamp.
6. Start distillation, and continue for 3 minutes, then lower the dish to allow distillate to drain freely into the Pyrex evaporating dish or beaker.
7. After 4 minutes, when 35 mL distillate or more is collected, turn off the steam supply and remove the distillation flask (first distillate).
8. Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NH_4 N from Diluted Stock Solution) and two blanks (pipette 20 mL 2 KCl solution). Recovery of NH_4 N should be at least 96 %.

To determine NO_3^-N and NO_2^-N

1. Place the second beaker underneath the condenser tip, with the tip touching the solution surface.
2. Immediately, add 0.2 g Devarda's alloy, with a calibrated spoon, to the same distillation flask, then attach back to distillation unit with a clamp, and start distilling.
3. After 4 minutes, when 35 mL distillate or more is collected, turn off the steam supply and remove the distillation flask (second distillate).
4. Wash tip of the condenser into Pyrex evaporating dish or the beaker with a small amount of distilled water.
5. Each distillation should contain at least two standards (pipette 20 mL 1.2 mg NO_3^- N from Diluted Stock Solution) and two blanks (pipette 20 mL distilled water). Recovery of NO_3-N should be at least 96 %.

Titration

Titrate the first distillate (for ammonia) and the second distillate (for nitrate), separately, to pH 5with standardized 0.01 *N* H_2SO_4using an Auto-Titrator.

Calculation

$$NH_4 \text{ or } NO_3\left(\text{mg L}^{-1}\right) = \frac{(V - B) \times N \times 14.01 \times 1000}{V_1}$$

Where:

V = Volume of 0.01 *N* H_2SO_4 titrated for the water sample (mL)

B = Distillate blank titration volume (mL)

N = Normality of H_2SO_4 solution

V1 = Volume of water sample used for distillation (mL)

14.01 = Atomic weight of N

Technical remarks

- If possible, NH_4^+ N and NO_3^- N should be determined immediately after sampling. If the analysis cannot be done immediately, water and wastewater samples may be kept refrigerated (4 °C).
- All water for reagents and dilution should be NH_3free. Distilled water is usually satisfactorily.
- Filter paper may contain traces of NH_4^+, so the first 20-25 mL of filtrate should be discarded.
- Never use acid preservation for samples to be analyzed for NO_3^- or NH_4^+.

B. Total Nitrogen

For the total nitrogen water samples can be analyzed after digesting the samples by persulphate digestion followed by colourimetric estimation of nitrate nitrogen. Alternatively water samples can also be analysed for total Kejldahl nitrogen.

Persulphate oxidizable total nitrogen

Alternately In this method salicylic acid is nitrated in an alkaline solution and light absorbance is measured at 410 nm

Apparatus and equipments:

Spectrophotometer, test tube shaker, autoclave, glass culture tube with Teflon lined screw cap.

Reagents

- **Oxidizing agent:** oxidizing agent is prepared by adding 25 g potassium persulphate AR grade ($K_2S_2O_8$) and 15 g of boric acid AR grade (H_3BO_4) in 50 mL of 3.75 M NaOH solution and the volume is made up to 500 mL with double distilled water. The reagent can be stored for upto 1 week at room temperature in dark bottle.
- **3.75 M NaOH:**Dissolve 75 g NaOH in 500 mL distilled water
- **4 M NaOH solution:** Dissolve 160 g NaOH cakes in 600 mL distilled water, make up to 1000 mL and mix well
- **5% Salicylic Acid:** Dissolve 5 g salicyclic acid in 95 mL concentrated H_2SO_4 (95-97%).
- **1000 mg NO_3^- L^{-1} solution**: Dissolve 7.223 g dry potassium nitrate in distilled water and make up to volume in a 1000 mL volumetric flask
- **Working standards:** Pipette 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution in 100 mL volumetric flaks and make up to volume to give the 0, 2, 4, 6, 8 and 10 mg NO_3^- L^{-1} working standards.

Procedure

1. Take 5 mL water sample in glass culture tube to this add 5 mL of the oxidizing agent, and immediately sealed the culture tube with screw caps containing Teflon liners.
2. Set a blank with 5 mL distilled water in place of aliquot
3. Autoclave the culture tube at 120 °C for 50 min.
4. After autoclaving cool the culture tube
5. Pipette 0.5 aliquot and each of the working standards in test tube for NO_3^- N determination
6. Add 1 mL of 5% salicylic acid slowely with stirring and wait for 30 min
7. Now add 10 mL of 4 *M* NaOH solution slowely and leave for 1 h for colour development
8. Mix the reagents properly using cyclomixer
9. Read the absorbance of the yellow colur at 410 nm using spectrophotomer
10. Plot the standard curve from absorbance versus concentration and find out the sample and blank concentration

Calculation:

NO_3^- N (mg L^{-1}) = (S-B) × 2

Where S= concentration of NO_3^- N (mg L^{-1}) in sample

B = concentration of NO_3^- N (mg L^{-1}) in blank

Factor "2" is the dilution factor during digestion of the sample

Total Kejldahl nitrogen

The Kjeldahl procedureis a good estimate of N content in the water or waste water samples. This procedure involves digestion and distillation. The water or waste water sample is digested in concentrated H_2SO_4 with a catalyst mixture to raise the boiling temperature and to promote the conversion from organic-N to ammonium-N, which is obtained by steam distillation, using excess NaOH to raise the pH. The distillate is collected in saturated H_3BO_3; and then titrated with dilute H_2SO_4 to pH 5.0.

Apparatus

- Block-digester
- Distillation unit
- Automatic titrator connected to a pH-meter
- Vortex tube stirrer

Reagents

- **Catalyst Mixture (K_2SO_4 – Se), 100: 1 w/ w ratio:** Grind reagent-grade chemicals separately and mix. If caked, grind the mixture in a porcelain pestle and mortar to pass a 60-mesh screen (0.250 mm), taking care not to breath *Se* dust or allow *Se* to come in contact with skin.
- **Sulfuric Acid (H_2SO_4), concentrated (98 %, sp. gr. 1.84)**
- **Sodium Hydroxide Solution (NaOH), 10 N:**Dissolve 400 g NaOH in distilled water, transfer to a 1 L heavy-walled Pyrex flask, let it cool, and bring to volume.
- **Boric Acid Solution (H_3BO_3), saturated:**Add 500 g H_3BO_3into a 5 L volume. Add 3 L DI water, and swirl vigorously. Leave overnight.
- **Tris Solution [hydroxymethylaminomethane] ($C_4H_{11}NO_3$), 0.01 N:**Dry reagent-grade Tris in an oven at 80 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 1.2114 g Trisin distilled water, and bring to 1 L volume
- **Sulfuric Acid Solution (H_2SO_4), 0.01 N:**Add 28 mL concentrated $H_2SO_4$to about 600 - 800 mL distilled water in a 1 L flask, mix well, let it cool, and bring to 1-L volume. This solution contains 1 *N* H_2SO_4 solution (Stock Solution). Pipette 10 mL Stock Solutionto 1-L flask, and bring to volume with DI water. This solution contains 0.01 *N* H_2SO_4.
- **Standard Stock Solution:**Dry reagent-grade ammonium sulfate $(NH_4)_2SO_4$in an oven at 100 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 5.6605 g dried $(NH_4)_2SO_4$in distilled water, and bring to 1 L volume. This solution contains 1.2 g NH_4 N L^{-1} (Stock Solution).

Procedure

A. Digestion

1. Pipette 20 mL aliquot of the water sample into a 100 mL calibrated digestion tube.
2. Add about 3.0 – 3.5 g catalyst mixture, a few pumice boiling granules, add 10 mL concentrated H_2SO_4 (in the fume hood) and then swirl carefully. Place the tubes in the rack and put a glass funnel in the neck of the tube.
3. Place the tubes rack in the block-digester, and slowly increase temperature setting to about 370 - 380 °C. The H_2SO_4 should condense about half-way up the tube neck; when solution clears, continue heating for about 3 hours.
4. Lift the tubes rack out of the block-digester, carefully place on a rack holder, and let tubes cool to room temperature.
5. Slowly add about 15 mL distilled water to the tubes, cool, and bring to volume with distilled water.
6. Each batch of samples for digestion should contain at least one reagent blank (distilled water sample), and one chemical standard (distilled water sample, pipette 1 mL Stock Solution).

Distillation

1. Before starting a batch for distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7-8 mL distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22 °C.
2. Calibrate pH meter with buffer solutions of pH 7.0 (buffer), and 4.0 (sensitivity), after setting for temperature. Then standardize the 0.01 *N* H_2SO_4 in the Auto-Titrator by titrating three separate 10 mL aliquots of the primary standard, 0.01 *N* Tris solution, to pH 5.0. The titrations should agree within 0.03 mL; if not; titrate further aliquots until agreement is found.

The H_2SO_4 normality is: $N\ H_2SO_4 = \frac{10 \times N_{Tris}}{V\ H_2SO_4}$

Carry out distillations as follows:

1. Dispense 1 mL saturated H_3BO_3 solution and 1 mL distilled water into a 100 mL Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface.

2. Pipette 20 mL aliquot into a 100 mL distillation flask, and add 10 mL 10*N* NaOH solution.
3. Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes, lower the dish to allow distillate to drain freely into the dish.
4. After 4 minutes when about 35 mL distillate is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.
5. Titrate the distillate to pH 5.0 with standardized 0.01 *N* H_2SO_4 using an Auto-Titrator.
6. Each distillation should contain at least two standards (pipette 10 mL digested water sample) and two blanks (pipette 10 mL digested water blank). Recovery of NH_4-N should be at least 96 %.

Notes

- After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish.
- Between different samples, steam out the distillations. Disconnect distillation flasks containing the digest sample and NaOH, and attach a 100 mL empty distillation flask to distillation unit. Place a 100 mL empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds.

Calculation

$$\% \textbf{ Recovery } = \frac{(V - B) \times N \times 14.01 \times 100}{V_3 \times C}$$

$$\textbf{N } \% = \frac{(V - B) \times N \times V_1 \times 14.01 \times 100}{V_2 \times 1000}$$

Where:

V = Volume of 0.01 N H_2SO_4 titrated for sample (mL)
V1= Total volume of the digest (mL)
V2= Volume of water digest used for distillation (mL)
B = Digested blank titration volume (mL)
N = Normality of H_2SO_4 solution
14.01 = Atomic weight of N
V3 = Volume of NH_4-N standard solution (mL)
C = Concentration of NH_4-N standard solution (µg mL^{-1})

10. Phosphorus

Phosphorus compounds are present in fertilizers and in many detergents. High concentration of P compounds may produce a secondary problem in water bodies. In such situations, the presence of additional P compounds can stimulate algal growth and enhances eutrophication.Groundwater rarely contains more than 0.1 mgL^{-1} phosphate if not polluted by organic matter. Phosphorus is essential to the growth of organisms and can be the nutrient that limits biological growth in water bodies. The water samples are analysed for the total as well as soluble reactive phosphorus

A. Soluble reactive phosphorus (SRP)

Soluble reactive phosphorus (SRP) describes the dissolved phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample and are termed 'reactive phosphorus'. Reactive phosphorus is largely a measure of orthophosphate (PO_4^{3-}); however, a small fraction of any condensed phosphate present is usually hydrolysed unavoidably in the analytical procedure. Reactive phosphorus occurs as both dissolved and suspended phosphorus.

Principle

SRP can be determined by vanadomolybdo phosphoric acid(colorimetric methods), where are the ammonium molybdate and potassium antimony tartrate react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid- that is reduced to an intensely colored molybdenum blue by ascorbic acid.

Apparatus

- Spectrophotometer or colorimeter
- Standard laboratory glassware: beakers, volumetric flasks, pipettes, funnels

Reagents

- **Sulfuric Acid Solution (H_2SO_4), 5 *N*:**Dilute 148 mL *concentrated* H_2SO_4 (in fume hood) with DI water, mix well, let it cool, and bring to 1-L volume.
- ***p*-nitrophenol Indicator, 0.25 % w/v**
- **Reagent A:**Dissolve 12 g ammonium heptamolybdate$(NH_4)6Mo7O_{24}.4H_2O$ in 250 mL distilled water **(a)**.Dissolve 0.2908 g antimony potassium tartrate$(KSbO.C_4H_4O_6)$ in 100 mL distilled water **(b)**.Add both dissolved Reagents **(a)** and **(b)** to a 2 L flask. Slowly add 1-L 5 *N* $H_2SO_4$to the mixture. Mix thoroughly, and dilute to 2-L volume.Store in a dark Pyrex bottle in cool place.
- **Reagent B:**Dissolve 1.056 g L-Ascorbic acid ($C_6H_8O_6$) in 200 mL Reagent A, mix well. This reagent should be prepared as required because it does not keep for more than 24 hours.
- **Standard Stock Solution:**Dry about 2.5 g *potassium* dihydrogen phosphate(KH_2PO_4) in an oven at 105 °C for 1 hour. Cool in desiccator, and store in a tightly stoppered bottle. Dissolve 2.197 g dried KH_2PO_4in distilled water, and bring to 1 L volume .This solution contains 500 ppm P (Stock Solution). Dilute 50 mL stock solutionto 250 mL volume by adding distilled water. This solution contains 100 ppm P (Diluted Stock Solution). Prepare a series of working standard solutions from the diluted stock solutionas follows:

Dilute 5, 10, 15, 20 and 25 mL Diluted Stock Solutionto 500 mL numbered flasks by adding distilled water, and then bring to volume. These solutions contain 1, 2, 3, 4, and 5 ppm P, respectively.

Procedure

1. Pipette a suitable aliquot of clear filter water sample (10 mL natural water sample) into a 50 mL Erlenmeyer volumetric flask add few drops of p-nitrophenol indicator
2. Add the required acid or base to all the water samples to bring the solution pH to 5.0.
3. Add 8 mL Reagent B, and dilute to 50 mL volume with distilled water, mix well.
4. Prepare a standard curve by pipetting 2 mL of each standard (1-5 ppm), and proceed as for the samples. Also make a blank with only distilled water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 10 minutes on the Spectrophotometer at 882 nm wavelength.
6. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations.
7. Read P concentration in the unknown samples from the calibration curve.

Calculation

$$P\ (mg\ L^{-1}) = P\ mg\ L^{-1}\ \text{from standard curve} \times \frac{V_1}{V}$$

Where:

V = Volume of water sample used for measurement (mL)

V1 = Volume of flask used for measurement (mL)

B. Total Phosphorus

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. In water it may be in several forms like orthophosphates (PO_4^{3-}), condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particle or detritus, or in the bodies of aquatic organisms. Phosphorus enrichment in water bodies may be from detergents in sewage and waste waters, fertilizers, animal wastes, sewage and some industrial wastes. High levels of phosphorus and/or other key nutrients may lead to eutrophication of the water bodies. Total phosphorus in water samples can be analyzed after digesting the samples by persulphate digestion followed by colourimetric estimation of phosphorus as given in section.

Reagents

1. **Oxidizing agent:** oxidizing agent is prepared by adding 10 g potassium persulphate AR grade ($K_2S_2O_8$) and 1.5 g of NaOH in 500 mL volumetric flask and the volume is made up to 500 mL with double distilled water. The reagent can be stored for upto 1 week at room temperature in dark bottle.
2. Reagents of the P estimation as given SRP estimation

Procedure

1. Take 5 mL water sample in glass culture tube to this add 5 mL of the oxidizing agent, and immediately sealed the culture tube with screw caps containing Teflon liners.
2. Autoclave the culture tube at 120°C for 30 min.
3. After autoclaving cool the culture tube and
4. Take suitable volume of aliquot and develop the colour as mentioned in soluble reactive phosphorus estimation section
5. Read the absorbance using spectrophotomer.

Calculation:

$$P\ mg\ L^{-1} = 2 \times P\ mg\ L^{-1}\ \text{from standard curve} \times \frac{V_1}{V}$$

11. Potassium

Although potassium (K) is a relatively abundant element, its concentration in natural fresh waters is usually less than 20 mgL^{-1}. Brines and seawater, however, may contain as much as 400 mgL^{-1} K or more. Potassium in water can be determined by flame photometry.

Principle

The estimation of K is based on the emission spectroscopy, which deals with excitation of electrons from ground state to a higher energy state and coming back to its original state with the emission of light.

Apparatus

- Flame photometer with accessories
- Beakers
- Pipettes and volumetric flasks, as required for dilution and tests of interference effects

Reagents

Standard Stock Solution

- Dry 3-5 g potassium chloride (KCl) in an oven at 120 °C for 1 -2 hours and cool in a desiccator, and store in a tightly stoppered bottle.
- Dissolve 1.907 g dried KClin distilled water, and bring to 1-L volume. This solution contains 1000 ppm K (Stock Solution).
- Prepare a series of Standard Solutions from the Stock Solutionas follows: Dilute 2, 4, 6, 8, 10, 15 and 20 mL Stock Solutionto 100 mL numbered flasks by adding distilledwater, and then bring to volume. These solutions contain 20, 40, 60, 80, 100, 150, and 200 ppm K, respectively.

Procedure

1. Filter a portion of water sample through Whatman no. 42 filter paper.
2. Calibrate Flame Photometer with a series of suitable K standards with distilled water as blank sample.
3. Measure the water samples, take the emission reading on the Flame Photometer at 767-nm wavelength, and record the readings.
4. Draw a calibration curve.
5. Calculate K concentrations according to the calibration curve.

Calculations

$$\mathbf{K\ (me\ L^{-1}) = K(ppm) from standard curve / 39.1}$$

Where:
39.1 = Atomic weight of K

Practical suggestions

- Check the performance of the photometer at frequent intervals by spraying some of the standard solutions and adjust the sensitivity as necessary.
- If K concentration is higher than the top standard, make an appropriate dilution. If this is a dilution of the original sample, multiply by the appropriate factor.

12. Chlorides

Important anions from water quality point of view are chloride, carbonate and biocarbonate, sulphates and nitrate. Concentration of chloride generally increases with increasing in electrical conductivity (EC) of waters. Therefore, magnitude of total salt may be predicted if chloride concentration is known.

Chloride (Cl^-) anions are usually present in natural waters. A high Cl^- concentration occurs in waters that have been in contact with Cl^-containing geological formations. Otherwise, high Cl^-content may indicate pollution by sewage or industrial wastes or by intrusion of seawater or saline water into a freshwater body or aquifer. A salty taste in water dependents on the ions with which the $Cl^-$are associated. With Na ions the taste is detectable at about 250 mgL^{-1}, but with Ca or Mg the taste may be undetectable at 1,000 $mg\ L^{-1}$. Chlorides being highly soluble is present in all waters but the amount is often very low in natural waters.

Principle

The determination of Cl^- is done by $AgNO_3$ (Mohrs's titration) method, which is based upon the fact that in solution containing Cl^- and chromate. Silver reacts with all the Cl^- and precipitates before the reaction with chromate begins. The appearance of the brick-red colour of the silver chromate precipitate is the end-point of the titration.

Apparatus

Burette and stand,Volumetric flask and Beakers

Reagents

- **Potassium Chromate Solution (K_2CrO_4), 5% in water:**Dissolve 5 g K_2CrO_4in 50 mL distilled water. Add dropwise 1 *N* silver nitrate($AgNO_3$) until a slight permanent red precipitate is formed. Filter, and bring to 100 mL volume with distilled water.
- **Silver Nitrate Solution ($AgNO_3$), 0.01 *N*:**Dry about 3 g $AgNO_3$in an oven at 105 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered and brown bottle. Dissolve 1.696 g dried $AgNO_3$in distilled water, and bring to 1 L volume.
- **Sodium Chloride Solution (NaCl), 0.01 *N*:**Dry about 3 g NaCl in an oven at 140 °C for 2 hours, cool in a desiccator, and store in a tightly stoppered and brown bottle. Dissolve 0.585 g NaCl in distilled water, and bring to 1 L volume.

Procedure

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) into a 250 mL Erlenmeyer flask.
2. Add 4 drops potassium chromate solution.
3. Titrate against $AgNO_3$ solution until a permanent reddish-brown color appears.
4. In order to standardize the $AgNO_3$ solution used in the determination of Cl^-:
    - Titrate 10 mL 0.01*N* NaCl solution against 0.01 *N* $AgNO_3$ after adding 4 drops potassium chromate solution until a permanent reddish-brown color appears.
    - Take the reading, and calculate $AgNO_3$ normality:

$$\boldsymbol{N\ AgNO_3} = \frac{10 \times N\ Nacl}{V\ AgNO_3}$$

Where:

$NAgNO_3$= Normality of $AgNO_3$ solution
$VAgNO_3$= Volume of $AgNO_3$ solution used (mL)
NNaCl= Normality of NaCl solution

Calculation

$$\mathbf{Cl\ (me\ L^{-1})} = \frac{V_1 \times N \times 1000}{V}$$

Where:

V1 = Volume of 0.01 N $AgNO_3$ titrated for the sample (mL)
N = Normality of $AgNO_3$ solution
V = Volume of water sample used for measurement (mL)

Practical suggestions

1. Natural waters are often low in Cl^-, and 10 mL is a suitable aliquot in most cases.
2. Saline waters may be high in $Cl^-$and 5 mL (or even less than 5 mL) may then be more appropriate aliquots.

13. Carbonate and Bicarbonate

Carbonate and bicarbonate in a sample can be determined by titrating the sample against standard acid using phenolphthalein and methyl orange or methyl red respectively as indicators. When the colour of phenolphthalein changes from pink to colorless, it indicates half the neutralization of carbonates. Now methyl red indicator is added and the titration continued. When the colour changes from yellow to rose red, end point reaches which show the complete neutralization of bicarbonates.

Principle

Carbonates

When the pH value of a sample of natural water is above 8.4, the CO_3^{--} is present, normally as sodium carbonate. The carbonate ion is converted to HCO_3. The amount of acid used a measure of the carbonate present.

$$CO_3^{--} + H^+ = HCO_3^-$$

Bicarbonate

Bicarbonate ions react with mineral acid and release carbon dioxide (CO_2) into the solution.

$$HCO_3^- + H^+ = H_2O + CO_2$$

Apparatus

Pipettes, Burette, Erlenmeyer flasks, Graduated pipette, Magnetic stirrer

Reagents

- **Standard Sulphuric acid (0.01 *N*):** Prepare 1 *N* acid from concentrated H_2SO_4 (36 *N*) (Dissolve approx. 27 mL L^{-1} in distilled water) and standardized it with 0.01 *N* sodium carbonate by taking methyl red as indicator.
- **Methyl red indicator (0.5%):** Dissolve 0.5 gram dry methyl orange powder in 100 mL. of 95% ethanol.
- **Phenolphthalein indicator (0.25%):** Dissolve 0.25 gram of pure phenolphthalein powder in 100 mL. of 60% ethanol.

Procedure

1. Pipette 5 mL. of water sample in a 125 mL conical flask and add 2-3 drops of phenolphthalein. If pink color appears it indicates the presence of carbonates.
2. Then titrate it with 0.01 *N* sulphuric acid with the help of a burette till the solution becomes colorless. Record this reading and designate this reading as Y.
3. Now add 3-4 drops of methyl red indicator and titrate again, till the colour changes from yellow to rose red and record this reading also and designate it Z.

Calculations

If N_1 and V_1 are normality (CO_3^{2-} conc.) and volume of aliquot and N_2, V_2 are the normality and volume of H_2SO_4 used respectively, then

$N_1V_1 = N_2V_2$

$$N_1 = \frac{N_2\,V_2}{V_1} = \frac{\text{Vol. of } H_2SO_4 \times \text{Normality of } H_2SO_4}{\text{ml of aliquot taken}}$$

Here N_1 = Normality = equivalents of CO_3^{2-} present in one Litre of aliquot.

Hence, milliequivalents of CO_3^{2-} per Litre:

$$\text{Carbonates (me L}^{-1}) = \frac{2y \times \text{Normality of } H_2SO_4 \times 1000}{V}$$

$$CO_3\ (\text{me L}^{-1}) = \frac{2y \times N \times 1000}{V}$$

$$HCO_3\ (\text{me L}^{-1}) = \frac{(Z - 2y) \times N \times 1000}{V}$$

Where:

2 = Valance of carbonate
Y = Volume of titrant against phenolphthalein indicator (mL)
Z = Volume of titrant against methyl red indicator (mL)
V = Volume of water sample used for measurement (mL)
N = Normality of H2SO4 solution

Practical suggestions

Standard HCl is used because H_2SO_4 may give rise to turbidity from calcium sulfate with Ca-rich samples.

14. Sulphate

Sulphate ions usually occur in natural waters. Many sulphate compounds are readily soluble in water. Most of them originate from the oxidation of sulphate ores, the solution of gypsum and anhydrite, the presence of shales, particularly those rich in organic compounds, and the existence of industrial wastes.The SO_4^{--} in water is determined normally by barium sulfate ($BaSO_4$) precipitation.

Turbidimetric method

Principle

This method is used for the determination of sulphate ions. 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. The reaction involved is given below:

$Ba^{2+} + SO_4^{2-} = BaSO_4$ (White suspension)

Light absorbance of the $BaSO_4$ suspension is measured by a photometer or the scattering of light by Nephelometer.

Apparatus

Magnetic stirrer, Colorimeter for use at 420 mm or turbidimeter/nephelometer, Stopwatch, Nessler tubes, 100 mL, Measuring spoon (0.2 - 0.3 mL)

Reagents and standards:

- **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%) in500 mL distilled water and make up to 1000mL.
- **Buffer solution B:** (required when the sample sulphate (SO_4^-) is less than 10mg/L). Dissolve 30 g Magnesium chloride, $MgCl_2.6H_2O$, 5 g sodium acetate, $CH_3COONa.3H_2O$, 1.0 g of
- **Potassium nitrate, KNO_3:** 0.111 g of sodium sulphate, Na_2SO_4 and 20 mL acetic acid (99%) in500 mL distilled water and make up to 1000 mL.
- **Barium chloride:** crystals, 20-30 mesh.
- **Standard sulphate solution:** dissolve 0.5434 g of oven dry AR grade K_2SO_4in distilled water and dilute to 1 L. this contains 100 mg L^{-1} sulphate.

Calibration

Prepare standard curve by carrying standard sulphate solution through entire procedure. Space standards at 5mg L^{-1} increment in the 0 to 40 mg L^{-1}range. Read mg SO_4^{2-} present in the sample from the standard curve.

Procedure

1. Take suitable volume of sample and dilute to 100 mL into a 250 mL Erlenmeyer flask
2. Add 20 mL buffer solution, mix well
3. Keep the flask constantly stirred with the help of stirrer. Add 1spatula $BaCl_2$ crystals withstirring. Continue stirring for 1 minute after addition of $BaCl_2$
4. Pour suspension into an absorption cell of photometer an measure turbidity at 5 ± 0.5 min
5. To correct for sample colour and turbidity, run a blank to which $BaCl_2$ is not added.

Calculation

$$\text{mg } SO_4^{2-}L^{-1} = (\text{mg } (SO_4^{2-}) \text{ form standard curve x } 1000\} / (\text{mL of sample})$$

15. Boron

In most natural waters boron(B) is rarely found in concentrations greater than 1 mg L^{-1}, but even this low concentration can have deleterious the effects on certain agricultural products. Water having B concentrations in excess of 2 mg L^{-1} can adversely affect many common crops. However, where levels are greater than 5 mg L^{-1}, toxicity may occur. Groundwater may have a greater B concentration, particularly in areas where the water comes in content with igneous rocks or other B-containing strata.

The hot-water procedure is still the most popular method for measuring B, and it was introduced by Berger and Truog (1939), and was modified by later researchers. The B is measured calorimetrically using Azomethine-H (Bingham, 1982).

Apparatus

Spectrophotometer, Erlenmeyer flasks, Polypropylene test tubes

Reagents

- **Buffer Solution:**Dissolve 250 g ammonium acetate(NH_4OAc), and 15 g EDTA disodium (ethylenediamine-tetraacetic acid, disodium salt)in 400 mL distilled water. Slowly add 125 mL glacial acetic acid(CH_3COOH), and mix well.
- **Azomethine-H Solution ($C_{17}H_{12}NNaO_8S_2$):**Dissolve1 g L-ascorbic acidin 100 mL distilled water, and then add 0.45 g Azomethine-H, and mix well. Fresh reagent should be prepared weekly and stored in a refrigerator.
- **Standard Stock Solution:**Dissolve 0.114 g boric acid(H_3BO_3) in distilled water, and bring to 1-L volume. This solution contains 20 ppm B (Stock Solution). Prepare a series of working Standard Solutions from the Stock Solutionby diluting 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL Stock Solutionto 100 mL numbered flasks by adding distilled water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively.

Procedure

1. Pipette 2 mL aliquot of the natural water sample in to a 10 mL polypropylene tube.
2. Add 4 mL buffer solution.
3. Add 4 mL Azomethine-H solution, and mix well.
4. Prepare a standard curve by pipetting 2 mL of each standard (0.5 – 3.0 ppm), and proceed as for the samples. Also make a blank with 2 mL distilled water, and proceed as for the samples.
5. Read the absorbance of blank, standards, and samples after 30 minutes on the Spectrophotometer at 420-nm wavelength.
6. Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations.
7. Read B concentration in the unknown samples from the calibration curve.

Calculation

B (mg L^{-1}) = (S-A)

Where S =B (mg L^{-1}) from standard curve in sample

A = B (mg L^{-1}) from standard curve in blank

16. Sodium

The main cations present in irrigation samples are those of calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K). In effluents, swage and waste waters heavy metals are also found.

Sodium (Na)is a common element, the sixth most abundant, and present to some extent in most natural waters. Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). Sewage, industrial effluents, sea water intrusion in coastal area, and the use of Na compounds for corrosion control and water-softening processes all contribute to Na concentration in water because of the high solubility of sodium salts and minerals. Sodium levels in groundwater vary widely but normally range between 6 and 130 mg L^{-1}.

Reagents

A. Standard stock solution (100 me Na L^{-1})

- Dissolve 5.845g of AR grade dried NaCl in distilled water and make the volume to 1 L

B. Working standard solution

- Dilute 5, 10, 15, 20, 30, 40and 50 mL portion of the stock solution (containing 100 me Na L^{-1}) to 100 mL in volumetric flask to get working standards of 5, 10, 15, 20, 30, 40, and 50 me Na L^{-1} concentrations.

Procedure

1. Filter a portion of water sample through Whatman filter paper No. 42.
2. Calibrate Flame Photometer with a series of suitable Na standards with distilled water as blank sample.
3. Measure the water samples, take the emission reading on the Flame Photometer at 589-nm wavelength, and record the readings.
4. Draw a calibration curve.

Calculations

$$\text{Na (me } L^{-1}) = \text{Na(ppm)from calibration curve}/23$$

Where:

23 = Atomic weight of Na

17. Calcium and Magnesium

Calcium (Ca) is dissolved easily out of almost all rocks and is, consequently, detected in most waters. Magnesium (Mg) are relatively abundant in the earth's crust and hence a common constituent of natural water. Waters associated with granite or siliceous sand usually contain less than 10 mg of calcium per litre and less than 5 mg magnesium per litre. Many waters from limestone areas may contain 30-100 Ca per litre, and those associated with gypsiferous shale may contain several hundred milligrams per litre. But for the water in contact with dolomite or Mg-rich limestone may content 10-50 mgL^{-1} and several hundred milligrams per liter may be present in water that has been in contact with deposits containing sulfates and chlorides of magnesium.

Calcium and Mg contribute to the hardness of water,it should be noted that the difference between total hardness and the Ca concentration can be used to calculate the magnesium concentration. However, some $CaCO_3$ is desirable for domestic waters because it provide a coating in the pipes which protects them against corrosion.

Principle

EDTA-disodium salt solution is used to chelate Ca^{2+} + Mg^{2+}. Calcium is separately estimated by the versenate method using ammonium purpuate (Murexide) indicator, when the pH is made sufficiently high, the Mg is largely precipitated as hydroxide and an indicator is used that combines with Ca only. Thus, Mg can be obtained by deduction of Ca from Ca+Mg content. Both cations can also estimated by atomic absorption spectrophotometer.

Apparatus

Burette (25 or 50 mL), Pipette, Stirring rods, Graduated cylinder, Beakers

Reagents

- **Buffer Solution (NH_4Cl-NH_4OH):**Dissolve 67.5 g NH_4Cl in 570 mL concentrated NH_4OH, and transfer the solution to a 1 L flask, let it cool, and bring to volume.
- **Eriochrome Black T Indicator:**Dissolve 0.5 g eriochrome black and 4.5 g hydroxylamine hydrochloride in 100 mL 95 % ethyl alcohol. Prepare a fresh batch every month.
- **Ethylene Diaminetetraacetic Acid Solution (EDTA), 0.01 *N*:**Dissolve 2 g EDTA, and 0.05 g magnesium chloride ($MgCl_2$) in distilled water, and bring to 1 L volume.
- **Sodium Hydroxide Solution (NaOH), 2 *N*:**Dissolve 80 g NaOHin about 800 mL distilled water, transfer the solution to a 1 L flask, cool, and bring to volume.
- **Ammonium Purpurate Indicator ($C_8H_8N_6O_6$):**Mix 0.5 g ammonium purpurate (Murexid) with 100 g potassium sulfate(K_2SO_4).
- **Standard Stock Calcium Chloride Solution ($CaCl_2.2H_2O$), 0.01*N*:**Dry about 3 g $CaCO_3$in an oven at 100 °C for 3 hours, cool in a desiccator, and store in a tightly stoppered bottle. Dissolve 0.5 g dried $CaCO_3$in 10 mL 3 *N* hydrochloric acid(HCL)and bring to1 L volume with distilled water. Standard stock can also be prepared by dissolving 0.735 g calcium chloride dihydrate($CaCl_2.2H_2O$) in 1 L volume with distilled water.

Procedure

A. Calcium

1. Pipette a suitable aliquot of water sample (10 mL natural water sample) into a 250 mL Erlenmeyer flask.
2. Dilute to 20 – 30 mL with distilled water, add 2 - 3 mL 2 *N* NaOH solution, and about 50 mg ammonium purpurate indicator.
3. Titrate with 0.01 *N* EDTA. The color change is from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 seconds since the color change is not instantaneous.

B. Calcium plus Magnesium

1. Pipette a suitable aliquot of water sample (10 mL natural water sample), dilute to 20-30 mL with distilled water. Then add 3-5 mL buffer solution. And a few drops eriochrome black indicator.
2. Titrate with 0.01 *N* EDTA until the color changes from red to blue.
3. In order to standardize the EDTA solution used in the determination of Ca and Mg:
    - Pipette 10 mL 0.01 *N* calcium chloridesolution, and treat it as in determining Ca and Ca+Mg procedure, respectively.
    - Take the reading, and calculate EDTA normality:

$$\textbf{NaEDTA}\ (\text{me L}^{-1}) = \frac{10 \times N\ CaCl_2}{V\ EDTA}$$

Where:

N EDTA = Normality of EDTA solution
V EDTA = Volume of EDTA solution used (mL)
N CaCl2= Normality of $CaCl_2$ solution

Calculation

$$\textbf{Ca or Ca + Mg}\ (\text{me L}^{-1}) = \frac{V_1 \times N \times 1000}{V}$$

$$\text{Mg}\ (\text{me L}^{-1}) = \text{Ca + Mg}\ (\text{me L}^{-1}) - \text{Ca}\ (\text{me L}^{-1})$$

Where:

V1 = Volume of EDTA titrated for the sample (mL)
N = Normality of EDTA solution
V = Volume of water sample used for measurement (mL)

Practical suggestions

- In most water nearly all of the hardness is due to Ca and Mg, which react with soap to form precipitates .This in increases soap consumption, and react with certain constituents to form scale. As a general rule, a value less than 60 is considered soft, and values above 200 are considered very hard.
- If an Atomic Absorption Spectrophotometer is used, a small aliquot of the water sample is sufficient to determine Ca and Mg.
- Orthophosphate precipitates Ca at pH of the test. Strontium (Sr) and barium (Ba) interfere with the Ca determination, and alkalinity in excess of 300 mg L^{-1} may cause an indistinct end-point with hard waters. Under the conditions of the test, normal concentration of the following ions causes no interference with the Ca determination: Cu, Fe, Mn, Zn, Al, Pb, Cu, and Sn.

18. Metals — Total and Dissolved Metals and Metalloids

Depending upon the source of origin water may contain a variety of metals and metalloids. If these metals rich water is used for irrigation they may enter in food chain and this has implications for human health as well as environmental health. Metals commonly determined include: aluminium (Al), silver (Ag), arsenic (As), boron (B), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg) , potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), titanium (Ti), uranium (U), vanadium (V) and zinc (Zn). Water samples can be analyzed for total as well as dissolved fraction of the metals depending. Total metals can be analyzed by digesting the sample using a concentrated nitric/hydrochloric acid added to an unfiltered water sample prior to analysis. The dissolved metals are determined by analyzing those metals in a filtered sample that passes through a 0.45 µm membrane filter. Before analysis of a field-filtered, field-acidified sample, some extra dilute acid is added to the filtered sample, to ensure dissolution of any precipitates formed after filtration.

Preliminary digestion for total metals analysis

To reduce interference by organic matter and to convert metal associated with particulate to a form (usually the free metal) that can be determined by atomic absorption spectrophotometer, use one of the digestion techniques. Use the least rigorous digestion method required providing complete and consistent recovery compatible with the analytical method and the metal being analysed. Nitric acid will digest most samples adequately. Nitrate is an acceptable matrix for both flame and electro-thermal atomic absorption.

Nitric Acid Digestion

Equipments and apparatus:

Atomic absorption spectrophotometer (AAS), hot plate, Conical (Erlenmeyer) flasks, 125 mL or Griffin beakers 150 mL, acid-washed and rinsed with double distilled water; volumetric flasks, 10 mL pipettes,

Reagents:

- **Nitric acid:** concentrated analytical grade or trace metal grade
- **Standard stock solution 'A':** Stock standard solutions are prepared from analytical reagent grade high purity metals, oxides, or nonhygroscopic salts using reagent water (double distilled water) and redistilled nitric or hydrochloric acids. Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions may also be used. Standard stock solutions of metals are prepared as given below:
    - Aluminum: Dissolve 1.000 g of aluminum metal in dilute HCl with gentle warming and dilute to 1 L with reagent water.
    - Antimony: Carefully weigh 2.743 g of antimony potassium tartrate, and dissolve in reagent water. Dilute to 1 L with reagent water.
    - Barium: Dissolve 1.779 g of barium chloride, analytical grade and dilute to 1 L with reagent water.
    - Beryllium: Dissolve 11.659 g of beryllium sulfate, BeSO4, in reagent water containing 2 mL of nitric acid (concentrated) and dilute to 1 L with reagent water.
    - Cadmium: Dissolve 1.000 g of cadmium metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water.

- Calcium: Suspend 2.500 g of calcium carbonate, $CaCO_3$, dried for 1 hr at 180 °C in reagent water and dissolve by adding a minimum of dilute HCl. Dilute to 1 L with reagent water.
- Chromium: Dissolve 1.923 g of chromium trioxide, CrO_3, in reagent water, acidify (to pH 2.0) with redistilled HNO3 (conc.), and dilute to 1 L with reagent water.
- Cobalt: Dissolve 1.000 g of cobalt metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water. Chloride or nitrate salts of cobalt(II) may be used. Although numerous hydrated forms exist, they are not recommended unless the exact composition of the compound is known.
- Copper: Dissolve 1.000 g of electrolytic copper in 5 mL of redistilled HNO_3 (conc.) and dilute to 1 L with reagent water.
- Iron: Dissolve 1.000 g of iron wire in 10 mL redistilled HNO_3 (conc.) and reagent water and dilute to 1 L with reagent water. Note that iron passivates in conc. HNO_3, and therefore some water should be present.
- Lead: Dissolve 1.599 g of lead nitrate, $Pb(NO_3)_2$, in reagent water, acidify with 10 mL of redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.
- Lithium: Dissolve 5.324 g of lithium carbonate, Li_2CO_3, in a minimum volume of 1:1 HCl and dilute to 1 L with reagent water.
- Magnesium: Dissolve 1.000 g of magnesium metal in 20 mL 1:1 HNO_3 and dilute to 1 L with reagent water.
- Manganese: Dissolve 1.000 g of manganese metal in 10 mL of redistilled HNO_3 (conc.) and dilute to 1 L with reagent water.
- Molybdenum: Dissolve 1.840 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, and dilute to 1 L with reagent water.
- Nickel: Dissolve 1.000 g of nickel metal or 4.953 g of nickel nitrate, $Ni(NO_3)_2.6H_2O$, in 10 mL of HNO_3 (conc.) and dilute to 1 L with reagent water.
- Osmium: Procure a certified aqueous standard from a supplier and verify by comparison with a second standard. If necessary, standards can be made from osmium compounds. However, due to the toxicity of these compounds, this approach is not advised.
- Potassium: Dissolve 1.907 g of potassium chloride, KCl, dried at 110 °C, in reagent water and dilute to 1 L with reagent water.
- Silver: Dissolve 1.575 g of anhydrous silver nitrate, $AgNO_3$, in reagent water. Add 10 mL of HNO_3 (conc.) and dilute to 1 L with reagent water. Store in a dark colored glass bottle in a refrigerator.
- Sodium: Dissolve 2.542 g of sodium chloride, NaCl, in reagent water, acidify with 10 mL of redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.
- Strontium: Dissolve 2.415 g of strontium nitrate, $Sr(NO_3)_2$, in 10 mL of conc. HCl and 700 mL of reagent water. Dilute to 1 L with reagent water.
- Thallium: Dissolve 1.303 g of thallium nitrate, $TlNO_3$, in reagent water, acidify (to pH 2) with 10 mL of conc. HNO_3, and dilute to 1 L with reagent water.
- Tin: Dissolve 1.000 g of tin metal in 100 mL conc. HCl and dilute to 1 L with reagent water.
- Vanadium: Dissolve 1.785 g of vanadium pentoxide, V_2O_5, in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.
- Zinc: Dissolve 1.000 g of zinc metal in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.
- **Standard working solutions:** At least 10 working standards in the range of 0 to 3.0 mg $L^{-1}$for Zn and Cu and 0-20 mg L^{-1} for Fe and Mn. The working standards should be prepared in the medium of the extracting solution after every few days as it cannot be preserved for long.

Procedure:

1. Transfer a measured volume (50 mL) of well-mixed, acid-preserved sample to a flask or beaker.
2. Add 5 mL conc. $HNO_3$and a few boiling chips or glass beads.
3. Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 to 20 mL).
4. Continue heating and adding conc. HNO_3 as necessary until digestion is complete as shown by a light-coloured, clear solution. Do not let sample dry during digestion.
5. Wash down flask or beaker walls with water and then filter if necessary.
6. Transfer filtrate to a 10 mL volumetric flask with two 5 mL portions of water, adding these rinsing to the volumetric flask.

7. Cool, dilute to mark and mix thoroughly.
8. Take portions of this solution for required metal determinations using Atomc absorption spectrophotometer.
9. Feed the standard working solutions and prepare a standard curve by plotting AAS readings against metal concentrations.

Calculations

Metals in water samples (mg L^{-1}) = $\frac{A \times D \times v}{V}$

Where,
A stands for the metal concentration in aliquot as read from X-axis of standard curve against the sample reading
D is the dilution factor
v final volume after digestion
V volume of water taken for digestion
Appendix-1

19. Water quality indices and suitability

After determination of water samples for different parameters like total salts (EC), cations and anions, it is imperative to calculate some indices in order to asses water quality and its subsequent effect on soil as well as plant growth.

Sodium Adsorption Ratio (SAR):

It is calculate to indicate the sodicity or alkalinity hazards of irrigation water.

$$SAR = \frac{Na}{\frac{\sqrt{Ca+Mg}}{2}}$$

Residual sodium carbonate(RSC):

The RSC may be calculated simply by subtracting the quantity of Ca + Mg from the total of the carbonates and bicarbonates determined in different samples and expressed in me L^{-1}

$$RSC = (CO_3^{--} + HCO_3^{-}) - (Ca^{++} + Mg^{++})$$

20. Further reading/reference books

For the preparation of this manual, the authors have drawn heavily from the following sources:

APHA, (1998).Standard methods for the examination of waters and wastewaters. APHAAWWA-WEF, Washington, DC.

Bremner, J. M. and Keeney, D. R. (1965). Steam distillation methods for determination of ammonium, nitrate, and nitrite. *Anal. Chem. Acta*. 32: 215 – 163.

Dhyan singh, Chhonkar, P. K. and Dwivedi, B.S. (2007). *Manual on plant and water Analysis.* Westville Publishing House, New Delhi, India. pp 199.

Estefan, G. Sommer, R. and Ryan J. (2013). *Methods of Soil, Plant, and Water Analysis***:** A manual for the West Asia and North Africa region -Third Edition, ICARDA, Beirut, Lebanon.

FAO, (1974). The Euphrates Pilot Irrigation Project. *Methods of soil analysis*, Gadeb Soil Laboratory (A laboratory manual). Food and Agriculture Organization, Rome, Italy.

Hesse, P.R. (1994). *A Text Book of Soil Chemical Analysis.*CSS Publishers and Distributors , *Delhi, pp 520.*

Jackson, M.L. (1958). Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ, USA.

Muhammed, S. (1996). Soil salinity, sodicity, and water logging. p. 472- 506. *In* A. Rashid and K. S. Memon *Soil Science*. National Book Foundation, Islamabad, Pakistan.

Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *U. S. Dep. Agric. Circ.* 939, USA.

Page, A. L. (ed.). (1982). *Methods of soil analysis*, *Agron. 9, Part 2: Chemical and mineralogical properties,* 2nd ed., *Am. Soc. Agron*., Madison, WI, USA.

Tandon, H.L.S (1993). *Methods of Analysis of soils, Plant, Waters and Fertilizers.* Fertilizer Development Consultation Organization, New Delhi, India. pp 144

APPENDIX-I

Guidelines for using saline waters (RSC <2.5 me L^{-1}) for irrigation in India

Soil texture (% clay)	EC_w(d Sm^1) limit for crops and rain fall region (mm)								
	Sensitive			Semi- tolerant			Tolerant		
	<300	350-500	>500	<300	350-500	>500	<300	350-500	>500
Fine>30	1.0	1.0	1.5	1.5	2.0	3.0	2.0	3.0	4.5
Moderately Fine (20-30)	1.5	2.0	2.5	2.0	3.0	4.5	4.0	6.0	8.0
Moderately Coarse (1-20)	2.0	2.5	3.0	4.0	6.0	8.0	6.0	8.0	10.0
Coarse (<10)	-	3.0	3.0	6.0	7.5	9.0	8.0	10.0	12.5

APPENDIX-II

Guidelines for using alkali/sodic waters

Soil texture (% clay)	Upper limits of	
	SAR (mole L^{-1})	RSC (me L^{-1})
Fine>30	10	2.5-3.5
Moderately Fine (20-30)	10	3.5-7.5
Moderately Coarse (1-20)	15	7.5-10.0
Coarse	20	7.5-10

APPENDIX-III

Percent concentration, specific gravity, normality and amount needed for making 1N solution of some commonly used liquid reagents.

Reagent	Conc. (%w/w)	Sp. Gr. at 20oC	Normality (approx.)	Amt. required for 1 L of 1N solution
Acetic acid glacial	99.0	1.06	17.5	58
Ammonium hydroxide	28.3	0.91	15.0	67
Hydrochloric acid	39.0	1.19	11.8	78
Nitric acid	71.0	1.42	15.6	62
Phosphoric acid	85.0	1.70	44.0	23
Sulphuric acid	96.0	1.84	36.0	28

APPENDIX-IV

Potential irrigation problem	Units	Degree of restriction on use
Element	Recommended max.conc.(mgL^{-1})	Remarks
As	0.10	Toxicity to plants varies widely, ranging from 12 mgL^{-1} for Sudan grass to less than 0.05 mg L^{-1} for rice.
Be	0.10	Toxicity to plants varies widely, ranging from 5 mg/l for kale to 0.5 mg L^{-1} for bush beans.
Cd	0.01	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg L^{-1} in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans.
Co	0.05	Toxic to tomato plants at 0.1 mg L^{-1} in nutrient solution. Tends to be inactivated by neutral and alkaline soils.
Cu	0.20	Toxic to a number of plants at 0.1 to 1.0 mg L^{-1} in nutrient solutions.
F	1.0	Inactivated by neutral and alkaline soils.
Fe	5.0	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings.
Li	2.5	Tolerated by most crops up to 5 mgL^{-1}; mobile in soil. Toxic to citrus at low concentrations (<0.075 mg L^{-1}). Acts similarly to boron
Mn	0.20	Toxic to a number of crops at a few-tenths to a few mg L^{-1}, but usually only in acid soils.
Mo	0.01	Not toxic to plants at normal concentrations in soil and water. Can be toxic to livestock if forage is grown in soils with high concentrations of available molybdenum.
Ni	0.20	Toxic to a number of plants at 0.5 mg L^{-1} to 1.0 mg L^{-1}; reduced toxicity at neutral or alkaline pH.
Pb	5.0	Can inhibit plant cell growth at very high concentrations.
Se	0.02	Toxic to plants at concentrations as low as 0.025 mg L^{-1} and toxic to livestock if forage is grown in soils with relatively high levels of added selenium. As essential element to animals but in very low concentrations
Ti	-	Effectively excluded by plants; specific tolerance unknown.
V	0.10	Toxic to many plants at relatively low concentrations.
Zn	2.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured.

Some important conversion factors

$N \times 1.286 = NH_4$
$N \times 4.43 = NO_3$
$P \times 2.29 = P_2O_5$
$K \times 1.20 = K_2O$
$Ca \times 1.40 = CaO$
$Ca \times 1.85 = Ca(OH)_2$
$S \times 3 = SO_4$
Acre $\times 2.471$ = hectare = 2.24×10^6 kg soil (plough layer)
Hectare $\times 0.404686$ = Acre
lb $acre^{-1} \times 1.121 = kg\ ha^{-1}$
meq $L^{-1} \times$ equivalent wt. = ppm
ppm = $\mu g\ mL^{-1}$ = mg L^{-1}