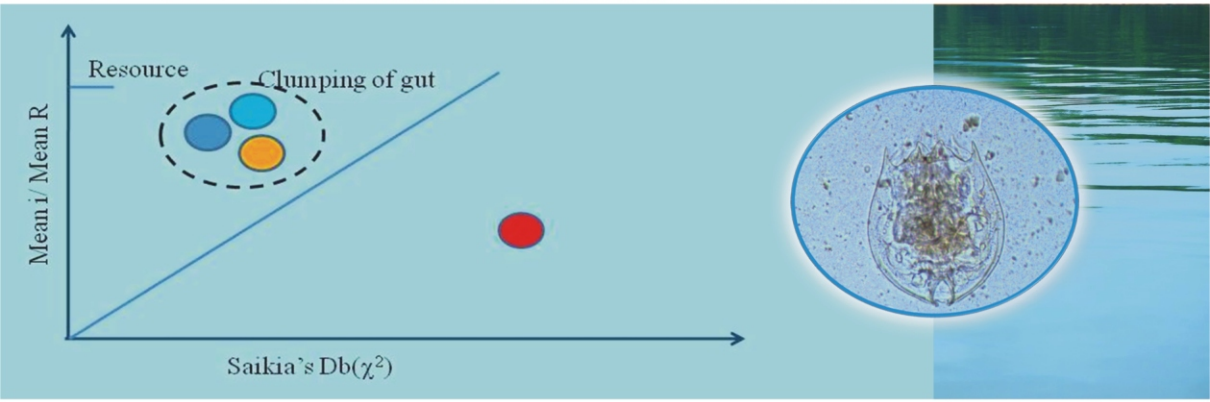
LABORATORY HAND BOOK ON BASIC ECOLOGY

(SOIL, WATER, PLANKTON AND FEEDING ECOLOGY WITH SPECIAL MENTION TO PERIPHYTON)

SURJYA KUMAR SAIKIA
DEBANGSHU NARAYAN DAS



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To

my parents…..

whose every memory is a living lesson

of struggle in life.

-Surjya Kumar Saikia

Preface

Laboratory work requires understanding and thorough knowledge of principles and working mechanism of experiments. Analysis of ecological parameters, especially soil and water involves skilled technical hand. The present book is a synthesized work to cover all these aspects in one place. The book is divided into four sections - Section I, II, III and IV. Each section is further subdivided into several Chapters for convenience. The part chemical analysis of soil and water include all principles and chemical reaction related to experiments concerned. The book has mathematical details for plankton and periphyton studies. The part diversity and feeding ecology studies denote special feature to the book. The experiments mentioned in this book are simple and fit for undergraduate and post graduate classes. In addition, it has been designed to help researchers working in aquatic ecological laboratories.

The authors would be grateful for any comments, suggestions and criticisms from the users of this handbook.

Dr. Surjya Kumar Saikia
Professor Debangshu Narayan Das

Acknowledgement

I would like to express my gratitude to the many people who provided support, offered comments, and reviewed the text, and assisted in the editing, proofreading and designing of this book.

Thanks to Visva Bharati University, Santiniketan, West Bengal, India - without your financial support this book would never find its way to the publisher and to so many people through Web.

I would like to thank my wife who supported and encouraged me in spite of all the time it took me away from them.

I would like to thank my parents for their unending love and support.

Apart from above, I would like to specially mention Sudarshana Nandi and Sandip Majumder, my PhD research scholars, who assisted me one way or other, while correcting and editing the book during its preparatory stages.

Last and not least, I beg forgiveness of all those who have been with me over the course of my research and whose names I have failed to mention.

Dr. Surjya Kumar Saikia

Contents

Section I

Water Analysis

Chapter 1

Chemical Analysis of Water

1.1 pH

The pH gives a scale of available hydrogen ion concentration in water. If free H^+ ions are more than OH^- ions, the water will be acidic, or otherwise alkaline. The most chemically pure water at 22℃ is partly dissociated into H^+ and OH^- ions. This quantity is exactly 10^{-14} g molecules of dissociated H^+ and OH^- ions. Therefore, the H^+ ions are 10^{-7}. This value is expressed in terms of negative logarithm of the total H^+ ion concentration. Hence, pH = 7 indicates neutral water, below this scale is acidic and above alkaline.

Fig. 1. *Laboratory and field pH meter.*

(1) Measurement Procedure

pH of water can be accurately measured using pH meter of different makes. Most of the pH meters come with following accessories/chemicals:

1. One digital pH meter with electrode.
2. Two sets of buffer solutions, mostly of pH 4.0 and 9.2.

BOX 1

However, buffers of these two ranges (4.0 and 9.2) can be prepared from different buffer solutions. Some of these are:

1. Phthalate buffer: A solution of 10.2 g Potassium hydrogenphthalate + 1L distilled water gives pH of 4.0 (±0.01).
2. Phosphate buffer: A solution of 3.4 g of KH_2PO_4+4.45 g of $NH_2HPO_4.2H_2O$ + 1L distilled water gives a pH of 6.9 (±0.05).
3. Borax buffer: A solution of 3.81 g of $Na_2B_4O_7.10H_2O$ + 1L distilled water gives a pH of 9.3 (±0.12).

Operation procedures for different pH meters are different. However, following procedure is described for most usual pH meters.

1. Switch on the pH meter and allow some time for stabilization.
2. Wash the electrode with distilled water and connect to electrode holder of the pH meter.
3. Dip the electrode in buffer solution of pH 4.0 and move the temperature knob to specified buffer temperature. Adjust the set buffer knob until it reads 4.0.
4. Turn to selector switch 0.
5. Wash the electrode with distilled water and dip in buffer pH solution 9.2.
6. Adjust the set buffer knob until it reads 9.2.
7. Turn selector switch to 0.
8. Wash the electrode with distilled water.
9. Read the pH of the sample by dipping the electrode into the sample. Fix the temperature knob to the temperature of the sample.
10. Wait for some time and note the reading.

(2) Caution

Prolonged dipping of electrode into the sample may cause variation in reading. Now a day, portable pH meters are available which are handy and does not require any buffer setting before use.

1.2 Electrical Conductivity

Electrical conductivity is a measurement required for brackish or sea water. In case of pollution studies, this parameter is required for fresh water.

(1) Principle

Electrical conductivity focuses on dissolved salts or their ions in water which are good conductors of an electrical current. It is measured by a probe that applies voltage between two electrodes, spaced a known distance apart, and records the drop in voltage. This drop reflects the resistance of the water, which is then converted to conductivity. Thus, conductivity is the inverse of resistance and is measured in the amount of conductance over a certain distance. The conductivity units are called "mhos" - the inverse of "ohms" used in resistance. For most natural waters, the units of mhos/cm are too large, so conductivity is reported as micro-mhos/cm where 10^6 micromhos is equal to one mho. Sometimes the units are expressed as microSiemens and 1 microS is equal 1 micromhos/cm.

(2) Requirements

Electrical Conductivity meter.

(3) Procedure

Most of the Electrical Conductivity meter comes with a temperature calibration knob and two probes (electrodes) for conductivity measurement. Electrical conductivity varies at different temperatures. Therefore, it is necessary to set the temperature of Electrical Conductivity meter as close as possible to the ambient temperature of the sampling station. The probes are then dipped to water for some time (15-20 minutes). Keeping probes under water for a longer time will give different results since salts or ions start depositing on the probes.

Electrical Conductivity also represents salinity of water. However, a g/l conversion can be made if the ratio of salts present in water sample is known.

1.3 Total Alkalinity

(1) Principle

The amount of acid required to titrate the bases in water are a measure of the alkalinity of water. Water contains a number of bases, including carbonates, bicarbonates, hydroxides, silicates, phosphates, ammonia and various organic compounds occur in water. However, bicarbonates (HCO_3^-), carbonates (CO_3^-) and hydroxides (OH^-) are considered as the predominant bases in natural waters. Since water with pH value of about 4.5 and above may contain bicarbonate, water sample that turn yellow upon the addition of methyl orange indicates alkalinity.

(2) Why $CaCO_3$ Alkalinity

Alkaline earth carbonates such as calcite or dolomite are the principal sources of bases in water, so alkalinity has been traditionally expressed as mg/L of $CaCO_3$. The standard H_2SO_4 used for alkalinity titration is often of such strengths that 1 ml is exactly equal to 1mg of $CaCO_3$.

(3) How to Express Alkalinity

Results of alkalinity titration is expressed as total alkalinity or as individual components of alkalinity, i.e. hydroxide, carbonate and bicarbonate alkalinity.

(4) Phenolphthalein and Methyl Orange Alkalinity

When, in addition of phenolphthalein indicator, water sample turns to pink, (pH above 8.4), they contain measurable carbonate ions. In that case, alkalinity titration is carried out in two steps.

(1) The sample is first titrated to the phenolphthalein end point which turns all CO_3^{2-} to HCO_3^-

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^-$$

(2) The sample is then titrated with H_2SO_4 until all of the HCO_3^- is converted to

carbon dioxide and water at the methyl orange end point.

$$HCO_3^- + H^+ \leftrightarrow H_2O + CO_2$$

(5) Reagents

Phenolphthalein indicator: Dissolve 0.5gm of phenolphthalein in 50ml of 95 % ethyl alcohol and add 50ml distilled water.

Methyl orange indicator: Dissolve 0.05gm of methyl orange in 100ml of distilled water.

Standard Sodium Carbonate Na_2CO_3, 0.0200N: Dissolve 1.0600gm of anhydrous Na_2CO_3 and dilute to 1000ml in CO_2 free distilled water. Boil distilled water for 10 to 15 minutes to expel CO_2 and cool before using. The Na_2CO_3 must be used within a few hours of preparation.

Standard H_2SO_4 titrant, 0.0200N: Prepare H_2SO_4 stock solution of approximately 0.1 N by diluting 2.8ml of conc. H_2SO_4 to 1000ml with CO_2 free distilled water. This solution is approximately 0.02N, but it must be carefully standardized to determine its exact normality. To standardize, pipet 10.00ml of 0.02N Na_2CO_3 into a 250ml beaker. Add 90ml of CO_2 free distilled water and 4 to 8 drops of methyl orange indicator solution. Select a number of drops of methyl orange which allows easy end point detection and use this number of drops in all subsequent titrations. Titrate over a white surface to the methyl orange end point with standard H_2SO_4. At the end point, one drop of acid will change the colour of methyl orange from yellow to faint orange. Calculate the normality of the sulphuric acid from the following equation:

$$NV = N'NV'$$

Where,

N= Normality of the standard;

V= Milliliter of the standard used in titration;

N' = Normality of the solution being standardized;

V ′= Volume of the solution in milliliters'.

(6) Procedure for Phenolphthalein Alkalinity

- Measure 100ml of water sample into a 250ml beaker.
- Add 2 drops of phenolphthalein indicator solution.

- If the sample turns pink, it contains phenolphthalein alkalinity. Titrate with standard H_2SO_4 solution until one drop of acid causes the pink colour of the sample to disappear.
- This sample can be saved for methyl orange alkalinity.
- Calculate the phenolphthalein alkalinity by following equation:

$$\text{Phenolphthalein alkalinity} = \frac{(\text{ml of titrant use})(\text{N})(50)(1000)}{\text{Sample volume in ml}}$$

(7) Total Alkalinity

- Take 100ml of fresh sample and add 4 to 8 drops of methyl orange indicator solution to it.
- Titrate with standard H_2SO_4 until the colour of the solution changes from yellow to faint orange.

(8) Measuring Total Alkalinity on Sample from Phenolphthalein Alkalinity

- Titration can be carried out on the sample used to measure phenolphthalein alkalinity.
- Add 4 to 8 drops of methyl orange indicator.
- Now titrate against the titrant used (in ml) in total alkalinity before calculating the total alkalinity of this sample.

$$\text{Total alkalinity} = \frac{(\text{ml of titrant used})(\text{N})(50)(1000)}{\text{Sample volume in ml}}$$

1.4 Total Hardness

(1) Introduction

The concentration of calcium plus magnesium ions expressed as equivalent $CaCO_3$ has traditionally been taken as a measure of total hardness. Other divalent metals also contribute to hardness, but their concentrations are negligible in natural waters.

(2) Principle

Calcium and magnesium ions are titrated with the complexing agent ethylene diamine tetra acetic acid (EDTA) to form the stable complexes CaEDTA and MgEDTA. The end point of the titration is signaled with a second complexing

agent, Erichromic Black -T.

If a small quantity of Erichromic Black-T is added to a water sample buffered at pH 10, it will form a soluble wine red complex with some of the calcium and magnesium ions. In the titration, the EDTA will first complex all of the free Ca^{2+} and Mg^{2+}, and then Calcium and Magnesium will dissociate from their complexes with Erichromic Black-T to form more stable complexes with EDTA. When all of the calcium and Magnesium has been complexed by EDTA, the colour of the solution turns blue.

$$Ca^{2+} + Mg^{2+} + \text{Erichromic Black} - T \rightarrow Ca \text{ and } Mg \text{ Erichromic Black} - T \text{ (Wine red)} + EDTA \rightarrow CaEDTA + MgEDTA + \text{Erichromic Blacl} - T \text{ (blue)}$$

(3) Reagents

Buffer solution: Dissolve 67.5gms of NH_4Cl in 570ml of concentration NH_4OH. Dilute to 1000ml in a volumetric flask with distilled water.

Erichromic Black-T indicator: Dissolve 4.5gms of hydroxylamine hydrochloride and 0.50gm of Erichromic Black-T in 100ml of 70% ethanol. This indicator should be prepared fresh every 2 to 3 months.

Standard Calcium Solution, 0.010M: Transfer 1000gm of anhydrous $CaCO_3$ to a 1000ml beaker. Add 1.1HCl slowly to dissolve the $CaCO_3$ and dilute to about 200ml with distilled water. Boil for 5 minutes to expel CO_2, cool and adjust to pH 7, as determined with a pH meter, with 3N NH_4OH. Transfer to a 1000ml volumetric flask and dilute to volume with distilled water.

Standard EDTA titrant: Dissolve 4.00gm of EDTA salt and 100mg of $MgCl_2.6H_2O$ in distilled water to 1000ml. This solution must be standardized against the standard calcium solution. Pipet 10ml of standard calcium solution into a 250ml beaker and add 90ml of distilled water. Add 8 drops of Erichromic Black-T. Titrate the calcium with EDTA.

Compute the normality of the EDTA from equation $NV = N'V'$ (See Total alkalinity).

(4) Procedure

- Measure 100ml of water sample into a 250ml Erlenmeyer flask.
- Add 2.0ml of the buffer solution and mix.
- Add 8 drops of Erichromic Black-T indicator.

- Titrate against EDTA solution. At the end point, the solution will change from wine red to pure blue.
- Calculate the total hardness by following equation.

$$\text{Total Hardness (mg/L as } CaCO_3) = \frac{(\text{ml of EDTA})(M)(100.0)(1000)}{\text{Sample volume in ml}}$$

(5) Comments

Sample for total hardness titration should not be stored for more than 2-3 days.

Note: Total hardness can also be measured partly as Calcium hardness and Magnesium hardness.

(6) Interpretation

Type of water	Hardness range (mg/L)
Soft water	0-60
Medium water	60-120
Hard water	120-180
Very hard water	>180

1.5 Calcium Hardness

(1) Principle

The calcium concentration in water is normally expressed as calcium hardness in terms of equivalent $CaCO_3$. Ethylenediamine tetra acetic acid (EDTA) which complexes Ca^{2+} is used as the titrating agent for calcium hardness. EDTA forms a stable complex with both Ca^{2+} and Mg^{2+},

$$Ca^{2+} + \text{EDTA} \rightarrow \text{CaEDTA}$$

$$Mg^{2+} + \text{EDTA} \rightarrow \text{MgEDTA}$$

Therefore a water sample is made mildly alkaline (pH 12 to 13) to precipitate magnesium as its hydroxide so that the EDTA titration is specific for calcium. Actually, others divalent metals also form stable complexes with EDTA, but Ca^{2+} and Mg^{2+} are the predominant divalent metals in normal natural waters.

The end point of the Calcium hardness titration is detected with murexide. This indicator forms a complex with Ca^{2+} and in the presence of Ca^{2+}, murexide imparts a pink colour to a solution. The complex formed by murexide with Ca^{2+} is not as stable as the complex formed by EDTA with Ca^{2+} . In titration, a small amount of murexide is added to the sample which complexes some of the calcium ions to produce a pink colour. As EDTA is added it reacts with Ca^{2+} in solution to form CaEDTA and, when all of the uncomplexed Ca^{2+} has been titrated, the calcium dissociate from the Ca-murexide complex to form more stable complex with EDTA. The murexide turns orchid purple upon loss of its calcium. The titration is described more simply as follows:

$$\underset{\text{(Pink)}}{Ca^{2+} + Ca - \text{murexide} + EDTA} \rightarrow \underset{\text{(Orchid purple)}}{CaEDTA + \text{Murexide}}$$

(2) Reagents

Sodium hydroxide (NaOH) Solution (1N): Dissolve 40g of NaOH and dilute to 1000ml with distilled water. Store in a tightly rubber stoppered bottle.

Standard EDTA: See hardness.

Murexide indicator: Mix 200mg of murexide indicator (Ammonium purpurate) with 100g of NaCl. Grind with a morter and pestle until grinding cause no further intensification of colour (40-50 mesh). Store the indicator in an opaque bottle.

(3) Procedure

- Measure a 100ml water sample into a 250ml beaker.
- Add 4.0ml of 1N NaOH and stir.
- Add 100 to 200mg of murexide and stir while titrating slowly with standard EDTA. The colour of the solution will gradually change from pink to orchid purple. At the end point, a single drop of EDTA will cause no further increase in the intensity of the colour.
- The volume of the last drop must be subtracted from the burette reading.

(4) Calculation

$$\text{Calcium Hardness } (\text{mg/L } CaCO_3) = \frac{(\text{ml of EDTA})(\text{M})(100.0)(1000)}{\text{Sample volume in ml}}$$

(5) Precaution

The titration must be conducted immediately after the addition of the hydroxide solution and the indicator to the sample.

1.6 Magnesium Hardness

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

$$\text{mg Mg/L} = [\text{Total hardness (as } CaCO_3\text{/L)} - \text{Calcium hardness (as mg } CaCO_3\text{/L)} \times 0.243]$$

1.7 Free Carbon Dioxide (Titrimetric Method with Na_2CO_3)

(1) Principle

Water having a pH value more than 8.34 (here Phenolphthalein end point) does not contain appreciable carbon dioxide dissolved in it. Therefore, the amount of base required to raise the pH of a water sample to the phenolphthalein end point is approximately equivalent to the CO_2 content of the sample.

CO_2 reacts with standard solution (NaOH, a base or Na_2CO_3, a bicarbonate) as follows:

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O \quad (1)$$

$$Na_2CO_3 + H_2O \rightarrow 2NaHCO_3 \quad (2)$$

These two reactions indicate two possible methods for determining CO_2 in water- titration either with standard NaOH or with standard Na_2CO_3 to the phenolphthalein end point. The use of sodium carbonate over NaOH for the titration is that Na_2CO_3 is a primary standard and it is not essential to standardize a solution of Na_2CO_3 before titration.

(2) Reagents

- Phenolphthalein indicator- Dissolve 0.5gm of phenolphthalein in 50ml of 95

% ethyl alcohol and add 50ml CO_2 free distilled water. Add 0.0454N sodium carbonate dropwise until a faint pink colour appears to remove all traces of CO_2 from the indicator.

- Standardize Sodium Carbonate (0.0454N) - Dissolve 2.407gms of anhydrous Na_2CO_3 and dilute to 1000ml with CO_2 free distilled water. This standard solution should be made free each day.

(3) Procedure

- Collect water sample carefully so that it does not come in contact with the atmosphere.
- Analyze for CO_2 immediately after collection or within 2 or 3 hours of sample collection.
- Take 50ml sample and add gently 2-3 drops of phenolphthalein indicator solution.
- If the sample turns pink, the pH is above 8.34 and free CO_2 is essentially absent. If the sample remains colorless, it contains free CO_2.
- Sample containing CO_2 must be titrated rapidly with 0.0454N Na_2CO_3 solution. Stir the sample gently while Na_2CO_3 is added. A faint pink colour which remains for 30 seconds marks the end point.

(4) Calculation

$$\text{Free } CO_2 \text{ in ppm} = \frac{\text{ml of N/44 } Na_2CO_3}{\text{Sample volume in ml}} \times 1000$$

$$\text{Free } CO_2 \text{ in mg/L} = \frac{\text{ml of } Na_2CO_3 \times N \times 22}{\text{Sample volume in ml}} \times 1000$$

1.8 Dissloved Oxygen (Winkler's Method)

(1) Principle

In the basic Winkler procedure (Winkler, 1888), a sample of water is treated with Manganous Sulphate $MnSO_4$, Potassium Iodide KI and NaOH. Under highly alkaline condition, the Mn^{2+} ion is oxidized by molecular oxygen to Manganous dioxide (MnO_2), a brown precipitate,

$$Mn^{2+} + 2OH + {}^{1}/_{2}\, O_2 \rightarrow MnO_2 + H_2O$$

Thus only one half of the oxygen in Manganous dioxide came from molecular oxygen. The H_2SO_4 is added to the sample to dissolve the precipitate and produce acid condition for the oxidation of iodide to iodine by Manganous dioxide. The reaction is:

$$MnO_2 + 2I^- + 4H^+ \rightarrow Mn^{2+} + I_2 + 2H_2O$$

The equation shows that the quantity of I_2 released is proportional to the amount of O_2 originally present i.e. one half of a molecule of O_2 resulted in the release of one molecule of iodine (I_2). The amount of I_2 is estimated by titration with standard Sodium thiosulphate. A starch indicator is used to determine the end point of the titration. As long as iodine is present, the solution is blue. When all the iodine has been titrated the solution becomes colourless.

$$I_2 + \text{Starch} - I_2 + 2Na_2S_2O_3.5H_2O \rightarrow Na_2S_4O_6 + 2NaI + 10H_2O + \text{Starch (Colourless)}$$

The amount of iodine used in reaction is taken to calculate the original Dissolved Oxygen concentration.

(2) Reagent

Manganous Sulphate solution: Dissolve 364gms of $MnSO_4.H_2O$ in distilled water, filter and dilute to 1000ml in a volumetric flask.

Alkali-Iodide-Azide solution: Dissolve 500gms of NaOH and 150gms of KI in distilled water and dilute to 1000ml in a volumetric flasks. Dissolve 10gmsof $NaNO_3$ in 40 ml of distilled water and add to the NaOH-KI solution.

Sodium thiosulphate solution: Dissolve 6.3gms of $Na_2S_2O_3.5H_2O$ in freshly boiled and cooled distilled water and dilute to 1000ml in volumetric flask. Add 5 drops of chloroform as preservative. This reagent must be standardized every few days and stored in the dark.

Concentrated Sulfuric acid: Analytical reagent grade.

Sulfuric acid solution 10%: Add 5ml of concentrated H_2SO_4 to 45ml of distilled water.

Potassium dichromate solution, 0.0250N: Dry 2 or 3gms of $K_2Cr_2O_7$ at 105 ℃ and cool in a volumetric flask to 500ml with freshly boiled and cooled distilled water.

Starch indicator: Add 2gms of soluble starch to 100ml of distilled water in a 250ml beaker. Heat while stirring until transparent and add 0.5ml of formalin as a preservative.

Standardization of Sodium thiosulphate solution: Dissolve 2gms of KI in a 500ml volumetric flask with 100ml of distilled water and add 10ml 10% H_2SO_4 solution. Add 10ml of 0.0250N $K_2Cr_2O_7$ into the flask and place the flask in the dark for 5minutes. Dilute to 250 or 300ml of distilled water. Titrate with sodium thiosulphate solution until a pale straw colour is reached. Add 8 drops of starch indicator and titrate until the blue colour of the starch suddenly disappears. Record the volume of the sodium thiosulphate used and calculate the normality by $NV = N'V'$ (See Total alkalinity).

(3) Procedure

- Collect water sample in a 300ml BOD bottle taking care so that no air buble remains in the bottle.
- Add 2.0ml of Manganous sulphate solution and 2.0ml of alkali-iodide-azide solution below the surface of sampler by dipping the pipette and stoppered with care to prevent air bubble.
- Mix the solution in the bottle by rapidly inverting it around twenty times and then let the sample stand until a precipitate settles to the bottom.
- Add 2.0ml of concentrated H_2SO_4 with a measuring pipette, stopper the bottle carefully, and invert several times to dissolve the precipitate.
- Take 50ml of sample in a volumetric flask.
- Add 4-5 drops of starch solution to it. It turns blue.
- Titrate against 0.025(N) Sodium thiosulfate till blue colour disappears.

(4) Calculation

$$\text{Dissolve } O_2\ (\text{mg/L}) = \frac{\text{ml of sodium thiosulfate} \times N \times 8 \times 1000}{\text{ml of sample titrated}}$$

Note: Factor 8 is multiplied because 1N Sodium thiosulfate is equal to 8 mg of O_2.

1.9 Dissolved Phosphorus (Soluble Orthophosphate)

(1) Principle

In an acid solution, orthophosphate reacts with ammonium molybdate to form an Ammonium phosphate-molybdate complex. The molybdenum in the complex can be reduced to a blue-coloured solution. The intensity of the blue colour formed in this solution increases in proportion to the amount of phosphate present since uncomplexed molybdenum in solution does not react with the reducing agent. A number of reducing agents may be employed, but stannous chloride has been widely used for this purpose in water analysis. Reaction may be summarized as:

$$PO_4^- + 12(NH_4)_2MoO_4 + 24H^+ \rightarrow (NH_4)_3PO_4.12MoO_3 + 21NH_4^+ + 12H_2O$$

The intensity of the blue colour can be measured by spectrophotometer (or colorimeter) and the concentration of orthophosphate is determined by reference to a calibration graph prepared from known concentration of orthophosphate.

(2) Special Apparatus

Suction flask, vacuum source, membrane filter holder for 47mm filters, glass fibre filter (Gelman Type A-E or equivalent, 47mm) and a spectrophotometer (or colorimeter), test tube, glass rod, physical balance, measuring cylinder.

(3) Reagents

Ammonium Molybdate, $(NH_4)6Mo_7O_{24}.4H_2O$ Reagent: Dissolve 25gm of $(NH_4)6Mo_7O_{24}.4H_2O$ in 175ml of distilled water. Cautiously add 280ml of concentrated H_2SO_4 to 400ml of distilled water in a 1000ml volumetric flask and let cool. Add the molybdate solution to the flask and dilute to 1000ml with distilled water.

Stannous Chloride, $SnCl_2$ Reagent: Dissolve 2.5gm of fresh $SnCl_2.2H_2O$ in 100ml of glycerol by heating in a water bath and stirring with a glass rod.

Standard Phosphate-Phosphorus Solution: Dissolve 0.2195gm of KH_2PO_4 in distilled water and dilute to 1000ml in a volumetric flask. This solution is too concentrated to use directly. Make a second solution containing 5.00mg/liter of phosphate-phosphorus by diluting 50ml of the first solution to exactly 500ml with distilled water. (1ml=1μgm of phosphate or 0.001mg of phosphate)

(4) Procedure

- Pipette approximate amount of phosphate solution to cover the range of 0.3-1.5mg/l into a series 100ml nessler tubes or test tubes. Dilute to 100mml using distilled water. Label the tube with the concentrations. These tubes serve as standards. Include one more tube containing 100ml distilled water as the blank.
- Take 100ml of the water sample in a nessler tube.
- To the blank, standards and sample, add 4ml of ammonium molybdate solution and mix well.
- Add 0.5ml stannous chloride to all the tubes and mix well.
- Wait for 10-12 minutes for the development of colour.
- Calibrate the spectrophotometer or colorimeter using blank (instrument reads 100% transmittance or 0% absorbance) and distilled water.
- Measure the intensity of blue coloured complex at 690nm using spectrophotometer.
- Prepare a standard curve by plotting the phosphate concentration of standard solutions on the x axis and the optical density (OD) on the y axis.
- Find the phosphorus content of the sample by matching its absorbance(s) with the standard curve.
- Express the result as mg/l phosphate as phosphorus. If it has to be expressed in terms of phosphates, multiply by a factor of 3.066.

(5) Precautions

- All glassware and containers must be carefully cleaned to prevent contamination with phosphorus. Prior to the initial use, rinse the glassware in 1 to 2N HCl, wash with detergent and tap water, and rinse in distilled water. The detergent must be phosphate free.
- Determine the orthophosphate within 2 to 3 hrs of sample collection. Collection bottles should be carefully chosen so that no additional phosphate is added to the sample. Bottles may be treated with a KI-I_2 solution to inhibit bacterial growth. To treat, fill the bottles with a solution containing 50gm I_2/l and 80gm KI/l and let stand for one week. Pour out the KI-I_2 solution (save this solution for later use) and wash the bottles thoroughly with distilled water. Any discolouration of the sample by I_2 will be destroyed by $SnCl_2$. Bottles may be filled with the KI-I_2 solution and stored until needed again.
- Use distilled water to read zero in the spectrophotometer. The reagent blank

(0.00mg/l Phosphorus) should read 100% transmittance or 0.00% absorbance on the spectrophotometer. If it doesn't, the reagents or distilled water contain phosphorus. If the amount of phosphorus contamination is slight, set the instrument at 100% transmittance with the reagent blank rather than with distilled water.

1.10 Total Phosphate

(1) Principle

The various forms of phosphorous are all hydrolyzed to orthophosphate by treatment with acid, heat and pressure. Orthophosphate is then measured by stannous chloride method.

(2) Special Apparatus

All materials required for the determination of orthophosphates; in addition, kjeldahl flask, volumetric flask, burette, Bunsen burner, tripod stand and wire gauge.

(3) Reagent

1N Sodium hydroxide: Dissolve 40gm of NaOH pellets in about 200ml of distilled water. Make up the volume to 1000ml in a volumetric flask.

Phenolphthalein: Dissolve 0.50gm of phenolphthalein in 50ml of 95% ethyl alcohol and 50ml of distilled water. Add 0.02N NaOH dropwise until a faint pink colour appears.

(4) Procedure

- Take 100ml of the sample in a kjeldahl flask.
- Add carefully 1ml of concentrated H_2SO_4 and 5ml concentrated HNO_3.
- Heat the sample until the solution becomes colourless.
- Cool and add 20ml of distilled water and 2 drops of phenolphthalein indicator.
- Titrate against sodium hydroxide until the appearance of a pale pink colour.
- Transfer the solution to a 100ml volumetric flask and dilute it upto the mark.
- Determine the total phosphates by the same procedure as described for orthophosphates.

1.11 Dissolved Nitrate (Brucine Sulphonic Acid Method)

(1) Principle

It is based on the reaction of nitrates of brucine sulphate in strong acidic condition. The intensity of colour produced by the reaction is proportional to the concentration of nitrate originally present in the sample, permitting nitrate analysis by spectrophotometer. The concentration of nitrate-nitrogen is estimated by reference to a calibration graph. The calibration graph is prepared plotting transmittance or absorbance values on the y axis versus their respective concentrations of nitrate-nitrogen on the x axis.

(2) Special Apparatus

A spectrophotometer, Whatman No 42 filter paper, Volumetric flask, Pipette, Beaker.

(3)Reagent

Coupling reagent: Dissolve 500mg of N-(I-napthyl)-ethylenediamine dihydrochloride in 500ml of distilled water. Store in a dark bottle and keep out of the light. This reagent gradually becomes dark brown and must be prepared fresh every 2 to 4 weeks.

Diazotizing reagent: Add 5g of Sulphanilamide and 50ml of concentrated hydrochlorine acid to 300ml of distilled water in a 500ml volumetric flask. Stir to dissolve and then dilute to volume.

Standard Nitrite-Nitrogen Solution (1.00mg/L): Dissolve 0.4925g of $NaNO_2$ in 1000ml of distilled water. This solution contains 100mg/L of NO_2-N. Pipette 1000ml of the 100mg/L NO_2-N solution into a 1000ml volumetric flask and dilute to volume with distilled water to give a 100mg/L NO_2^--N solution. These solutions deteriorate rapidly.

(4) Procedure

- Filter the water sample through Whatman No 42 or equivalent filter paper.
- Measure 50ml of it in a 100ml beaker.
- Add 1.0ml of coupling reagent and stir.
- Let the solution stand for 10 minutes to form the azo compound.
- Transfer to a cuvette and measure the pink colour by a spectrophotometer at 543nm.

- Use a reagent blank to set the spectrophotometer at 0.0 absorbance (100% transmittance).
- Prepare a series of NO^{2-}-N concentration from standard solution. For this, take 2.0, 4.0, 6.0, 8.0, 10.0, 15.0 and 20.0ml of 1.0mg/L NO^{2-}-N solution in 25ml test tube and add reagent as in the sample. This will give a NO^{2-}-N concentration of 0.02, 0.04, 0.06, 0.08, 0.10, 0.15 and 0.20mg/L respectively.
- Evaluate the pink colour at 543nm.
- Use 0.0mg/L solution to set the spectrophotometer to 0.0 absorbance or 100% transmittance.

(5) Calculation

$$\text{mg/L } NO_2^- - \text{N} = \frac{\text{ml } NO_2^- - \text{N} \times 1000}{\text{sample taken for estimation}}$$

$$\text{mg/L } NO_2^- = 3.28 \times \text{mg/L } NO_2^- - \text{N}$$

1.12 Sulphate Estimation

(1) Principle

Sulphate ion is precipitated in an Acetic acid medium with Barium chloride ($BaCl_2$) so as to form Barium sulphate, $BaSO_4$ crystals. Light absorbance of the $BaSO_4$ suspension in measured using spectrophotometer and the SO_4^{2-} concentration is determined by comparing with a standard curve. The minimum detectable concentration is 1mgSO_4^{2-}/L.

(2) Special Apparatus

Magnetic stirrer, Spectrophotometer, Stopwatch or electric timer, Measuring spoon (0.2 to 0.3ml).

(3) Reagents

Buffer Solution A: Dissolve 30g of Magnesium chloride $MgCl_2.6H_2O$, 5g Sodium acetate, $CH_3COONa.3H_2O$, 1.0g potassium nitrate, KNO_3 and 20ml acetic acid, CH_3COOH (99%), in 500ml distilled water and make up to 1000ml.

Buffer Solution B (Required when the sample contain less than 10mg SO_4^{--}

/L): Dissolve 30g of $MgCl_2.6H_2O$, 5g $CH_3COONa.3H_2O$, 1.0g KNO_3, 0.111g Sodium sulphate, Na_2SO_4 and 20ml Acetic acid (99%) in 500ml distilled water and make upto 1000ml.

Barium Chloride, $BaCl_2$.

Standard Sulphate Solution: Prepare a standard sulphate solution as described in (1) or (2) below:

1. Dilute 10.4 ml standard 0.0200N H_2SO_4 titrant to 100ml with distilled water.
2. Dissolve 0.1479g anhydrous Na_2SO_4 in distilled water and dilute to 1000ml.

$$\text{It gives an estimation of } 1.00\text{ml} = 100\mu\text{g } SO_4^{2-}$$

(4) Procedure

- Measure 100ml sample or a suitable portion made up to 100ml into a 250ml Erlenmeyer flask.
- Add 20ml of buffer solution A and mix in stirring apparatus.
- While stirring add a spoonful of $BaCl_2$ crystals and begin timing immediately. Stir for around 60s at constant speed.
- After stirring period has ended, measure turbidity.
- 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.

(5) Precautions

- Space standards at 5mg/L increments in the 0 to 40mg/L SO_4^{2-} range. Above 40mg/L accuracy decreases and $BaSO_4$ suspensions lose stability.
- Check reliability of calibration curve by running a standard with every three or four samples.

(6) Calculation

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

Chapter 2

Physical Analysis of Water

2.1 Potability of Water

(1) Principle

Colour is determined by visual comparison of the sample with known concentration of coloured solutions. Comparison also may be made with special, properly calibrated glass colour disks. The platinum-cobalt method measuring colour is the standard method, the unit of colour being that produced by 1mg platinum/L in the form of the Chloroplatinum ion.

(2) Chemicals Required

Potassium Chloroplatinum (K_2PtCl_4), Cobaltons Chloride ($CoCl_2.6H_2O$), Concentrated HCl.

(3) Materials Required

Nessler tube (50ml), pH meter, Aluminium foil, measuring cylinder (50ml), graduated pipette.

(4) Preparation of Stock Solution

Dissolve 1.246g pottassium Chloroplatinum (equivalent to 500mg metallic Pt) and 1.00g crystallized Cobalton Chloride (equivalent to about 250mg metallic Co) in distilled water. Carefully add 100ml concentrated HCl and make up the volume to 1litre (1000ml) with distilled water. This stock solution gives a colour value of 500 colour units.

(5) Preparation of Standards

Prepare standards having colour value of 5, 10, 15, 20, 25, 30, 25, 40, 45, 50, 55, 60, 65 and 70 by diluting 0.5, 1.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0ml of the stock solution with distilled water. Label each tube and cover with aluminum foil to protect from evaporation and contamination.

(6) Pre-Treatment of Sample

Even a slight turbidity in sample may cause deviation of apparent colour (colour due to suspended matter and other substances in the solution) from the original colour (colour from which turbidity has been removed). Therefore, removal of turbidity is necessary before putting samples into experiment. The pretreatment includes removal of turbidity by centrifugation or filtration. While centrifuging, the sample, it should be kept in mind excessive centrifugation or centrifugation at high speed may alter results.

(7) Procedure

- Take 50ml of the sample in a nessler tube.
- Hold the standard tubes vertically above white surface and match the colour of the sample with that of the nearest colour of the standard. Record the value.
- If the colour exceeds 70 units, dilute sample with distilled water in known proportions until the colour is within the range of the standard.
- Measure pH of each sample.

(8) Calculation

$$\text{Colour (unit)} = \frac{A \times 50}{B}$$

Where,

A = estimated colour of a diluted sample,

B = Volume of the sample taken for dilution in ml,

50 = Total volume taken for reading,

(Comment= Drinking water usually shows colour unit below 20.)

2.2 Turbidity

(1) Introduction and Principle

Turbidity of water can be accurately measured for any water sample that is free from debris and rapidly settling coarse sediment with the help of an instrument called 'Nephalo-turbiditymeter'. The method is based on a comparison of the intensity of light scattered by a standard reference suspension under the same condition. The higher the intensity of light, higher the turbidity. Formazine polymer is used as the primary standard reference suspension. The turbidity of a specific concentration of formazin suspension is defined as 4000NTU (Nephalo Turbidity Unit).

(2) Reagent

Hydrazine Sulphate, $(NH_2)2H_2SO_4$, Hexamethylene tetramine $(CH_2)_6N_4$, Distilled water.

(3) Apparatus

Nephalo turbiditymeter, Sample tube (made of colour free glass), 100ml volumetric flask, measuring cylinder.

(4) Stock Solutions

Solution I: Dissolve 1.0g hydrazine sulphate in distilled water and dilute to 100ml in a volumetric flask.

Solution II: Dissolve 10.0g hexamethylene tetramine in distilled water and dilute to 100ml in a volumetric flask.

Composite solution: In a flask, mix 5.0ml solution I and 5.0ml solution II. Let stand for 24h at 25±3 ℃. This solution is 4000NTU suspension. Transfer stock suspension to an amber glass or other UV-light-blocking bottle for storage. Make dilution from this stock suspension. The stock suspension is stable for upto 1year, if properly stored.

(5) Procedure

- Switch on the nephalo-turbiditymeter and keep 15minutes to warm up.
- Set nephalo-turbiditymeter to 100 using 100 NTU standard suspension (Standard solution of 50, 10 or more than 100 NTU can be used as per the level of turbidity of the sample).

- Shake the sample thoroughly and let the air bubbles subside.
- Take the sample in a nephalo-turbiditymeter tube and find out the value.
- If the turbidity of the sample exceeds 100 NTU, the sample can be diluted further with distilled water (rather than using other standard suspension) so that its turbidity can be read within 100NTU.
- Note down the reading.

(6) Calculation

$$\text{Turbidity (NTU)} = \frac{A \times (B+C)}{C}$$

Where,

A= NTU reading in diluted sample,

B= Volume of distilled water,

C= Sample volume taken for dilution in ml.

(7) Precautions

- Shake the sample thoroughly before measuring. Do not allow sample to stand for long time to remove air bubbles.
- Hydrazine sulphate is a carcinogen, avoid inhalation, ingestion and skin contact. Formazin suspensions may also contain residual hydrazin sulphate.

2.3 Temperature

(1) Introduction

Turbid water absorbs more sunlight and hence the temperature of water increases.

(2) Apparatus

Alcohol or mercury thermometer with 110 ℃ as maximum graduation.

(3) Procedure

- Record the surface temperature by holding the thermometer upright in the surface.
- Rerecord the water temperature immersing the bulb of the thermometer in it.

See that the bulb is completely immersed in water.

- Observe the mercury or alcohol column and note down the reading in Celsius when the reading becomes constant.

(4) Precautions

- Do not expose the bulb of the thermometer to direct source of heat or sunlight while taking the reading.
- It is important to take temperature reading at the same time of the day and season, if the readings have to be composed over a period.
- Take minimum and maximum temperature and mention it in your record if temperature is measured over a period of time i.e. Day, Month, etc.

Note: Conversion of temperature

$$℃ = \frac{(℉ - 32.0)}{1.80}$$

$$℉ = (℃ \times 1.80) + 32.0$$

Where, ℉ = degree Fahrenheit.

2.4 Dissolved Solids

(1) Principle

The solids represent the portion of a water sample, which is not lost upon evaporation. Solids include dissolved organic matter, particulate organic matter, dissolved inorganic substances except gases and the CO_2 contained in bicarbonate and particulate inorganic substances.

The Total Solid (TS) concentration represents dissolved and particulate organic and inorganic matter. It is determined by evaporating a raw water sample, weighing the residue and expressing the weight of the residue as mg/L in the sample.

The Total Volatile Solid (TVS) concentration is measured by determining the weight loss upon ignition of the residue from the TS analysis. It is also expressed as mg/L in the water sample and is an estimate of dissolved and particulate organic matter.

To measure the Total Dissolved Solids (TDS) concentration, a sample is

filtered to remove the particulate matter, the filter is evaporated and the residue weighed. It indicates the mg/L of dissolved organic and inorganic matter in a sample.

When the residue from the TDS analysis is ignited and the weight loss determined, one may obtain the Total Volatile Dissolved Solids (TVDS), which is expressed as mg/L of the dissolved organic matter in a sample.

(2) Apparatus

Porcelain dish/glass beaker, oven or bunsen burner, analytical balance and dessicator, glass fibre filter, funnel and muffle furnace.

(3) Procedure

a) TS and TVS

- Weigh a glass beaker or a porcelain dish. This is initial weight (w_1).
- Take a known volume of the water sample in the weighed dish or beaker.
- Evaporate the sample to dryness at 103°C to 105°C for 24h in an oven. If an oven is not available, heat the beaker on a bunsen burner using a sand bath or directly, until the water evaporates and a residue is left behind.
- Cool the container in a dessicator if available or make air cool. Weigh the container and record the reading. This is the final weight (w_2).

b) Calculation of TS

$$\text{TS in mg/L} = \frac{\text{Final weight } (w_2) - \text{Initial weight } (w_1)}{\text{Volume of the sample taken}}$$

For TVS, the sample processed for TS can be further treated as follows:

- Place the evaporating dish (porcelain dish) in a muffle furnace at 550°C for 30 minutes.
- Cool in a dessicator and weigh. This is w_3.

c) Calculation of TVS

$$\text{TVS in mg/L} = \frac{\text{Final weight } (w_2) - \text{Initial weight } (w_1)}{\text{Volume of the sample taken}}$$

d) TDS and TDVS

The procedure is same as TS and TDS. But the sample is to be filtered before

measuring TS.

TDS can be used as a measurement of Salinity in brackish or sea water and expressed as g/L (equivalent to parts per thousand, ppt). However, one has to be careful for that measurement of TDS gives all dissolved solids including salts and other organic/inorganic materials in water.

Section II

Soil Analysis

Chapter 3

Chemical Analysis of Soil

3.1 Available Nitrogen (Alkali $KMnO_4$ Method)

(1) What is Available Nitrogen

Nitrogen (N) is found in the horizon of the soil, mostly in organic materials. In the soil solution, organic N is gradually transformed into ammoniacal (NH_4^+), nitrite (NO_2^-) and nitrate nitrogen (NO_3^-)-N by microbial processes. Organic-N is, in itself, of very little use to plants, as it cannot be absorbed as such. It is therefore, necessary to estimate the different forms of mineralized or available N. The NO_2^- - N and NO_3^- - N together, hardly, exceed 1% of the total N in normal soil.

The available N in soil refers to a fraction of the total N which is converted into forms accessible to the plants. This constitutes, on an average, only 0.5-2.5 % (rarely 5%) of the total N in a soil at any given time.

(2) Principle

Potassium permanganate ($KMnO_4$) in alkaline medium (in presence of NaOH here) acts as a weak oxidizing agent. Hence when $KMnO_4$ and NaOH are added to a soil, $KMnO_4$ oxidizes the organic forms of N (e.g. protein) to amines. Thus in presence of alkali (NaOH), NH_3 volatilizes. During distillation NH_3 comes out from the distillation flask and is absorbed in a known volume of a standard acid, i.e. H_2SO_4, the excess of which is titrated with a standard alkali (0.02NaOH) using methyl red as the indicator. The known volume of H_2SO_4 taken in the conical flask is in excess so that only a portion reacts with NH_3 and another portion remains unchanged. The volume of this unchanged H_2SO_4 is measured by titration with the standard NaOH (N/50) solution. By mathematical deduction of this volume from the volume taken, the volume of H_2SO_4 that

reacts with NH_3 is found out.

(3) Reactions

(a) Distillation

$$2KMnO_4 + H_2O \xrightarrow{\text{Alkaline Medium}} 2MnO_4 + 2KOH + 3O^-$$

$$RCH.NH_2COOH + O^- \xrightarrow{\text{Oxidative deamination}} R.CO.COOH + NH_3$$

$$NH_3 + H_2O \xrightarrow{\text{Distillation}} NH_4OH$$

$$2NH_4OH + H_2SO_4 \xrightarrow{\text{Absorption}} (NH_4)_2SO_4 + 2H_2O$$

(b) Titration

$$H_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O$$

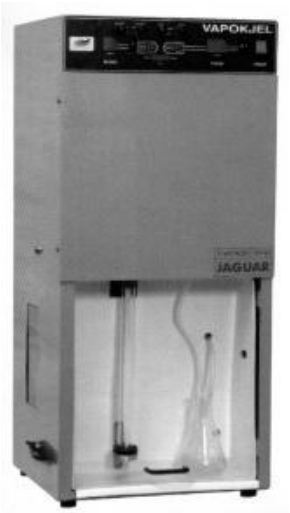

Fig. 2. *Portable Kjeldahl distillation unit.*

(4) Materials Required

(i) Kjeldahl distillation set
(ii) Measuring cylinders
(iii) Pipette

(iv) burette

(v) Conical flask (less than 250ml)

(vi) Heater

(5) Reagents Required

0.32% $KMnO_4$ solution: Dissolve 3.2gm of $KMnO_4$ in distilled water, make up the volume to 1 litre.

2.5% NaOH solution: Dissolve 25gm of NaOH pellete in distilled water. Make up the volume to 1 litre.

N/50 (or 0.02N) H_2SO_4: Add water upto 1000ml to 0.55ml concentrated H_2SO_4.

N/50 NaOH : Dissolve 0.80gm NaOH in a 600ml distilled water. Stir to dissolve NaOH. Add distilled water upto 1000ml.

Methyl red indicator (0.15%): Dissolve 0.15%gm of methyl red indicator powder in 50ml ethyl alcohol and make up the final volume to 100 with distilled water.

(6) Procedure

- Place 20gm of soil in a distillation flask.
- Moisten the sample by adding 20ml of distilled water.
- Add 100ml of 3.2% $KMnO_4$ solution.
- In conical flask, take 30ml N/50 H_2SO_4. Add 2-3drops of methyl red indicator and dip the end of the delivery tube into it.
- Pour 100ml of 2.5% NaOH solution into the distillation flask and cork it immediately.
- Distill ammonia by steadily heating the distillation flask and collect enough distillate in a conical flask.
- Titrate the excess of the standard H_2SO_4 in the conical flask with 0.02% N NaOH (or N/50 NaOH)). The end point is reached when the colour changes from pink to yellow.

(7) Calculation

Weight of the soil taken = 20gm

Volume of N/50 H_2SO_4 taken = 30ml

Volume of N/50 NaOH used in titration = Xml

Volume of N/50 acid used for NH_3 absorption = (30-x)ml

(1ml of N/50 H_2SO_4 = 0.02mg of N = 0.028mgN= 0.00028gm N)

(a) % available N = (30-x) × 0.00028 ×100/20

(b) Available N in the soil (kg/ha) = percentage N value × 22400

(For expressing available N in kg/ha, I ha/g furrow slice (0-15cm) of the soil is assumed to be 2.24 million kg in weigh.)

(8) Interpretation

Amount of available N (kg/ha)	Comments
< 272	Low
272-544.1	Medium
> 544	High

3.2 Organic Carbon and Organic Matter (Walky and Black, 1934)

(1) What is Soil Organic Matter

All plants, animal and microbial residues both decomposed and endecomposed in the soil constitute the organic matter, most of which is of plant origin.

(2) Importance

The Organic Matter (OM) is the source of plant nutrients which are released in assimilable forms during microbial degradation. A major proportion of N (95-99% of the total), occurs in organic combinations, which mineralize to release the nutrients in inorganic forms to be used by plants. Thus OM is an index of the productivity. It is also a source of cation exchange capacity besides retention and aeration of soils.

3.2.1 Titrimetric Method

(1) Principle for Titration Method

The soil is digested with chromic and sulphuric acid making use of the heat of dilution of strong H_2SO_4 so as to oxidize the humus. The highest temperature

attained by the heat of dilution reaction, produced on the addition of H_2SO_4 is approximately 120^0C, which is sufficient to oxidize the active forms of the soil organic C. On oxidation potassium dichromate is reduced to potassium sulphate. Cr^{6+} is reduced to Cr^{3+}. The colour of the oxidized form of chromium Cr^{6+} is yellow and that of reduced form Cr^{3+} is green. Appearance of yellow (or organic) colour after completion of oxidation (i.e. after 30 minutes of addition) indicates that only a small fraction of dichromate is reduced and major fraction remain unchanged. An appearance of green colour indicates that entire amount of Cr^{6+} has been reduced to Cr^{3+}. There might have some possibility that an amount of C remain unoxidized. (Therefore, in this case the procedure is repeated taking small amount of soil or increased volume of $K_2Cr_2O_7$ so that after completion of oxidation, a considerable amount of $K_2Cr_2O_7$ exists unchanged that shows yellow or orange colour) The volume of unchanged $K_2Cr_2O_7$ solution is determined by titration with ferrous iron sulphate. During titration, ferrous iron sulphate reduces unchanged or excess of $K_2Cr_2O_7$ present in the solution. In the procedure, H_3PO_4 and NaF are used as a redox titration. Diphenylamine added is oxidized first to colourless diphenylbenzidine which is further oxidized to diphenylbenzidine violet.

(2) Reactions

(i) The oxidation of Carbon

$$[K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3O^-] \text{ X } 2$$
$$[C + 2O \rightarrow CO_2] \text{ X } 3$$

$$2K_2Cr_2O_7 + 8H_2SO_4 + 3C \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 - 8H_2O + 3CO_2$$

(ii) The titration procedure

$$[FeSO_4(NH_4)2SO_4.6H_2O \rightarrow FeSO_4 + (NH_4)_2SO_4 + 6H_2O] \text{ X } 2$$
$$2FeSO_4 + H_2SO_4 + O \rightarrow Fe_2(SO_4)_3 + H_2O$$

$$2FeSO_4.(NH_4)2SO_4.6H_2O + H_2SO_4 + O \rightarrow 2(NH_4)_2SO_4 + Fe_2(SO_4)_3 + 13H_2O$$

(iii) The action of diphenylamine indicator

$$2C_6H_5NH_6H_5 \rightarrow 2(C_6H_5.NHC_6H_4) \rightarrow C_6H_5N\text{-}C_6H_4C_6H_4N\text{-}C_6H_5$$

(3) Materials Required

(i) Conical flask
(ii) Pipette
(iii) Burette
(iv) Analytical balance

(4) Reagents Required

1. 1N Potassium dichromate: Dissolve 49.04gm of AR grade $K_2Cr_2O_7$ in distilled water and make up the volume to 1 litre.
2. 0.5N ferrous ammonium sulphate (Mohr's salt): Dissolve 392gm of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 15ml of conc.H_2SO_4 and make up the volume to 2litre with distilled water.
3. Conc.H_2SO_4. For saline soil, 1.25gm of Ag_2SO_4 in 100ml of conc.H_2SO_4 is dissolved and the solution (1.25% $AgSO_4$ in H_2SO_4) is used instead of only conc.H_2SO_4.
4. Orthophosphoric acid (H_3PO_4) 85%.
5. Sodium fluoride.
6. Diphenylamine indicator: Dissolve 0.5 gm of diphenylamine indicator in a mixture of 20ml of water and 100ml of conc.H_2SO_4.

(5) Procedure

- Take 1gm of soil in 500ml conical flask.
- Add accurately 10ml of $K_2Cr_2O_7$ solution with a 10ml pipette. Gently rotate the flask to mix them.
- Add 20ml conc. H_2SO_4 with a measuring cylinder and swirl the flask 2 or 3 times.
- Allow the flask to stand for 30minutes on an asbestos sheet for the reaction to complete. After 30 minutes, a yellow colour would be observed. If green colour appears, through the content and repeat with less amount of soil (<1g) or adding more volume of $K_2Cr_2O_7$ solution.
- Add 200ml of distilled water to dilute the suspension. Filter, if it is expected that the end point of the titration will not be clear.
- Add 10ml of Orthophosphoric acid and 2gm of sodium fluoride. Shake vigorously to mix.
- Add about 1ml of diphenylamine indicator.
- Immediately back-titrate the solution with 0.5N ferrous ammonium sulphate, till the colour flashes from violet through blue to bright green.
- Note the volume of the ferrous ammonium sulphate used.
- Carry out a blank titration (i.e. without soil) in a similar manner.

Since dichromate is reduced to oxidize diphenylamine, an error appears if diphenylamine is allowed to stay with potassium dichromate for long time.

Therefore, diphenylamine should be added just before titration.

Note: If the titration value is <4ml, repeat the experiment with <1g soil.

(6) Calculation

Weight of soil = Wgm

Volume of 0.5NFe$(NH_4)_2(SO_4)_2$ solution used = Bml for blank solution

Volume of 0.5NFe $(NH_4)_2(SO_4)_2$ solution used = Sml for sample solution

Volume of 1N $K_2Cr_2O_7$ used for oxidation of C = 0.5 × (B-Sml (or B-S/2ml)

(1 ml of 1N $K_2Cr_2O_7$ oxidizes 3gm of C or 0.003g C)

% of Organic C in the soil (uncorrected) = 0.5 × (B-S) × 1 × 0.003 × 100/W = Q

% Organic C in the soil (Corrected) = Q × 1.3 =R

(Walky averaged a 77% recovery of organic carbon by this method. Thus the correction factor is 100/77 = 1.3.)

Hence, % of organic matter in the soil = R × 1.724.

(Since organic matter, on an average, contains 58% carbon, the percent organic matter is multiplied by 1.724 (100/58) is known as the 'Von Bemmlen factor.)

3.2.2 Colorimetric Method

(1) Principle

The oxidation of soil organic matter is carried out by dichromate sulfuric acid mixture. The intensity of the chromium sulphate found is measured to give directly the amount of carbon oxidized.

(2) Materials Required

Photoelectric colorimeter, conical flask, volumetric flask, pipette, AR grade potassium dichromate, concentrated H_2SO_4, sucrose (anhydrous).

(3) Procedure

- Take 1gm of soil in 100ml conical flask.
- Add 10ml of 1N$K_2Cr_2O_7$, swirl the flask, followed by addition of 20ml

H_2SO_4, swirl again.

- Allow the flask to stand for 30minutes and then centrifuge the contents to clear state.
- Read the green colour of the chromium sulphate on the supernatant layer on a colorimetet after, adjusting the blank solution to zero, using 660nm red filter.

(4) Standard Curve Preparation

Take 1 to 25mg of anhydrous sucrose in 100ml conical flask, develop the colour and read on a colorimeter as per the procedure outlined for the sample. Draw a curve by plotting the concentration of carbon as sucrose on the abscissa and the colorimeter reading (%T) on the ordinate in log scale.

(5) Interpretation

Organic C (%)	Comments
<0.50	Low
0.50-0.75	Medium
> 0.75	High

3.3 Available Phosphorous (Olsens et al., 1954)

(1) Introduction

The term available Phosphorous (P) refers to the inorganic form, occurring in the soil solution, which is almost exclusively orthophosphate. This orthophosphate occurs in several forms and combinations, and only a small fraction of the total amount present may be available to plants, which is of direct relevance in assessing P fertility level.

The phosphate concentration in soil solution is governed by heterogeneous equilibrium in which it takes part. This situation can be represented as:

P adsorbed in solid phase ↔ P in soil solution ↔ P precipitate

The phosphorous absorbed by plants from soil solution comes as inorganic orthophosphate ions, viz. $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}. The most available ion is H_2PO^{4-}, followed by HPO_4^{2-}. The accessibility of P by plants is influenced by a series of soil properties. The relative abundance of ions is, however relatively dependent on the soil pH. For soil having a pH between 4.7 and 7.5, ions of $H_2PO_4^-$ as well as of HPO_4^{2-} exists in soil solution. At a pH of 7.2, $H_2PO_4^-$ and

HPO_4^{2-} ions have an equal activity, and when the pH is strongly alkaline (>8.3) ions of HPO_4^{2-} predominates the solution. Above pH of 9.0, the trivalent ion (PO_4^{3-}) becomes more important than $H_2PO_4^-$, but even at a pH of 12, the HPO_4^{2-} concentration is still greater than that of PO_4^{3-}.

(2) Importance

The available P indicates fertility level of soil. The knowledge of critical limits of soil is necessary for better soil - crop response. The evaluation of the soil critical limit of available P would help in developing P-fertility ranges for effective fertilizer recommendation schedule.

(3) Principle

The soil is shaken with 0.5M $NaHCO_3$, at a nearly constant pH of 8.5, in 1:20 ratio for half an hour, in presence of Darco-G60 (which absorbs the dispersed organic matter in the sample and helps giving a clear extract) and the extract is obtained by filtering the suspension. Phosphorous in the extract is treated with ammonium molybdate (a complexing agent), which results in the formation of a heteropoly complex, known as 'phosphomolybdate' (faint yellow colour). This 'faint yellow colour' is not visible, and hence, it is unsuitable for colorimetry or spectrophotometry. To make it suitable, the heteropoly complex is partially reduced by stannous chloride that given a blue colour. With increasing P concentration in the soil extract, the amount of molybdo-phosphoric acid complex increases and hence, on partial reduction of the complex, the intensity of blue colour increases. Thus, a faint blue colour indicates low P content and a deep blue colour indicates high P content of soil.

1. Extraction

$$Ca_3(PO_4)_2 + 6NaHCO_3 \rightarrow 3Ca(HCO_3)_2 + 2Na_3PO_4$$
$$3Ca(HCO_3)_2 \rightarrow 3CaCO_3 + 3H_2CO_3$$
$$2Na_3PO_4 + 3H_2CO_3 \rightarrow 2H_3PO_4 + 3Na_2CO_3$$

$$Ca_3(PO_4)_2 + 6NaHCO_3 \rightarrow 3CaCO_3 + 2H_3PO_4 + 3Na_2CO_3$$

2. Colour development

$$(NH_4)_6Mo_7O_{24}4H_2O + 6H_2O \rightarrow 7H_2MoO_4 + 6NH_4Cl$$
(Ammonium molybdate) (Molybdic acid)

$$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$
(Phosphate) (Phosphomolybdate, yellow colour)

3. Reduction

$$\text{Phosphomolybdate} + SnCl_2 \rightarrow \text{Reduced Phosphomolybdate (Molybdenum blue)}$$

(4) Apparatus

Photoelectric colorimeter, pipette (5ml), volumetric flask (25 or 50ml), funnel, Whatman No 42 or 44 filter paper.

(5) Reagents

0.5M $NaHCO_3$: Dissolve 42gm of Sodium bicarbonate in distilled water and make up the volume to 650ml approximately. Adjust the pH of the solution to 8.5 with dilute NaOH solution and make up the volume to 1liter with distilled water.

Darco-G60 (activated charcoal): This is to be made free from soluble P first by following procedure:

1. Take about 1g of charcoal in a 250ml conical flask.
2. Take about 15-20ml of 0.5M $NaHCO_3$ (pH 8.5) solution in the flask.
3. Stoppered the flask and shake for about 30 minutes.
4. Filter through Whatman No1 filter paper.
5. Take 10ml of the filtrate in a 50ml volumetric flask.
6. Add 1-2 drops of 2, 4 dinitrophenol. It turns filtrate to yellow in colour which indicates pH above 3.
7. Add 4N HCl drop by drop until yellow colour disappears to adjust pH at 3.
8. Add 5 drops of $SnCl_2$ and shake.
9. Wait for 5-6 minutes.

If no blue colour appears, then it may be concluded that the charcoal is free from phosphorous. If blue colour appears, it indicates the presence of excess P in charcoal. Such charcoal is made free from P by the method as follows:

1. Take required amount of charcoal in the beaker.
2. Add 0.5M $NaHCO_3$ (pH 8.5) to submerge the charcoal and stir for 30 minutes.
3. Keep it overnight and decant the $NaHCO_3$ solution next day.
4. Again add 0.5M $NaHCO_3$ (pH 8.5) solution to submerge the charcoal, stir with glass rod, decant the $NaHCO_3$ solution. Repeat the decantation process 6-8 times.

5. Place a whatman No1 filter paper on a buckner funnel and transfer the entire amount of charcoal from the beaker to the buckner funnel.
6. Connect the buckner funnel with a water suction apparatus.
7. Repeatedly leach the charcoal with 0.5M $NaHCO_3$ (pH 8.5) solution.
8. Take about 10ml of the leachate in a 50ml volumetric flask.
9. Follow the procedure to test the presence of P in the leachate by the method as already described.
10. After the charcoal is made free from P, the excess of $NaHCO_3$ solution present in charcoal is removed by leaching the charcoal with P free warm water several times.
11. Dry the charcoal in an oven at low temperature.

Dickman and Brays' reagent in excess of acid: Dissolve 15g of ammonium molybdate (AR grade) in 300ml of warm water (about 60^0C), cool and filter, if turbidity exists. Add to it 400ml g 10N HCl, and make up the volume to 1 litre.

40% Stannous Chloride (Stock solution): Dissolve 10g of $SnCl_2.2H_2O$ (AR) crystals in 25ml of concentrated HCl. Warm the solution, if necessary to dissolve Stannous Chloride. A watery look of solution indicates $SnCl_2$ solution. Store it in a brown bottle. This is 40% $SnCl_2$ stock solution. Addition of a piece of tin metal (AR), will keep the stock solution for long time.

Stannous Chloride working solution: Dissolve 0.5ml of the 40% $SnCl_2$ stock solution to 66ml with distilled water, Prepare this solution just before use.

Standard P solution: Dissolve accurately 0.439g of Potassium dihydrogen Phosphate (AR), KH_2PO_4, in about half a liter of distilled water. Add to it 25ml of 7N H_2SO_4 (approx.) and make up the volume to 1 litre with distilled water. This gives 100ppm stock solution of P.

(6) Other Necessary Reagents

1. 4N NH_4OH: Take 27ml of ammonium hydroxide in a 100ml volumetric flask and add distilled water upto 100ml mark.
2. 4N HCl: Take 34.5 ml of concentrated HCl in a 100ml volumetric flask and make the volume to 100ml by adding distilled water.
3. 2,4-dinitrophenol indicator: Take an amount of (app 1g) 2,4, dinitrophenol indicator in abeaker and add approximately 200ml distilled water and stir for sometime. Filter or decant the solution to obtain a clear solution.
4. 7N H_2SO_4 : To 50ml of distilled water pour 19.5ml of concentrated H_2SO_4

very slowly in a 100ml volumetric flask. Cool and then add distilled water upto 100ml mark.

(7) Procedure for Standard Curve

- Take 9 numbers of 50ml volumetric flasks.
- Take a little volume of distilled water in one flask (blank) and 1, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 20.0 of 2ppm P solution in individual flask. (For 2ppm standard solution of the volume 500ml, 20ml of 50ppm P solution is diluted to 500ml.)
- Add 1-2 drops of 2,4-dinitrophenol in each flask. No colour appears in P solution except yellow colour in distilled water.
- Add 4N NH_4OH drop wise to each solution of P until yellow colour appears.
- Add drop wise 4N HCl to each flask until yellow colour disappears.
- Add 10 ml ammonium molybdate solution to each flask.
- Fill each flask with 20ml of distilled water.
- Add 1ml of $SnCl_2$ working solution to each and gently stir to mix. Fill each flask with distilled water upto 50ml mark. Blue colour appears within 4-5 minutes and the colour intensity remains unchanged for 20 minutes. The flask in which only distilled water was taken instead of P solution doesn't show blue colour. This is blank.
- Place red filter in the colorimeter or set it to 660nm.
- Set zero with blank and take readings of each P solution.
- Complete the work within 20 minutes.
- Find out the concentration of P solution (i.e. ppm of P) in each flask. Plot P concentration on a graph paper on X axis and optical density of each corresponding P solution on Y axis. The graph obtained will be stratight line and it is the standard curve of P.

(8) Procedure of P Estimation

- Take 25g soil in a 250ml conical flask.
- Add 50ml of 0.5M $NaHCO_3$ (pH 8.5) solution with a 50ml bulb type pipette in the flask.
- Add 1-2g of P free charcoal.
- Close tightly the mouth of the flask with a rubber cork and shake for 30 minutes. Filter with a Whatman No 42 filter paper till a colourless filtrate is obtained. (P free charcoal can be added while filtering the extract through

filter paper.)

- Whatman No 1 filter paper may be used, if a clear filtrate is not obtained.
- Take two 50ml volumetric flasks. (For more samples, a maximum of 10 volumetric flasks can be arranged. More than 10 flasks may take more time in colorimetric reading. The colour may be deteriorated after 20 minutes of development.)
- Take 10ml of extract (filtrate) with a 10ml bulb type pipette in one flask and 10ml of distilled water in another flask (blank).
- Adjust their pH to 3 with 2, 4 dinitrophenol, 4N NH_4OH and 4N HCl by the method as described earlier for standard curve preparation.
- Add 10ml of ammonium molybdate solution to each flask.
- Add distilled water (about 15ml) so that the total volume of solution becomes about 35ml in each flask.
- Add to each 5 drops of stannous chloride stock solution (For $NaHCO_3$ extract), and shake gently.
- Add distilled water upto 50ml mark to each flask.
- After 5 minutes, set the colorimeter to zero optical density reading using red filter (i.e. 660nm) with blank. Take reading of all samples within 20minutes of $SnCl_2$ addition.
- Put the values of OD in standard curve and find the corresponding P.

(9) Calculation

a. Weight of soil taken = Agm
b. Volume of 0.5M NaHCO3 solution added = Xml
c. First dilution = A/X times
d. Volume of soil extract taken = Bml
e. Final volume following colour development=50ml (Volume of volumetric flask)
f. Second dilution = 50/B times
g. Total dilution = A/X × 50/B times
h. Concentration of P as read from standard curve = S ppm (say)
i. Available P in ppm = S × A/X × 50/B ppm
j. Available P in kg/ha = S × A/X × 50/B × 2.24 kg/ha
k. Available P_2O_5 in kg/ha = S × A/X × 50/B × 2.24 × 2.29 kg/ha

(10) Interpretation

P (kg/ha)	Rating
<11.2	Low
11.2 - 22.4	Medium
>22.4	High

(11) Precaution

- Always use fresh $SnCl_2$ working solution.
- Before P testing, glasswares should be washed with 6N HCl or Chromic acid followed by washing with tap water and finally dips in distilled water for 24hrs. Washing powder containing P should not be used for cleaning the glass wares.
- After completion of P estimation, the 50 ml volumetric flasks should be washed with tap water at least 4-6 times and then filled with Chromic acid cleaning solution. (16g of $K_2Cr_2O_7$ in about 60ml of hot water + 200ml L.R. grade H_2SO_4 slowly with stirring) and kept overnight. Next day the volumetric flasks should be washed with tap water several times (4-6 times) followed by washing with distilled water at least 3 times.
- If blue colour appears in blank though all reagents are P free, the 50ml volumetric flasks should be washed properly. If blank colour still appears, this may be due to poor quality of Ammonium molybdate reagent.

3.4 Available Potassium

(1) Introduction

The total K content of a soil varies from 0.05 to 2.5 %. The total K is distributed in mineral from (lattice K, 90-98%) fixed non-exchangeable or temporary retrograded K (1-10%), and exchangeable plus water-soluble K (1-2%). Available K in soils is generally the sum of water soluble and exchangeable K which can be readily taken by plants.

Extraction of K with Ammonium acetate (CH_3COONH_4 or NH_4OAc): A solution of neutral (pH 7.0) normal (1N) Ammonium acetate (CH_3COONH_4) is generally used for extracting both water soluble and exchangeable K. This extract contains only water-soluble and exchangeable K. K extraction by this extractant is considered as a suitable index of K availability in most soils, based on crop response correlation study, and, thus, K extract by this method is

equated as the available K.

(2) Principle

The method is based on the principle of equilibrium of soils with an exchangeable cation made of the solution of neutral normal NH_4OAc, in a given soil: solution ratio. During the equilibrium, ammonium ions exchange with the exchangeable K ions of the soil. The K content in the equilibrium solution is estimated with a flame photometer. Since NH_4^+ holds highly charged layers together just as K, the release of the fixed K, in an exchangeable form, is retarded during NH_4OAc extraction.

(3) Reaction

$$\text{Clay}\begin{vmatrix}K\\K\\K\\K\\K\end{vmatrix} + 5CH_3COONH_4 \leftrightarrow \text{clay}\begin{vmatrix}NH_4\\NH_4\\NH_4\\NH_4\\NH_4\end{vmatrix} + 5CH_3COOK$$

(4) Materials Required

Flame photometer with potassium filter, conical flask, Pipette, Mechanical shaker, Funnel, Filter paper (Whatman No 42 or 1), balance.

(5) Reagent

Neutral 1N Ammonium acetate (1N CH_3COONH_4, pH 7.0): Dissolve 77.08g of solid Ammonium acetate in distilled water and make up to one litre. Adjust its pH to 7.0 with dilute NH_4OH or dilute CH_3COOH with universal indicator or pH paper or glass electrode pH meter.

Standard Potassium (K_2O) solution: Dissolve 1.5829g of AR grade KCl in the flask (if possible dry at 60^0C for 1hr) and make up the volume upto 1000ml. It gives 1000ppm of K solution.

Butyl alcohol: Available in the market.

(6) Preparation of Standard Curve

From the stock solution take measured aliquots and dilute with NH_4OAc solution to give 15 to 50 ppm of K. This can be done using the following formula:

$$S_1V_1 = S_2V_2$$

Here,

S_1 = Final strength of solution (in ppm);

V_1 = Final volume of standard solution to be prepared;

S_2 = Strength of K solution taken (1000ppm here);

V_2 = Volume of K solution (1000ppm) to be taken to make solution of desired strength.

How to prepare desired solution: Example of preparation of aliquots from stock solution is given in Table 1.

Table 1. *Preparation aliquots of K solution from 1000ppm stock solution.*

S_2 in ppm (Known)	S_1 in ppm (desired)	V_1 in ml (Known)	V_2 in ml (to be taken)
1000	50	50	2.5
1000	40	50	2.0
1000	30	50	1.5
1000	20	50	1.0
1000	15	50	0.5

(7) Procedure

- Take five numbers of 50ml (V_1) volumetric flask.
- The range of standard K solution to be prepared is 15-50ppm (S_1). Now, from the calculations of table 1, take 0.5, 1.0, 1.5, 2.0 and 2.5ml of 1000ppm solution in volumetric flasks.
- Make the volume of each flask to 50ml by adding NH_4OAc solution.
- Take 50ml of NH_4OAc in another 50ml volumetric flask as blank.
- Set up the flame photometer by atomizing blank in 0 and 50 in 100 reading scale.
- Atomize the other solutions and record the readings.
- On a graph paper, plot the readings against the respective ppm. The graph thus drawn will be a straight graph.

(8) Extraction of K_2O

- Take a 250ml conical flask.

- Take accurately 5g of soil sample in the flask.
- Take 25ml of 1N CH_3COONH_4 (pH 7.0) solution in the flask. The soil extracted ratio is 1:5.
- Close tightly the mouth of the flask with a rubber cork.
- Shake the flask for about 30 minutes on the mechanical shaker.
- Filter only through Whatman No 42 filter paper.
- Collect the filter (i.e. soil extract) to estimate K_2O concentration.

(9) Estimation of K_2O

- Add 2 drops of butyl alcohol to the filtrate. It improves spraying properties of the solution.
- Place the K filter in the flame photometer.
- Atomize the filter and record the reading.
- Find the K_2O in ppm by putting the reading in standard curve.

(10) Observation and Calculation

The K_2O content is calculated as follows:

a. Weight of the soil =5g
b. Volume of the neutral 1N NH_4OAc =25g
c. Reading of the flame photometer for the test solution=R (say)
d. Concentration (ppm) as read from the standard curve= c(say)
e. Dilution factor= 25/5= 5 times
f. Now, available K in soil (ppm) = $C \times 5$
g. Available K in soil (kg/ha) = $C \times 5 \times 2.24$
h. Available K_2O in soil (kg/ha) = $C \times 5 \times 2.24 \times 1.20$

(11) Interpretation

Available K	Available K_2O	Comments/rating
<120	<136	Low
120-280	136-337.5	Medium
>280	>337.5	High

(12) Precaution

- See that filtrate is purely clear.

- Maintain the air pressure in flame photometer between 0.4 to 0.6 kg/cm^2. Air pressure must not fluctuate during analyses.

3.5 Soil pH

(1) Principle

The potentiometer determination of pH is based on the measurement of the electrical potential developed by an electrode whose potential depends on the hydrogen ion concentration of the solution. The single electrode potential of any electrode should be measured with the second electrode (reference electrode) dipping into the same solution. Glass electrode is the most convenient among different pH electrodes. The reference electrode commonly used is the saturated calomel type. The pH meter measures the voltage developed by the combination of glass electrode and reference electrode and the scale is graduated in pH units as well as in milivolts. The pH meter is to be calibrated using standard buffer solution.

(2) Materials Required

Balance, Beaker, (100ml/250ml), measuring cylinder, Glass rod, pH meter, Buffer solution (pH 4.0, pH 7.0 and pH 9.2).

(3) Procedure

- Take a 100ml beaker and place 20gm of air dry soil into it.
- Add 50ml distilled water in the beaker (soil:water = 1:25). Stir with a glass rod occasionally for about 30 minutes. If mechanical shaker is available, shake the suspension continuously for 4-5 minutes.
- Make the pH meter ready for pH measurement following the instructional manual.
- Standardize the pH meter at 4.0 by buffer solution. First take 7.0 solution and then 4.0. Repeat the procedure two or three times. Wash the electrode after each immersion with strong stream of distilled water.
- Immerse the glass rod into soil-water suspension in the beaker. Note the reading.
- Wash the electrode immediately after reading.

(4) Interpretation

pH	Interpretation
<6.5	Acidic reaction
6.5-7.5	Normal
>7.5	Alkaline

(5) Comment

- Drying changes the soil pH. For convenience, the air-dried soil samples are used for pH determination. In soil testing report, whether the dried or field-moist samples were used must be mentioned.
- The pH value in soil-water suspension increases with increasing dilution. The soil:water ratio may vary from 1:1, 1.0:2.5, 1:5 and 1:10. Therefore, in soil testing report the ratio of soil:water should also be mentioned.

3.6 Electrical Conductivity of Soil

(1) Principle

The measurement is based on the principle that ions being the carriers of electricity the electrical conductivity of a solution increases with soluble salt concentration. Thus, it is possible to measure electrical conductivity of a soluble salt.

(2) Equipment

Beaker (100ml/250ml), glass rod, Balance, Conductivity meter.

(3) Procedure

- Take 20g of soil in a 100ml beaker.
- Add 50ml of distilled water into it (soil: water =1:2.5).
- Stir with glass rod occasionally for about 30 minutes. If mechanical shaker is available shake for 4-5 minutes.
- Stand the suspension for few minutes (2-3) so that soil particles settle down.
- Use the supernatant liquid to measure conductivity by conductivity meter.

(4) Interpretation

Electrical Conductivity (mmhos/cm)	Interpretation
<0.8	Normal for all crop
0.8-1.6	Critical for salt sensitive crop
1.6-2.5	Critical for salt tolerant crop
>2.5	Injurious to all crop

(5) Comment

- The supernatant solution of soil:water suspension used for pH determination can be used for electrical conductivity measurement.
- Soil: water ratio and type of soil used (air dry/moist) must be mentioned in the report.

Chapter 4

Physical Analysis of Soil

4.1 Collection and Preservation of Soil

(1) Composite Soil Sample

Soil samples are collected from a number of sites (cores/furrow slices) of a soil unit (8-10 or 20-30 sites depending on the area of the soil unit). The samples are thoroughly mixed. This mixture sample is termed composite soil sample. It represents the properties of the soil unit. Its analytical value is equivalent to the mean analytical value of the individual sites (i.e. cores or furrow slices).

(2) Collection

- Core or furrow-slices should have the uniform volume.
- Cores or furrow-slices should be taken at random or in a zigzag or criss-cross manner.
- The cores or furrow-slices should not be positioned on the rows or on the crop hills.
- Sufficient numbers of cores or furrow-slices are to be taken throughout the entire soil.

(3) Preparation

Drying: The soil samples are air dried under shade at room temperature. On drying the ferrous iron (Fe^{2+}) oxidize to ferric iron (Fe^{3+}), exchangeable K content increases in some soils (May be increased more than two folds) and decreases in some soils high in exchangeable K and to some extent hydrogen ion activity (i.e. soil pH) changes. Hence, iron (Ferrous), potassium, soil pH etc.

should be determined on field moist soils.

Grinding: The soil samples are grinded by wooden mortar, roller, motorized grinder etc. to break the soil aggregates. Care should be taken so that primary sand and gravel particles are not crushed.

Mixing: The grinded soil samples are spread uniformly over a piece of polythene paper or cloth (size 2ft × 2ft) or as required.

(4) Steps for Mixing Soil Samples

1. The corner (1) is grasped and pulled diagonally across the sample slowly so that the soil particles roll over towards the opposite corner no (3). The corner no (1) is pulled back to its original position.
2. The corner no (3) is grasped and pulled diagonally across the sample slowly so that the soil particles roll over towards the opposite corner no (1). The corner no (3) is pulled back to its original position.
3. Thus a rod or ribbon of soil extending from corner no (2) to (4) if formed. Following the same method, the other two corners (2 and 4) are pulled. The whole process is repeated at least five times.

Sieving: Sieves of 20 and 80 meshe sized made of brass or nylon possessing round hole are used. Fine sieve (i.e. 80 meshes) is used for determination of oxidizable organic carbon and elements. Coarse sieve (i.e. 20 meshes) is used for determination of soil pH and electrical conductivity. The entire soil volume should pass through the sieve. Sieving only a fraction of the sample volume increases the concentration of elements in the sample.

Storage: The prepared soil sample should be kept in tightly sealed dry polythene jar or poly packet with label. The sample should be kept away from laboratory fumes and ammonia gas.

4.2 Moisture Content of Soil (Gravimetric Method)

(1) Principle

Even after drying, the soil retains variable quantities of moisture. This determination provides a means for comparing the analytical values of samples with different moisture contents on moisture free basis.

(2) How to Express Moisture

Soil moisture is normally expressed as percentage on weight basis (g of water per 100g of oven dry soil). It can, however, be expressed on volume basis.

Moisture on dry weight basis M_w = Wm/Ws × 100

Moisture on volume basis Mv = Vm/Vs × 100

Where, Wm = Weight of moisture box in g

Ws = Weight of oven dry soil

Vm = Volume of moisture in cubic cm

Vs = Volume of soil in cubic cm

(3) Materials Required

Aluminium moisture box with lid.

1. Physical balance with weight box.
2. Dessication with dessicant viz. Anhydrous or fused $CaCl_2$.
3. Oven with thermometer 0-110^{0}C.

(4) Procedure

- Take weight of empty moisture box with lid.
- Place about 50 to 70g of soil and weigh (upto 2nd decimal place).
- Dry the soil for 24 hours at 105^{0}C. This can be done in 2 or 3 stages of 8 to 12 hours each.
- Cover the box with lid.
- Cool it in dessicator.
- Weigh it.
- Repeat the above procedure till the consecutive weights are constant.

(5) Calculation

Weight of empty box with lid = Xg

Weight of box + lid + moist or air dry soil = Yg

Weight of box + lid + oven dry soil = Zg

Thus, weighing water loss during drying = (Y-Z)g

Weight of oven dry soil = (Z-X)g

Percentage moisture by dry weight = (Y-Z)/(Z-X) × 100

Percentage moisture by volume = (Y-Z)/(Z-X) × 100 × Db

Here, Db = Bulk density

Or

$$\text{Percentage moisture by volume} = \frac{\text{Moisture volume in cm}^3,\ (Y-Z)\text{cm}^3}{\text{Soil volume in cm}^3\ (Z-X)\text{cm}^3}$$

4.3 Bulk Density of Soil (Core Tube Method)

(1) The Bulk Density

The bulk density (also known as apparent density) is defined as the mass per unit volume which include volume (space) occupied by solid as well as pore spaces. This is expressed as:

Bulk density (Bd) = Mass (w) in g /Volume (V) in cc

The Bd is influenced by a variety of factors such as particle size and distribution, pore space, organic matter content, depth of soil and mechanical manipulation like tillage, plaughing, compaction etc. Increase in clay and organic matter content decrease the bulk density. The bulk density of the soil generally increases with depth which is due to low organic matter content and compaction resulting from overburden pressure.

(2) Significance of Bulk Density

Bulk density is used in estimating porosity, void ratio, weight of the furrow slice (0-15cm) and converting soil moisture from weight to volume basis.

(3) Materials Required

(i) Balance
(ii) Weight box
(iii) Moisture box
(iv) Core tube with hummer
(v) Vernier caliper
(vi) Oven
(vii) knife
(viii) Dessicators

(4) Procedure

- Measure accurately the length and diameter of the core tube with the help of vernier caliper.
- Select a representative area in the field, clean the surface litter, if any and press the core tube vertically into the soil by hand; place the wooden block and press further by steady hammering till the core sampler is driven down to the surface of the soil, filling it completely.
- Remove the soil from outside the core tube to facilitate easy withdrawal of the tube along with the core from the field.
- Put your palm at the bottom of the core tube while withdrawing it from the field in order to check falling of soil from tube.
- Remove the excess soil sticking to the core tube with the help of a knife.
- Remove the soil from the core tube and weigh it.
- Take a homogenous sample of core soil, say 20-30g in a weighed moisture box.
- Weigh the moisture box and place it in an oven for drying at 105^0C to constant weight. (Normally this may take 24 hours)
- Take out the moisture box and determine the percent moisture content in the soil.
- Calculate the moisture content for the entire core.
- Subtract the moisture content from the original weight of the core and get the weight of the dry core.

(5) Calculation

Length of the core tube = h (cm)

Diameter of the core tube = D(cm)

Weight of the dry core = W(g)

Thus, volume of the core tube = $\Pi (D/2)^2h$ in cc

Bulk density (Bd) = $W/ \Pi(D/2)^2h$ = g/cc

(6) Precautions

- Do not take soil core sample in very wet or dry soil condition.
- Place a block of wood over the core while hammering.
- Use metal core tube to reduce the error arising due to compression of soil

during sampling.

- Weigh immediately after taking the core sample.

4.4 Particle or Real Density of Soil

(1) Particle Density

Particle density refers to the actual density of soil solids. It is defined as the mass per unit volume of soil solids only. Particle density of soil is expresses in g/cc. Since the volume is exclusive of poor spaces, the volume of particle density is higher than that of bulk density.

(2) Factors Affecting Particle Density

Particle density of most ranges between the narrow limits of 2.50 to 2.75 g/cc with an average value around 2.60 g/cc. This is because most mineral particle are quartz, feldspars and other silicates whose densities also vary within the same limits. However, particle density of soil differs from the usual range due to presence of large amounts of organic matter and heavy minerals. Unlike bulk density, particle density of a soil is not altered by mechanical manipulation.

(3) Principle

Particle density requires the measurement of two variables i.e. mass and volume of soil. Mass is determined by weighing oven dry soil. Volume of soil is determined by the volume of water displaced when soil is immersed therein. The accuracy of method depends upon how closely the volume of displaced water approaches that of the true volume of soil solids.

(4) Materials Required

(i) Pychnometer (specific gravity bottle)
(ii) Analytical balance and weight box
(iii) Pipette
(iv) Blotter/ordinary filter paper/piece of clean cloth

(5) Procedure

- Weigh a clean dry pycnometer with stopper on.
- Transfer 10g of oven dry soil into the pycnometer.

- Fill the pycnometer to about half its volume with water using a pipette, washing into the flask any soil particles sticking inside the neck. Allow enough time for the water to completely soak into the soil.
- Remove the entrapped air by filling the bottle with distilled water to the brim.
- Insert the stopper and wipe out the outer surface of the pycnometer with a blotter to remove water adhering to it and weigh the pycnometer, record this weight.
- Empty the pycnometer, fill it again with distilled water replace the stopper, wipe out water adhering on the outside with a blotter, weighs the pycnometer and record this weight.

(6) Calculation

Weight of ovendry soil =W= 10g

Weight of Pycnometer with soil and water =W_1g

Weight of Pycnometer filled with water alone =W_2g

Thus particle density (Pd) = Wt of oven dry soil/volume of oven dry soil
= 10g/W_2+10g-W_1) × water density (g/cc)

Here, volume of oven dry soil = Volume of displaced water
= (W_2 + 10g –W_1) × water density

(7) Precaution

- Take organic debris free soil.
- Use boiled and cooled distilled water/rain water.
- Ensure the removal of all entrapped air (bubbles) from the soil submerged in water.

4.5 Pore Space or Porosity of Soil

(1) Introduction

Pore space refers to the portion of soil volume not occupied by the solid particles. It is filled with air, water or both. The amount of pore space in a soil is expressed as a percentage of the total volume.

Several important soil and plant processes such as retention and movement of water in soil, get exchanged between the soil and the atmosphere, solute movement and proliferation and penetration of roots depend on the amount and

size distribution of pores.

In general two types of pores–micro and macro pores are recognized. Generally, the micropores hold water while air is held in macropores. In order to maintain a favourable air-water relationship for plant growth, there should not only be sufficient total pore space but also a proper balance between the two kinds of pores. In an ideal soil, total pore space should be more or less equally distributed between two types of pores.

(2) Materials Required

All materials required for bulk density and particle density are necessary.

(3) Procedure

Determine bulk density by bulk density procedure and particle density by particle density procedure.

(4) Calculation

Particle density of soil (g/cc) =Pd

Bulk density of soil (g/cc) = Bd

Therefore, Percent pore space = (1-Bd/Pd) × 100

Section III

Plankton and Periphyton Studies

Chapter 5

Collection, Preservation and Quantification (Plankton)

5.1 What are Plankton

Plankton are free floating organisms without powers of movement or with very feeble locomotion abilities. *Net plankton* are planktonic organisms which are caught in a fine meshed net and *nanoplankton* are organisms too small to be caught in nets and must be extracted from water.

The two wide categories of plankton are animal plankton (zooplankton) and plant plankton (phytoplankton). However, depending on the size, following classification has been made:

Ultra nanoplankton	below 2 µ
Nanoplankton	2-20 µ
Microplankton	20-200 µ
Mesoplankton	200-2000 µ
Megaplankton	above 2000 µ

5.2 Foreworks of Plankton Sampling

(1) Preliminary Preparation

Before plankton sampling, following fore works are to be done.

- Determine sampling location (also called ‘station’), its depth and prepare a

brief note.

- Label sample container with date, time, serial number, sampling station, study area, type of sample, depth and if surface plankton are concerned record surface temperature.
- If samples are to be preserved, make fixative ready so that it can be used immediately after collection.
- In the field record book, note sample location, depth, type, time, meteorological conditions, turbidity, water temperature, conductivity. Use leak proof vials or containers and waterproof labels.

5.3 Preservation of Plankton

(1) Preparation of Fixatives

Most commonly used preservatives are

1. 2-5% formalin
2. Lugol's solution
3. Formalin acid-alcohol (FAA)
4. Formal alcohol
5. 80% methyl alcohol

(a) 2-5% Formalin solution: Commercially available 40% formalin is a saturated solution for formaldehyde gas in water. This is suitably diluted with water for preservation of plankton.

(b) Lugol's solution: Water samples containing nano-plankton (especially zooplankton) are best fixed and preserved in lugol's iodine to which acetic acid or acetate has been added. Iodine fixes, preserves and colours the plankton while acetic acid preserves the flagella and cilia. To about 100ml of water sample containing nano-plankton add 2-3 drops of lugol's iodine solution. Keep the bottles tightly closed and store in dark. Such complex will keep good for a very long time. Allow at least 24 hrs for the nano-plankton to settle down by sedimentation.

BOX 2

Preparation of x% formalin

$$S_1V_1=S_2V_2$$

Here, S_1= x S_2 = 40
V_1 = 100 V_2 = ?

Since $S_1V_1=S_2V_2$, $V_2 = S_1V_1/ S_2 = 100x/40 = 2.4x$
If x = 5, 2.5X5 =12.5

Add 87.5ml distilled water to it. This is 5% formalin.

Preparation of Lugol's solution: Prepare lugol's solution by dissolving 20g potassium iodide (KI) and 10g iodine crystals in 200ml distilled water containing 20ml glacial acetic acid.

(c) Formalin Acid Alcohol (FAA): FAA is a good preserving and killing agent when the material is to be used for cytological studies of planktonic algae.

(d) Preparation of FAA: FAA is prepared by mixing together 50ml 95% alcohol, 5ml glacial acetic acid or 10ml 40% formalin and 35ml distilled water.

(e) Formal alcohol: It is prepared by equal parts of 5% formalin and 70% alcohol.

(f) 80% methyl alcohol: Methyl alcohol is diluted to make 80% to be used for preservation of plankton.

5.4 Plankton Sampling

5.4.1 Plankton Samplers

(1) Selection of Plankton Sampler

Samplers usually are referred to as 'surface' or 'depth' (or 'subsurface') sampler. The later can collect samples from some 'stated depth'. Samplers are specifically designed to collect samples from surface desired depth. Moreover, standardized samplers are required for qualitative and quantitative study of plankton. Depending on the objective and depth, plankton samplers can be of two types:

1. Water sampler bottles
2. Plankton net

(2) Water Sampler Bottles

Water sampler bottles are used for quantitative and qualitative study. It consists of a cylindrical tube or bottle of known volume with stoppers at each end. It is fixed to a graduated tube and sent down to the required depth. Then a weight called 'messengers' is released which slides down the supporting rope and performs the closing mechanism. The commonly used sampler on this principle is Kemmerer sampler.

(3) Plankton Net

Plankton nets are preferred where plankton are few and plankton samples are required for qualitative study. However, modifications are required for quantitative work. A typical plankton net is a bolting cloth constructed in a conical shape with circular mouth. The bolting cloth is made of silk, nylon or other synthetic fibres and is available in a variety of mesh sizes.

(4) Classification of Plankton Net Depending on the Mesh Size

Plankton nets are classified to different numbers (Silk No.) depending on the mesh size. This is important to know the mesh size of the plankton net since it affects the size of plankton collected. Table 2 gives a detail characteristic of commonly used plankton nets with the type of plankton can be collected.

Table 2. *Classification of plankton nets on their mesh sizes.*

Silk No	Mesh Size(μm)	Mesh^{-cm} (app.)	App. open area(%)	Type of plankton to be collected
000	1024	9	58	Largest zooplankton and Ichthypoplankton
00	752	11	54	Largest zoo- and ichthyoplankton
0	569	15	50	Largest zoo- and ichthyoplankton
2	366	21	46	Large microcrustacea
6	239	29	44	Microcrustacea
10	158	43	45	Microcrustacea and rotifers
20	76	68	45	Net phyto and zooplankton
25	64	79	33	Nannoplankton

APHA, 1998

BOX 3

How to determine unknown mesh size of a plankton net

The simple procedure to determine mesh size of a plankton net is as follows:

- With a millimeter scale, count the units of a plankton net up to an accurate whole number. Magnifying glass can be used for counting such units.
- Find the size of one unit in μm scale.

For example

Let 20 smaller units of plankton net = 2mm in scale

$$1 \text{ smaller unit} = \frac{2}{20} \times 1000\ \mu \quad (1\text{mm} = 1000\ \mu\text{m})$$

$$= 100\ \mu$$

The mesh size of the plankton net is 100μ. Therefore, only micro plankton (Table 1) can be collected by this plankton net.

5.4.2 Plankton Sampling Methods from Surface Water

Usually plankton are collected from the surface of water body. The commonly used tool for plankton collection from surface water body is plankton net.

(1) Equipment

1. A no. 5 or 20 plankton net
2. Plastic mug of accurately one litre capacity
3. Collecting vials (preferable 25ml)
4. Record book and label
5. Pen/pencil

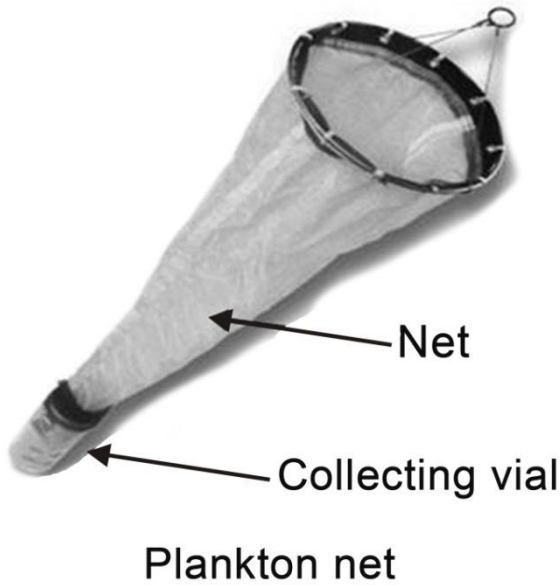

Fig. 3. *A simple plankton net for collection of plankton from surface water.*

(2) Reagents

Formalin (determine with a dropper the amount to be added to 25ml water to make 4%) or lugol's solution.

(3) Procedure

- Select sites for plankton collection.
- Collect water samples from selected sites with a mug of known capacity in volume.
- Collect full mug of water every time and count the number of full-mug water collection.
- Strain every mug of water through plankton net immediately after collection.
- Transfer collected volume of sample to collecting vial and pour required drops of formalin to make 4%. Lugol's solution can be used to make 1%. It will give a light tea colour.

(4) Precaution

- Before collection, the water surface should not be disturbed. A disturbance makes water turbid which block the collecting vial attached to net.

- Do not forget to label the vials with (i) time of collection, (ii) Date of collection (iii) Number of collection or total number in litre (volume) filtered (iv) Site of collection (v) Temperature at the time of collection.

(5) Sedimentation of Sample

Though there are different techniques of sedimentation of plankton, most commonly used technique is by centrifugation. In centrifugation, a pre-processing is done by allowing all preserved samples to settle down keeping the collecting vials on a plan surface for at least 1hr. This settlement is followed by gentle removal of approximately 10ml of water from the top of the sample bottle. The sample is then transferred to centrifuge for further sedimentation. However, this step can be skipped if a centrifuge tube of greater than 15ml capacity is used as collecting vial. In this case, the centrifuge tube that is used as collecting vial can directly be placed on to a centrifuge for further sedimentation. The rotor is balanced and centrifugation is done at 1000-1500 rpm for 15-20 minutes. After centrifugation, the volume in centrifuged tube can be reduced further to desired level. All processed centrifuge tube must be transferred to good quality glass vials with approximate volume of fixative. Labeling with initial volume (in L when sampled from surface water) and final volume (in ml after centrifugation) must be done for quantification.

5.5 Quantitative Analysis of Plankton

(1) Preparation of Slides

1. Agitate the settled sample after centrifugation or stored in vials.
2. With the help of a calibrated pipette, withdraw a required amount (See counting technique, Lackys' drop count) on the slide.
3. Calibrated pipette can be replaced by a standardized dropper. For this, a dropper with capacity 1.0ml is used. One can count the number of drops in 1.0ml and thereby volume of single drop can be calculated out. One drop of the sample placed on the glass slide is counted for plankton and then converted to 1.0ml subsequently.
4. Add one to two drops of glycerin to the slide. This prevents the organisms from drying after water evaporation.
5. Place a cover slip gently so that no air bubble forms.
6. Allow the slide to stand for few minutes. It helps in settling organisms for study.

(2) Counting Technique

Two counting techniques are widely used:

(1) The Sedgwick-Rafter (S-R) cell counting and

(2) Lacky's drop count.

5.5.1 The Sedgwick Rafter (S-R) Cell Counting

S-R cell is a device approximately of 50ml long by 20mm wide by 1mm deep. The total bottom area is approximately 1000mm or 1ml.

(1) Procedure

- Place the cover glass diagonally across the cell.
- Transfer sample (1ml) with a large-bore pipette or dropper. Mix thoroughly the sample before transfer.
- Allow cover slip to rotate slowly with a fine needle.
- Allow the S-R cell to stand for at least 15minutes to settle plankton.
- Now, count plankton on the bottom of S-R cell.

(2) Strip Count in S-R Cell

- Strip counting is done when few plankton are preserved.
- A strip is approximately 50mm long, 1mm deep, and the width of the total whipple grid.
- More strip count will give closer to the real value of plankton present.
- The number of plankton per ml can be derived from the following equation.

$$\text{Number/ml} = \frac{\text{C}\times 1000\ \text{mm}^3}{\text{L}\times\text{D}\times\text{W}\times\text{S}}\ (\text{APHA}, 1998)$$

Where,

C = Number of organisms counted;

L = Length of each strip (S-R cell length, 50mm) in mm;

D = Depth of strip (S-R cell length, mm) in mm;

W = Width of a strip (whipple grid width) in mm and;

S = Number of strips counted.

Number of plankton per litre of water can be determined as:

$$\text{Number/ml} = \frac{\text{Number/ml} \times 1000}{\text{Concentration factor (cf)}} \text{ (APHA, 1998)}$$

where

$$\text{cf} = \frac{\text{Volume of pond water filtered (ml)}}{\text{Volume of concentrate}}$$

(3) Field Count in SR Cell

- Field counting is performed for samples containing large number of plankton in a field.
- In this method, count plankton in random fields each consisting of one whipple-grid. More field selection gives more accuracy to the count.
- The number of plankton per milliliter can be determined as:

$$\text{Number/ml} = \frac{\text{C} \times 1000\ \text{m}^3}{\text{A} \times \text{D} \times \text{F}} \text{ (APHA, 1998)}$$

Where,

C = Number of organisms counted;

A = Area of a field (S-R cell depth) in mm;

F = Number of fields counted;

D = Depth of the field (1mm deep) in mm.

Number/ml can be converted to number/L following the conversion formula described for strip counting.

5.5.2 Lacky's Drop Count Method

This is a simple method for counting dense plankton population. Nano plankton can also be enumerated by Lacky's drop count method.

(1) Procedure

- Take one drop of sample by a pre-standardized pipette or dropper from a homogenized sample.
- Place a cover slip gently to avoid any overflow or air bubble.
- Allow 15 minutes to settle down all organisms.
- Start counting through strips.
- Calculate number of organisms per milliliter as follows:

$$\text{Number/ml} = \frac{C \times A_C}{A_S \times S \times V} \text{ (APHA, 1998)}$$

Where,

C = Number of organisms counted;

A_c = Area of cover slip in mm^2;

A_s = Area of one strip, mm^2;

S = Number of strips and;

V = Volume of sample under cover slip in ml.

Note: An easier method is to count the whole cover slide and present the organisms as number/drop. Since each drop is pre standardized before counting, the total count in a drop can be converted to any desired volume for quantitative expression of plankton analysis.

Chapter 6

Collection, Preservation and Quantification (Periphyton)

6.1 What is Periphyton

Periphyton is any aquatic planktonic (phyto- or zoo-) organism attached to some submerged substratum. They are direct nutrient as well as pollution indicator of any aquatic body. Wetzel (1983) defined it as the micro 'floral' community living attached to the substrate inside water. For its property in substrate selectivity, it commonly prefers submersed plants or plant parts, rocks and sediments for growth. Saikia (2011) featured it as a mid successional biofilm community mainly dominated by floral members i.e. phytoplankton.

6.2 Periphyton Collection and Quantification

Periphyton can be collected from two available substrates:

1. Natural substrate and
2. Artificial substrate

However, the basic procedure of sample collection is same for both substrates.

6.2.1 Periphyton Collection from Natural Substrate

- Submerged portions are usually cut in a required length. The cut-length is noted.
- The cut portion is directly preserved in fixative, mostly in 4% formalin in a vial containing distilled water.

- In laboratory, the surface of the substrate portion is scrapped using a fine razor or scalpel upto visibility by naked eye in the same distilled water where it was preserved.
- This is then transferred to centrifuge tube (preferably of the volume 15 ml) and centrifuge at 1500 rpm for 20 minutes.
- Volume is reduced to desired level and kept in a glass container (vol <15ml) and labeled properly along with all information required for the determination of surface area scrapped.

6.2.2 Periphyton Collection from Artificial Substrate

Artificial substrates like glass slide, cellophane paper, PVC pipe etc. are placed in water for periphyton collection. These substrates are fitted in suitable depth of water. However, glass or plastic slides are frequently preferred over other artificial substrates. A design of the glass slide sampler for shallow water body has been shown in Fig. 4.

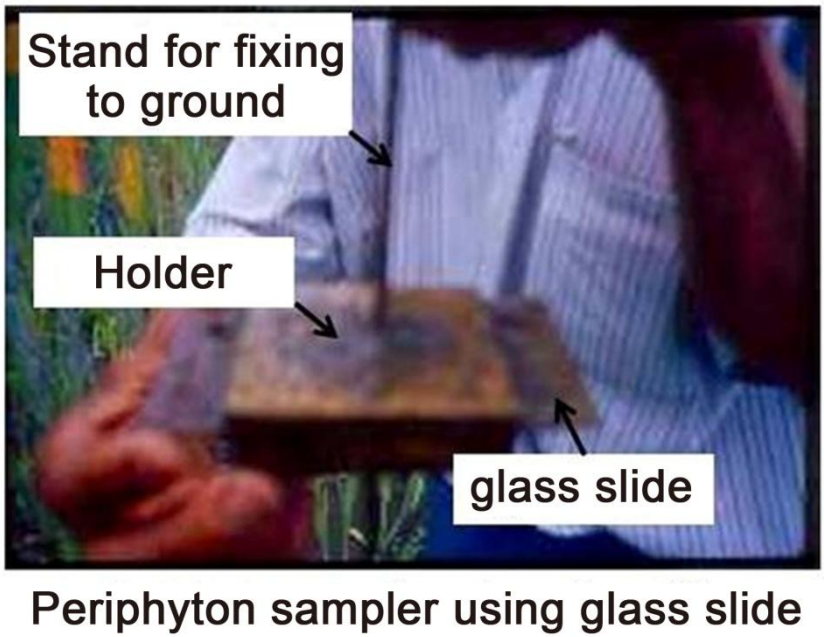

Fig. 4. *A device for collection of periphyton from shallow water body using glass slide.*

6.3 Quantitative Analysis of Periphyton

Organisms are counted using an S-R cell according to the procedure described for plankton. Periphyton numbers were estimated using the formula

$$\text{Periphyton cell or unit/cm}^2 = \frac{P \times C \times 100}{S}$$

Where

P = total number of periphyton units counted in desired fields (minimum 10);

C = Volume of final concentrate of the sample (ml);

S = Area of scrapped surface (cm^2).

Where periphyton organisms are counted by Lacky's drop count method, periphyton numbers are enumerated as:

$$\text{Number/cm}^2 = \frac{C \times A_C}{A_S \times S \times V \times A_P}$$

Where C, A_c, A_s,S and V are same as plankton counting.

A_p = Scrapped area for periphyton collection.

Periphytic organisms in artificial substrate can be quantified as per the methodology described for natural substrates.

Chapter 7

Plankton and Periphyton Productivity

7.1 What is Productivity

Productivity is the amount of organics substance acquired by an individual, a population, or a system per unit time.

(1) Categories of Productivity

Productivity falls into two major categories:

1. Primary productivity and
2. Secondary productivity

Primary productivity is exhibited by green plants which are otherwise photo synthetically active. On the other hand, Secondary productivity is exhibited by organisms which are not capable of photosynthesis.

(2) Terminology

Gross Primary Production (GPP): The total amount of organic matter fixed by photosynthetic plant is called Gross production.

Net Primary Production (NPP): Part of gross production is used in metabolic activities by plants through respiration. Gross production minus respiratory loss is called net production.

(3) Primary Production and Net Primary Production

According to Boyd (1979), this is the rate of formation of new organic matter. If Q = primary production (= increase of plant biomass over a period) and T = time, primary productivity is Q/T. Net primary productivity or production is the total

amount of new organic matter created by photosynthesis minus the amount of organic matter used in respiration.

7.2 Measurement of Productivity

When we measure primary productivity, we actually measure net primary productivity. Net primary productivity in water is measured chiefly by:

1. Dark and light bottle method,
2. Chlorophyll a method,
3. Biomass estimation method and,
4. Carbon -14 light bottle method.

Being the cheapest and easiest to operate, only first three methods have been given here.

7.2.1 Dark and Light Bottle Method

(1) Principle

The basic reactions in algal photosynthesis involve uptake of inorganic carbon and release of oxygen, which cen be summarized as:

$$CO_2 + H_2O = (CH_2O)_n + O_2$$

The dissolve oxygen concentration is determined at the beginning and end of incubation period. Productivity is calculated on the assumption that one atom of carbon is assimilated for each molecule of oxygen released.

In light bottle, photosynthesis activity is allowed to occur while in the dark bottle it is restricted. The decrease in the dissolve oxygen in the dark bottle, as compared to initial value represents the amount of dissolved oxygen consumed by the biomass in the bottle through the process of respiration. The increase in dissolved oxygen in the light bottle indicates the amount of dissolved oxygen in water which exceeds oxygen consumption by respiration.

(2) Reagents Required

1. All reagents required for DO estimation.
2. One light and one dark bottle (volume 125 or 250ml).

(3) Procedure

- Select relatively shallow water (photic zone). For euphotic zones, record the depth and measure light penetration.
- Take water sample carefully and uniformly in three bottles for initial DO, light bottle DO and dark bottle DO.
- Add 1ml of $MnSO_4$ followed by 1ml of alkaline iodide (where water volume is 125ml) to initial bottle. Invert it with stopper for 1-2 minutes.
- Fixed the other two bottles (light and dark) under water wherefrom water samples were collected.
- Incubate the bottles under water for desired time which should not be less than 3 hrs.
- After expiry of the desired time, fixed the DO in both the bottles as followed in case of the initial bottles.
- Determine DO values as described earlier.

(4) Calculation

$$\text{Gross primary productivity (GPP)} = \frac{\text{LB}-\text{DB}}{\text{H}} \times 1/1.2 \times 12/32 \times 1000 \text{ mg cm}^{-3}\text{h}^{-1}$$

$$\text{Net Primary Productivity (NPP)} = \frac{\text{LB}-\text{IB}}{\text{H}} \times 1/1.2 \times 12/32 \times 1000 \text{ mg cm}^{-3}\text{h}^{-1}$$

$$\text{Gross respiration} = \frac{\text{IB}-\text{DB}}{\text{H}} \times 1/1.2 \times 12/32 \times 1000 \text{ mg cm}^{-3}\text{h}^{-1}$$

Where,

LB = DO in light bottle

DB = DO in dark bottle

IB = DO in initial bottle

H = Hours of incubation

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

1.2 = Photosynthetic co-efficient

Note: During incubation, a measure of temperature, turbidity, and solar radiation (light intensity) should be recorded. These measures help in expressing the productivity for the entire daily photoperiod.

7.2.2 Cholorophyll – a

(1) Introduction

The concentration of photosynthesis pigment is used to estimate plankton productivity. All green plants contain chlorophyll a which constitutes approximately 1 to 2% of dry weight of planktonic algae. It is the main centre of photosynthetic reactions (Buttery and Buzzell, 1977). Algal biomass can also be expressed by multiplying a factor 67 to Chl-a content in terms of ash free weight.

(2) Principle

The pigment (Chlorophyll a) is extracted from the plankton concentrate with freeze cold acetone or methanol and the optical density (absorbance) of the extract is determined with a spectrophotometer. The results are expressed, as if all chlorophyll were chlorophyll-a, as chlorophyll-a equivalent (Golterman et al., 1978).

(3) Reagents

Freeze cooled acetone or methanol.

(4) Apparatus

Spectrophotometer, centrifuge, 25 or 50ml volumetric flask.

(5) Procedure

a) Collection of Chlorophyll Material

A suitable volume of water can be filtered through plankton net. The filtered amount is kept in dark vial. For periphyton, organisms (algae) are collected from a known surface area and kept in dark vial.

b) Pigment Extraction

- Transfer the volume into a centrifuge tube.
- Centrifuge the volume to settle plankton at bottom of the centrifuge tube.
- Decant the supernatant water and raise the volume with cold acetone and transfer to the volumetric flask.
- Wash the tube with acetone two or three times and transfer the solvent to volumetric flask.
- Stopper the flask and keep in a refrigerator in the dark or near 4 ℃.

- After 24 hours, bring out the flask and measure the absorbance at 630nm, 664nm and 750nm.

Note: Every time, before reading the absorbance for different wavelengths, set the spectrophotometer to zero by cold acetone (blank).

c) Calculation for Chlorophyll-a (C_a)

$$C_a = 11.85(OD\ 664) - 1.54(OD\ 647) - 0.08(OD\ 630)\ (APHA, 1998)$$

Where OD 664, OD 647 and OD 630 are the corrected optical densities (with a 1cm light path).

Note: The OD reading 750nm is a correction for turbidity. Subtract this reading from each of the pigment OD values of the other wavelengths before using them into the equation.

d) Chl-a Value for Plankton

$$\text{Chlorophyll} - a(mg/m^3) = \frac{C_a \times \text{extract volume (L)}}{\text{Volume of sample } (m^3)}\ (APHA,\ 1998)$$

e) Chl-a Value for Periphyton

$$\text{Chlorophyll} - a(mg/cm^2) = \frac{C_a \times \text{extract volume (L)}}{\text{Area of the substrate } (cm^2)}\ (APHA,\ 1998)$$

Note: if processing is delayed, keep sample in ice at 4℃ and protect from exposure to light. This way, it can be preserved for 3 weeks.

7.2.3 Biomass (Dry Weight and Ash Free Dry Weight)

(1) Introduction

Biomass is a quantitative estimation of the total mass of living organisms within a given area or volume. It includes the mass of populations or communities but does not give any information on community structure and function. The most accurate methods are Dry weight (DW), Ash free dry weight (AFDW) and volume of living organisms. Expression of biomass in volume has been described earlier.

(2) What is Dry Weight (DW)

Dry weight is the concentrate of inorganic or organic matter at 105 ℃.

(3) Equipment

Oven (max. 105 ℃), porcelain crucible (approximately 30ml capacity), analytical balance (sensitivity 0.1mg), dessicator.

(4) Procedure

- A large number of sample volume is concentrated (either centrifuged or settled gravitationally) to 20ml volume.
- Concentrated sample is taken in a pre-weighed (at 105 ℃) porcelain crucible.
- Dry it at 105 ℃ to constant weight.
- Keep it in a dessicator and then weigh.

(5) Calculation

a) Measurement of DW

$$\text{Planktocic } D_W(\text{mg/L}) = \frac{(\text{Porcelain crucible+sample at 105℃}) - (\text{Porcelain crucible at 105℃})}{\text{Original sample volume in L}}$$

$$\text{Periphytic } D_W(\text{mg/L}) = \frac{(\text{Porcelain crucible+sample at 105℃}) - (\text{Porcelain crucible at 105℃})}{\text{Scrapped surface area (cm}^2\text{)}}$$

b) Measurement of AFDW

The D_w is cooled in a dessicator and ignite in a muffle furnace at 500 ℃ for 1hr. It is then colled and weighed.

$$\text{Planktonic AFDW} = \frac{(\text{Porcelain crucible+sample at 500℃}) - (\text{Porcelain crucible at 500℃})}{\text{Original sample volume in L}}$$

$$\text{Planktonic AFDW} = \frac{(\text{Porcelain crucible+sample at 500℃}) - (\text{Porcelain crucible at 500℃})}{\text{Scrapped surface area (cm}^2\text{)}}$$

Section IV

Diversity and Feeding Ecological Studies

Chapter 8

Diversity Indices

8.1 What is Diversity

Diversity is the degree of heterogeneity in a community. The purpose of measuring diversity in an ecosystem is to judge the relationship of a community to its own members and also to other community properties (e.g. productivity) or to prevailing environmental conditions (Pielou, 1975). High taxon (e.g. species) diversities are related to high community composition, high environmental stability, high environmental predictability and high productivity. It is claimed as an effective statistics to understand and to predict a changed environment (Wilhm and Dorris, 1968; Cairns and Dickson, 1971).

8.2 Indices for Diversity Measures

Diversity can be measured using diversity measures or indices. Diversity indices are mathematical functions that combine richness and evenness in a single measure. These indices provide information about community composition rather than simply explaining the richness of a community. Most diversity indices are easy to compute and are widely used for community as well as ecosystem assessment.

Species diversity or α-diversity is measured in species level (Mc Arthur, 1964). However it can be measured upto any taxonomic unit. The species diversity measures included here are:

(i) Species richness

(ii) Relative Comparison Index

(iii) Shannon-Weaver diversity index

(iv) Simpsons' diversity index
(v) Margalef's diversity index
(vi) Berger-parker index

8.2.1 Species Richness(s)

The number of species in a community or ecosystem is called richness. It is the simplest of all diversity indices and gives only the number of species present irrespective of their distribution or evenness. However, chances are there for biasness if richness is considered since all species, especially animals in an area cannot be counted during the time of sampling.

However, other diversity indices like Shannon-Weaver or Simpson combine richness and evenness (homogeneity) to a single measure.

8.2.2 Relative Comparison Index (Saikia and Das 2012)

Relative Comparison Index (CI) is a rapid method of comparing planktonic communities on a gradient (e.g. temporal, spatial etc.). This index assuming '1' for every absence data in a sample, instead '0' (which is adopted in most of other indices) while comparing with other samples of same environment. The advantage over such assumption in relative CI is that it makes the comparison more sensible than other indices with less sensibility when results are on the basis of virtual presence or absence of the taxon in the sample. An explanation on how to compute this index is given in Table 3. The table displays phytoplankton records of five sampling dates, e.g. Day A, Day B, Day C, Day D and Day E. On day A (p=A), the measure M_A/n_{iA} reveals the comparative value of plankton abundances to other samples (i.e. upto M_E/n_{iE}) on monocaculative terms. The value for ratio M_A/n_{iA} where p=A has been termed as Comparison Index (CI).

For sample p, mathematical expression of CI is,

$$CI_p = M_P / \sum n_{ip} \tag{1}$$

or simply,

$$\log CI_p = \log M_P - \log \sum n_{ip} \tag{2}$$

where $\sum n_{ip}$ is total count of i^{th} taxon employing '1' for each absence event in sample p ($\sum n_{ip} > 0$). In table 3, p = A or B or C or D or E. The $M_p = N_p - \sum n_{ip}$ with N_p as total overall count of taxa present employing '1' for each presence

event of a taxon in overall sample.

To make CI as more justified ecological index, an explanation of evenness in the sample can also be counted as log CI.

The evenness values based on CI (or E_{CI}) from *p* sample was computed out as

$$E_{CIp} = \log CI_p/2 \text{ or } \log M_P - \log \sum n_{ip}/2 \quad (3)$$

where $\sum n_{ip}$ is total count of i^{th} taxon employing '1' for each absence event in sample *p* and $M_p = N_p - \sum n_{ip}$ with N_p as total count of taxa present employing '1' for each presence event of a taxon in overall sample.

Table 3. *Calculation of relative comparison index (Saikia and Das 2012).*

N	A	B	C	D	E
1	Scenedesmus	Scenedesmus	Scenedesmus	1	1
2	Pediastrum	1	1	Pediastrum	Pediastrum
3	Oedogonium	Oedogonium	Oedogonium	1	Oedogonium
4	Euastrum	Euastrum	1	1	1
5	Closterium	Closterium	Clsterium	1	1
6	Pleurotaenium	1	Pleurotaenium	Pleurotaenium	1
7	Cylindrocapsa	1	Cylindrocapsa	1	1
8	Closteriopsis	Closteriopsis	Closteriopsis	Closteriopsis	Closteriopsis
9	Cosmarium	Cosmarium	Cosmarium	Cosmarium	Cosmarium
10	Micrasteria	1	Micraseria	1	1
11	Euglena	Euglena	Euglena	1	1
12	1	Docidium	1	1	1
13	1	Macrospora	Microspora	1	Microspora
14	1	Ankistrodesmus	1	Ankistrodesmus	Ankistrodesmus
15	1	Ankistrodesmus	1	Tetraspora	1
16	1	Tetraspora	1	Chlorella	1
17	1	Chlorella	1	Staurastrum	Staurastrum
18	1	Staurastrum	Mesotaenium	1	1
19	1	1	Actinotaenium	1	1
20	1	1	Gonatozygon	1	1
21	1	1	1	1	Geminella
22	1	1	1	1	Hormidium
23	1	1	1	1	Zygnema
n_{ip}	12	09	10	15	13
M_p	11	14	13	8	10
CI_p	0.9167	1.5556	1.3	5.3333	0.7692
$\log CI_p$	-0.03779	0.19189	0.11394	-0.273	-0.11394

The graphical presentation for CI is shown in Fig 5.

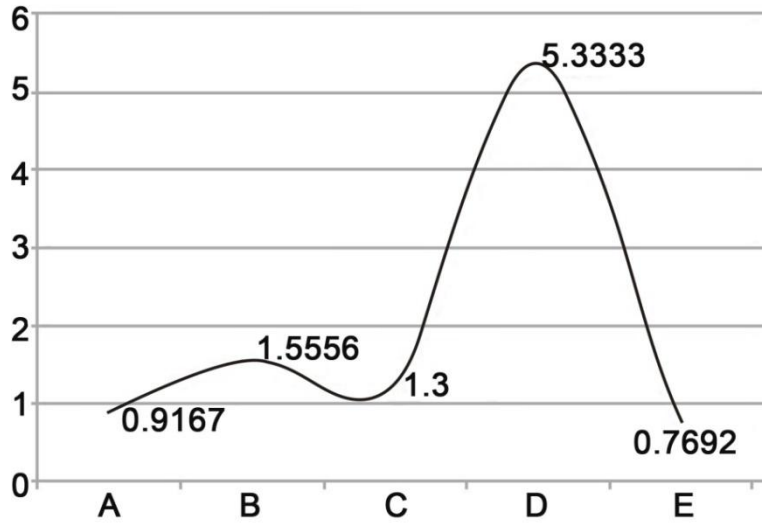

Fig. 5. *Graphical presentation of CI on temporal gradients. The minor differences of planktonic richness on different sampling dates (e.g. B and C) are prominent.*

8.2.3 Shannon-Weaver diversity (Shannon 1948, Shannon-Weaver 1949)

This is one of the most commonly used measures of species diversity. It accounts the relative abundance and evenness of a species in the community.

The measure is based on two information:

(1) the number of species (i.e. richness) and

(2) The number of individuals in each species

The index is used only on random samples drawn from a large community in which the total number of species is known.

$$H = -\sum_{i=1}^{s} Pi \ln Pi \quad \text{where } Pi = ni / N$$

Here,

H is Shannon-Weaver species diversity.

ni is the total number of individual of species.

N is the total number of all species in stand.

S is the number of species in the sample.

BOX 4

Calculation of H

The following table is prepared from plankton diversity from a freshwater pond.

Sl no.	Name of organism	individual count (ni)	ni/N	ln(ni/N)	ni ×ln(ni/N)
1	*Scenedesmus sp.*	22	0.0503	-2.9889	-0.1505
2	*Pediastrum sp.*	56	0.1281	-2.0546	-0.2633
3	*Oedogonium sp.*	36	0.0824	-2.4964	-0.2057
4	*Spirogyra sp.*	10	0.0229	-3.7773	-0.0864
5	*Bulbochaete sp.*	4	0.0092	-4.6936	-0.0430
6	*Closterium sp.*	26	0.0595	-2.8218	-0.1679
7	*Chlorella sp.*	18	0.0412	-3.1896	-0.1314
8	*Pleurotaenium sp.*	60	0.1373	-1.9856	-0.2726
9	*Triplocera sp.*	20	0.0458	-3.0842	-0.1412
10	*Xanthidium sp.*	14	0.0320	-3.4409	-0.1102
11	*Cosmarium sp.*	10	0.0229	-3.7773	-0.0864
12	*Staurastrum sp.*	8	0.0183	-4.0005	-0.0732
13	*Gonatozygon sp.*	30	0.0686	-2.6787	-0.1839
14	*Mesotaenium sp.*	25	0.0572	-2.8611	-0.1637
15	*Euastrum sp.*	98	0.2243	-1.4950	-0.3353
	S=15		N = 437		∑2.4146

Theoretical maximum value of

$$H = \ln(S) = \ln(15) = 2.70805$$

Theoretical minimum value of

$$H = \ln[N/(N-S)] = \ln[437/(437-15)] = 0.03493$$

Calculated H from

$$\text{data} = -\sum pi \ln pi \text{ or } -\sum ni/N \ln ni/N = -(-2.4146) = 2.4146$$

Comment

As calculated H is nearer to theoretical maximum value of H, the diversity in the pond can be said high.

(1) Interpretation

1. H increases with the number of species (i.e. richness) in the community. However, increasing the number of species in a community will not necessarily increase diversity.
2. More complex the community, the greater is the species diversity and stability (Mc Arthur, 1965).
3. When diversity is studied in a time scale, greater the function in H, less is its stability.
4. High diversity when there is no numerically dominant species.
5. Shannon-Weaver diversity index is less sensitive to rare species.

(2) Comment

- In practice, for biological communities H does not seem to exceed 5.0 (Washington, 1984). The theoretical maximum value is ln(S), and the minimum value in ln[N/(N-S)] (Fager, 1972).
- At least 10 replications are to be analyzed.

8.2.4 Simpson's Diversity Index (D) (1964)

Simpson's diversity index (D) can be measured for finite and infinite population. Simpson diversity is less sensitive to richness than to evenness.

(1) D for Finite Population

A finite population is a population in a closed ecosystem. It can be measured as:

$$D = 1 - \sum_{i=1}^{S} \left[\frac{ni\,(ni-1)}{N\,(N-1)} \right] \quad \text{(Pielou, 1969)}$$

(2) D for Infinite Population

$$D = 1/\sum ni/N$$

Here,

ni = Number of individual species.

N = Total number of species.

BOX 5

The following table is prepared from plankton diversity from a freshwater pond (finite population).

Sl no.	Name of organism	individual count (ni)	ni-1	ni(ni-1)	ni(ni-1)/N(N-1)
1	*Scenedesmus sp.*	22	21	462	0.0024
2	*Pediastrum sp.*	56	55	3080	0.0162
3	*Oedogonium sp.*	36	35	1260	0.0066
4	*Spirogyra sp.*	10	9	90	0.0005
5	*Bulbochaete sp.*	4	3	12	0.0000
6	*Closterium sp.*	26	25	650	0.0034
7	*Chlorella sp.*	18		306	0.0016
8	*Pleurotaenium sp.*	60	59	3540	0.0186
9	*Triplocera sp.*	20	19	380	0.0020
10	*Xanthidium sp.*	14	13	182	0.0010
11	*Cosmarium sp.*	10	9	90	0.0005
12	*Staurastrum sp.*	8	7	56	0.0003
13	*Gonatozygon sp.*	30	29	870	0.0046
14	*Mesotaenium sp.*	25	24	600	0.0031
15	*Euastrum sp.*	98	97	9506	0.0499
	N = 437		N-1=436		0.1107

Here, N(N-1) = 190532

Simpson D = 1-∑[ni(ni-1)/N(N-1)] = 1.0000-0.1107 = 0.8893

Maximum value of D = 1- (1/S)= 1-(1/15) = 0.9333

(3) Interpretation

1. Simpson's index ranges from 0 to 1.0.
2. 0 diversity, when community complexity is less and 1 diversity when community complexity is high.
3. Low diversity (near to 0) indicates presence of dominant species or taxa, high diversity (near to 1) indicates presence of all constituent taxa in more or less uniform state.

(4) Comment

Simpsons' index is most sensitive to the changes in the more abundant species.

8.2.5 Margalef's Diversity Index, 1968

This index is used for small samples. It can be measured as:

$$H = S\text{-}1/InN$$

Here,
H = Margalef's index
S = Number of species
N = Total number of individuals

8.2.6 McIntosh Diversity Index

It was suggested by McIntosh in 1967. The values are between 0 – 1. When the value is getting closer to 1, it means that the organisms in a community are homogeneously distributed (McIntosh 1967).

$$Mc = [\ N - \sqrt{(\sum ni^2)}\ ] / [\ N - \sqrt{N}\ ]$$

Here,
Mc = McIntosh Diversity Index
ni = Number of individuals belonging to species i
N = Total number of individuals

8.3 Indices for Evenness Studies

Evenness measure represents the equitability of species in a community. A measure of evenness is supportive for the diversity index. Lloyed and Ghelardi (1964) suggested to measure the evenness component separately for the study of diversity. The two highly popular evenness measures are:

(i) Shannon-Weaver evenness measure.

(ii) Simpson's evenness measure or Evenness measure based on D.

8.3.1 Shannon-Weaver Evenness Measure (J) (Pielou, 1966)

It is calculated on the basis of H value.

$$J = H / H_{max}$$

Here,
H = Shannon-Weaver index
H_{max} = Maximum value of H. H_{max} = ln S

For example, in BOX 4, the H is 2.4146 and H_{max} is 2.70805. Therefore, J = 2.4146/2.70805 = 0.8916 which reflects the species are more or less equally homogenous distribution in the pond environment at the time of sampling.

8.3.2 Simpsons' Evenness Measure (E)

It is calculated on the basis of D value.

$$E = D / D_{max}$$

Here,
D = Simpsons' diversity index
D_{max} = Maximum possible value of Simpsons' diversity. D_{max} = 1/S.

From BOX 5, D is 0.8893 and Dmax is 0.9333. Hence, E = 0.8893/0.9333 = 0.9528 which indicates more or less equal distribution of species in pond environment at the time of sampling.

(1) Interpretation

(i) Low evenness value indicates less equitability among the species, high evenness value indicates equal distribution of species.

(ii) Less evenness value indicates dominance of a single species in the community.

(2) McIntosh Evenness Index

It was derived from McIntosh index. The values are between 0 – 1. When the value is getting closer to 1, it means that the individuals are distributed equally (Heip and Engels 1974).

$$McE = [N - \sqrt{(\sum ni^2)}] / [N – (N / \sqrt{S})]$$

Here,
McE = McIntosh evenness index

ni = Number of individuals belonging to i species

S = Total number of species

N = Total number of individuals

8.4 Dominance Index

It gives the magnitude of dominance of a species in a community. Two popular dominance indices are:

1. Concentration of dominance.
2. Berger-Perker dominance index.

8.4.1 Concentration of Dominance (C)

It is the most common and easiest index to calculate. It is calculated as:

$$C = (Ni/N)^2 \text{ (Odum, 1971)}$$

Here

Ni = the total number of individuals of species and

N = the total number of individuals of all species in a stand.

8.4.2 Berger-Perker Index (d) (1970)

It is calculated as:

$$d = N_{max}/N$$

Here

N = total number of individuals of all species in a stand

N_{max} = Maximum number of species counted.

8.5 Variety Index (V)

Variety index helps in comparing one community or group of population with another. It is calculated as:

$$V = S/InN^* \text{ (Odum, 1971)}$$

(*Odum used $\log_2$)

Here

S = Number of species recorded

N = total number of individual recorded

8.6 Similarity and Dissimilarity Indices

8.6.1 Similarity Index

Similarity index helps in understanding the diversity level of two ecosystems. It indirectly indicates the magnitude of similarity and dissimilarity of the environmental conditions of two different ecosystems.

The similarity index can be computed according to Sorensen (1948).

$$SIMI = 2C/ A+B$$

Here

A and B are the number of taxa recorded to different ecosystems,

C is the number of taxa common to A and B ecosystems.

8.6.2 Index of Dissimilarity (DI)

$$DI = 1\text{-}SIMI$$

(1) Comment

The values of SIMI and DI are generally expressed in terms of percentage.

Chapter 9

Feeding Ecological Studies

9.1 What is Feeding Ecology

Food and feeding habit of any heterotrophs in and around its habitat is an important parameter for assessing its functional role in the ecosystem. It also influences as well as co-relates all other aspects of habitat ecology for that particular organism. The functions of community dynamics for organism can be best studied on the basis of food and feeding habits. Therefore, to generate information on available feed in and around its dwelling environment, the study of feeding ecology of an organism is must. However, there still exists confusion towards the extent of using most appropriate approaches for obtaining complete information on feeding ecology of an organism. Lack of such approach is mainly due to appropriate feeding ecology parameter to be considered to draw up conclusions on food habit of studied organism. The following is a description of some feeding ecology indices used to obtain information on feeding ecology of fish.

9.2 Simple Indices for Gut Analysis

9.2.1 Fullness and Fullness of Gut

Fullness of gut predicts the foraging pattern, feeding intensity and environmental effect on feeding rate of fish for a given time. There is a controversy regarding appropriate fullness index. However, commonly used method is visual scale method. In this method following scale is used for organisms with considerable length of gut.

Empty stomach = 0

$1/4^{th}$ full stomach = 0.25

$1/2^{nd}$ full stomach = 0.50

$3/4^{th}$ full stomach = 0.75

Completely full stomach = 1.0

In visual methods, the results may be biased due to inappropriate or unequal length of the gut. Partial fullness of the gut may not fall within the specified scale. Presence of trace amount of food in the gut is generally ignored. Fullness index is studied only to support some other food studies result.

However, Herbold (1986) proposed an alternative method where he calculated the fullness index as percentage of observed gut content mass to expected maximum gut content mass.

$$\% \text{ Fullness Index} = \frac{\text{Observed gut content mass}}{\text{Expected maximum gut content mass}} \times 100$$

Fullness index is also expressed as Percent Fullness of gut.

$$\% \text{ Fullness of gut} = \frac{\text{Number of guts with food}}{\text{Total guts examined}} \times 100$$

Higher value of Percent fullness of gut indicates more intensive feeding while lower values indicate less intensive feeding. Higher value with less number of fish indicates higher activity and lower value with more number of fish indicates low feeding activity.

9.2.2 Stomach Content Index

Stomach content is the difference of the wet weight of the stomach before and after emptying it. It is expressed as

Stomach Content = Gut weight before emptying – Weight of empty gut

For this gut of organism is preserved immediately to avoid losing any food content from the stomach. The excess water from the gut is removed with a blotting paper and weight of full gut is taken. The stomach is then cut to open it and gut contents are collected carefully with a brush. All stomach contents are removed till it looks empty to naked eyes. The weight of the empty gut is taken to measure Stomach content.

Stomach content can also be expressed as Percent Stomach content weight to body weight. This is expressed as

$$\% \text{ Stomach Content} = \frac{\text{Stomach content}}{\text{Total weight o the fish}} \times 100$$

9.2.3 Stomach Index

Stomach Index is expressed as the percentage of the ratio of weight of the full stomach to fish body weight when the fish lacks a sizable stomach to store a considerable amount of feed.

$$\text{Stomach Index} = \frac{\text{Stomach Weight}}{\text{Fish body weight}} \times 100$$

9.2.4 Percent Composition of Food Items in the Gut

The percentage composition of food item is the total value of a food item observed in all stomach (Tf_p) divided by the total number of all food item (Tf) for a particular length-group of fish or time.

$$\% \text{ Composition of food} = \frac{Tf_P}{Tf} \times 100$$

9.3 Dietary Breadth

Study of dietary breadth determines the way the fish utilize the resource (or food) from its environment. The most common indices to measure diet breadth in ecology were niche breadth of Levins (1968), Hulbert (1978) and Smith (1982). These metrics use observed food category in the gut of studied organism as the basis of calculation.

9.3.1 Levin's Diet Breadth

Levin's diet breadth is the modification of Simpson's diversity index. It is calculated as:

$$B = 1/\sum(p^2_j)$$

where, B is Levin's Diet Breadth, p_j is fraction of items in the diet that are of food category j.

B value ranges from 1.0, when the population under study uses one resource state exclusively and equal to R (i.e. the number of taxonomic identity or size category or anything categorizing resource or food) when the population uses all resource states. B can be normalized by R as follows:

$$B = 1/(R\sum p^2_j)$$

The normalized B ranges from 1/R when the population uses one resource state to 1.0 when the population uses all resource state in equal proportions. Hulbert suggested the following standardized measure of niche breadth:

$$B_A = \frac{\left(1/\sum P_j^2\right)-1}{n-1}\times 100$$

BOX 6

Calculation of Levin's Diet Breadth

Sl no.	Name of organism	individual count in he gut	p_j	p^2_j
1	*Scenedesmus sp.*	22	0.0503	0.0025
2	*Pediastrum sp.*	56	0.1281	0.0164
3	*Oedogonium sp.*	36	0.0824	0.0068
4	*Spirogyra sp.*	10	0.0229	0.0005
5	*Bulbochaete sp.*	4	0.0092	0.0000
6	*Closterium sp.*	26	0.0595	0.0035
7	*Chlorella sp.*	18	0.0412	0.0017
8	*Pleurotaenium sp.*	60	0.1373	0.0189
9	*Triplocera sp.*	20	0.0458	0.0021
10	*Xanthidium sp.*	14	0.0320	0.0010
11	*Cosmarium sp.*	10	0.0229	0.0005
12	*Staurastrum sp.*	8	0.0183	0.0003
13	*Gonatozygon sp.*	30	0.0686	0.0047
14	*Mesotaenium sp.*	25	0.0572	0.0033
15	*Euastrum sp.*	98	0.2243	0.0503
		N = 437		∑ 0.1127

Levin's measure or $B = (1/\sum p^2_j)$. Here $\sum p^2_j = 0.1127$. Therefore, B = 1/0.1127 = 8.8731.

Normalized B using R i.e. $B = 1/R(\sum p^2_j) = 1/15(0.1127) = 1/0.1127 = 0.5915$.

Normalized B using n i.e. $B_A = [(1/\sum p^2_j)-1]/n-1 = 8.8731-1 / 14 = 0.5624$.

9.3.2 Hulbert's Diet Breadth

However, Hulbert (1978) proposed an index that accounts resources available in the environment. It is calculated as:

$$B' = 1/(\sum p^2_j/a_j)$$

Where B ´is Hulbert's standardized diet breadth, p_j is fraction of items in the diet that are of food category j ($\sum p = 1.0$), a_j is proportion of total available resources consisting of resource j ($\sum a_j = 1.0$). B ´ranges from 1/n to 1.0.

Hulbert's diet breadth can be standardized as:

$$B'_A = \frac{[1/\sum(p_j^2/a_j)] - a_{min}}{1 - a_{min}}$$

Where B'_A is Hulbert's standardized diet breadth, p_j and a_j are as described in B ´. The a_{min} is smallest observed proportion of all the resources or minimum a. B'_A ranges from 0 to 1.0.

The variance of Hulbert's diet breadth can be estimated by delta method (Smith, 1982). It is

$$Var(B') = \frac{4B'^4[\sum(p_j^2/a_j)] - (1/B')^2}{Y}$$

Where,

Var (B ʹ) = Variance of Hulbert's measure of diet breadth (B ʹ);

p_j and a_j = As described in B ʹ;

Y = Total number of individuals studied = ∑N.

Statistically, this Var (B ʹ) can be used to measure 95% confidence limit for B ´ using following expression:

$$B' \pm 1.96\sqrt{var(B')}$$

BOX 7

Calculation of Hulbert's diet breadth

From the p_j and a_j values, Smith's diet breadth can be calculated as follows:

Sl no.	Name of organism in the gut	Individual count from resource	Individual count from gut	a_j	p_j	$(p_j)^2$	p^2_j/a_j
1	*Scenedesmus sp.*	22	0	0.0503	0	0	0
2	*Pediastrum sp.*	56	25	0.1281	0.1269	0.0161	0.1257
3	*Oedogonium sp.*	36	18	0.0824	0.0914	0.0083	0.1007
4	*Spirogyra sp.*	10	0	0.0229	0	0	0
5	*Bulbochaete sp.*	4	0	0.0092	0	0	0
6	*Closterium sp.*	26	20	0.0595	0.1015	0.0103	0.1731
7	*Chlorella sp.*	18	5	0.0412	0.0254	0.0006	0.0146
8	*Pleurotaenium sp.*	60	24	0.1373	0.1218	0.0148	0.1078
9	*Triplocera sp.*	20	8	0.0458	0.0406	0.0016	0.0349
10	*Xanthidium sp.*	14	6	0.0320	0.0305	0.0009	0.0281
11	*Cosmarium sp.*	10	0	0.0229	0	0	0
12	*Staurastrum sp.*	8	0	0.0183	0	0	0
13	*Gonatozygon sp.*	30	10	0.0686	0.0508	0.0026	0.0379
14	*Mesotaenium sp.*	25	15	0.0572	0.0761	0.0058	0.1014
15	*Euastrum sp.*	98	66	0.2243	0.3350	0.1122	0.5002
	N = 437			∑1.2244			

Hulbert's $B \leftleftharpoons 1/(\sum p^2_j/a_j)$. Here, $B' = 0.8167$. The diet breadth is closure to 1.0. Hence, the gut has wider diet breadth. The a_{min} is 0.0092. Hence B'_A is 0.8150.

9.3.3 Smith's Diet Breadth

Smith (1982) diet measure is calculated as:

$$FT = \Sigma\sqrt{(p_j a_j)}$$

Where,
FT is Smith's diet breadth, p_j and a_j are as described in B_A and B'_A.

BOX 8

Calculation of Smith's diet breadth

From the p_j and a_j values, Smith's diet breadth can be calculated as follows:

Sl no.	Organism in the gut	Individual count from resource	Individual count from gut	a_j	p_j	$\sqrt{(p_j a_j)}$
1	*Scenedesmus sp.*	22	1	0.0503	0	0
2	*Pediastrum sp.*	56	25	0.1281	0.1269	0.1275
3	*Oedogonium sp.*	36	18	0.0824	0.0914	0.0868
4	*Spirogyra sp.*	10	1	0.0229	0	0
5	*Bulbochaete sp.*	4	1	0.0092	0	0
6	*Closterium sp.*	26	20	0.0595	0.1015	0.0777
7	*Chlorella sp.*	18	5	0.0412	0.0254	0.0323
8	*Pleurotaenium sp.*	60	24	0.1373	0.1218	0.1293
9	*Triplocera sp.*	20	8	0.0458	0.0406	0.0431
10	*Xanthidium sp.*	14	6	0.0320	0.0305	0.0312
11	*Cosmarium sp.*	10	1	0.0229	0	0
12	*Staurastrum sp.*	8	1	0.0183	0	0
13	*Gonatozygon sp.*	30	10	0.0686	0.0508	0.0590
14	*Mesotaenium sp.*	25	15	0.0572	0.0761	0.0660
15	*Euastrum sp.*	98	66	0.2243	0.3350	0.2741
	N = 437		0.0	$\sum\sqrt{(p_j a_j)}$=0.9270		

Smiths FT = $\sum\sqrt{(p_j a_j)}$. Here, FT ´= 0.9270. The diet breadth is closure to 1.0. Hence, the gut has wider diet breadth.

9.3.4 Saikia's Diet Breadth or DB (χ^2) (Saikia, 2012)

The recent method of measurement of diet breadth is Saikia's diet breadth (Saikia, 2012) which follows χ^2 (Chi square) statistics. The χ^2 expression for diet breadth is computed as follows:

$$DB(\chi^2) = \sum_{i}^{n} \frac{(\log O_i - \log E_i)^2}{\log E_i}$$

Here, the DB(χ^2) is diet breadth, $logO_i$ and $logE_i$ are the log value of observed and expected food abundances of i^{th} category. DB(χ^2) considers that $logO_i \neq 0$ for $logE_i > 0$. Rather, in such cases where $logO_i$ for $logE_i$ is 0, a minimum representation '1' is considered in gut. This will not affect the result since log(1)=0. The expected food abundance in DB(χ^2) is constituted of available food resources in the environment.

When food categories in the diet show equal representation as in the resource, DB(χ^2) is 0, and greater variation of result from 0 indicates avoidance of the resource food categories by the organism.

BOX 9

Calculation of Saikia's diet breadth

From the p_j and a_j values of Box 7, Smith's niche breadth can be calculated as follows:

Sl no.	Organism in the gut	Individual count from resource	Individual count from gut	$(logO- logE)^2$	$(logO- logE)^2/logE$
1	*Scenedesmus sp.*	22	1	1.802	1.3424
2	*Pediastrum sp.*	56	25	0.1227	0.0702
3	*Oedogonium sp.*	36	18	0.0906	0.0582
4	*Spirogyra sp.*	10	1	1.000	1.000
5	*Bulbochaete sp.*	4	1	0.3625	0.6021
6	*Closterium sp.*	26	20	0.1298	0.0092
7	*Chlorella sp.*	18	5	0.3095	0.2464
8	*Pleurotaenium sp.*	60	24	0.1584	0.0891
9	*Triplocera sp.*	20	8	0.1584	0.1217
10	*Xanthidium sp.*	14	6	0.1354	0.1181
11	*Cosmarium sp.*	10	1	1.000	1.000
12	*Staurastrum sp.*	8	1	0.8156	0.9031
13	*Gonatozygon sp.*	30	10	0.2276	0.1541
14	*Mesotaenium sp.*	25	15	0.0492	0.0352
15	*Euastrum sp.*	98	66	0.0295	0.0148
	N = 437		202	$\sum(logO- logE)^2/logE=5.7647$	

DB($\chi 2$)= 5.7647. Since 5.7647>>0.0, the diet breadth of the organism is narrow. It does not feed wholly on the resources considered.

9.3.5 Czekanowski's Proportion of Similarity Index

Feinsinger et. al. (1981) advocated Czekanowski's Proportion of Similarity Index for diet breadth analysis of a species. It takes into account the resource items availability in the environment for the species studied. It is calculated as:

$$Ps_x = 1 - 0.5\Sigma\left|Px_i - q_i\right|$$

Where, Ps_x is the Czekanowski's Proportion of Similarity Index, Px_i is the proportion of resource items in category i out of all items used by species x and q_i is the proportion of i^{th} items available in the resource base for the population of the species studied. A Czekanowski's index closure to 1.0 indicates wider diet breadth of organisms. An explanatory example is shown in BOX 10.

BOX 10

Calculation of Czekanowski's Proportion of Similarity Index

From the p_j and a_j values of BOX 7, Smith's diet breadth can be calculated as follows:

Sl no.	Organism in the gut	Individual count from resource	Individual count from gut	a_j	p_j	$\lvert p_j-a_j \rvert$
1	*Scenedesmus sp.*	22	0	0.0503	0	0.0503
2	*Pediastrum sp.*	56	25	0.1281	0.1269	0.0012
3	*Oedogonium sp.*	36	18	0.0824	0.0914	0.0090
4	*Spirogyra sp.*	10	0	0.0229	0	0.2290
5	*Bulbochaete sp.*	4	0	0.0092	0	0.0092
6	*Closterium sp.*	26	20	0.0595	0.1015	0.0420
7	*Chlorella sp.*	18	5	0.0412	0.0254	0.0158
8	*Pleurotaenium sp.*	60	24	0.1373	0.1218	0.0155
9	*Triplocera sp.*	20	8	0.0458	0.0406	0.0051
10	*Xanthidium sp.*	14	6	0.0320	0.0305	0.0015
11	*Cosmarium sp.*	10	0	0.0229	0	0.0229
12	*Staurastrum sp.*	8	0	0.0183	0	0.0183
13	*Gonatozygon sp.*	30	10	0.0686	0.0508	0.0173
14	*Mesotaenium sp.*	25	15	0.0572	0.0761	0.0192
15	*Euastrum sp.*	98	66	0.2243	0.3350	0.1107
	N = 437				$\sum \lvert p_j-a_j \rvert$=0.3615	

Czekanowski's Proportion of Similarity Index is $Psx = 1-0.5 \sum \lvert Pxi - qi \rvert = 1-0.5\ (0.3615) = 1-0.18075 = 0.81925$.

9.4 Dietary Overlap

Diet overlap among species or size classes of a single species helps to explain the community structure or to clarify competitive relationship (Fig 6). This is an ecological measure through which competition between two organisms for similar diets can be assessed. Hence, diet overlap simply means food organisms from natural environment shared by both competitors in an ecosystem. Narrow is the diet breadth, less is the competition between the two.

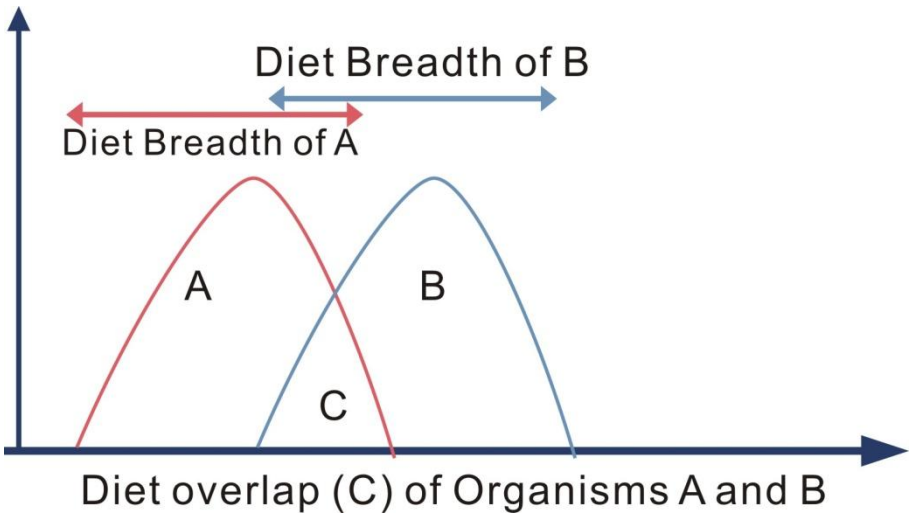

Fig. 6. *Diet overlap of two organisms (A and B) in an environment. The overlap (C) is moderate. Hence, both the organisms are not good competitor.*

9.4.1 Schoener's (α) Index

The most satisfactory method in absence of any estimate of food available is Schoener's (α) index. It is calculated as

$$\alpha = 1 - 0.5\left|Px_i - Py_i\right|$$

Where α is Schoener's index, Px_i is the proportion out of all resource items in category i used by species x, Py_i is propotion out of all resource items in category i used by the species y.

Schoener's (α) index ranges from 0.0 (representing no overlap) to 1.0 (complete overlap).

BOX 11

Calculation of is Schoener's (α) index

Sl no.	Organism in the gut	Individual count from gut of X	Individual count from gut if Y	Px_j	Py_j	$\lvert Px_j - Py_j \rvert$
1	*Scenedesmus sp.*	10	40	0.0265	0.0946	0.0681
2	*Pediastrum sp.*	20	22	0.0529	0.0520	0.0009
3	*Oedogonium sp.*	14	10	0.0370	0.0236	0.0134
4	*Spirogyra sp.*	6	20	0.0159	0.0473	0.0314
5	*Bulbochaete sp.*	22	8	0.0582	0.0189	0.0393
6	*Closterium sp.*	40	40	0.1058	0.0946	0.0113
7	*Chlorella sp.*	60	50	0.1587	0.1182	0.0405
8	*Pleurotaenium sp.*	38	32	0.1005	0.0757	0.0249
9	*Triplocera sp.*	10	70	0.0265	0.1655	0.1390
10	*Xanthidium sp.*	8	85	0.0212	0.2009	0.1798
11	*Cosmarium sp.*	50	10	0.1323	0.0236	0.1086
12	*Staurastrum sp.*	5	15	0.0132	0.0355	0.0222
13	*Gonatozygon sp.*	15	7	0.0397	0.0165	0.0231
14	*Mesotaenium sp.*	35	6	0.0926	0.0142	0.0784
15	*Euastrum sp.*	45	8	0.1190	0.0189	0.1001

$N = \sum | Px_j - Py_j | = 0.8811$

Schoener's (α) index is $\alpha = 1 - 0.5 \left| Px_i - Py_i \right| = 1 - 0.5\ (0.8811) = 1 - 0.4406 = 0.5595$. The species X and Y has moderate overlap of diets in the environment studied.

9.4.2 Clumping of Gut and Possible Diet Overlap

DB(χ^2) can give guts with possible diet overlap through clumping of gut (Fig 7). Clumping of guts means grouping of DB(χ^2) values of organisms on foods available in same resource environment. It helps in understanding magnitude of competition among different organisms assessed on similar food resource in an ecosystem.

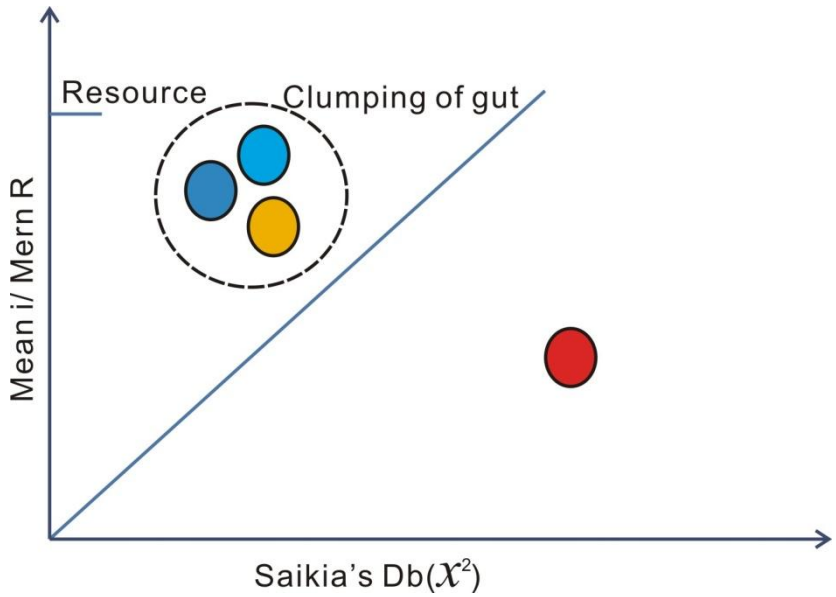

Fig. 7. *Clumping of guts (dashed circle) shows these organisms are competitive for food resources (on y axis). However, the gut represented by single circle (red) shows it does not feed on the food resources studied for.*

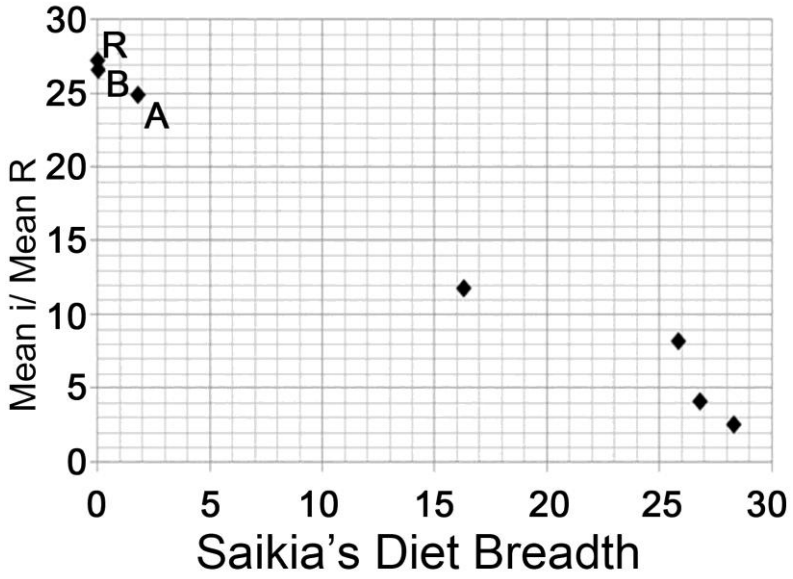

Fig. 8. *Clumping of gut index (A and B, Saikia's Diet Breadth) near resource (R, food) in the environment.*

In Fig 8, clumping of gut has been explained for six fishes of which A and B showed strong competition for food resource R. This is shown by plotting DB(χ^2) values on a graph against mean i/meanR where mean i represents mean value of abundances of gut category i and mean R represents mean value of abundances in R or resource. Co-ordinates for R on the graph is (0,1.0) which means complete preference of food from the environment by the organisms (DB(χ^2) =0) and mean i (mean value of abundances of gut category i) = mean R (mean value of abundances in R).

9.5 Feeding Strategy Study

(1) Ivlev's Electivity Index

Ivlev's (1961) electivity index is used to measure the selection of available food organisms by fish.

$$Ei = St_i - P_i / St_i + P_i$$

Where

E_i = Ivlev's electivity index for species i;

St_i = Relative proportion of species i in the diet;

P_i = Relative proportion of species i in the environment;

E value varies from 0 to 1. E value around to 0 indicates random ingestion, +1.0 or around to +1.0 indicates strong ingestion and 0 to -1.0 indicates weak to strong avoidance.

Since Ivlev's electivity (E) values are sensitive to the relative densities of the food types (Jacob, 1974), feeding strategy is analyzed by plotting E values against relative proportion of resource available in the environment studied (Fig 9).

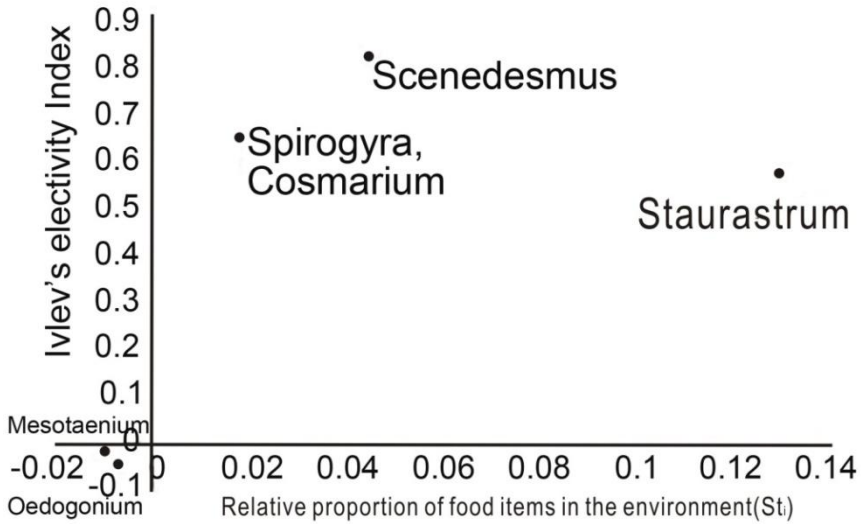

Fig. 9. *Ivlev's electivity index on a graph.*

BOX 12

Calculation of Ivlev's electivity Index

	Name of organism	Individual count from environment	St_i	Individual count from gut	P_i	$St_i + P_i$	$St_i - P_i$	$St - P_i / St_i + P_i$
1	*Scenedesmus sp.*	22	0.05	1	0.005	0.055	+0.045	+0.81
2	*Pediastrum sp.*	56	0.128	25	0.124	0.252	+0.004	+0.016
3	*Oedogonium sp.*	36	0.082	18	0.089	0.171	-0.007	-0.041
4	*Spirogyra sp.*	10	0.023	1	0.005	0.028	+0.018	+0.64
5	*Bulbochaete sp.*	4	0.009	1	0.005	0.014	+0.004	+0.285
6	*Closterium sp.*	26	0.059	20	0.099	0.158	-0.04	-0.253
7	*Chlorella sp.*	18	0.041	5	0.025	0.066	+0.016	+0.242
8	*Pleurotaenium sp.*	60	0.137	24	0.119	0.256	+0.018	+0.070
9	*Triplocera sp.*	20	0.046	8	0.039	0.085	+0.007	+0.082
10	*Xanthidium sp.*	14	0.032	6	0.029	0.061	0.003	+0.049
11	*Cosmarium sp.*	10	0.023	1	0.005	0.028	+0.018	+0.64
12	*Staurastrum sp.*	8	0.018	1	0.005	0.023	+0.013	+0.565
13	*Gonatozygon sp.*	30	0.068	10	0.049	0.117	+0.019	+0.162
14	*Mesotaenium sp.*	25	0.057	15	0.074	0.131	-0.01	-0.017
15	*Euastrum sp.*	98	0.224	66	0.326	0.55	-0.102	-0.185
	N =	437	0.997	202	0.998			

The graphical presentation of Ivlev's electivity index is shown in Fig 9. *Scenedesmus* were preferred over *Spirogyra* and *Cosmarium*. Though abundant, *Staurastrum* were preferred moderately. *Mesotaenium* and *Oedogonium* were avoided.

The above results can be presented in a more meaningful way using following graphical analysis (Fig 10). The E value beyond a level of +0.4 and –0.4 represents a biologically significant selection and avoidance. While between -0.4 and +0.4 indicates generalization (Amundsen et al. 1996).

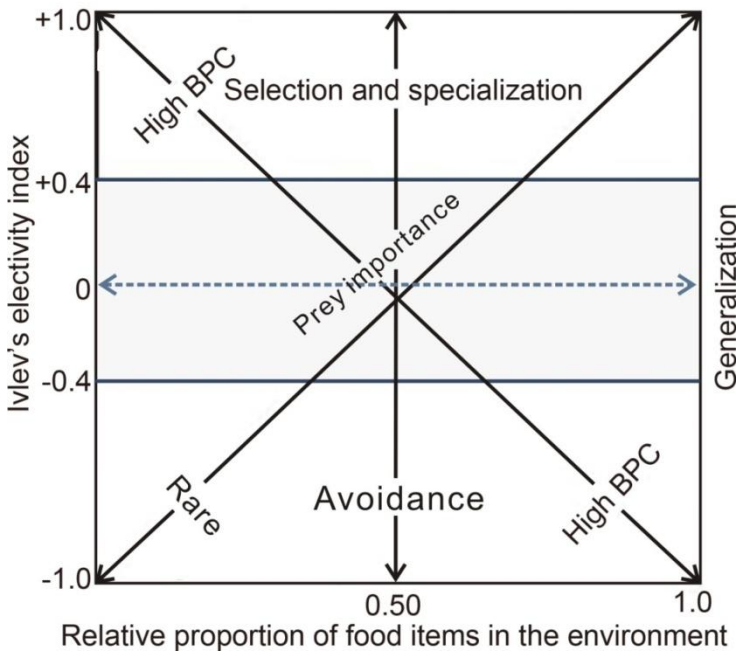

Fig. 10. *Explanatory diagram of feeding strategy and prey selectivity and avoidance (Amundsen et al. 1996). BPC, between phenotype component; WPC, within phenotype component.*

On the basis of graphical explanation in Fig 10, the results from the BOX 12 can be interpreted as follows:

- The organism highly prefers *Cosmarium* sp, *Spirogyra* sp, *Scenedesmus* sp and *Staurastrum* sp.
- It specially prefers *Staurastrum* sp. This reference is proportionate to the availability of prey item in the environment.
- *Cosmarium* sp and *Spirogyra* sp, though not available in the environment, preferred over other food items.

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Appendices

Metric Units

Volume

1milliliter (ml) = 1000 microliter (µl)
1litre = 1000ml
1cubic meter (cm^3) = 1000 litres
1cubic centimeter (1cc) = 1ml
1 ft^3 = 0.02832m^3

Weight

1milligram (mg) = 1000 microgram (µg)
1 gram (g) = 1000 (mg)
1 kilogram (kg) = 1000 gram
1 metric ton (mt) = 1000 kg
1lb = 453.6g = 0.4536kg
1 kg = 2.205 lb

Area

1 square centimeter (cm^2) = 100 square millimeter (mm^2)
1 square meter (m^2) = 10,000 cm^2
1 hectare (ha) = 10,000 m^2
1 acre = 0.4047 hectare = 100 m^2

Length

1 millimeter (mm) = 1,000 micrometer (microns)
1 centimeter (cm) = 10mm
1 meter (m) = 100 cm
1 kilometer (km) = 1000 m
1 foot (ft) = 12 inches (in) = 0.305m
1 inch = 2.54 cm

Prefix

Mega - (M) = 10^6
Kilo – (K) = 10^3
milli - (ml) = 10^{-3}
micro – (μ) = 10^{-6}
nano – (n) = 10^{-9}
pico – (p) = 10^{-12}

Strengths of Some Common Acids/Bases

Acids/Bases	Normality
1. Hydrochloric acid (HCl)	11.6
2. Acetic acid (CH_3COOH)	17.5
3. Nitric acid (HNO_3)	16.0
4. Sulphuric acid (H_2SO_4)	36.0
5. Orthophosphoric acid (H_3PO_4)	45.0
6. Ammonium hydroxide (NH_4OH)	15.0

Index

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Professor Debangshu Narayan Das is currently holding the office of the Dean, Faculties of Sciences, Engineering & IT, Rajiv Gandhi University, Ronohills, Doimukh, India. His broad areas of research cover finfish biology and fish and fisheries of eastern Himalayas. Currently his laboratory is involved in the documentation of fish genetic diversity and conservation of threatened wild fishes, eco-biology of wild food and ornamental fishes, aquaculture development in hill wet rice lands, application of fisheries IKS and location specific refinement and fish biotechnology. Professor Das has to his credit several nationally funded projects and memberships to reputed biological societies. His total publication in national and international journals is more than 80.

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