ECOLOGY AND TOOLS & TECHNIQUES IN BIOLOGY

PRACTICAL (206MA24)

M.Sc., ZOOLOGY First Year

Semester –II, Paper-VI

Lesson Writer

Prof. G. Simhachalam

Dept. of Zoology & Aquaculture

Acharya Nagarjuna University

Editor

Prof. K. Veeraiah

Dept. of Zoology & Aquaculture

Acharya Nagarjuna University

.

Academic Advisor

Prof. K. Veeraiah

Dept. of Zoology & Aquaculture

Acharya Nagarjuna University

Director I/c

Prof. V. VENKATESWARLU

MA.,M.P.S., M.S.W.,M.Phil., Ph.D.

CENTREFORDISTANCEEDUCATION

ACHARAYA NAGARJUNA UNIVERSITY

NAGARJUNA NAGAR – 522510

Ph:0863-2346222,2346208,

0863-2346259 (Study Material)

Website: www.anucde.info

e-mail:anucdedirector@gmail.com

M.Sc., ZOOLOGY - ECOLOGY AND TOOLS & TECHNIQUES IN BIOLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A^{+}' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the doorstep of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech.,Ph.D.,
Vice-Chancellor I/c Acharya Nagarjuna University

M.Sc.–Zoology

SEMESTER-II

206ZO24 - ECOLOGY AND TOOLS & TECHNIQUES IN BIOLOGY

PRACTICAL SYLLABUS

ECOLOGY

1) Area species curve.
2) Quadrate species curve.
3) Determination of frequency.
4) Analysis of soil – temperature, colour, texture, pH, moisture content, phosphorus content,
5) Carbonate content and nitrate content.
6) Estimation of primary productivity (light and dark bottle method).

TOOLS AND TECHNIQUES IN BIOLOGY

1) Microscopy - description and working methodology.
2) Spectrophotometer - principle and working methodology.
3) Paper chromatography - separation of molecules.
4) Thin layer chromatography - isolation of molecules.
5) Calculation of mean, median, mode, standard deviation and standard error.
6) Analysis of Variance (ANOVA).

PRACTICAL-1

AREA SPECIES CURVE

AIM:

To determine the minimum size of the sample or quadrate for adequate sampling.

REQUIREMENTS:

- 1x1m quadrate
- Graph
- Scale and Pencil
- Grassland ecosystem.

SAMPLE:

A sample representative is taken from a large plot characteristic of a plot. It has same characteristics of a plot. The sample must be reasonably small but represent all the species thus the size of the sample must be studied for area species curve.

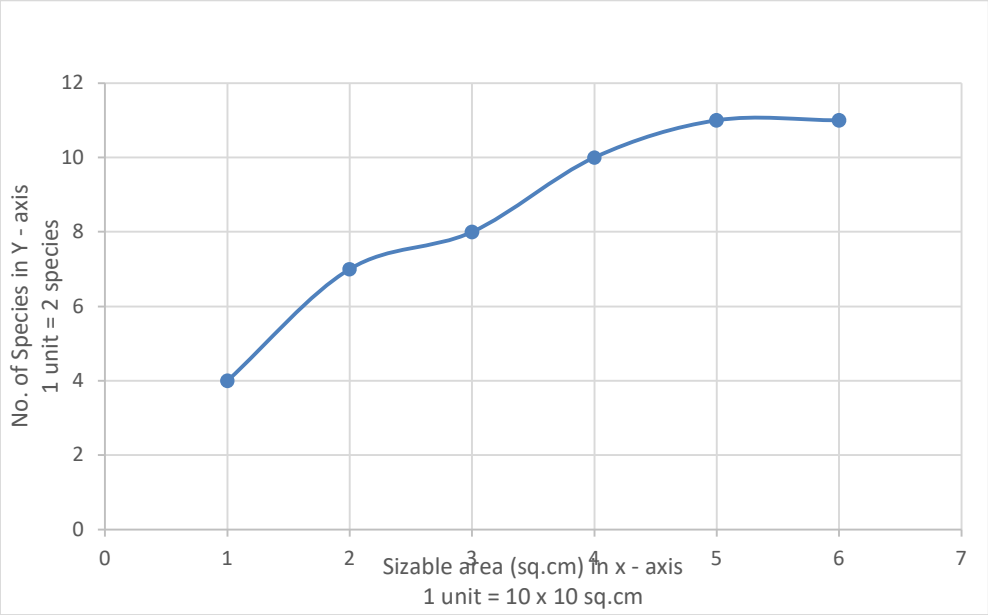
PROCEDURE:

A metal quadrate of 1m x 1m is placed on heterogeneous plot. By taking an area of 10x10 sq.cm the number of species of various plots is counting. The plantation in the area is notified. In the same way by increasing the area in the multiples of 10 sq.cm the number of new species are counted and the process is continued till no further increase in species in that area with the increasing the size of quadrate. A graph is drawn by plotting area on x-axis and number of species on y-axis. Here, it can be understandable that the area is an independent variable and no of species are dependent variables. The number of species at first increase to some extent, later remain constant as shown by graph.

Table: EXAMPLE FOR AREA SPECIES CURVE

S. No	Sizable Area (Sq.cm)	Total no.of Species	Additional no.of species if any
1	10 x 10	4	-
2	20 x 20	7	+3
3	30 x 30	8	+1
4	40 x 40	10	+2
5	50 x 50	11	+1
6	60 x 60	11	-

GRAPH:



RESULT:

Minimum sizeable area for adequate sampling of given grassland ecosystem is50 x 50 Sq.cm

DISCUSSION:

1. The graph shows, for the species detail analysis of vegetation and suitable size of quadrate.So as to study the existing species within the given area by area species curve as given by 'Garstang' (1950)
2. The flattening of the curve shows that the number of species in the consecutive quadrate remains same. It is also identified by fact that the increase in number of species is rapid at initial stage.
3. Later the rate gradually falls down and curve tends to be horizontal. At that point the size of quadrate and the minimum size of sample for adequate sampling is notified.
4. The size of quadrate varies with type of vegetation for grassland of sandy area, pore type 50sq.cm quadrate seaves the purpose.
5. In the grassland ecosystem with great density 1 x 1m or more maybe needed. In forest ecosystem quadrate may be large 10x10 m and bigger in size.
6. Depending upon the size of objective quadrates may be circle, square or rectangular.

Quadrates are of Four Types:

1. List Quadrate
2. Count Quadrate
3. List Count Quadrate
4. Chart Quadrate

The quadrate in the field must be laid down in a set pattern and in random position but the aim should be to cover the entire range of vegetation. The determination of quadrate size is necessary.

CONCEPT OF AREA SPECIES CURVE:

Species area curve shows how the number of species in an area depends on size of that area. It is clear that greater the area it contains than small one. Here the habitat size does affect species the richness. Larger the area more the inhabitants will be in the area, so that a greater number of species will find suitable niches to be occupied.

The second alternative argument is arrived from MC Arthur.

Another commonly observed pattern of species distribution is that species which have a wide range of distribution tend to be locally abundant. Restriction distribution to be rare. The first is that wide spread species in any area able to occupy a relatively wide range of conditions and they have wide niche according to niche breadth hypothesis.

The second explanation shown like an equilibrium theory. It argues that that common species has low extinction rate that migrate from larger population and comes to occupy above small population thus forms a meta population..The distribution advances the species thus, distribution abundant curve roughly similar to species area curve.

- Hanski and Gyllenberg (1997) form a mathematical model which combined species area and species distribution pattern.

- The model conserves as survive of islands of different size and incidence of the species of abundance.

- The model shows the number of species of an individual island constitute the speciesarea and thedistribution of species.

- Thus,thisspecies distribution and species abundance curve is more important to generate a realistic area species curve because species distribution and species abundant in turn dependent on colonization and extinction rate, species abundance thus reflected species richness and species distribution depends on habitat selection.

- Thus, the species area curve in turn depends upon species distribution and abundance as such both the factors responsible to enhance our understanding of observes species with abundance area species curve.

PRACTICAL-2

QUADRATE SPECIES CURVE

AIM:

To determine the number of samples for adequate sampling in a given grassland ecosystem.

REQUIREMENTS:

- One meter quadrate (1m x1m)
- Graph and pencil
- Grassland Ecosystem

PROCEDURE:

A quadrate of size 1x 1m is taken and placed on heterogeneous plot. The various species occurrence in that 1 x 1m are noted. Then, the quadrate is removed and placed on another area and all new species present in that quadrate area are noted. This process is continued till no new species found in the consequent quadrate. A graph is drawn by taking, number of quadrates on x-axis and number of species on y-axis.

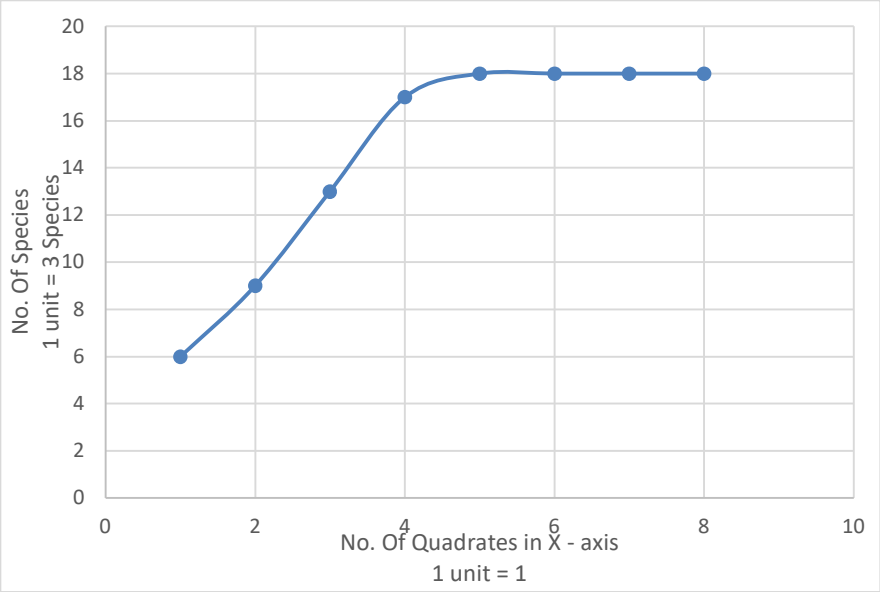
OBSERVATION AND DISCUSSION:

Quadrate is the name given to the sample is of definite shape and size in this experiment. We will determine the individual size of the quadrate to be used to study the vegetation of grassland ecosystem. Such community consists of different species, group of individual species together known as population. The structure of plant community of each population is to be done individual of plant species. Therefore rough estimation of population is done by quadrate method.

Table: EXAMPLE FOR QUADRATE SPECIES CURVE

S. No	No. of Quadrates	Total no.of Species	Additional no.of species if any
1	1	6	-
2	2	9	+3
3	3	13	+4
4	4	17	+4
5	5	18	+1
6	6	18	-
7	7	18	-
8	8	18	-

GRAPH:



RESULT:

Minimum number of samples for adequate sampling in given grassland ecosystem is6 quadrates.

SIGNIFICANCE:

1. Quadrate method is the most conventional method to study the settlement of terrestrial ecosystem. It cannot be used in aquatic environment because the surface of waterbody cannot be precisely marked.

2. Precise sample and accurate measurement & quadrate method is mostly suitable. This is advantage for quantitative estimation of community structure.

3. Quadrate method is to be implemented in small area. It saves the time and but at the same time to make comprehensive study of entire area is difficult.

4. This technique of quadrate which yield quite significant results. In this method, the community represented by the species whose dispersion, numerical strength etc. are in terms of quantitative type.

5. The size of the quadrate varies with the type of vegetation. Quadrate may be circular or rectangular. The shape is actually square type.

6. The small plants like mosses, lichens and liverworts grow in small area where,1m quadrate of 20x20 cm size.

7. In grassland with relatively modern species diversity 50x50 cm size quadrate is useful.

8. Many workers fixed 1x0.5m rectangular quadrate for grassland which is most suitable.

9. In forest ecosystem quadrate may be quite large, i.e.,10 x10m or even bigger number of sample quadrates should be done in such a way to cover at least 5-10% area.

10. In mixed community with a number of larger strata of vegetation, the quadrate size differs for each of life from at the same place.

11. At ground level, small quadrate should be laid down for shrubs.

12. For trees, large size quadrates are to be preferred.Thus, each stratum is separately sampled in its own way and each super imposed quadrates of different size are called nested quadrates.

NOTE: The quadrate should be lay down in field at random set pattern but the aim should be to or in the cover entire range of vegetation.

PRACTICAL-3

DETERMINATION OF FREQUENCY

AIM:

Determination of the degree of dispersion of an individual species in an area.

The term frequency refers to the degree of dispersion an individual species in an area and is usually expressed in terms of percentage of occurrence.

1. It can be defined as a chance of probability of an individual of agiven species to be present in randomly placed quadrate.

2. It can be studied by sampling the study area of several places. They may be land or desert pattern so as to cover the site adequately and record the number of species that occur in each sample.

3. The study of sample is referred to as sampling. A group of individuals chosen from a population is termed as sample.

4. The sample should exhibit characteristic features of population.

   For instance, a species occurs in 5 out of the total 20 quadrates studied its frequency is,

$$\text{Frequency} = \frac{\text{Number of quadrates in which species occur}}{\text{Total no of quadrates sampled}} \text{ x100}$$

FOR EXAMPLE:

Number of quadrates in which species occur = 5

Total no of quadrates sampled = 20

Frequency = $\frac{5}{20}$ x 100 = 25.

PROBLEM:

Weed plants occurrence of in the given area of agriculture plots of 36 are as follows determines the frequency.

Range of Variable	Total no. of occurrence of variable	Percentage Frequency of Variable
2	2	$\frac{2}{36}$ x 100 = 5.6
3	6	16.6
4	9	2.5
5	3	8.3
6	3	8.3
7	4	11.1
8	4	11.1
9	5	13.8

Variable: Weed Plant

Occurrence of Species: 36

Range of Variable: 2 - 9

If a species exists in all randomly placed quadrates, then its frequency will be 100% that means, particular species spread abundantly in all over the area.

A poorly spread species in one corner will have a chance of occurrence in only few quadrates and its frequency value will be low that, the lower frequency value shows that the lower uniformity of its spread or dispersion.

For recording the data for frequency studies, simply the presence and absence of species in quadrate is notified and not the number of individuals of each species. Such a study has helped for establishing the relative significance of different grass and weed species which naturally grow there and has important rolein management of grassland.

Raunkiaer (1934) made extensive frequency studies, grouped species into free frequency classes. Accordingly, the species content showed variations and same species have low and some have high and other have intermediate values.

Raunkiaer showed five frequency classes are as follows:

- Class with 1-20%
- Class with 21-40%
- Class with 41-60%
- Class with 61-80%
- Class with 81-100%

Raunkiaer further propounded low class of frequency on the basis of studies made on 8,000 quadrates accordingly the number of species in A>B, B>C, C ≥ D, D>E

As such, the number of species exist and recorded in A class = 53%, B=14%, C=9%, D=11% & E = 16%.

It means species with poor dispersion of frequency higher in number than that of species with higher frequency. Here the quadrate is more important matter. If the quadrate size differs the frequencies of A&B is followed by difference in frequencies of C, D&E.

So, the quadrate is critical factor to obtainthe required data of different size,same at different time. The frequency is different from normal.

$$\text{Relative frequency} = \frac{\text{Number of occurance of species}}{\text{Total number of occurance of species}} X\ 100$$

PRACTICAL-4

ANALYSIS AND ESTIMATION OF SOIL PH

AIM:

To estimate the P^H of given sail sample.

APPARATUS:

- Soil sample
- P^H meter
- Beaker
- Glass rod
- Buffer tablet of Frown value
- P^H paper
- Filter paper

PROCEDURE:

STEP-1

About 500 grams of given soil sample was taken and powdered finely. Then the soil sample istaken into the glass beaker. Distilled water is added to the sample so that the soil turns into paste form. Then 50gms of paste form is taken into small beaker and add required amount of distilled water and stirred the sample very well by a glass rod as such, the sample is ready to find out the pH of the given sample.

STEP-2

The known value of buffer tablet was taken into a small beaker and added 100 ml of distilled water into it while taking glass rod the sample was stirred completely. As such the buffer solution is prepared to find out pH of given soil sample.

- To standardize the P^H meter with the buffer solution of P^H

The pH meter was used by lifting the electrode of P^H meter from ordinary beaker and washed it by means of distilled water followed by the application of filter paper so as to make the electrode free of moisture completely.

Then put the electrode of P^H meter is laid down within the known value of buffer solution. While rotating the knob, keep the electrode in the buffer solution for 15 min So as to standardize the P^H of ^{PH} meter to that of buffer.

After that electrode of P^H meter lifted up and washed by distilled water followed by filter paper.

Then put the electrode within the given soil sample to find out P^H

RESULT:

P^H of given soil sample is 7.5

SIGNIFICANCE OF SOIL P^H IN SEDIMENT WATER INTERFACE:

1) Soil influences productivity.

2) It enriches the overlying water

3) It helps in dyke construction and to laydown the foundation structure.

4) It promotes white revolution indirectly and green Revolution directly and blue revolution

TYPES OF SOIL

- Clay Soil
- Loamy soil
- Slit soil
- Sandy soil
- Sandy loamy soil
- Heavy clay soil
- Silt clay soil
- Sandy clay soil
- Silt clay loamy soil
- Heavy clay loamy soil
- Sandy clay loamy soil
- Black soil
- Alluvial Sol

Heavy clay loamy soil, sandy clay loamy soils are best Studied for construction of the dyke in shrimps and fish ponds. Because these soils show high degree of compaction, devoid of seepage and less subjected to erosion. These soils show high water retention capacity and our in impermeable nature.

Some soils are light textured. These soils show many interstitial pores. as such to let out the water and to go out easily by means of seepage. These soils are not fit for the farm construction with some remedial measures known as pudding. Pouring organic wastes, pond wastes, dead algal material retention into pond bottom blocks the pores of the soil, so as to minimize the seepage nature.

Acid sulphate soils are also known as catalysis. These soils are much more prevalent in the brackish water areas adjoining to seas, special type of rooted vegetation, Rhizophores, Nepa, melaneuca and mangroves etc. are coexisting of aluminum and iron content due to that these elements not only make phosphorous unavailable to the plankton as such water

becomes unproductive. In addition to this, acid sulphate soil consists of pyrite. When it is exposed to into the, atmosphere release the H_2S0_4into the dyke, so that soils and water become acidic,due to this reason, these soils are not fit for the culture of fish and shrimp.

These soils are prevalent at high sulphate and Iron content areas of limited aeration. Mangroves rooted vegetation and accompanied by sulphate reducing bacteria disulpho-vibrio, disulfuricans, disulfo-maculatum.

INFLUENCE OF P^H: -

Negative logarithm of hydrogen in concentration is known as p^H. If the P^H value is 7, it is neutral. If the value is less than 7 it is acidic. The P^H should be in between 7-11 which is desirable for aquatic and terrestrial organisms. If the value is more than 11 it is basic. The difference of, If the P^H gradient should not be more than 0.5

P^H IS DEPENDENT ON VARIABLE FORMS:

1) Photosynthesis of aquatic organism and followed by forms of Co_2

2) Forms of Co_2 are of four types

(a) Carbonic form ($C0_3$) - Bound state (precipitous form)

(b) Bicarbonate form (HCO_3)- Half bound state (dissolved form)

(C) Gaseous form (free $C0_2$) -Free gaseous

(d) Hydrated form (H_2CO_3) - Hydrated form

The above form influences the P^H value of the aquatic ecosystem. The presence of CO_2 at a given atmospheric pressure reduces the P^H to an extent of 5. In case, if any further reduction occurs that is mainly due to some other factor. Here the pressure of Co_2 and carbonic acid and organic acids (Tannic acid) reduces the P^H value to 5 therefore, up to 3 or 4.

INFLUENCE OF P^H VALUE ON SEDIMENT WATER INTERFACE:

Increasing P^H value is accompanied by dissociation of the carbonic acid (weak acid). Co_2 forms involving in natural water influence the p^H in many ways.

1) In acid conditions:

$$CO_2 + H_2O \leftrightarrow H_2CO_3H^+ + HCO_3^- \leftrightarrow H^+ + Co_3^-$$

2) In highly basic condition.

$$H_20 + base^+ + HCO_3^-$$

3) With dissociation of calcium bicarbonate.

$$Ca\,(HCO_3)_2 \leftrightarrow Ca^{++} + HCO_3^-$$

4) Further dissociation of HCO_3^-

$HCO_3^- \leftrightarrow H^+ + Co_3^-$at higher pH value.

CARBONIC ACID IS HYDROLYSED AS UNDER:

$$H_2O + HCO_3^- \leftrightarrow H_2CO_3 + OH^-$$

$$H_2O + CO_3^- \leftrightarrow HCO_3^- + OH^-$$

$$Ca(HCO_3)_2 \leftrightarrow CaCO_3\downarrow + H_2O + CO_2$$

The presence of CO_2 when it interacts with the water it forms carbonic acid. When it dissociates, further into the H^+& HCO_3^-, the p^H value increases to an extent of 8.3. After that further dissociation of HCO_3^-into H^+& HCO_3.The value of p^H goes to an extent of 10 or above 10. As such p^H fluctuations occur in aquatic ecosystem are apparently accompanied by forms of CO_2

In a chemical buffer system p^H value remains constant. This is mainly due to the fact, the H^+ ions form the dissociation of H_2CO_3 and OH^- ions released from hydrolysis of bicarbonate. As such the value in a system remains constant.sea water acts as a buffer solution because P^H remains up to 8.3, however the fresh water differs P^H from time to time because of variable factors.

IMPACT OF P^H ON SEDIMENT WATER INTERFACE:

1. Proliferations of aquatic organisms
2. Loss of appetite.
3. Lack of biogenic capacities.
4. Susceptibility to disease, heavy metals, H_2S& othertoxic gases
5. Influence of metabolic activities.
6. Feeding, breeding, migration, behaviour, growth and other activities

7 To provide congenital atmosphere for aquatic organisms.

8. The p^H should be maintained between 7-9.

EXPERIMENT-5

ESTIMATION OF SOIL CARBONATE CONTENT

AIM:

To estimate the carbonate content in the soil sample

PRINCIPLE:

The soil sample is treated with a strong acid. The volume of the carbon dioxide produced is measured by using a calciminer (Scheibler unit), and is compared with the volume of carbon dioxide produced by pure calcium carbonate. Alternatively, when the laboratory is determining the total organic carbon by dry combustion, using the indirect method, the measurement of the total inorganic carbon can be used to report the carbonate content. In case the direct method for TOC is used, the TIC content can be derived by analyzing the sample twice. Once with acid treatment and once without acid treatment. The TIC content is derived indirectly by TIC = TC – TOC.

APPARATUS:

- Calcimeter (Scheibler unit)
- Analytical balance (accuracy 0.0001 g)
- Reaction vessels (capacity 150 ml)
- Plastic cups (which can pass through the neck of the reaction vessel)
- Tong Watch glass

REAGENTS:

- Distilled water
- Hydrochloric acid (HCl), conc. 4 mol/l
- Dilute 340 ml of concentrated hydrochloric acid (ρ = 1.19 g/ml) to 1000 ml with water.
- Calcium carbonate (CaCO3), pure.

PROCEDURE:

PREPARATION:

The mass of the test portion is determined based on the carbonate content. For a preliminary test on carbonate content, add some hydrochloric acid to a portion of the soil on a watch glass. The carbonate content of the sample can be estimated on the basis of the intensity and duration of effervescence. Determine the mass of test portion (air-dried soil fraction < 2 mm).

Table: MASS OF TEST PORTION FOR DETERMINATION OF CARBONATE CONTENT BASED ON INTENSITY OF EFFERVESCENCE

Intensity of effervescence	Carbonate content (g/kg)	Mass of test sample (g)
None or only limited	< 20	10
Clear, but for a short time	20 – 80	5
Strong, for a long time	80 – 160	2.5
Very strong, for a long time	> 160	≤1

MEASUREMENT:

1. Transfer the sample into the reaction vessels and add 20 ml of water.
2. Fill the plastic cup with 7 ml of hydrochloric acid and place this, using tongs in the reaction vessel containing the test portion.
3. Take care that there is no contact between the hydrochloric acid and the soil before the reaction vessel is connected to the calcimeter (Scheibler unit).
4. Warm the reaction vessel by hand. Connect the reaction vessel to the calcimeter.
5. Carefully add the hydrochloric acid from the cup to the soil by tilting the reaction vessel at an angle. The gas produced will lower the water level in the tube on the right and at the same time will raise the water level in the tube on the left.
6. Shake for 5 min and note the volume when it no longer varies. If it still varies, continue shaking until the volume is stable, but not longer than 1 h.
7. At the end of the shaking period, bring the water level in both tubes to the same height and measure the volume of gas in the calibrated tube with an accuracy of 0.1 ml.

CALIBRATION:

Determinations of samples, blanks and the calcium carbonate used as standard material, shall be performed simultaneously in a room where temperature and pressure do not vary too much during the measurement.Weigh the standards of 0.200 g and 0.400 g of calcium carbonate, transfer these amounts into the reaction vessels and add 20 ml of water. For the blank determinations, use reaction vessels containing 20 ml of water.

CALCULATIONS:

$$w(CaCO_3) = 1000 \times \frac{m_2 (V_1 - V_3)}{m_1 (V_2 - V_3)}$$

($CaCO_3$) = carbonate content of sample (g/kg) on basis of air-dried soil

m_1 = mass (g) of test sample

m_2 = mean mass (g) of standards

V_1 = volume (ml) of CO_2 produced by test sample

V_2 = mean volume (ml) of CO_2 produced by standards

V_3 = volume change (ml) in blank determinations (can be negative)

In case the total inorganic carbon content was determined by dry combustion, following formula needs to be used to convert between TIC and $CaCO_3$, all expressed in g/kg.

Table: EXAMPLE VALUES

Symbol	Meaning	Example Value
(m_1)	Mass of soil (g)	10 g
(V_1)	Volume of acid added (mL)	20 mL
(V_2)	Normality of acid	0.5 N
(m_2)	Blank factor	1
(V_3)	Volume used in back titration (mL)	4 mL
(m_3)	Constant or correction (often =1)	1

Step 1: $m_1V_2 = 10\times0.5=5$

Step 2: $m_2V_3=1\times4=4$

Step 3: $m_1V_2-m_2V_3=5-4=1$

Step 4:$m_3V_1V_2=1\times20\times0.5=10$

$$CaCO_3(\%) = 1000 \times \frac{(10 \times 0.5)-(1 \times 4)}{1 \times 20 \times 0.5} = 100$$

RESULT:

$CaCO_3$=100%**-** the soil sample contains 100% calcium carbonate equivalent according to the example values used.

EXPERIMENT- 6

ESTIMATION OF NITRATE CONTENT

AIM:

To estimate the nitrate (NO_3^--N) content in a soil sample using a simple water-extraction method followed by measurement with nitrate test strips.

PRINCIPLE:

Nitrate ions present in soil are water-soluble. When soil is mixed with distilled water (commonly at a 1:5 soil-to-water ratio), the nitrate dissolves into the solution (extract).

When a nitrate test strip is dipped into this extract:

- The reagent zone on the strip reacts specifically with nitrate.
- This reaction produces a color change.
- The intensity of the color is proportional to the amount of nitrate in the extract.
- The strip's color is compared to a standardized chart to get the nitrate concentration (mg/L NO_3^--N) in the extract.

This concentration can then be converted to soil nitrate concentration (mg/kg or ppm) based on the extraction ratio.

REQUIREMENTS / REAGENTS:

MATERIALS-

- Clean plastic or glass container
- 1 cup or measuring spoon
- Distilled water
- Funnel
- Coffee filter / clean cloth
- Clean bottle or jar with lid
- Notepad for recording
- Gloves (optional)

REAGENTS / EQUIPMENT

- Nitrate test strips (range 0–50 or 0–500 mg/L NO_3^--N)

PROCEDURE:

A. SOIL PREPARATION

1. Collect soil samples from 5–15 spots in the field at required depth (usually 0–30 cm).
2. Mix all subsamples thoroughly in a clean container.

3. Remove stones, sticks, and debris.
4. Take about 1 cup of the mixed soil for the test.

B. EXTRACTION OF NITRATE

5. Add 1 part soil into a container (e.g., 1 cup soil).
6. Add 5 parts distilled water (e.g., 5 cups water).
7. Close the container and shake vigorously for 1–2 minutes.
8. Allow to settle for 10–15 minutes, or filter the mixture through a coffee filter to obtain clear extract.

C. TESTING THE EXTRACT

9. Dip a nitrate test strip into the clear extract for the time indicated on the strip instructions (usually 1–2 seconds).
10. Remove the strip and wait for the color to develop (usually 30–60 seconds).
11. Compare the strip's color to the color chart provided with the kit.
12. Record the reading in mg/L NO_3^--N.

CALCULATION:

Most nitrate strips give results in mg/L nitrate-nitrogen (NO_3^--N) in the water extract. To convert this to soil nitrate concentration (mg/kg or ppm):

IF USING 1:5 SOIL-TO-WATER EXTRACTIONS:

Soil nitrate (ppm NO_3^--N)=Strip Reading (mg/L NO_3^--N)×5

EXAMPLE CALCULATION

Extraction ratio = 1:5

8×5=40 ppm NO_3^--N in soil

RESULT:

- Nitrate concentration in extract: 8 mg/L NO_3^--N
- Soil nitrate concentration**:** 40 ppm NO_3^--N
- Interpretation:
  - 0–10 ppm → Low
  - 10–25 ppm → Moderate
  - 25–50 ppm → High
  - 50 ppm → Very high

REPORT:

"The soil sample contains 40 ppm NO_3^--N, indicating a high level of plant-available nitrate."

EXPERIMENT-7
PRIMARY PRODUCTIVITY

AIM:

To estimate the primary productivity by dark and light bottle method in a given area of water body

PRINCIPLE:

Primary productivity of a water body usually determined from the difference in dissolved oxygen (DO) values of a water samples incubated in bottles under light and dark conditions.Thus,allowingpHotosynthetic activity to take place in one bottle and the same to be restrictedunder other. The decrease in DO content in dark bottle as compare to initial value represented the amount consumed by respiration by all the biomass in the bottle. The increase in the no light bottle indicates the amount of DO in water which exceeded oxygen consumption by respiration both GPP and NPP can be calculated from the difference in DO values.

REQUIREMENTS:

- I liter volumetric flask
- Pipette
- Burette
- 100ml conical flask
- 125 ml bottle

LIGHT AND DARK BOTTLES

Use 125 ml stopper transparent glass bottles as initial and light bottles for using as dark bottle, paint a similar bottle with black color and then wrap with black cloth or polythene sheet in such way that light penetration in bottle can be prevented.

REAGENTS:

Wrinkler-A:

Dissolve 250gms of $MnSO_4$in 500ml ofdistilled water.

Wrinkler-B:

Dissolve 900grams of potassium iodide (KI) and 400 grams of sodium hydroxide (NaOH) or potassium hydroxide (KOH) in distilled water.

Conc. H_2SO_4:

100% Conc. $H_2SO_4$to is taken into the reagent bottle.

Hypo ($Na_2S_20_3$):

Normality - 0.025N, Dissolve 6.2 gms of Hypo in one litre of distilled water.

Starch Solution:

Dissolve 1gm of starch in 100ml of boiled water at 80^0c

PROCEDURE:

1. Take in water sample continuously as well as uniformly in three bottles (Initial, light & dark) from the desired depth of water.
2. Add 1ml of manganous sulphate and alkaline iodide each in initial bottle.
3. Invert the stopped bottle a few times and take the other 2 bottles and dip them in water samples are collected.
   .
4. Incubate the water sample under water for some period, which should not normally to be less than 3hrs.
5. After expiry of the time taken up not normally be less than 3 hrs.
6. After expiry of the time, take up both of the bottles fix oxygen as before determined DO value of water in all the bottles as stated for examination of primary productivity.

CALCULATION:

1. Determination of DO values of bottles in all the bottles

Gross primary productivity (GPP)= $\frac{LB-DB}{T}$ x$\frac{0.375}{1.2}$x 1000mgc/m^{-3}/hr

Net primary productivity (NPP) = $\frac{LB-IB}{T}$ x $\frac{0.375}{0.2}$x1000 mgc/m^{-3}/hr

LB = Dissolved oxygen (ppm) in light bottle.

DB = Dissolved oxygen (ppm) in dark bottle.

IB = Dissolved oxygen (ppm) in initial bottle.

T = Time (hrs) of incubation

0.315 = Ratio of weight of carbon and oxygen

1.25= Photosynthetic coefficient.

2. **Amount of DO present in given bottle=**

$$\frac{\text{Volume of hypo run down x No.of hypo x Eq.Wt of O2}}{Amount of sample taken in conical flask} \text{ x } 1000$$

COMMENTS:

In some situations, it may be feasible to carryout incubation in the site of study itself. In such cases the bottles filled with water samples may be brought to a convenient place and incubated under of desired depth in a plastic pod.Transparency of this watershould be as possible to that of pond water under study so as to enable the sunlight penetrate the water body in similar manner as should have been in case ofpond water

CONCEPT:

Dissolved oxygen is probably the most widely analysed chemical parameter in fish pond water quality.A part from it directly effecton respiration of direct biotic organisms. It

controls directly or indirectly many other limnological properties of pond and thus governs the productivity of the ecosystem to a great extent.The most popular method of DO estimation is wrinkler's method

The productivity of a water body determines the living substances that is manufactured through interactions of the constituents of the natural environment.
The self-sufficient ecosystem some steps in the operation include

- Respiration of energy
- Production of energy
- Consumption of these materials and its further.
- Elaboration
- Decomposition to inorganic compounds.

1) Transformation of inorganic compound into suitable forms for nutrition of producers.

2) Non-living constituents' nutrients are light energy and inorganic nutrients both required for the photosynthetic activity.

3) Living components of the ecosystem of producer and consumer.

4)Producers include the chlorophyll bearing phytoplankton large green plants and p^{h}otosynthetic bacteria which are capable of synthesizing energy containing organic substances through utilization of solar radiation and inorganic materials.

5) Consumers include all other types of consumers include herbivores which feed directly on the grass, plants and other carnivores.

6) Decomposers like heterophilic bacteria and fungi whichreduce the organic substances to their elemental state by recycling the nutrients the cycles for the use of producers.

7) Plants and animals that depend one upon the other forms that links in a food chain.

8) Links of different food chains trophic levels is known as trophic levels.

SIGNIFICANCE OF PRIMARY PRODUCTIVITY:

Primary productivity is defined as the ratio of which radiant energy is stored by pHotosynthetic activity of producer organisms (chiefly green plants) in the form organic substances which can be used as food materials (Odum 1971).

1. The gross primary productivity is the total rate of pHotosynthesis including organic matter used up in respiration during the measurement period while net primary productivity (NPP) indicates the amount of organic matter that is stored in the plant tissue after meeting the demand of respiration.

2. Both the rates of primary production, Gross and Net measured on basis of organic carbon present in the unit volume (or) beneath unit area of pond surface.

3. Productivity could be expressed asmgc/m^{-3}/day or mg c/ m^{-2}/ day

4. The word day refers to day-lighthours during in surface water which gross and net

photosynthesis occur in surface water.

5. The term day is usedwhen full 24 hoursperiod declared.

6. Various methods for measuring the rate of primary and secondary producers production have been valued out of these:

   1) Role of change of standing

   2) Production of oxygen.

   3) Uptake of Carbon dioxide

   4) Concentration of chlorophyll

   5) Uptake of nutrients.

   6) Transformation of energy by consumers.

   7) Water bodies are classified basing on their production.

Wrinkler's Method:

This method (1948) is the most widely adapted technique for estimation of DO and has been in use throughout the world for long time.

AIM:

Estimation of dissolved oxygen to determine primary productivity of given water body.

PRINCIPLE:

Manganous (Mn^{2+}) ions form manganous hydroxide [$Mn(OH_2)$] under highly alkaline condition. This [$Mn(OH_2)$]reacts with dissolved oxygen of water and an equivalent amount of (Mn^{2+}) is oxidized to higher manganous (Mn^{4+}) again reveres to divalent Mn^{2+} and iodide (I_2) liberated in equivalent amount of DO. This iodide estimated by titration with sodium thiosulphate ($Na_2S_20_3$) indicates the amount of DO in water sample.

REQUIREMENTS & REAGENT:

As already mentioned above in the primary productivity methodology

PROCEDURE:

Collect water sample ina 125ml bottle. Add 1ml each of $MnSO_4$ and KI solution through pipette, dipping slowly in the bottom of the bottle.Care should be taken to create minimum disturbance to the sample during the exercise. Close the bottle and invert it for few minutes to ensure throughmixing of water with the reagents. This will result in development of flocculent precipitation which will gradually settle at the bottom. Whitish colour ppt indicates poor status of DO, while reddish to brown colour represents moderate to high value.

Add 1ml of conc. H_2SO_4 and invert the stopper bottle a few times to dissolve the precipitate. Take 50ml of the solution to a 250ml conical flask and add 0.5 ml of starch indicator to form a blue colour solution. Add it, titrate with freshly prepared 0.025 N $Na_2S_2O_3$ till the colour of the solution changesto colourless, which is endpoint.

CALCULATION:

Amount of DO present

in given bottle = $\frac{\text{Volume of hypo run down x No.of hypo x Eq.Wt of O2}}{Amountofwatertakeninconicalflask}$x100

1. AMOUNT OF DO PRESENT IN INITIAL BOTTLE

S.No	Vol. of Sample taken	Vol. of Hypo rundown		Vol. of Hypo rundown
		Initial	Final	
1.	50ml	0	1.6	1.6ml
2.	50ml	0	1.6	1.6ml
3	50ml	0	1.6	1.6ml

Volume of Hypo. Rundown = 1.6 ml

Normalityof Hypo. = 0.025 N

Eq. Wt. of O_2 = 8

Volume of sample to be taken in conical flask = 50 ml

Amount of Do present in given bottle = $\frac{1.6 \text{ x } 0.025 \text{ x } 8}{50}$x1000 = 6.4 ppm

2. AMOUNT OF DO PRESENT IN LIGHT BOTTLE

S.No.	Vol of Sample taken	Vol of hypo rundown.		Vol of hypo rundown.
		Initial	Final	
1.	50ml	0	2.2	2.2ml
2.	50ml	0	2.2	2.2ml
3.	50ml	0	2.3	2.3ml

Volume of Hypo. Rundown = 2.2 ml

Normality of Hypo. = 0.025 N

Eq. Wt. of O_2 = 8

Volume of sample to be taken in conical flask = 50 ml

Amount of Do present in given bottle = $\frac{2.2 \times 0.025 \times 8}{50}$x1000 = 8.8 ppm

3. AMOUNT OF DO PRESENT IN DARK BOTTLE

S.NO	Vol of Sample taken	Vol of Hypo rundown		Vol of hypo rundown.
		Initial	Final	
1.	50ml	0	1.4	1.4ml
2.	50ml	0	1.4	1.4ml
3.	50ml	0	1.4	1.4ml

Volume of Hypo Rundown = 1.4 ml

Normality of Hypo = 0.025 N

Eq. Wt. of O_2 = 8

Volume of sample to be taken in conical flask = 50 ml

Amount of Do present in given bottle = $\frac{1.4 \times 0.025 \times 8}{50}$x1000 = 5.6 ppm

4. GROSS PRIMARY PRODUCTIVITY = $\frac{LB-DB}{T}$ x$\frac{0.375}{1.2}$ x 1000mgc/m^{-3}/hr

Light Bottle: 8.8

Dark Bottle: 5.6

Time: 3

$$GPP = \frac{8.8-5.6}{3} x\frac{0.375}{1.2} \times 1000mgc/m^{-3}/hr$$

GPP = 33.3mg of carbon perm^{-3}/hr

5. NET PRIMARY PRODUCTIVITY (NPP) = $\frac{LB-IB}{T}$x $\frac{0.375}{0.2}$x1000 mgc/m^{-3}/hr

Light bottle: 8.8

Initial bottle: 6.4

Time: 3

NPP = $\frac{8.8-6.4}{3}$ x$\frac{0.375}{1.2}$ x 1000mgc/m^{-3}/hr

NPP = 250 mg of carbon per m^{-3}/hr

RESULT:

The gross primary productivity of the given water body is 333.3 mg
The Net primary productivity of the given water body is 250mg

EXPERIMENT-1

SIMPLE MICROSCOPE

AIM:

To study the principle and applications of simple microscope

SIMPLE MICROSCOPE:

A simple microscope is one that uses a single lens for magnification, such as a magnifying glass while a compound microscope uses several lenses to enhance the magnification of an object.

PRINCIPLE:

A simple microscope works on the principle that when a tiny object is placed within its focus, a virtual, erect, and magnified image of the object is formed at the least distance of distinct vision from the eye held close to the lens.

PARTS OF MICROSCOPE:

These parts support the optical parts and help in their adjustment for focusing the object. They include the following components:

1. **METAL STAND**

It has a heavy base plate and a vertical rod fitted to it, which provide support and stability to other parts of the microscope.

2. **STAGE:**

It is a rectangular metal plate fitted to the vertical rod. It has a central hole for light to pass from below. Slide with the specimen to be observed is kept on the stage, in such a way that, the specimen remains just on the central hole. Some microscopes have a pair of slanting wings projecting from both sides of the stage. They provide support to handfor manipulating the object.

3. **MIRROR:**

A plano-concave mirror is fitted below the stage to the vertical rod using a frame. It focuses the surrounding light on the object to be observed.

4. **LENS:**

A biconvex lens is fitted above the stage to the vertical rod using a frame. It magnifies the object's size, and the enlarged virtual image formed is observed by keeping the eye above it. For proper focusing, the lens can be moved up and down by the frame.

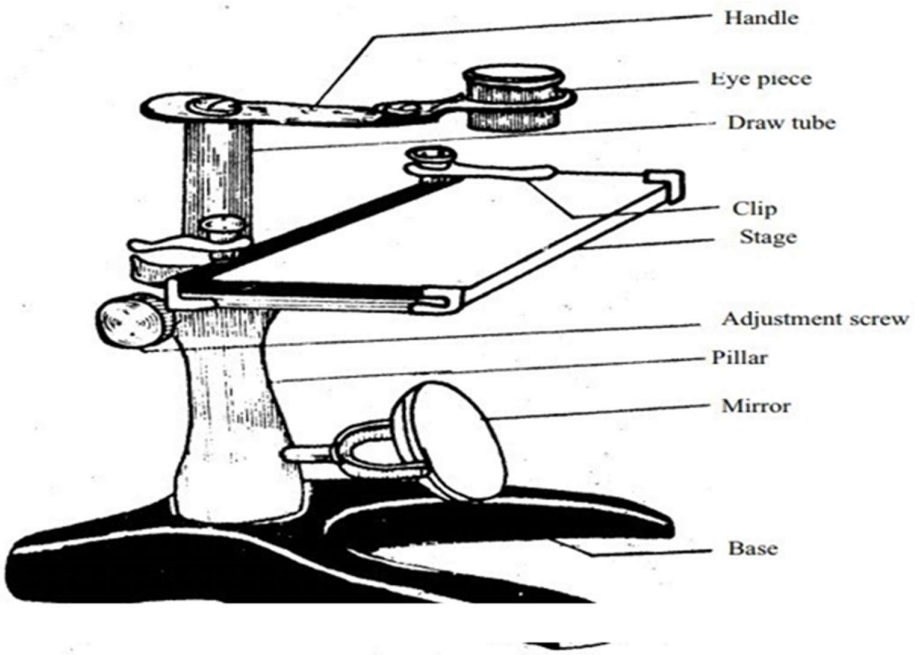


Fig. P.2A Simple Microscope

APPLICATION:

It is usually used to study microscopic algae, fungi, and biological specimens. It is used to see the magnified view of different particles of different types of soils. Skin specialists use it to find out various diseases of the skin.

EXPERIMENT-2
COMPOUND MICROSCOPE

AIM:

To study the principle and applications of compound microscope

COMPOUND MICROSCOPE:

A compound microscope is defined as a microscope with a high resolution and uses two sets of lenses providing a 2-dimensional image of the sample.

PRINCIPLE:

A compound microscope is considered to be one of the standard microscopes that can be used for general purposes. The arrangement of the lens is such that it magnifies the objects from the complex system.

There are two types of lenses that are used in the compound microscope:

- The objective lens is placed close to the object that needs to be examined.
- The eyepiece allows the image to be viewed. The eye piece is also known as the ocular lens.

PARTS OFMICROSCOPE:

Optical Parts: There are 5 optical parts of a compound microscope. They are:

- The **diaphragm** controls the amount of light falling on the object. It is present below the stage. Disc and Iris are the two types of diaphragms.
- The **condenser** of the compound microscope is located below the diaphragm and facilitates the focusing of light.
- A **reflector** is attached above the base and helps in directing the light falling on the object with the help of the diaphragm and condenser.
- The **objective lens** is placed above the nose piece, and forms a real inverted image of the object inside the tube. These are of three types- low power, high power, and oil
- **Occular Lens** is also called the eye piece and facilitates four types of magnifications - 5, 10, 15, and 20X.

NON-OPTICALPARTS:

The non-optical parts of a compound microscope are as follows: Arm, Pillar, and Base
The compound microscope's Body Tube is connected to the Microscope's base by the Arm.

- The Microscope and its Illuminator are supported by the Base.

- The base and the arm are connected by a pillar.

ILLUMINATOR AND STAGE:

The illuminator is the light source of a compound microscope.

- The illuminator in a compound light microscope is usually a low-voltage bulb.
- The slide is positioned on the stage, which is a level platform.

NOSEPIECE AND APERTURE:

The objective lenses are the in place by a revolving turret known as the nosepiece.

- Theviewerrotatesthenosepieceofthecompoundmicroscopetochangetheobjectivelens.
- The aperture is the opening in the middle of the stage that permits illuminator light to reach the specimen.

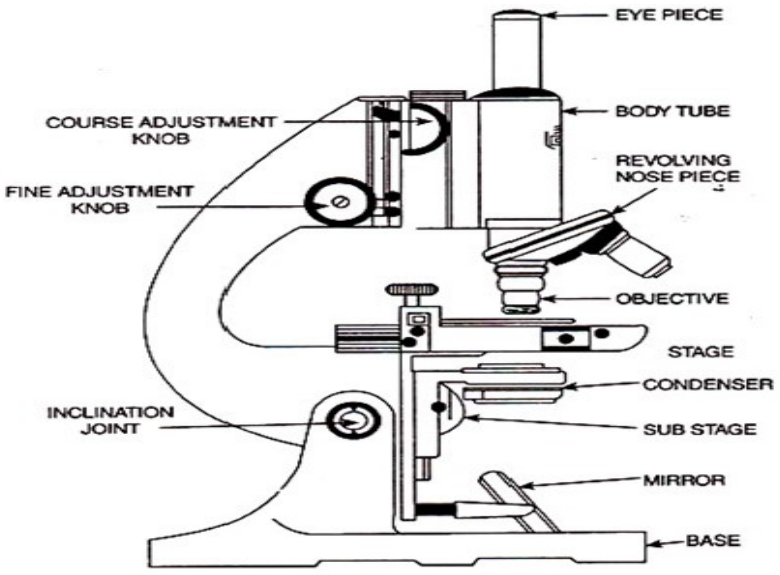


FIG. 15.1. The compound microscope showing its various parts.

APPLICATION:

- In pathology labs**,** a compound microscope is extremely useful for detecting illnesses.
- Human cells are drawn and examined under a microscope in forensic laboratories to detect and solve various crime cases.
- It helps in the visualization and comprehension of the micro-biological world of bacteria and viruses, which is otherwise undetectable to the naked eye.

EXPERIMENT-3
BINOCULAR MICROSCOPE

AIM:

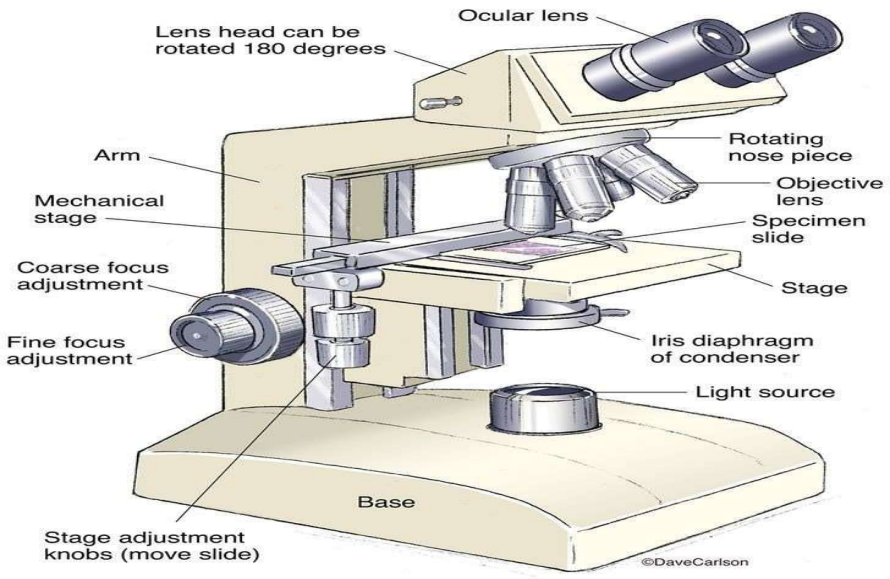
To study the principle and applications of Binocular microscope

BINOCULAR MICROSCOPE:

A binocular microscope is any microscope that has two eyepieces instead of the traditional monocular (single) eyepieces.

PRINCIPLE:

In this microscope, a single image is magnified by the objective lenses and then is split so that it can be observed by both eyes, creating a "flat" 2D image. The microscope also comes with three objective lenses, a sub stage condenser lens, a built-in lamp and a micrometer stage.



APPLICATION OF BINOCULAR MICROSCOPE:

It has various uses in the botanical, biological, investigation, medical and educational fields.

1. To understand the detail structure of the cell and cell organelles from the animal tissue.
2. It helps in the analysis of different types of tissue from animal body.
3. It helps in the examination of forensic evidences.
4. To understand the specific structure and different atomic structures.
5. Due to its high magnification, can be viewed in 2D

EXPERIMENT-4

SPECTROPHOTOMETRY
PRINCIPLE AND WORKING METHODOLOGY

AIM:

To identify the main features of the spectrophotometer and define their functions, Use a spectrophotometer to obtain an absorbance spectrum.

SPECTROPHOTOMETER:

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
- Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer.
- Before the light passes through the sample in the cuvette, an adjustable prism and diffraction grating filters the light so that only a single wavelength of light can be selected and allowed to pass through the sample.
- All molecules differ in how strongly they absorb each wavelength of light in the visible spectrum because of differences in their molecular structure and composition.
- This allows us to use a specific wavelength of light to detect the presence of, and quantify, one molecular compound in a simple or complex liquid mixture.
- Spectrophotometers are also calibrated by using a "blank" solution that we prepare containing all of the components of the solution to be analyzed except for the one compound we are testing for so that the instrument can zero out these background readings and only report values for the compound of interest.

PRINCIPLE:

The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a grapH on the detector and then on the display device.

1. In the spectrophotometer, a prism (or) grating is used to split the incident beam into different wavelengths.
2. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm.
3. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

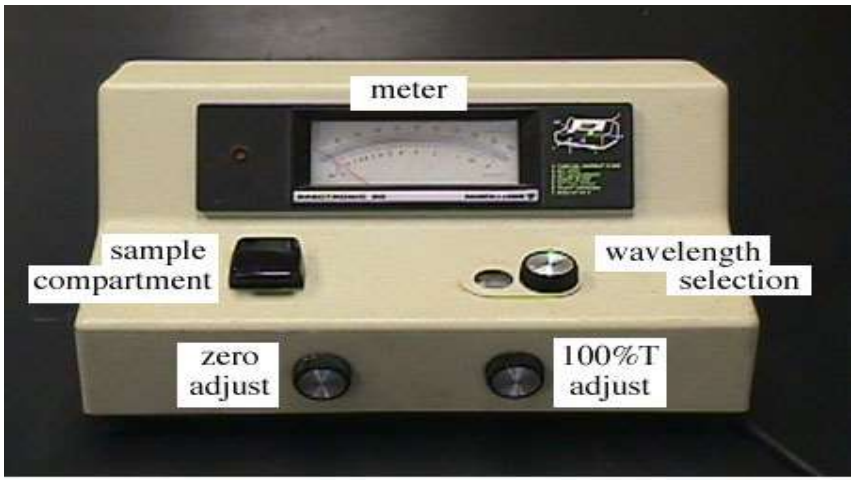


Fig. Labeled parts of a spectropHotometer

https://www.ncbionetwork.org/sites/default/files/1280x720-images/spectropHotometer.jpg

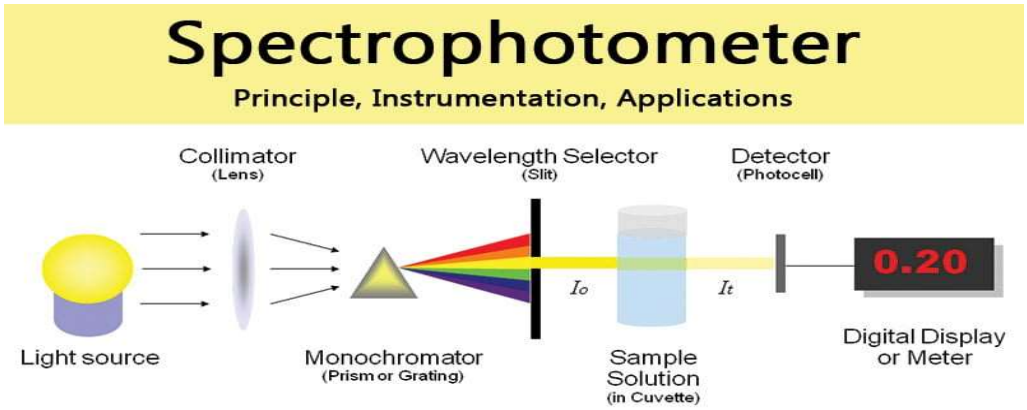


Fig.2: Functional parts of a SpectropHotometer

https://microbenotes.com/wp-content/uploads/2018/10/SpectropHotometer-Principle-Instrumentation-Applications.jpg

A visible spectrophotometer works by passing monochromatic visible light through a sample and measuring the amount of light absorbed by the sample, a principle based on Beer-Lambert Law. The absorption of specific wavelengths of light causes electrons in the sample's molecules to move from a lower to a higher energy state. The instrument detects the transmitted light using a photoelectric cell and converts it into an electrical signal, allowing for the measurement of a substance's concentration based on how much light it absorbs at a given wavelength.

A spectrophotometer works by passing a monochromatic beam of light through a sample and measuring the amount of light that is absorbed or transmitted, using the Beer-Lambert Law to relate this to the sample's concentration. The basic principle involves a light source, a mono-chromator to select specific wavelengths, a sample holder (cuvette), a detector to measure the light intensity after passing through the sample, and a readout device to display the results.

A visible spectrophotometer typically measures light in the visible light spectrum, which humans can see, ranging from approximately 380 to 700 nanometers (nm). Some instruments may have a slightly broader visible range, such as 340 nm to 1000 nm, while other UV-Vis spectrophotometers cover both ultraviolet (UV) and visible light, often from about 190 nm or 320 nm up to 900 nm or 1100 nm.

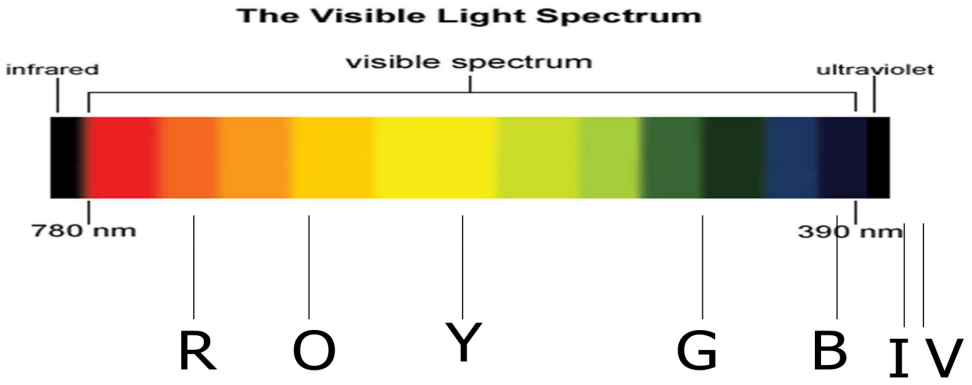


Fig: Instrumentation of Spectrophotometer

The essential components of spectrophotometer instrumentation include:

1. A table and cheap radiant **energy source**

- Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.

2. A **monochromator**, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.

- A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands.

PRISMS:

- A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent

- Two types of Prisms are usually employed in commercial instruments. Namely, 600 cornu quartz prism and 300 Littrow Prism.

GRATING:

- Gratings are often used in the monochromators of spectropHotometers operating ultraviolet, visible and infrared regions.

3. **Transport vessels** (cuvettes), to hold the sample

- Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as “CUVETTES”.

- Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz.

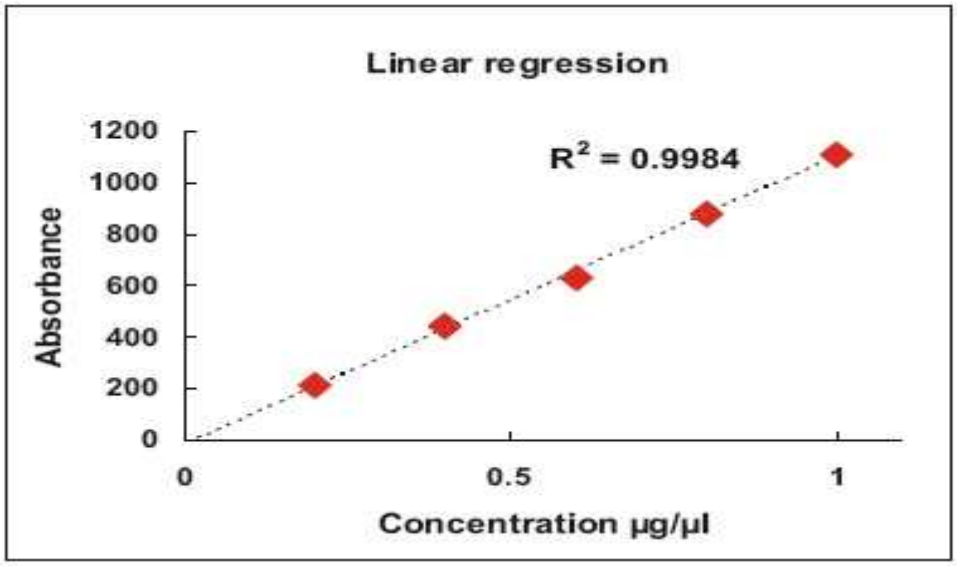
4. A PHOTOSENSITIVE DETECTOR **AND AN ASSOCIATED** READOUT SYSTEM:

- Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it.
- Radiation detectors generate electronic signals which are proportional to the transmitter light.
- These signals need to be translated into a form that is easy to interpret.
- This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.

To use a spectrophotometer, first turn it on and allow it to warm up. Next, select the desired wavelength and calibrate the instrument with a blank solution (usually solvent) to set a baseline. Then, replace the blank with your test sample, taking care to wipe the cuvette clean and place it in the holder. Record the absorbance or transmittance reading and then repeat the process for any other samples, ensuring you rinse the cuvette thoroughly between uses.

QUANTITATIVE DETERMINATION USING STANDARD GRAPH:

A standard graph for a spectrophotometer is a calibration curve, which plots the absorbance of several solutions of known concentrations against their corresponding absorbance values. Typically, concentration is on the x-axis and absorbance on the y-axis, forming a linear relationship as described by Beer-Lambert Law. This grapH is then used to determine the unknown concentration of a sample by comparing its measured absorbance to the curve.



https://www.news-medical.net/image-handler/picture/2016/2/Epp3.jpg

Fig. Plotting standard graph for Quantitative determination of sample concentration

APPLICATIONS:

Some of the major applications of spectrophotometers include the following:

- Detection of concentration of substances
- Detection of impurities
- Structure elucidation of organic compounds
- Monitoring dissolved oxygen content in freshwater and marine ecosystems
- Characterization of proteins
- Detection of functional groups
- Respiratory gas analysis in hospitals
- Molecular weight determination of compounds
- The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

EXPERIMENT-5

PAPER CHROMATOGRAPHY - SEPARATION OF MOLECULES

AIM:

To separate the components (molecules) of a mixture, using paper Chromatography:

APPARATUS REQUIRED:

1. Filter paper
2. Developing chamber
3. Developing solvent
4. Wooden sticks
5. Sample of mixed molecules

PAPER CHROMATOGRAPHY:

Paper chromatography is a type of chromatographic technique that employs a porous paper strip or sheet as the stationary phase and a liquid solvent as the mobile phase. This technique takes advantage of the differential solubility and adsorption properties of the components in a mixture, enabling their separation and identification. It relies on a simple principle: different substances in a mixture will move at different speeds through a piece of special paper when they're dissolved in a liquid. This paper is called chromatography paper, and it's designed to absorb the liquid and let it travel.

PAPER CHROMATOGRAPHY PRINCIPLE:

The principle of paper chromatography lies in the concept of partitioning. The mixture is applied near one end of the paper strip, the mobile phase (solvent) moves through capillary action, carrying the mixture along with it. As the solvent travels up the paper, it interacts with the compounds in the mixture. Depending on their solubility and affinity for the paper, the components are selectively carried at different rates. This differential movement allows the separation of the mixture into its individual components.

PAPER CHROMATOGRAPHY PRINCIPLE

Well, it relies on a simple principle: different substances in a mixture will move at different speeds through a piece of special paper when they're dissolved in a liquid. This paper is called chromatography paper, and it's designed to absorb the liquid and let it travel.

- **Sample Application:** First, you take a small drop of your mixture and place it near the bottom of the chromatography paper. This is where the magic begins.
- **Developing Solvent:** Next, you dip the bottom of the paper into a liquid, called the developing solvent. This liquid moves up the paper by a process called capillary action, and it carries the different components of your mixture along with it.

- **Separation Time:** As the solvent travels up the paper, it carries the mixture's components with it. But here's the cool part – because each substance in the mixture interacts with the paper and solvent differently, they move at different speeds. This causes them to spread out along the paper, creating colorful bands.
- **Identification:** Once the solvent reaches the top of the paper, you've got a beautiful chromatogram. Now, you can see the different components of your mixture separated out. It's like having a secret code that tells you what's in your mixture!

TYPES OF PAPER CHROMATOGRAPHY:

There are several types of paper chromatography, each with its own specific applications and techniques. Here are some commonly used variations:

- **Ascending Paper Chromatography:** In this method, the solvent is allowed to rise up the paper strip by capillary action, separating the mixture components as it progresses. It is particularly useful for separating small molecules and organic compounds.
- **Descending Paper Chromatography:** Unlike ascending paper chromatography, the solvent is applied at the top and allowed to flow downwards, carrying the mixture components. This technique is advantageous when analyzing non-volatile substances or those that are more soluble in the solvent.
- **Two-Dimensional Paper Chromatography:** This method involves the sequential application of solvents in two directions, allowing better separation and analysis of complex mixtures.
- **Preparative Paper Chromatography:** In this technique, larger quantities of separated components are obtained for further analysis or use. It is commonly used for isolating pure compounds.

R_F VALUE, SOLUTES, AND SOLVENTS:

The retention factor (R_f) may be defined as the ratio of the distance travelled by the solute to the distance travelled by the solvent. It is used in chromatography to quantify the amount of retardation of a sample in a stationary phase relative to a mobile phase. R_f values are usually expressed as a fraction of two decimal places.

- If R_f value of a solution is zero, the solute remains in the stationary phase and thus it is immobile.
- If R_f value = 1 then the solute has no affinity for the stationary pHase and travels with the solvent front.

For example, if a compound travels 9.9 cm and the solvent front travels 12.7 cm, the R_f value = (9.9/12.7) = 0.779 or 0.78. R_f value depends on temperature and the solvent used in experiment, so several solvents offer several R_f values for the same mixture of compound. A solvent in chromatography is the liquid the paper is placed in, and the solute is the ink which is being separated.

CHROMATOGRAPHY DIAGRAM:

To help visualize the paper chromatography process, refer to the following diagram:

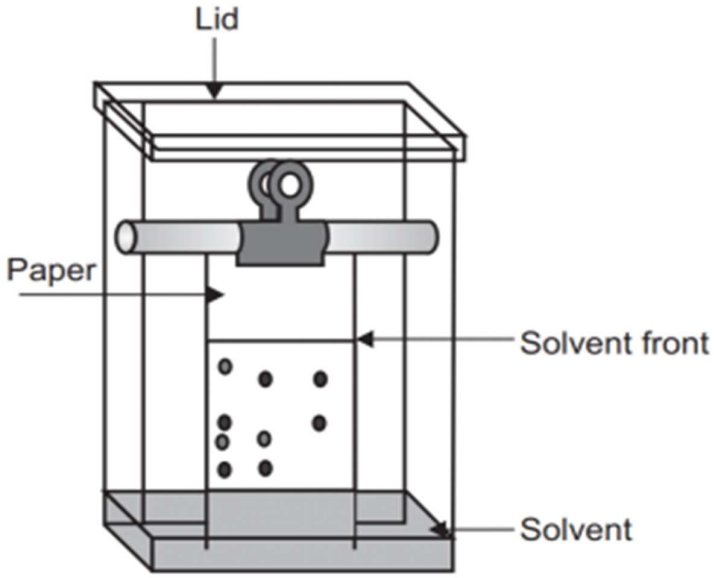


Fig: Paper chromatography process

https://www.tutoroot.com/blog/wp-content/uploads/2023/10/Diagrammatic-representation-of-paper-chromatograpHy-min.png

The diagram illustrates the setup of paper chromatograpHy, with the mixture applied near one end of the paper strip. The solvent migrates through the paper, carrying the components at different rates. As the solvent progresses, distinct bands or spots corresponding to each component are formed, allowing easy identification.

PAPER CHROMATOGRAPHY EXPERIMENT AND PROCEDURE:

Carrying out a paper chromatography experiment requires careful attention to detail. Here is a general procedure to follow:

- **Prepare the mixture:** Obtain a small quantity of the mixture to be analyzed and ensure it is properly dissolved or suspended in a suitable solvent.
- **Prepare the paper strip:** Cut a piece of chromatography paper to the desired size. It is crucial to handle the paper strip with clean hands or gloves to avoid contamination.
- **Apply the mixture:** Using a capillary tube or dropper, carefully apply a small spot or line of the mixture near one end of the paper strip. Allow it to dry completely to avoid smudging.
- **Prepare the solvent:** Choose an appropriate solvent based on the nature of the mixture components. Pour the solvent into a suitable container, ensuring it is deep enough to allow the solvent front to reach the desired height on the paper strip.

- **Perform the chromatography:** Immerse the paper strip vertically into the container, ensuring the mixture spot is above the solvent level. Cover the container to prevent evaporation. Capillary action will cause the solvent to rise through the paper, separating the components. The developed chromatogram can take anywhere from minutes to hours.
- **Interpret the results:** Once the solvent front reaches the desired distance (usually about 2/3 of the paper strip height), remove the paper strip from the container and allow it to air dry. Observe the separated components as distinct bands or spots. Identify and mark them as needed for further analysis.

EXPERIMENT- 6

THIN LAYER CHROMATOGRAPHY- ISOLATION OF MOLECULES

AIM:

Separation and Purification of organic mixture by extraction techniques (TLC monitoring)

THEORY:

In Thin Layer Chromatography component of a mixture of substances are located by flow of mixture of two solvents which are immiscible or partially miscible on alumina coated silica gel. The solvents rise up on the alumina coated silica bed owing to capillary action and the component of the organic mixture are separated by the differential migration and adsorption on the plate.

APPARATUS REQUIRED:

Thin layer platform,

Spreader,

TLC chamber,

TLC plate,

Pencil,

Scale,

measuring cylinder.

CHEMICALS REQUIRED:

Organic solvent (Ethyl acetate &petroleum Ether),
Acetone
Hexane
Acetonitrile
Ethanol
Methanol
Aniline,
Acetanilide.

PRINCIPLE:

Thin layer chromatography (TLC) is a widely used separation technique for quantitative and qualitative analysis. It uses a thin layer of a stationary phase coated on a glass, plastic, or aluminum plate. A liquid solvent called the mobile phase carries the sample and separates it as it moves across the plate. It offers the advantages of simplicity, sensitivity, and rapid analysis over other separation techniques.

PROCEDURE:

A small amount of the mixture to be identified or to be separated in a small scale (Preparative TLC) is dissolved in suitable solvent. The solution is spotted near one edge of the plate covered with a thin layer of adsorbent. The spot is allowed to dry and spotted plate is placed in a solvent chamber containing a suitable solvent or solvent mixture so that the lower end of the plate is dipped 1-2 cm into the liquid. The solvent rises through the adsorbent owing to capillary attraction and the various components of the mixture ascend at different rates depending on their affinities to the adsorbent causing the separation of individual component. The principle is similar to that of column chromatography when the solvent front almost reaches the top of the adsorbent layer or three-fourths of it. The plate is taken out of solvent chamber, dried and sprayed with suitable reagent to locate individual component. The choice of solvent is very much similar to that of column chromatography.

Fig. Developing chamber with TLC plates
https://media.sciencepHoto.com/image/c0259051/800wm/C0259051-Thin-layer_chromatograpHy.jpg

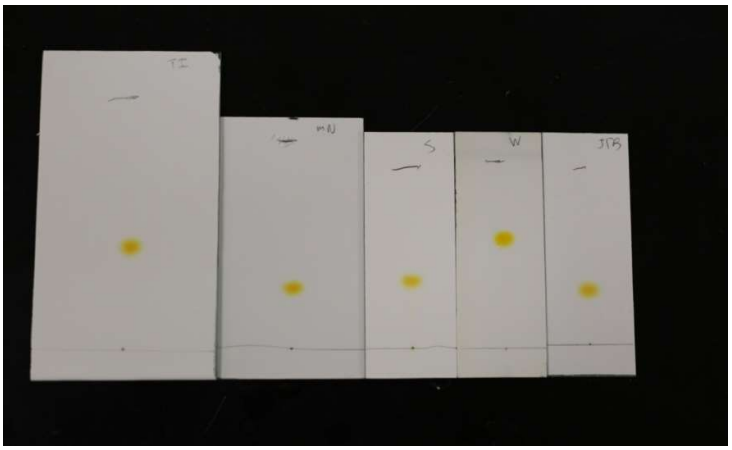


Fig:Dimethyl yellow run on various TLC plates from various vendors in 10% ethyl acetate in hexanes.
https://www.teledyneisco.com/enus/ChromatograpHy_/Blog_/PublishingImages/Dimethyl%20yellow%20run%20on%20various%20TLC%20plates%20from%20various%20vendors.jpg

RETENTION FACTOR:

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent. The formula is;

R_f = Distance traveled by sample/ Distance traveled by Solvent front

The R_f value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower value.

RESULT:

Calculate the Rf value of the compound in the organic mixture.

Conclusion: Report the Rf value.

EXPERIMENT-7

CALCULATION OF MEAN, MEDIAN, AND MODE

AIM:

To define and determine the mean, mode and median for the given data

MEASURES OF CENTRAL TENDENCY:

In biology we usually encountered with large set of data's, because whatever our approach is for data collection, we have to collect data at least in replicates so that more reliable conclusion can be drawn. In order to describe the whole mass of unwieldy data a single value is required. Thus, biostatistics provides us the tool to get a single value which can better describe the group of data i.e. central value or an average or in other words we can say that, from the collected data the values of the variable tend to concentrate around some central value of observation, that value can be used as the representative value. So this tendency of distribution is known as central tendency. Under measures of central tendency Mean, median and mode are the most popular averages that are studied.

TYPES OF MEASURE OF CENTRAL TENDENCY

1. Arithmetic Mean

2. Geometric Mean

3. Harmonic Mean

4. Median

5. Mode

STUDY OF MEAN:

Arithmetic Mean Arithmetic Mean is the most popular and commonly used measure of central tendency. It is defined as the number obtained by dividing the total values of different items by their number and it's denoted by, in general if arithmetic mean for ungrouped data or individual observation is, , , ,…… be 'n'observations for a variable X, the arithmetic mean $\bar{x}$ is given by

$$\bar{X} = \frac{X_1 + X_2 + X_3 + ... + X_n}{n}$$

To further simplify the writing of a sum, the Greek letter Σ (sigma) is used. The sum $X_1 + X_2 + X_3 + ... + X_n$ is denoted,

$$\bar{X} = \sum_{i=1}^{n} \frac{X_i}{n}$$

- **CALCULATION OF ARITHMETIC MEAN**

1. Series of individual observation

2. Discrete series

3. Continuous series

1. SERIES OF INDIVIDUAL OBSERVATIONS

The calculation of arithmetic mean for such data which are present in individual series, the calculations is easy. Generally we have to get the total of values and divide this total by number of observation.

Suppose we have five values for stomata's on leaves of a plan t is 14 for first leaf, 17 for second leaf, 13, 15, and 19 for third, fourth and fifth leaf respectively.

The arithmetic mean therefore is

$$\frac{14+17+13+15+19}{5}=15.6$$

So the average number of stomata for the plant is 15.6

SYMBOLICALLY

Leaf's	**Stomata's**
A	14
B	17
C	13
D	15
E	19
$N=5$	$\Sigma X=15.6$

Further, the arithmetic mean for series of individual observation can be calculated by two methods:

A. Direct method

B. Assumed mean method or short cut method

A. DIRECT METHOD:

EXAMPLE:

Calculate the arithmetic mean of the following marks in Hindi obtained by 10 students in a unit test.

Student	A	B	C	D	E	F	G	H	I	J
Marks	15	17	13	16	18	19	14	16	17	18

WORK PROCEDURE:

Obtained ΣX by adding all the variables and divide the total by number of observation (N) symbolically.

$$\Sigma X = 15+17+13+16+18+19+14+16+17+18$$
$$\Sigma X = 163$$
$$N = 10$$
$$Thus, \bar{X} = \Sigma X / N$$
$$\bar{X} = 163/10 = 16.3$$

The average marks in Hindi is 16.3

B. ASSUMED MEAN METHOD OR SHORT CUT METHOD:

EXAMPLE:

Calculate the arithmetic mean of the marks in Hindi obtained by 10 students in a unit test given in illustration by assumed method.

Student	Marks (X)	X-A (d)
A	15	-4
B	17	-2
C	13	-6
D	16	-3
E	18	-1
F	19	0
G	14	-5
H	16	-3
I	17	-2
J	18	-1
$N = 10$		-27

WORK PROCEDURE:

In this example, first we have to assume a mean, suppose assume mean=19.

Calculate the deviation from assumed mean (X-A)=d

Get the total, of deviation from data using the following formula,

Mean $\bar{X} = A + \frac{\Sigma d}{N}$, where "A" is assumed mean and "d" is deviation

=19+ (-27/10)

= 19-2.7

=16.3 marks

Thus the average marks in Hindi= 16.3.

Thus from results of both methods we got the same result (16.3 marks).

B. DISCRETE SERIES:

In case of discrete series, frequency against each variable (observation) is multiplied by the value of the observation. The values, so obtained, are summed up and divided by the total number of frequencies.

SYMBOLICALLY,

Where, ΣfX = sum of the product of variables and frequencies.

Σf = sum of frequencies.

EXAMPLE:

The number of seeds produced by 120 plants in garden were given in table. Calculate the arithmetic mean.

Seed's (X)	Number of Plants (f)	fX
200	4	800
190	12	2280
180	15	2700
170	37	6290
160	22	3520
150	6	900
140	10	1400
	N=106	$\Sigma fX = 17890$

WORK PROCEDURE:

Multiply the frequency with the variable X and get the sum of the product (ΣfX).

Divide ΣfX with the total number of observation ΣfX or N

Number of class: 200, 190, 180, 170, 160, 150, 140.

Frequency (f) = 4+12+15+37+22+6+10

$$\Sigma fX \text{ or } N = 106$$

$$fX = 800+2280+2700+6290+3520+900+1400$$

$$\Sigma fX = 17890$$

$$\text{So, } \bar{X} = \frac{\Sigma fX}{\Sigma f} = 17890/106 = 168.77$$

SHORT CUT METHOD OR ASSUMED MEANMETHOD:

We can use this method for discrete series also, the formula to be used for this method is

$$Mean = A + \frac{\Sigma fd}{N}$$

Here, A = assume mean

N = Number of observation

d = deviation of variable taken from assumed mean

Σfd = Sum of the product of frequencies and their respective deviations.

Seed's (X)	Number of Plants (f)	X-A=d	fd
200	4	30	120
190	12	20	240
180	15	10	150
170	37	0	0
160	22	-10	-220
150	6	-20	-120
140	10	-30	-300
	106		**-130**

WORK PROCEDURE:

In this example, first we have to assume a mean, suppose assume mean=170. Calculate the deviation from assumed mean $X - A = d$

Get the total, of deviation from data using the following formula,

$$Mean = A + \frac{\Sigma fd}{N},$$

where "A" is assumed mean and "d" is deviation

$$Mean = A + \frac{\Sigma fd}{N}$$

$$= 170 + \frac{-130}{106}$$

$$= 170 + (-1.23)$$

$$= 168.77$$

The average number of seeds for the plant is 168.77 and again the answer we got by both method is same.

C. CONTINUOUS SERIES:

In this approach of calculating arithmetic mean, class intervals are given. The method of calculating arithmetic mean in case of continuous series is same as that of a discrete series. The only difference is that the mid-points of various class intervals are obtained. We have already known that class intervals may be exclusive or inclusive or of unequal size. The following equation may be used to derived or get the mid-points.

$$\text{Mid-point (m)} = \frac{l_1 + l_2}{2}$$

Here, l_1 = lower limit and l_2 = upper limit.

After obtaining the midpoint we calculate arithmetic mean as we calculated in case of discrete series.

EXAMPLE:

Find out the mean of the following distribution in a class Marks

Marks	4-8	8-12	12-16	16-20
Student	4	8	6	3

WORK PROCEDURE:

In first step: obtain mid-point (m) of the classes by using following formula

$$\text{Mid-point (m)} = \frac{l_1 + l_2}{2}$$

In second step: Multiply the frequency (f) with mid-point (m) and get the product (fm)

In third step: Divide the Σfm by the total number of observation (N), i.e.,

Mean = Σ**fm** / **N**

Marks (X)	**Number of Student (f)**	**Mid-point (m)**	**fm**
4-8	4	6	24
8-12	8	10	80
12-16	6	14	84
16-20	3	18	54
	N=21		**Σfm =242**

Mean = Σ**fm** / **N** = 242/21 = 11.52.

Thus, the mean mark of the students in class among 21 students was 11.52.

EXAMPLE:

Calculate the mean of the following distribution in an experimental garden for fruit production.

Number of fruits	100-120	120-140	140-160	160-180	180-200	200-220
Number of plants	10	20	25	30	15	5

In first step: obtain mid-point (m) of the classes by using following formula

$$\text{Mid-point (m)} = \frac{l_1 + l_2}{2}$$

In second step: Multiply the frequency (f) with mid-point (m) and get the product (fm).

In third step: Divide the Σfm by the total number of observation (N), i.e.,

Mean = Σ**fm** / **N**

Marks (X)	**Number of Student (f)**	**Mid-point (m)**	**fm**
100-120	10	110	1100
120-140	20	130	2600
140-160	25	150	3750
160-180	30	170	5100
180-200	15	190	2850
200-220	5	210	1050
	N=105		**Σfm =16450**

Mean = Σ**fm** / **N** = 16450/105= 156.67.

Thus, the mean mark of the students in class among 21 students was 156.67.

ASSUMED OR SHORT-CUT METHOD:

EXAMPLE:

Find out the mean of the following distribution in a class

Marks	4-8	8-12	12-16	16-20

Student	4	8	6	3

In first step: obtain mid-point (m) of the classes by using following formula

$$\text{Mid-point (m)} = \frac{l_1 + l_2}{2}$$

In second step: Decide an assumed mean from the data $A = 10$.

In third step: calculate the deviation from assumed mean $m - A = d$, and then multiply the deviation(d) with mid-point (m) and get the product (fd)

In fourth step: Divide the Σfd by the total number of observation (N), i.e., **Mean = A+ Σfd/N**

Marks (X)	**Number of Student (f)**	**Mid-point (m)**	**m-A=d**	**fd**
4-8	4	6	0	0
8-12	8	10	4	32
12-16	6	14	8	48
16-20	3	18	12	36
	N=21			$\Sigma fd = 116$

$$Mean = A + \Sigma fd / N$$

$$= 6 + 116 / 21$$

$$= 6 + 5.52$$

$$= 11.52.$$

Thus, the average mark in a class among 21 students is 11.52.

SOME OTHER TYPES OF MEAN'SARE;

1. GEOMETRIC MEAN:

DEFINITION:

The geometric mean of a set of 'n'observations is the nth root of their product. It is denoted by G.

GEOMETRIC MEAN FOR AN UNGROUPED DATA:

If $x_1.x_2.....x_n$ are 'n'observations, then Geometric mean G is calculated by

$$G = (x_1.x_2.....x_n)^{1/n}$$

$$\log G = \frac{1}{n}\left(\log x_1 + \log x_2 + ... + \log x_n\right)$$

or

$$\log G = \frac{1}{n}\sum_{i=1}^{n} \log x_i$$

$$G = Anti\log\left[\frac{1}{n}\sum_{i=1}^{n} \log x_i\right]$$

EXAMPLE1:

Find the geometric mean of 16, 64 and 108.

Solution: The geometric mean G is given by

$$g = \sqrt[3]{16\ X\ 64\ X\ 108}$$

$$g = 48$$

EXAMPLE 2:

The birth rate of a country in the years 1980 and 1990 are 8% and 10% respectively. Find average birth rate.

Solution: The geometric mean of the birth rates in the country is

$$g = \sqrt{108\ \text{X}\ 110}$$

$$g = 104.9$$

$\therefore$ The average birth rate according to geometric mean is 4.9%

GEOMETRIC MEAN FOR A GROUPED DATA:

If $x_i / f_i, i = 1, 2, ..., n$ is a grouped frequency distribution, the Geometric mean G is calculated by the following formula

$$G = \left[x_1^{f_1}.x_2^{f_2}.....x_n^{f_n}\right]^{\frac{1}{N}}$$

$$Where\ \text{N} = \sum_{I=1}^{n} f_i$$

$$\log G = \frac{1}{N}\left(f_i \log x_1 + f_2 \log x_2 + ... + f_n \log x_n\right)$$

or

$$\log G = \left[\frac{1}{N}\sum_{i=1}^{n} f_i \log x_i\right]$$

$$\therefore G = Anti\log\left[\frac{1}{N}\sum_{i=1}^{n} f_i \log x_i\right]$$

EXAMPLE 1: Find the geometric mean to the following.

x	3	8	10	12
f	15	20	24	8

Solution:

x_i	f_i	$\log x_i$	$f_i \log x_i$
3	15	0.4771	7.1565
8	20	0.9031	18.0620
10	24	1.0000	24.0000
12	8	1.0792	8.6336
	N=67		$\sum_{i=1}^{n} f_i \log x_i = 57.8521$

$$Where,\ \mathrm{N}=\sum_{I=1}^{n} f_i = 67$$

$$\therefore G = Anti\log\left[\frac{1}{N}\sum_{i=1}^{n} f_i \log x_i\right]$$

$$G = Anti\log\left[\frac{57.8521}{67}\right]$$

$$G = Anti\log\left[0.8635\right]$$

$$G = 7.303$$

$\therefore$ The geometric mean G = 7.303

EXAMPLE 2: Find the geometric mean to the following data.

Class Interval	4-8	8-12	12-16	16-20	20-24
Frequency	2	4	8	5	3

Solution:

Class Interval	Frequency f_i	Mid-value x_i	$\log x_i$	$f_i \log x_i$
4-8	2	6	0.7782	1.5564
8-12	4	10	1.0000	4.0000
12-16	8	14	1.1461	9.1688
16-20	5	18	1.2553	6.2765
20-24	3	22	1.3424	4.0272
	N=22			$\sum_{i=1}^{n} f_i \log x_i = 25.0289$

$$Where,\ \mathrm{N} = \sum_{I=1}^{n} f_i = 22$$

The geometric mean G is given by

$$\therefore\ G = Anti\log\left[\frac{1}{N}\sum_{i=1}^{n} f_i \log x_i\right]$$

$$G = Anti\log\left[\frac{25.0289}{22}\right]$$

$$G = Anti\log\left[1.1377\right]$$

$$G = 13.7309$$

$\therefore$ The geometric mean of the given data is G = 13.7309

2. HARMONIC MEAN:

DEFINITION:

The Harmonic mean of a number of observations is the reciprocal of the arithmetic mean of the reciprocals of the given values. It is denoted by H.

HARMONIC MEAN FOR AN UNGROUPED DATA:

Let $x_1.x_2.....x_n$ are $'n'$ observations, then Harmonic mean H is calculated by

$$H = \frac{1}{\frac{1}{N}\sum_{i=1}^{n}\left(\frac{1}{x_i}\right)}$$

GEOMETRIC MEAN FOR A GROUPED FREQUENCY DISTRIBUTION:

If $x_i / f_{i,}\ i = 1,2,.....,n$ is a grouped frequency distribution, then Harmonic mean H is calculated by

$$H = \frac{1}{\frac{1}{N}\sum_{i=1}^{n}\left(\frac{f_i}{x_i}\right)}$$

$$Where\ \mathrm{N} = \sum_{I=1}^{n} f_i$$

EXAMPLE 1:

A person travel from Vijayawada to Tirupati at 80 km per hour and returns at 70 km per hour, find the average speed of the journey?

Solution: The average speed of the journey by using harmonic mean is

$$H = \frac{1}{\left[\frac{1}{N}\sum_{i=1}^{n}\left(\frac{1}{x_i}\right)\right]} = \frac{1}{\left[\frac{1}{2}\left[\frac{1}{80}+\frac{1}{70}\right]\right]} = 74.67$$

The harmonic mean (average speed of the journey) H is 74.67 km per hour.

EXAMPLE 2:

Find the harmonic mean to the following frequency distribution

Class Interval	10-20	20-30	30-40	40-50	50-60
Frequency	8	15	30	17	10

Solution:

Class Interval	Frequency f_i	Mid-value x_i	$f_i \log x_i$
10-20	8	15	0.5333
20-30	15	25	0.6000
30-40	30	35	0.8571
40-50	17	45	0.3778
50-60	10	55	0.1818
	N=80		$\sum_{i=1}^{n}\left(\frac{f_i}{x_i}\right) = 2.5500$

$$Where,\ \mathrm{N}=\sum_{I=1}^{n} f_i = 80,$$

The harmonic mean H is given by

$$H = \frac{1}{\left[\frac{1}{N}\sum_{i=1}^{n}\left(\frac{1}{x_i}\right)\right]} = \frac{1}{\frac{2.5000}{80}} = 31.3725$$

The harmonic mean H =31.3725

3. MEDIAN:

Median may be defined as the *middle value* (half of the observations are smaller and half are larger than this value) in the data set when elements are arranged in a sequential (either ascending or descending) order of magnitude.

Thus, median is a measure of the *location* or *centrality* of the observations. The median can be calculated for both ungrouped and grouped data sets.

The median is helpful in understanding the characteristic of a data set when
• Observations are qualitative in nature.

• Extreme values (outliers) are present in the data set.

• At a glance estimate of an average is desired.

METHODS OF CALCULATING MEDIAN:

UNGROUPED DATA:

Arrange elements in the data set in a sequential (either ascending or descending order) of magnitude.

(i) If the number of observations (n) are *odd number*, then the median (Med) value is

Med = Size or value of $\left(\frac{n+1}{2}\right)$ observation in the data array.

(ii) If the number of observations (n) are *even number*, then the medianvalue is the arithmetic mean of the numerical values of $(n/2)^{th}$ and $(\frac{n}{2}+1)^{th}$ observations in the data array.

$$\text{That is, } Med = \frac{\frac{n}{2}th\ observation + \left(\frac{n}{2}+1\right)th\ observation}{2}$$

EXAMPLE 1:

Find the median of the values 25, 30, 35, 15,10.

Solution:

After arranging the data into ascending order it is 10, 15, 25, 30, 35.

The observations are odd,

then the median is middle value of the arrangement.

$$\text{Med} = \text{Size or value of } \left(\frac{n+1}{2}\right) \text{ observation in the data array.}$$

$$= \{(5 + 1)/2\}\text{th} = 3^{rd} \text{ observation in the data array} = 25$$

$\therefore$Median = 25.

EXAMPLE 2:

Calculate the median of the following data that relates to the service time (in minutes) per customer for 7 customers at a railway reservationcounter: 3.5, 4.5, 3, 3.8, 5.0, 5.5, 4.

Solution:

The data are arranged in ascending order as follows:

Observations in the data array	1	2	3	4	5	6	7
Service time (in minutes)	3	3.5	3.8	4	4.5	5	5.5

Since number of observations are odd, the median for this data would be

$$\text{Med} = \text{Size or value of } \left(\frac{n+1}{2}\right) \text{observation in the data array.}$$

$$= \{(7 + 1)/2\}\text{th} = 4\text{th observation in the data array} = 4$$

Thus, the median service time is 4 minutes per customer.

MEDIAN FOR A GROUPED DATA:

For grouped data, first find

If $x_i / f_i, i = 1, 2, ..., n$ is a grouped frequency distribution, then

CASE 1:

For discrete frequency distribution, we can find the median through the following steps.

1. Arrange the data in ascending or descending order of magnitude
2. Find cumulative frequencies
3. Find $\frac{N}{2}$, consider the cumulative frequency just greater than $\frac{N}{2}$.
4. The corresponding value of x is median.

CASE 2. For continuous frequency distribution, the median is obtained by considering the median class. The class corresponding to cumulative frequency *(c.f.)*just greater than $\frac{N}{2}$ is called median class. The value of median is obtained by the following formula:

$$Median = l + \left(\frac{\frac{N}{2} - c}{f}\right) X \ h$$

Where l = lower limit of median class

f = frequency of median class

h = magnitude of the median class

c = cumulative frequency preceding to the median class.

EXAMPLE 1:

Obtain median to the following data:

x	5	8	11	14	17	20	23
f	2	8	12	20	10	6	3

Solution:

x	f_i	Cumulative Frequency
5	2	2
8	8	10
11	12	22
14	20	**42← Median class**
17	10	52
20	6	58
23	3	61
	N=61	

$$Where,\ \text{N}=\sum_{I=1}^{n} f_i = 61,\ \frac{N}{2} = \frac{61}{2} = 30.5$$

Cumulative frequency is greater than $\frac{N}{2}$ is 42, and the value of x corresponding to 42 is 14.

$$\therefore Median = 14$$

EXAMPLE 2:

Find the median to the following data:

Class Interval	40-50	50-60	60-70	70-80	80-90
Frequency	5	12	23	8	2

Solution:

Class Interval	Frequency f_i	Cumulative Frequency (C.f.)
40-50	5	5
50-60	12	**17**c
l **60-70**	**23** *f*	40
70-80	8	48
80-90	2	50
	N=50	

$$Where,\ \text{N}=\sum_{I=1}^{n} f_i = 50,\ \frac{N}{2} = \frac{50}{2} = 25$$

Cumulative frequency is greater than $\frac{N}{2}$ is 40, and the value of x corresponding class is 60-70.

$\therefore$ The median class is 60-70

$\therefore l = 60$, c = 17, $f = 23$, $h = 10$

$$Median = l + \left(\frac{\frac{N}{2} - c}{f} \right) X\ h$$

$$Median = 60 + \left(\frac{25 - 17}{23} \right) X\ 10$$

$$Median = 60 + 3.4782 = 63.4782$$

5. MODE:

Mode is defined as the value which occurs most frequently in a set of observations

MODE FOR AN UNGROUPED DATA:

Mode for an ungrouped data is calculated by the value of the variable which repeats maximum number of times.

EXAMPLE 1:

Obtain mode of the values 10,**12**,15,20,**12,**16,18,15,**12,**10,16,20,12,24

SOLUTION:

Mode is the value which occurs the maximum number of times. In this series the value (observation) 12 repeated 4 times which is maximum frequently occurred than any other observations.

$$\therefore Mode = 12$$

MODE FOR A GROUPED DATA:

CASE 1.

In case of discrete frequency distribution, mode can be obtained by inspection. Thevalue of the variable having the maximum frequency is known as modal value.

CASE 2.

In case of continuous frequency distribution, the mode can be calculated by the following formula.

$$Mode = l + \left(\frac{f_1 - f_0}{2 f_1 - f_0 - f_2} \right) X\ h$$

Where l : lower limit of modal class

h : Width of the modal class interval

f_1 : Frequency of modal class

f_0 : Frequency of preceding the modal class

f_2 : Frequency of succeeding the modal class

EXAMPLE 2:

Find the mode to the following frequency distribution:

x	1	2	3	**4**	5	6	7	8
f	4	9	16	**25**	22	15	7	3

Solution:

The maximum frequency is 25

The value of x corresponding to maximum frequency is 4. Hence mode is 4.

EXAMPLE 3:

Find mode to the following data:

Class Interval	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40
Frequency	5	7	10	18	20	12	8	2

Solution:

Class Interval	Frequency (f_i)
0-5	5
5-10	7
10-15	10
15-20	**18** f_0
l **20**-25	**20** f_1
25-30	**12** f_2
30-35	8
35-40	2

The maximum frequency is 20 in the class interval 20-25, therefore model class is 20-25.

$\therefore l = 20,\ f_1 = 20,\ f_0 = 18, f_2 = 12, h = 5$

$$\therefore Mode = l + \left(\frac{f_1 - f_0}{2f_1 - f_0 - f_2} \right) X\ h$$

$$Mode = 20 + \left(\frac{20 - 18}{2X20 - 18 - 12} \right) X\ 5$$

$$Mode = 21$$

EXPERIMENT-8

STANDARD DEVIATION AND STANDARD ERROR

AIM:

Defining Standard Deviation and Standard Error and calculating the both for the exemplary data

DEFINITION:

Standard Deviation is the positive square root of the arithmetic mean of the squares of the deviations of the given values from their arithmetic mean. It is denoted by σ or S.D.

STANDARD DEVIATION FOR AN UNGROUPED DATA:

For ungrouped data, the Standard Deviation σ is given by

Where $\bar{x}$ is the mean of the given data.

STANDARD DEVIATION FOR A GROUPED DATA:

If $x_i / f_{i,}\ i = 1,2,.....,n$ is a grouped frequency distribution, then the Standard Deviation

σ is given by $\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{n} f_i \left(x_i - \bar{x}\right)^2}$

$$Where,\ \mathrm{N} = \sum_{I=1}^{n} f_i$$

EXAMPLE 1:

Compute standard deviation of the values 10,6,8,12,20

Solution:

$$mean = \bar{x} = \frac{10 + 6 + 8 + 12 + 20}{5}$$

$$\bar{x} = \frac{56}{5} = 11.2$$

$$S.D. = \sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}$$

$$\sigma = \sqrt{\frac{1}{5}\left[(10-11.2)^2 + (6-11.2)^2 + (8-11.2)^2 + (12-11.2)^2 + (20-11.2)^2\right]}$$

$$\sigma = \sqrt{\frac{116.8}{5}}$$

$$\sigma = 4.83$$

EXAMPLE 2:

Find the standard deviation of 14,15,16,17,18,19,20

Solution:

$$mean = \bar{x} = \frac{14+15+16+17+18+19+20}{7}$$

$$\bar{x} = \frac{119}{7} = 17$$

$$S.D. = \sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

$$\sigma = \sqrt{\frac{1}{7}\left[(14-17)^2 + (15-17)^2 + (16-17)^2 + (17-17)^2 + (18-17)^2 + (19-17)^2 + (20-17)^2\right]}$$

$$\sigma = \sqrt{\frac{28}{7}}$$

$$\sigma = 2$$

EXAMPLE 3:

Calculate standard deviation to the following grouped frequency distribution:

Class Interval	0-10	10-120	20-30	30-40	40-50	50-60
Frequency	2	8	16	28	12	4

Solution:

Class Interval	Frequency f_i	Mid-value x_i	$f_i x_i$	$f_i(x_i - \bar{x})^2$	$f_i x_i^2$
0-10	2	5	10	1508.81	50
10-20	8	15	120	2430.44	1800
20-30	16	25	400	883.28	10000
30-40	28	35	980	184.94	34300
40-50	12	45	540	1906.06	24300
50-60	4	55	220	2037.62	12100
	N=70		$\sum_{i=1}^{n} f_i x_i = 2270$	$\sum_{i=1}^{n} f_i(x_i - \bar{x})^2 = 8937.15$	$\sum_{i=1}^{n} f_i x_i^2 = 82550$

$$Where,\ N = \sum_{I=1}^{n} f_i = 70,$$

$$\overline{x} = \frac{1}{N}\sum_{i=1}^{n} f_i x_i = \frac{2270}{70} = 32.43$$

$$S.D. = \sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{n} f_i \left(x_i - \overline{x}\right)^2}$$

$$\sigma = \sqrt{\frac{8937.15}{70}}$$

$$\sigma = 11.29$$

STANDARD ERROR:

In statistics, the standard error is the standard deviation of the sample distribution. The sample mean of a data is generally varied from the actual population mean. It is represented as SE. It is used to measure the amount of accuracy by which the given sample represents its population. Statistics is a vast topic in which we learn about data, sample and population, mean, median, mode, dependent and independent variables, standard deviation, variance, etc. Here we will learn standard error formula along with *SE* of the mean and estimation.

STANDARD ERROR FORMULA:

The accuracy of a sample that describes a population is identified through SE formula. The sample mean which deviates from the given population and that deviation is given as;Where 'S' isthestandarddeviationand 'n' isthenumberofobservation.

STANDARD ERROR OF THE MEAN(SEM):

The standard error of the mean also called the standard deviation of mean, is represented as the standard deviation of the measure of the sample mean of the population. It is abbreviated as SEM. For example, normally, the estimator of the population mean is the sample mean. But, if we draw another sample from the same population, it may provide a distinct value.

Thus, there would be a population of the sampled means having its distinct variance and mean. It may be defined as the standard deviationof such sample means of all the possible samples taken from the same given population. SEM defines an estimate of standarddeviation which has been computed from the sample. It is calculated as the ratio of the standard deviation to the root of sample size, such as:

$$SEM = \frac{s}{\sqrt{n}}$$

Where's' is the standard deviation and 'n' is the number of observation.

The standard error of the mean shows us how the mean changes with different tests, estimating the same quantity. Thus if the outcome of random variations is notable, then the standard error of the mean will have a higher value. But, if there is no change observed in the data points after repeated experiments, then the value of the standard error of the mean will be zero.

STANDARD ERROR OF ESTIMATE (SEE):

The standard error of the estimate is the estimation of the accuracy of any predictions. It is denoted as SEE. The regression line depreciates the sum of squared deviations of prediction. It is also known as the sum of squares error. SEE is the square root of the average squared deviation. The deviation of some estimate from intended values is given by standard error of estimate formula.

$$SEE = \sqrt{\frac{\Sigma\left(x_i - \bar{x}\right)}{n-2}}$$

Where x_i stands for data values, $\bar{x}$ is the mean value and n is the sample size.

HOW TO CALCULATE STANDARD ERROR:

- Note the number of measurements (n) and determine the sample mean (μ). It is the average of all the measurements.
- Determine how much each measurement varies from the mean.
- Square all the deviations determined in step 2 and add altogether: $\Sigma\left(x_i - \mu\right)^2$
- Divide the sum from step 3 by one less than the total number of measurements $\left(n-1\right)$. Take the square root of the obtained number, which is the standard deviation $\left(\sigma\right)$.
- Finally, divide the standard deviation obtained by the square root of the number of measurements (n) to get the standard error of your estimate.

STANDARD ERROR EXAMPLE:

CALCULATE THE STANDARD ERROR OF THE GIVEN DATA:

y: 5, 10, 12, 15, 20

SOLUTION:

First we have to find the mean of the given data;

Mean = (5+10+12+15+20)/5 = 62/5 = 10.5

Now, the standard deviation can be calculated as;

S = Summation of difference between each value of given data and the mean value/Number of values.

Hence,

$$S = \sqrt{\frac{(5-10.5)^2 + (10-10.5)^2 + (12-10.5)^2 + (15-10.5)^2 + (20-10.5)^2}{5}}$$

After solving the above equation, we get;

$S = 5.35$

Therefore, SE can be estimated with the formula;

$$SE = \frac{s}{\sqrt{n}}$$

$$SE = \frac{5.35}{\sqrt{5}} = 2.39$$

ANOVA - ONE-WAY CLASSIFICATION:

MEANING:

ANOVA (Analysis of Variance) is a statistical technique used to test whether there are any significant differences between the means of three or more groups. The one-way classification (or one-way ANOVA) is used when there is only one factor (independent variable) that affects a continuous response variable.

PURPOSE:

For determination whether the means of different groups (classified under one factor) are equal or significantly different.

EXAMPLE SITUATION

Suppose we want to test whether three different fertilizers (A, B, and C) produce the same mean yield of wheat.

Factor (Independent variable): Type of Fertilizer

Levels of factor: A, B, C

Response variable: Yield of wheat (quantitative)

ASSUMPTIONS:

1. The samples are independent.
2. Each sample is drawn from a normally distributed population.
3. The populations have equal variances (homoscedasticity).

MATHEMATICAL MODEL:

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where:

X_{ij} : observation for the j^{th} unit in the i^{th} group

μ : overall mean

α_i : effect of the i^{th} treatment (factor level)

ε_{ij} : random error component, assumed $N(0, \sigma^2)$

HYPOTHESES:

$$H_0 : \mu_1 = \mu_2 = \mu_3 = ...\mu_k$$
$$H_1 : At\ least \text{ one } \mu_i \text{ differs}$$

STEPS IN ONE-WAY ANOVA:

1. Calculate group means and overall mean.

2. Compute the Sum of Squares:

- Between Groups (SSB) = $\sum n_i \left(\bar{X}_i - \bar{X} \right)^2$

- Within Groups (SSW) = $\Sigma \sum n_i \left(\bar{X}_{ij} - \bar{X}_i \right)^2$

- Total Sum of Squares (SST) = SSB + SSW

3. Compute Mean Squares: MSB = SSB / (k - 1), MSW = SSW / (N - k)

4. Compute F-ratio: F = MSB / MSW

5. Decision Rule: Compare F calculated with F tabulated from F-distribution table.

- If F calculated > F tabulated → Reject H_0

- If F calculated < F tabulated → accept H_1

ANOVATABLE FORMAT:

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-ratio
Between Groups	SSB	k – 1	MSB = SSB / (k – 1)	F = MSB / MSW
Within Groups	SSW	N – k	MSW = SSW / (N – k)	-
Total	SST	N – 1	-	-

INTERPRETATION

If the F-test is significant, it means that at least one group mean differs.

EXAMPLE 1: FERTILIZER YIELDS

Three different fertilizers (A, B, and C) were tested to determine their effect on crop yield (in quintals per acre).

The data obtained are as follows:

Fertilizer A: 10, 12, 13

Fertilizer B: 9, 8, 10

Fertilizer C: 15, 14, 13

STEP 1:

Calculate Group Totals and Means

$$\Sigma A = 10 + 12 + 13 = 35 \rightarrow \bar{Y}A = 35/3 = 11.6667$$

$$\Sigma B = 9 + 8 + 10 = 27 \rightarrow \bar{Y}B = 27/3 = 9.0$$

$$\Sigma C = 15 + 14 + 13 = 42 \rightarrow \bar{Y}C = 42/3 = 14.0$$

$$\text{Overall Mean } (\bar{Y}) = 104/9 = 11.5556$$

STEP 2:

Between-Group Sum of Squares (SSB)

$$SSB = \Sigma\, n_i(\bar{Y}_i - \bar{Y})^2$$

$$= 3[(11.6667-11.5556)^2 + (9-11.5556)^2 + (14-11.5556)^2]$$

$$= 3(0.0123 + 6.5309 + 5.9753) = 37.5556$$

STEP 3:

Within-Group Sum of Squares (SSW)

Group A: $(10-11.667)^2 + (12-11.667)^2 + (13-11.667)^2 = 4.667$

Group B: $(9-9)^2 + (8-9)^2 + (10-9)^2 = 2$

Group C: $(15-14)^2 + (14-14)^2 + (13-14)^2 = 2$

SSW $= 4.667 + 2 + 2 = 8.667$

STEP 4:

Degrees of Freedom and Mean Squares

Between Groups: df = 2, MSB = 37.5556 / 2 = 18.7778

Within Groups: df = 6, MSW = 8.667 / 6 = 1.4444

STEP 5:

F-Ratio and Decision

$$F = MSB / MSW = 18.7778 / 1.4444 = 13.00$$

$$F(0.05, 2, 6) = 5.14 \rightarrow \text{Since } 13.00 > 5.14, \text{ Reject } H_0$$

Conclusion: There is a significant difference among the fertilizer means.

EXAMPLE 2:

Student Exam Scores (Three Teachers)

A principal wants to know whether there is a significant difference in the average marks of students taught by three different teachers: A, B, and C.

Teacher A: 78, 85, 82, 88

Teacher B: 72, 70, 68, 65

Teacher C: 90, 95, 88, 92

STEP 1:

Calculate Group Means and Overall Mean

$\bar{Y}A = 83.25$, $\bar{Y}B = 68.75$, $\bar{Y}C = 91.25$, Overall Mean $\bar{Y} = 81.0833$

STEP 2:

Between-Group Sum of Squares (SSB)

$$SSB = \Sigma\, n_i(\bar{Y}_i - \bar{Y})^2$$

$$= 4[(83.25-81.0833)^2 + (68.75-81.0833)^2 + (91.25-81.0833)^2]$$

$$= 4(4.6944 + 152.1111 + 103.1944) = 1040.6667$$

STEP 3:

Within-Group Sum of Squares (SSW)

Teacher A: $(78-83.25)^2 + (85-83.25)^2 + (82-83.25)^2 + (88-83.25)^2 = 54.75$

Teacher B: $(72-68.75)^2 + (70-68.75)^2 + (68-68.75)^2 + (65-68.75)^2 = 26.75$

Teacher C: $(90-91.25)^2 + (95-91.25)^2 + (88-91.25)^2 + (92-91.25)^2 = 26.75$

$$SSW = 54.75 + 26.75 + 26.75 = 108.25$$

STEP 4:

Mean Squares and F-Ratio

$$MSB = 1040.6667 / 2 = 520.3333$$

MSW = 108.25 / 9 = 12.0278

F = 520.3333 / 12.0278 = 43.26

STEP 5:

DECISION

F(0.05, 2, 9) = 4.26 → Since 43.26 > 4.26, Reject H_0

CONCLUSION:

There is a significant difference among the mean scores of students taught by the three teachers.