PREFACE

In the curricular structure introduced by this University for students of Post-Graduate Degree Programme, the opportunity to pursue Post-Graduate course in any subject introduced by this University is equally available to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post-Graduate level in different subjects are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing and devising of proper lay-out of the materials. Practically speaking, their role amounts to an involvement in 'invisible teaching'. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials, the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so that they may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great deal of these efforts is still experimental–in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

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POST GRADUATE ZOOLOGY

[M.Sc]

PAPER : GROUP
PGZO - 5 : A

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PGZO–5
Laboratory Course–5

Group A

Unit 1 ▫ Identification and analysis of common soil (terrestrial) and aquatic micro arthropods.

Structure

1.1 Terrestrial biota

Soil animals make a significant contribution to soil formation. The numbers of microorganism in the soil are diverse and produce a profound effect on the quality of the substratum. The soil community is highly variable in form and function and is of mainly three kinds on the basis of size.

a) **Microbiota :** These are small organisms, such as bacteria, algae and protozoa.

b) **Mesobiota :** These are medium sized animals like nematodes, small oligochaetes, insect larvae, and microarthropods.

c) **Macrobiota :** It includes earth worm, burrowing rodents and moles etc.

Soil animals may further be divided into burrowing forms and nonburrowing forms. Burrowing forms live within their own tunnels and non-burrowing forms inhabit existing crevices of the soil. Soil microarthropods may be burrowing and non-burrowing types.

1.2 Common micro arthropods as indicator of soil fertility

Extraction of soil micro arthropods :

Microarthropods of the soil are usually extracted by tullgren funnel technique. In this device heat is used to extract the animals from a given area of soil.

Some common microarthropods of the soil :

(A) Insecta :

a) ***Collembola (springtail) :*** Abdomen with six segments, usually with three sets of appendages; a retaining hook on segment 3 which functions with a powerful springing organ on fourth abdominal segment.

b) ***Isoptera (termite) :*** Contains a number of castes including winged fertile males and females, and wingless sterile workers and soldiers. Wings when present are long, similiar, membranous and capable of being shed at a basal fracture : Biting and chewing mouth parts. Tarsi 4 segmented.

c) ***Hymenoptera : (Ants) :*** Contains a member of castes including winged fertile males and dealated fertile females and wingless sterile workers and soldiers. Males with well developed genitalia and with smaller and rounded head. The fertile females possess large gaster and well developed reproductive organs. Workers and soldiers possess well developed mandible and small gaster. Junction between the thorax and abdomen is very much constricted.

d) ***Coleoptera (Beetles) :*** Four wings are modified into horny or leathery clytra : hind wings membranous. Biting mouth parts. Tarsi 3 to 5 segmented.

(B) **Mites :** Unsegmented body, usually avoid and flattened. Six pairs of appendages.

Chelicerae are chelate, pedipalpi are leg like with 5 joints or less. Generally four pairs of legs.

1.3 Aquatic biota

Animal forms found in aquatic habitats are most fascinating organisms because of their diversity and behaviour. Animals provide an important indication of the health of freshwater ecosystems because they integrate stresses and sensitive species are generally not found in polluted habitats. Food webs are a major pathway of energy flow through ecosystems and generally are dominated by animals.

Common microorganisms as indicator of water quality :

The most common organisms in all kinds of aquatic bodies are zooplanktons. Animals forms of relatively small size, mostly microscopic which have either relatively small power of locomotion or not at all and which drift in water subject to waves, currents and other forms of water motions are termed zooplankton.

(A) Phylum : *Rotifera*

Aquatic microscopic animals with the anterior end modified into a ciliary organ, the corona, Pharynx provided with internal jaws, with a pair of flame bulb protonephredia.

- **Key to classes of rotifera**

(1) a) Rotifera with paired generative organs.

b) Rotifera with single generative organ, males present but mostly reduced.

Hence, Class :Monogonata

(2) a) Marine, corona not with two trochal discs, reduced, males fully developed.

Class : seisonidea

b) Freshwater, corona with two trochal discs, latter rarely modified in some forms, males not known.

Class : Belelloidea

Class – *Monogonata*

Swimming or sessile Rotifera, with a single germovitellarium, males usually present, reduced with one testis, lateral antennce present, foot present or absent, when present, with two toes or without toes.

- **Key to orders of class Monogonata**

1. a) Free swimming, never fixed, foot, when present, with toes ..... order ploima.

   b) Adults rarely free - swimming, foot when present without toes.

   ..... order : 2.

2. a) Mastax malleoramate ..... order : Fiosculariacea

   b) Mastax uncinate order : Collothecacea.

Order – *Ploima*

Body shape vermiform, sacciform or dorsovertrally flattened; foot, with two toes, or reduced, even absent in some, eyes present or absent, when present one or two.

- **Key to the genera**

1) Free turing 2

   Parasitic, commensal or epizoic

2) With well-developed lorica in which foot and corona are retracted 3
   Without distinct lorica 9

3) Without foot . . . . . 4.

   With foot . . . . . 7.

4) With lorical spines 5

   With no lorical spines 6

5) Dorsal plate of lorica with polygonal facet pattern . . . . . genus *Keratella*.

7) Foot with toes, annulated, vermiform and retractile into lorica 8

   Foot without toes, annulated and distally ciliated, lorica dorsoventrally compressed and flat.

   . . . . . Genus *Testudinella*

8) Anterior ventral margin of lorica with central sinus, lorica with or without spines, eyes present foot forked

..... Genus *Brachionus*

9) In colonies or solitary, animals in cases ..... 10.

Solitary and no cases 11

11) Without foot 12

Foot present

12) With movable long spines or appendages ..... 13.

With no long spines or appendages, no intestine, with large body cavity, small stomach, transparent ..... *Genus Asplanchna*

13) Appendages not arm like and hollow ..... 14.

Appendages arm like, hollow with setae .... Genus *Hexartha*

14) Appendages as long setiform spines ..... Genus *Filinia*

Swordlike appendages as flattened paddles under anterior end ..... Genus *Polyarthra*

Family–*Brachionidae*

Heavily loricated, corona bearing strong cilia.

Genus — *Brachionus*

i) Heavily lonicate forms, lorica broad and covers the trunk completely, may be one piece when it continues around the body or two pieces united through flexible cuticle.

ii) Antero-dorsal edge of lorica always with even number of spines.

iii) Antero ventral edge rigid or flexible but may be wavy or smooth with 'V' or 'U' shaped notch.

iv) Pesterolateral spines present or absent depending upon the species and may seasonally appear or disappear even in the same species.

v) Posteromedian spines mostly present and flank the foot.

vi) Anterior portion of the body projects from lorica in the form of coronal disc which bears a circlet of cilia.

vii) Foot slender with two toes with no spine, highly contradile and projects from the posteroventral edge of lorica.

- **Description of different species**

(1) ***B. Calyciflorus***

a) Lorica Flexible, not seperated into dorsal and ventral plates.

b) Anterior dorsal margin with four broad based spines of variable length, medians longer than laterals.

c) Posterior spines present or absent.

d) Posterolateral spines usually absent.

(2) ***B. Caudatus***

a) Lorica firm with a pattern of cuticular ridges, divided into dorsal and ventral plates.

b) Anterodorsal margin with 2 median spines separated by 'v' on 'U' shaped notch.

c) Laterals mostly longer than medians, inter-mediates

d) Posterolateral spines well developed.

(3) ***B. falcatus***

a) Lorica firm, composed of dorsal and ventral plates.

b) Anterodorsal margin with 6 spines, intermediate spines considerably longer than laterals and medians curving ventrally towards the head of the animal, medians mostly equal to laterals but may be smaller.

c) Posterior spines widely separated basally, long, their width much more than anterior spines, parallel on bow outwards and then converge completing a full arch.

(4) ***B. forficula***

a) Lorica firm, divided into dorsal and ventral plates.

b) Occipital margin with 4 spines, laterals always longer than medians, no intermediate spine, occipital spines rounded at tips, rarely pointed.

c) Lorica terminates posteriorly in two stout, long and subsquare spines, widely separated basally and tapering to blunt points.

(5) ***B. angularis***

a) Lorica firm, divided into dorsal and ventral plates, dorsal plate with pattern of cuticular ridges.

b) Anterodorsal margin with two median spines flanking a V shaped notch, lateral and intermediate spine usually obliterated.

c) Mental margin rigid, somewhat elevated with a shallow median notch.

d) Foot aperture in ventral plate flanked by cuticular protruberances, no posterior spines.

(6) ***B. rubeus***

a) Lorica firm, a notch on the posterior side.

b) Anterior dorsal margin with 6 spines, medium longest, intermediates somewhat longer than laterals, medians and intermediates asymmetrical.

c) Posterior spines absent.

Genus – *Keratella*

i) Lorica composed of dorsal and ventral plates, dorsal plate convex, sculptured with varying pattern for different species. ventral plate flat or slightly concave.

ii) Both plants of lorica usually covered with fine areolate networks and postulated.

iii) One or two posterior spines often present, when single usually median in position.

iv) Foot wanting.

Family : *Asplanchnidae* : Illoricate sacciform body with a large body cairty.

Genus — *Asplanchma*

i) Large transparent form with sacciform body, greatly contractile, without lorica.

ii) Foot on any other extensions of cuticle wanting.

iii) Corona reduced to a thin course of cilia around the head.

iv) Often viviparous with one or several embryos.

Family : *Synchaetidae* : Body in some with flattened cuticular appendages, corona with several prominences, each bearing setae or a long pencil of cilia.

Genus — *Polyarthra*

i) Body more or less oval on subsquare with flattened cuticular appendages attached in 4 groups to dorsolateral and ventro– lateral surfaces near anterior end.

Family : *Testudinellidae :* Body cylindrical, circular or oval; foot usually absent but present in some and retractile.

Genus – *Filinia*

i) Lorica thin, flexible, fusiform, barrel shaped or cup shaped, appendages long.

ii) Two long anterolateral spines and one lone posterior spine, may be terminal or lateral.

iii) Foot wanting.

(B) Phyhum : *Arthropoda*

Body covered by an exoskeleton of chitin and protein; Body segments carry paired jointed appendages.

- **Key to sub-phylum**

Subphylum – Crustacea - Head with 2 pairs of antennae, mostly aquatic arthropods.

Sub-phylum – Chelicerata - Antennae absent

Sub-phylum – Uniramia - Head with one pair of antennae, mostly terrestrial.

- **Key to classes of crustacea**

Freshwater or marine forms with leaflike. setose appendages.
— Class Branchiopoda

Entire body is enclosed within a bivalved carapace; marine or freshwater forms.
— Class Ostracoda

Very small marine and freshwater forms having a cylindrical tapered body with long. first antennae — Class - Copepoda

Marine sessile crustaceans in which the body is enclosed within a bivalved carapace that is typically covered with calcoreous plates
— Class - cirripedia

Trunks composed of eight segmented thorax on which legs are located and a six-segmented abdomen. — Class - Malacostraca

- **Key to orders of branchiopoda**

1. (a) Carapace absent, eyes stalked – Order Anostraca

   (b) Carapace present ; eye or eyes sessile — (2)

2. (a) Single eye and an ocellus, 4 - 6 thoracic appendages – order cladocera

   (b) Two eyes – Order Notostraca, Conchrostraca

- **Description of cladoceran species :**

Superfamily : *Daphnoidea :* Five or six pairs of dissimilar legs (first and second pairs more or less prehensile, others leaf - like).

Family – *Daphnidae*

Antennae with 3 or 4 Jointed rami, post-abdomen distinctly demarcated from body, usually more or less compressed, always with anal spines, eyes large, ocellus usually small, sometimes wanting.

- **Key to genera**

1. Presence of beak — 2
   Absence of beak — 3

2. Presence of postanal spine – Genus *Daphnia*
   Absence of Postanal spine – Genus *Ceriodaphnia*

3. Absence of postanal spine
   Presence of supraocular depression – Genus *Moina*

- **Description of genera :**

Genus – *Daphnia*

Form oval or elliptical, dorsal and ventral margins rounding over towards each other posteriorly, posterior shell spine present, dorsal and ventral edges of the shell equipped with spinules, antennules small or rudimentary, placed behind rostrum, fixed; in males, head large with rostrum, antennules long & movable.

Genus – *Ceriodaphnia*

General form more or less oval, small rarely exceeding 1 mm, head small and depressed with or without spine, antennule small, not freely movable, eye large, nearly filling the head, ocellus prominent reduced, dorsal shell margin more or less straight. Ventral shell margin circular, and insignificant acute shell spine occurs at the junction of dorsal and ventral shell margins.

Genus – *Moina*

Head and body rounded and transparent. The head is without a rostrum, large and bent downwards. The antennules are long and movable which arise from the ventral surface of the head. Most species have a depression above the eye known as supraocular depression. The post abdomen invariably has a bident tooth and a number of ciliated or feathered structures. The abdominal setae is long.

Class – *Copepoda*

The freeliving copepods are separable into three distinct groups : calanoida, cyclopoida, and Harpacticoida.

Copepods are characterized by :

1. Body consists of the anterior metasome (Cephalothorax), which is divided into the head region, bearing five pairs of appendages, representing antennae and mouth parts, and the thorax, with six pairs of mainly swimming legs.

2. The posterior urosome consists of abdominal segments, the first of which is modified in females as the genital segment, and terminal caudal rami bearing setae.

Calanoida

1. Anterior part of body much broader than posterior.

2. Marked constriction between somite of 5th leg and genital segment.
3. One egg sac, carried medially.
4. First antennae long, extend from end of metasome to near end of caudal setae, 23-25 segments in female.
5. Fifth leg similar to other legs.
6. Planktonic, rarely littoral.

Cyclopoida

1. Anterior part of body much broader than posterior.
2. Market constriction between somites of fourth and fifth legs.
3. Two egg sacs, carried laterally.
4. First antennae short, extend from proximal third of head segment to near end of metasome, 6 - 17 segments in female.
5. Fifth leg vestigial
6. Littoral, a few species planktonic.

Harpacticoida

1. Anterior part of body a little broader than posterior.
2. Slight or no constriction between somites of fourth and fifth legs.
3. Usually one egg sac, carried medially
4. First antennae very short, extend from proximal fifth to end of head segment, 5-9 segments in female.
5. Fifth leg vestigial.
6. Exclusively littoral, on macrovegetation and sediments.

Unit 2 ▫ Estimation of gross and net primary productivity (GPP & NPP) of an aquatic system by using light and dark bottle technique

Principle : This is based on the estimation of oxygen (O_2) released by the producers over a period of time. O_2 produced is simultaneously used up in respiration. Photosynthesis depends on light which varies with the time of the day, clarity or transparency of the water and also with the concentration of the chlorophyll type of plant organisms.

This method consists of taking the water sample containing natural plankton populations in a glass bottle and exposing the bottle (light bottle - LB) to light in the euphotic zone. In a parallel experiment, a portion of the initial sample is held in a dark bottle (DB) for the same length of time and same temperature as the light bottle sample. The initial oxygen content (IB) of the sample is determined by modified Winkler Method. Difference between this concentration and the concentration found from freshwater in the light bottle after a suitable period of exposure (L. B.) is calculated. (LB - IB) is a measure of the net evolution of O_2 due to photosynthesis. This is not necessarily equal to true net photosynthesis of the plant enclosed in the LB as O_2 may have been consumed by respiration of the plant cell proper. It is more common to the dark and light bottle technique to measure gross photosynthesis. This is done by finding the difference betwen initial oxygen content of IB of water and O_2 remaining in the DB, that is (IB - DB). Such a difference is assumed to be equal to the total respiration occurring in the light bottle over the same period of time and thus if added to the net value obtained from LB - IB above, it gives a measure of gross photosynthesis from the relationship.

Procedure : Light and dark bottles (150 ml capacity) are fixed in the morning into the studied eaberbody at surface. At this point, initial bottle oxygen is measured. Analysis of oxygen is done from LB and DB after removing them out of the pond following a time exposure (say 4 hours). The following prodedure is followed for O_2 measurements :

i) Sample bottle stopper is removed carefully. 1 ml of manganous sulphate and 1 ml of alkaline iodide reagents are added into each of the bottles.

ii) 1 minute time is allowed for precipitation. The stoppers are replaced and each bottle is inverted 3-4 times for thorough mixing of reagents. A precipitate is formed.

iii) 1 ml of cocentrated H_2SO_4 is added to each of the bottles. The bottles are agitated well to dissolved the precipitate.

iv) The total volume of the water in each of the 3 bottles are measured by a measuring cylinder.

v) 50 ml of water sample from each bottle is transferred to a conical flask and placed against a white background. 0.025 (N) Na_2SO_3 is added dropwise till the colour turns pale yellow.

3-4 drops of 1% starch solution is added to give a blue colour and the titration is terminated when the solution turns colourless.

vi) The value of titrant required for titration is noted. Three observations are usually taken for each bottle for mean result.

Result : The results are represented in tabular form as shown below —

Initial bottle				Light bottle				Dark bottle			
V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4	V_1	V_2	V_3	V_4
Mean				Mean				Mean			

$$\text{Dissolved oxygen (mgL}^{-}) = \frac{V_1 \times N \times 8 \times 1000}{V_4 (V_2 - V_3) / V_2}$$

When V_1 = Volume of titrant required,

V_2 = Volume of sample water after placing stopper.

V_3 = Volume of alkaline iodide & manganous sulphate added.

V_4 = Volume of sample water used for titration.

IB, LB and DB oxygen concentration is thus calculated.

Calculations : $\text{Gross primary productivity} = \frac{LB - DB \times 12 \times 1000}{T \times 32}$ mg c/m^3 / hr

$$\text{net primary productivity} = \frac{LB - IB}{T} \times \frac{12}{32} \times 1000 \text{ mgc/m}^3 / \text{hr}$$

Where IB = O_2 content in initial bottle

DB = O_2 content in dark bottle

LB = O_2 content in light bottle

T = Tune (hrs) of incubation period.

1,000 = Conversion factor to change litres to cubic metres

$$\frac{12}{32} = \frac{\text{atomic weight of carbon}}{\text{molecular weight of oxygen}}$$

We use the factor $\frac{12}{32} = 0.375$ to convert O_2 to carbon.

1 molecule of O_2 (32g) is released for each molecule of carbon (12 gm) that is fixed.

General ranges of primary productivity of phytoplankton and different trophic categoreis (Revised by WETZEL, 1983 in 'Limnology')

Trophic type		**Mean NPP (mg c/m²/day)**
Ultra oligotrophic	→	< 50
Oligotrophic	→	50 – 300
Mesotrophic	→	250 – 1,000
Eutrophic	→	>1,000
Dystrophic	→	<50 – 500

Comments : Give your own comments.

Unit 3 ▫ Estimation of dissolved oxygen, dissolved CO_2, alkalinity and hardness of water bodies

Structure

3.1 Estimation of dissolved oxygen in water by modified Winkler Iodometric method

Principle : Manganous sulphate reacts with KOH or NaoH to give a white precipitation of white manganous hydroxide. In presence of oxygen brown manganic basic oxide is formed. Addition of H_2SO_4 dissolves the brown manganic oxide yielding manganic sulphate which reacts instantly with iodide to yield iodine. Iodine is then determined by sodium thiosulphate with 1% starch solution as an end point indicator.

$2KOH + MnSo_4 \rightleftharpoons K_2SO_4 + Mn(OH)_2$ (white ppt.)

The dissolved oxygen of the sample reacts with $Mn(OH)_2$ forming a brown precipitate of $MnO(OH)_2$

$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2$

With the addition of H_2SO_4, $Mn(SO_4)_2$ is produced.

$MnO(OH)_2 + 2H_2SO_2 \rightleftharpoons Mn(SO_4)_2 + 3H_2O$, $Mn(SO_4)_2 + 2KI \rightleftharpoons Mn\ SO_4 + 2K_2SO_4 + I_2$

The quantity of Iodine liberated is equivalent to the quantity of O_2 present in the sample. The quantity of iodine is determined by titration with a standard $Na_2S_2O_3$ solution using starch as indicator;

$2\ Na_2S_2O_3 + I_2 \rightleftharpoons Na_2S_4O_6 + 2\ NaI$

Reagents : i) Alkaline iodide

ii) Manganous sulphate

iii) Concentrated sulphuric Acid

iv) Starch solution (1%)

Prodecure : i) In a narrow mouthed bottle (for stopping mixing of surface oxygen into the bottle content), the subsurface water is collected preferably at or before 8 A.M.

ii) Collected sample is fixed immediately & taken to the laboratory for analysis.

iii) Carefully the stopper of the sampling bottle is removed.

iv) 1 ml of manganous sulphate & 1 ml of alkaline iodide reagents are added by means of a pippette dipped into the sample bottle.

v) One minute is allowed for precipitation.

vi) The stopper is replaced & the sample bottle is inverted 3 or 4 times for a thorough mixing of the reagents.

vii) A precipitation is formed which settle at the bottom (if the ppt is whitish in color oxygen content is poor; light brown color indicates less oxygen while brown to red brown color means medium to high amount of dissolved oxygen.)

viii) For quantitative estimation 1 ml of conc. H_2SO_4 is added & the bottle is shaken well to dissolved the ppt.

ix) 50 ml of the solution is transferred to a conical flask placed against a white background.

x) 0.025 N sodium thiosulphate is added drop by drop till the colour turns into pale yellow.

xi) Then 3-4 drops of 1% starch solution is added to give a blue colour & the .itration is terminated by turning this solution into colourless one.

Result : The results are represented in tabular form.

No. of observation	Vol. of Sample used for titration	Burette reading (ml)		Difference in burette readins (ml)	Mean (ml)
		Initial	Final		
1					
2					
3					

Calculations : The amount of dissolved oxygen is calculated by the following formula :-

$$\text{Oxygen (ml/L) or ppm} = \frac{V_1 \times N \times 8 \times 100}{\frac{V_4 (V_2 - V_3)}{V_2}}$$

Where, V_1 = volume of titrant (ml) ($Na_2S_2O_3$)

N = normality of titrant (0.025) ($Na_2S_2O_3$)

V_2 = Vol of sample bottle after placing stopper (ml).

V_3 = Vol. of manganese sulphate & concentrated H_2SO_4 added (ml).

V_4 = Vol. of fraction of the sample water as per titration (ml).

8 = Equivalent wt. of oxygen.

Significance : Dissolved oxygen is one of the most important parameters in water quality assessment & reflects the physical and biological processes prevailing in water.

i) It is a measure of one of the important environmental factors affecting the aquatic life & the capacity of water to receive organic matter without causing hazards.

ii) Little dissolved oxygen values indicated a very high organic pollution.

iii) Almost all plants & animals need oxygen for respiration. So, dissolved oxygen gives an idea of total plants present in water, also it helps to evaluate the gross production of water bodies.

iv) Dissolved oxygen value also helps to find out the BOD values indicating the pollution condition in water.

v) The concentration of O_2 will also reflect whether the process undergoing are aerobic or anaerobic. Low O_2 concentration are usually associated with heavy contamination of organic matter. In such conditions oxygen sometimes totally disappear from the water.

vi) The level of sufficient oxygen concentration is a limiting factor in the distribution of aquatic organisms.

vii) The permissible level of dissolved oxygen in tropical climate is 5 ppm.

Comment : Give your own comments.

3.2. Estimation of dissolved free CO_2 in a water sample

Principle : Free carbondioxide can be determined by titrating the sample using a strong alkali (NaOH) at PH 8.3. At this pH all the free CO_2 is converted into bicarbonates. The phenolpthalein indicator turns into faint pink. Amount of alkali needed to produce the pink colour indicates the amount of free CO_2 in the sample solution.

Reagents : i) N/44 sodium hydroxide

ii) Phenolphthalein

Procedure : i) Water samples are collected in different ways for different times & analysed. For dissolved gasses, bubbling or mixing with air or other gasses are avoided. A kemmer's or friedinger water sample may be used. Water may be collected in a large beaker or in a plastic bucket & transferred to a sampling bottle by a siphon tube. The sample for dissolved CO_2 should be fixed immediately after collection because CO_2 is liable to escape easily from the sample.

ii) 50 ml of water sample is taken in a conical flask or in a Nessler's tube.

iii) A few drop (3-4) of phenophthalein indicator are added to the sample.

iv) The flask is placed against a white background.

v) If the colour turns pink free CO_2 is absent.

v) If the sample remains colorless titrate it against N/44 NaOH. At the end point a faint pink color appears.

vii) The end point reading of burette is noted.

Precaution : Since atmospheric CO_2 is readily soluble in H_2O, methods of determing the amount of free CO_2 is always subjected to more or less 10% error. The degree of accuracy of the result increases if the following precautions are taken.

i) While collecting the sample care should be taken to avoid contact of sample with air. This can be achieved by collecting the sample from surface water by opening the stopper of empty sample bottle.

ii) The sample should not be agitated.

iii) The surface of water sample exposed to air during titration should be kept as small as possible.

iv) The sample should be stirred gently & not agitated during titration.

Result : In tabular form

Calculations : Free CO_2 (ppm) = $\dfrac{\text{ml of NaOH} \times \text{(N) of NAOH} \times 1000 \times 44}{\text{Volume of sample taken for titration}}$

Significance :

i) Dissolved CO_2 is a measure of one of the important environmental factors affecting aquatic life. Higher concentration of CO_2 have inhibitory effect on plants and animals.

ii) CO_2 signify the rate of decomposition of organic matters and the respiratory activity of aquatic plants and animals.

iii) Dissolved CO_2 is inversely related with the pit value of water as when CO_2 dissolves in water, carbonic acid is formed and pH is lowered.

iv) CO_2 is one of the most essential raw materials, necessary for photosynthesis of green plants. Thus productivity of a water system can be measured.

v) The pH of the blood as well as O_2 carrying capacity of vertebrate haemoglobin and the respiratory pigment of invertebrates are affected with increase of CO_2 concentration.

Comment : For pisciculture more than 15 ppm dissolved CO_2 is harmful to culture operation and sometime cause even mortality of fish. (Please justify your result)

3.3 Estimation of alkalinity of water sample

Introduction : In water analysis generally 3 types of alkalinities are differentiated — carbonate, bicarbonate and hydroxide alkalinity. These are determined by using two separate indicators phenolphthalein and methyl orange. For all practical purpases methyl orange alkalinity, known as 'MOA', gives a measure of the acid combining capacity of water.

Principle : The amount of acid required to titrate the bases of the given water sample is a measure of alkalinity. Bicarbonate, carbonate and hydroxides are considered to be the chief bases of natural water. Water sample containing bases turn yellow by the addition of methyl orange indicator.

Reagents : (i) Methyl orange indicator.

(ii) 0.02 (N) H_2SO_4

Procedure :

i) 50 ml of sample is taken in a conical flask and placed against a white background.

ii) 2-3 drops of methyl orange indicator is added to it.

iii) The sample turns yellow.

iv) It is then titrated with 0.02(N) H_2SO_4 from a burette. The end point is indicated by a joint orange colour.

v) The end point is recorded.

Results : In tabular form.

Calculation : Methyl orange alkalinity —

$$(MOA)\ mgL^{-1} = \frac{\text{volume of titrant} \times N \times 50 \times 1000}{\text{volume of the sample}}$$

Significance :

i) Alkalinity of water is its acid nutralizing capacity and it is the sum of all the titrable bases.

ii) Alkalinity is significant of treatment of neutral water and waste water.

iii) The value gives an idea about the productivity of water.

iv) Alkalinity value correlate with the pH value of water.

v) Alkaline water are generally known to show high biological productivity.

vi) The presence of $CaCO_3$ improves aeration and permeability indirectly by increasing the particle size of the soil due to the precipitation of different doses.

Comments : Give your own comments

3.4. Estimation of total hardness of water

Principle : Hardness of water is caused mainly by calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions. The ions are generally present in water as sulphates, chlorides and bicarbonated. In most natural water hardness is entirely due to bicarbonates mainly calcium bicarbonates ($CaHCO_2$) to a lesser degree magnesium bicarbonates.

Hardness caused by bicarbonates is called carbonate hardness. It is also called temporary hardness, since it can be removed by heating. Hardness caused by sulphates, chlorides of calcium and magnesium is called permanent hardness. The sum of these two types is collectively termed as total hardness of water. Hardness values like that of alkalinity and acidity are expressed in ppm of $CaCO_3$. The following table shows the commonly acceptable standard for degree of hardness in ppm of $CaCO_3$

Reagents :

1. Standard calcium carbonate ($CaCO_3$) solution.
2. Buffer solution.
3. Eriochrome black T- indicator solution.
4. EDTA solution.

Experimental procedure :

i) 50 ml of sample is taken in a conical flask.

ii) 0.5 ml of buffer solution is added to the sample and mixed thoroughly.

iii) 1-2 drops of indicator solution is mixed and it is then mixed well.

iv) In presence of calcium or magnesium the solution will become red.

v) If the water is exceptionally hard, the indicator should be added before the introduction of buffer solution.

vi) The solution is titrated immediately with EDTA solution.

vii) The end point is indicated by a colour change from red to blue.

viii) The end point is gradual & not sudden or sharp.

The blue colour develops before the end point, but the reddish tinge could still be seen. The end point is determined by complete disappearance of this reddish tinge.

Results : In tabular form

Calculations :

$$\text{Total hardness (ppm)} = \frac{\text{ml of EDTA solution} \times 1000}{\text{ml of water sample taken}}$$

Significance : Excess hardness of water is undesirable in pisciculture ponds. The problems are basically for the presence of Ca^{2+} and Mg^{2+} ions in water, which reacts with different manures and toxicants to reduce their action.

Comments : Give your own comments.

Unit 4 ▫ Measurement of soil pH and organic carbon

Structure

4.1 Soil pH

4.2 Organic carbon

4.1 Soil pH

A. Electrometric method : This method gives direct reading and because of its accuracy and rapidity it is considered the best.

Procedure : Take 10 gm of soil in a 50 ml beaker and add 25 ml of glass distilled water (soil : water ratio as 1 : 2.5). The suspension is stirred at regular intervals for 20 minutes. Now the pH meter is set, electrodes are immersed into the samples and the pH is determined. This pH meters are mostly direct readings recording pH in 1 unit interval.

B. Colorimetric method : Colorimetric indicators are most useful for field testing kit and for soil testing laboratories. Though approximate, they give satisfactory results if properly and carefully used.

Reagents : (1) Neutral barium sulphate A. R. Grade

(2) Indicator solutions; viz,

Bromophenol blue	—	3.0 – 4.6	pH range
Bromo cresol green	—	3.8 – 5.4	„
Bromo cresol purple	—	5.2 – 6.8	„
Bromo thymol blue	—	6.0 – 7.6	„
Phenol red	—	6.8 – 8.4	„
Cresol red	—	7.2 – 8.8	„
Thymol blue	—	8.0 – 9.6	„

Procedure : Place a layer of neutral barium sulphate 1 cm thick in a 50 ml dry test tube, add 10 g of air dried powdered soil and 0.25 ml of distilled water. Shake well for 10 minutes and keep if for settling. Take 10 ml of clear aliquot in a small clear glass tube and add 0.5 ml of indicator. To know which of the above indicators is to be used, a preparatory test with a universal indicator may be done which gives a very approximate value of the pH; otherwise phenol red would be used first and then, if necessary, indicators of higher or lower ranges. After adding the indicator to the sample,

it is stirred gently and the colour developed is matched against colour discs in a comparator or standard colour charts.

4.2 Organic carbon

Organic carbon can be determined very rapidly with fair accuracy by the method described below :

Reagents :

a) Normal Potassium dichreomate sollution : Exactly 49.04 g of reagent grade $K_2Cr_2O_7$ is dissolved in distilled water and the solution is diluted to one litre.

b) Normal Ferrous solution : Dissolve 278.0 g of reagent grade $FeSO_4$, $7H_2O$ or 392.13 g of $FeSO_4$, $(NH_4)_2SO_4$. $6H_2O$ in distilled water. Add 15 ml of conc. H_2SO_4 and make up the volume to one litre.This should be standardized against N $K_2Cr_2O_7$ so that 1 ml of Ferrous solution = 1 ml N $K_2Cr_2O_7$ Solution.

c) Diphenyl amine indicator : Dissolve 0.5 g of diphenyl amine in 10 ml conc. H_2SO_4 and 20 ml distilled water.

d) 85% Phosphoric acid.

e) Conc. H_2SO_4 (sp.gr. 1.84)

Procedure : Place 1 g of soil sample in a 500 ml conical flask. Add exactly 10 ml of N $K_2Cr_2O_7$ and mix the two by swirling the flask. Then add 20 ml of conc. H_2SO_4 and mix by gentle rotation for one minute. Allow the mixture to stand for 30 minutes. Dilute with water to 200 ml and add 10 ml of 85% phosphoric acid. The excess dichromate is titrated with N $FeSO_4$ solution using 1 ml diphenyl amine as indicator.

(10 – No. of ml of $FeSO_4$ soln. required) × 0.003 × 100 = organic carbon (%)

Unit 5 ▫ Toxicity tests – LC_{50}/LD_{50} determination

Procedure :

A) For a toxic chemical or material of unknown toxicity, conduct short term (24 h/48 h) range finding best to determine approximate concentration range to be used in definitive tests.

B) Expose test organism to a wide range of concentrations of the test substance, usually in a logarithmic ratio, such as 0.01, 0.1, 1, 10 and 100% of the sample. Attempt to find and the concentrations that killed no or only a few test organism and the lowest concentration that killed most or all test organisms.

C) Conduct short term definitive test with such concentrations. For this, select a geometrically spaced series of concentrations between the highest concentration that killed no or only a few and the lowest concentration that killed most or all test organisms. The bests may be static, renewal or flow through depending upon the choice of test organism. Exposure periods usually are 48 h or 96 h. Test duration is determind by the toxicant and the test objectives.

D) Record the concentration which kills 50% of the test organisms in the experiment within the specified time period LC_{50}/LD_{50} is the incipient dose of the toxic chemical for 50% mortality or an estimate of the true median lethal concentration/dose of the test material for the entire test species.

E) Provide a measure of statistical confidence in the point estimate. values other than 50% can be used to characterize toxicity.

F) To analyze LC_{50}/LD_{50} data, parametric procedures such as probit analysis, Logit and generalized linear models (GLM) are available. The probit method is the most widely used LC_{50}/LD_{50} calculation procedure and uses the probit transformation of mortality data in combination with a standard-curve-fitting technique.

However, single chemical toxicity tests can be useful with described range finding tests and the short term definitive test. Record results in tabular or graphical form.

NETAJI SUBHAS OPEN UNIVERSITY

STUDY MATERIAL

POST GRADUATE ZOOLOGY

PAPER - 5
GROUP : B

Laboratory Course - III

PREFACE

In the curricular structure introduced by this University for students of Post Graduate degree programme, the opportunity to pursue Post Graduate course in Subjects introduced by this University is equally available to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post Graduate level in different subjects are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing and devising of a proper lay-out of the materials. Practically speaking, their role amounts to an involvement in invisible teaching. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so that it may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great part of these efforts is still experimental—in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

Professor (Dr.) Subha Sankar Sarkar

Vice-Chancellor

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POST GRADUATE ZOOLOGY

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Group B

UNIT 1 ❐ HANDLING OF DROSOPHILA, CHROMOSOME MAPPING, GENETIC CROSSES

Structure

1.0 INTRODUCTION

Drosophila melanogaster is commonly referred to as the "fruit fly" because it is so frequently found associated with over ripe fruit such as banana, apple etc. The optimal temperature for breeding *Drosophila melanogaster* is between about 15 and 30°C; the "standard" laboratory temperature is 23-25°C. Several species of *Drosophila* other than *melanogaster* have been used in research, and in recent years, each of the other species has come to occupy a particular research niche. Some are favoured for ecological studies, some for evolution, and some for population biology, but for studies

of genetics, development, and molecular biology, *Drosophila melanogaster* is unequaled. Its primary advantage lies in the reliability of its growth in the laboratory– it is small and grows on simple media, so it is cheap and takes up little space; in addition, it has a short generation time of less than two 'weeks at 25°C and a single female will produce hundreds of offspring during her lifetime. These features make it possible to generate adequate numbers for genetic studies. One can even obtain tile huge numbers of genetically homogeneous individuals at defined stages of their life cycle required for biochemical studies.

Here we will use *Drosophila* to illustrate some of the basic principles of inheritance. Genes, chromosomes, and meiosis are so fundamental to eukaryotic life that the principles we will be observing in tile fruit fly can be directly applied to most eukaryotes, including humans. In order to get the most out of these exercises, it will be important to apply the principles learned in class to the inheritance of the phenotypes we will be following in *Drosophila*.

1.1 LIFE CYCLE OF DROSOPHILA

Drosophila is a holometabolous dipteran insects with two wings. Holometabolous refers to the fact that when it goes through metamorphosis it changes completely in form; butterflies and moths are also holometabolous. An understanding of the life cycle will help us to follow the events occurring in the fly cultures. There are four stages: the embryo, the larva, the pupa, and the adult. The duration of each of these stages is well established, and is generally given for incubation at 25°C unless otherwise indicated. If we consider the moment at which the egg is laid as "time 0", embryonic development lasts for 22 hours. Development begins when the egg is fertilized, but fertilization does not necessarily occur immediately after copulation. The female will store sperm from a single copulation and use it for all her ovulations if necessary. If the female perceives conditions to be optimal, will lay the egg immediately after fertilization. If, however, the temperature is too low, there is no suitable site for laying, or conditions are sub optimal for some other reason, the female will hold the, eggs and development will begin in utero. (Fig. 1, 2 & 3)

The egg has a few characteristics that are visible to the naked eye. It is about 0.4 mm long, oblong, and slightly flatter on its dorsal side than on its ventral side. It has two filaments coming out of the dorsal surface very near the anterior end that are used for gas exchange. At even low power magnification, a tough outer membrane, the chorion, can be seen to be imprinted with a hexagonal cellular pattern. Beneath the chorion is a thin layer of air, then a tough vitelline membrane surrounding the

plasma membrane of the egg itself. At low power, a small protrusion called the micropile can be seen at the anterior end - this is the tube through which the sperm

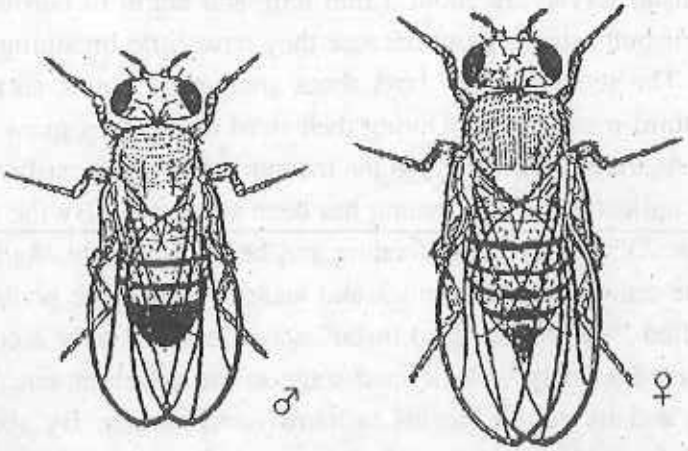


Fig. 1 : Adult *Drosophila* (Male & Female)

enters, After 22 hours of development, the larva emerges from the egg. There are three larval stages called instars separated by moults when the larva sheds its cuticle. Larvae spend their time only by eating; the amount they eat is the major determinant of their size as adults. The first instar larva is about 1 mm long, and stays on the

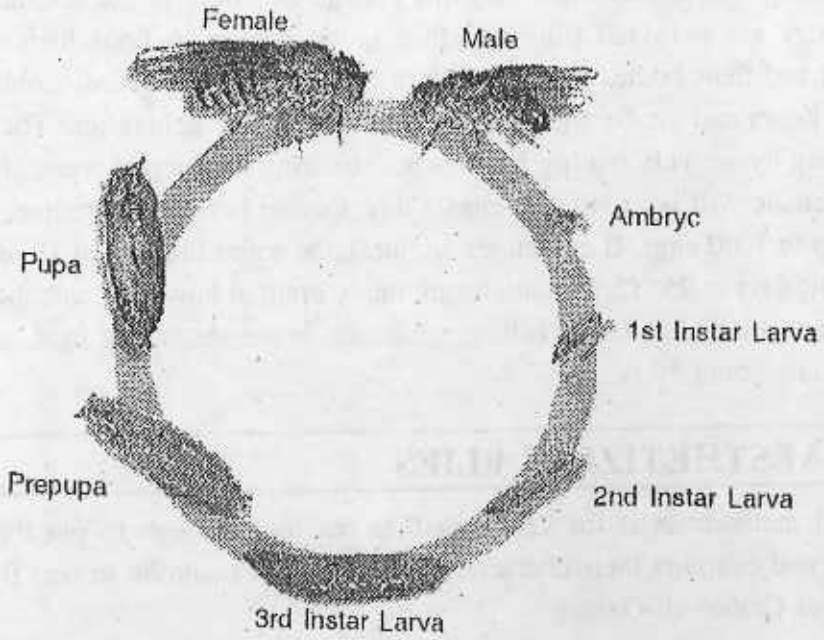


Fig. 2 : Life Cycle of *Drosophila melanogaster*

surface of the medium. The first instar lasts for about 24 hours, so at about 2 days after egg laying it molts to a second instar larva.

The second instar larvae are about 2 mm long and begin to burow into the food, often leaving their butts sticking out because they have little breathing tubes on back, called spiracles. The second instar' lasts about another 24 hours, so at about 3 days they moult to a third instal larvae. During their third instar, they grow to more than 4 mm, if well fed. As they burrow through the medium, they leave easily visible channels that are an early indicator that the mating has been successful. By the end of the fifth day (110 hours at 25°C) they cease feeding and begin to pupate. As they prepare to pupate, the larvae crawl out of the muck and wander around the walls of the culture tube. This so-called "wandering third instar" stage lasts for only a couple of hours, and so is considered a fairly well defined stage in the development. The larva then stops wandering and its cuticle begins to harden and darken. By about 122 hours, the pupa is fully formed and have a dark brown colour; pupation takes about 4.5 days. There is little to be seen during this remarkable stage from outside. However, inside pupal case, inost of the cells that made up the larva are auto-digested and a whole new animal is rebuilt from special sets of cells called imaginal disks. At the end of pupation, the adult emerges from the pupa by splitting it lengthwise and crawling out; this process is called "eclosion". Part of this process involves blowing their heads and bodies up with air, so when they first emerge they have big heads and abdomens. Their wings are wrinkled little wet things, but within an hour their wings have expanded and their bodies develop mature colouration. The sexual maturation takes about 12 hours and the fly then ready to initiate the new generation. The production of offspring by actively mating couples peaks during the second week after ecosion, and the female will lay upto 100 eggs a day. During her entire lifetime, one female can lay up to 1000 eggs. If conditions are ideal, the entire lifespan of *D. melanogaster* is about 50 days at 25° C. Conditions are rarely optimal however, and about a month is more common under normal culture conditions. In the absence of food, an individual will last only about 50 hours.

1.2 ANAESTHETIZING FLIES

Several methods exist for knocking flies out long enough to put them under a binocular and examine their characteristics. The most common in real fly labs is to use ether or Carbon-di-Oxide.

1.2.1 Procedure :

Take a food vial with adults in it, tap it lightly but firmly on the table top a couple times to knock the flies toward the bottom. Then in one smooth motion (by practice!) remove the cotton plug and invert the vial over a second empty vial through a small funnel the base of which is wrapped with cotton where a few drops of ether is applied. Let this setup sit there for 2-5 minutes – watch the flies —when they are all down at the bottom and stand still. Tap them out onto a 3×5 card and place them under the binocular. The flies will stay out for at least 10 minutes, during which time we can sex them, pick virgins, and score phenotypes.

1.2.2 Preparation of Culture medium :

For the preparation of culture medium the following ingredients are used :

Water — 500 ml

Agar powder — 4.1 gm

Maize powder — 45 gm

Brown Sugar — 45 gm

Dried yeast — 13 gm

Nipagin (antifungal) — 500 mg [Na methyl r hydroxybenzoate]

Propionic acid — 0.5 ml

The following procedure may be followed for the preparation of culture medium :

1. Agar in cold water dissolved by boiling.
2. Add maize powder & brown sugar in water.
3. Mix them and stir continuously to avoid clumping. Boil it for 20 minutes.
4. Add dried yeast powder and boil the mixture for another 10 minutes.
5. Add 0.5 ml propionic acid to the mixture & mix it thoroughly.
6. Dissolve nipagin in ethanol and add it to the mixture.
7. Continue boiling until the mixture become viscous.
8. Pour the mixture allow it to settle & plug it with non absorbent cotten.

1.3 SEXING FLIES

A number of sexually dimorphic characters exist in Drosophila, but we will rely on a couple simple ones.

1. The posterior of the abdomen is heavily pigmented in males and more rounded than females.

2. Females have a couple narrow bands of pigment through the region occupied by solid pigmentation in males, and are more pointed at the end. But in these labs you will be expected to confirm the sex of any flies you use by examining the genitalia.
3. The genital arch and anal plate are easily visible in males, whereas in females more discrete.
4. Male have sex comb in left fore leg.

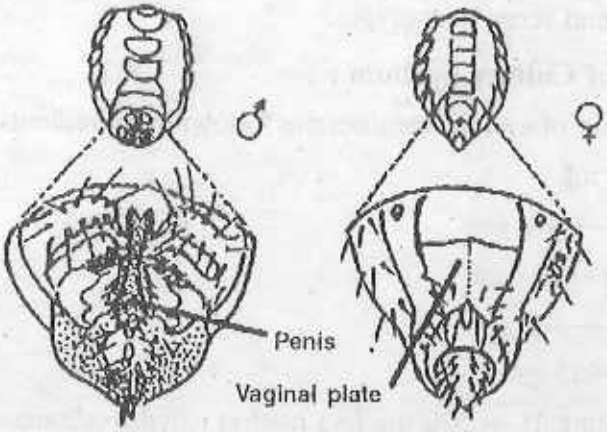


Fig. 3 : Ventral view of genetilia of male (left) and female (right) *Drosophila :* below is enlarged view

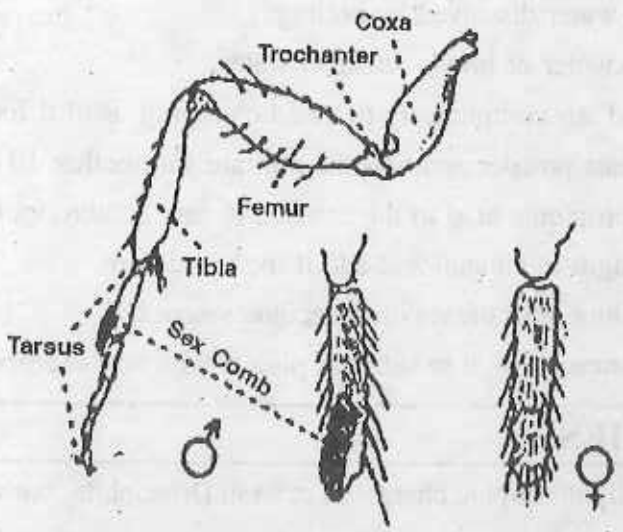


Fig. 4 : Left foreleg of male left and female right *Drosophila; The sex comb on tarsus of male fly, absent in female* (from W. Hewitt).

1.4 COLLECTING VIRGINS

The female stores sperms from a single mating and use it several times during her own ovulations. Therefore, once a fly has mated with one genotype, she cannot be "cleared" and used for matings with other genotypes that will be of any use for genetics. Although the techniques for collecting virgins are straight-forward, it is surprisingly difficult to get through a set of genetic crosses without making a mistake. Therefore, it is essential that the possibility of a non-virgin in the matings be kept in mind when the results of crosses are being interpreted. Typically, the result of a non-virgin mating will be a mixture of the offspring expected from a potential parent present in the vial in which the female eclosed, and the offspring expected from the intended mating. Once a series of crosses has begun, this mixture can be bafflingly complex. It is therefore essential that at each generation, the results we get can be rationalized on the basis of basic genetic principles, and are reproducible.

The simplest way to collect virgin females is to take advantage of the fact that newly eclosed flies will not mate for about 12 hours. Simply clearing the vials of all adults in the morning will ensure that any females collected during at least the next 8-10 hours will be virgins. It is not necessary to use virgin males for mating so, in practice, it is convenient to collect some males into one vial and put them aside to use for mating to virgins females as they are collected. The separated sexes can be kept in vials with food for several days and still are useful for mating.

The other way to collect virgin females is to find newly enclosed individuals based on the characteristics mentioned above : relatively unpigmented bodies, exploding heads, or wet, wrinkled wings. None of these should be considered foolproof however. The only foolproof visible marker of the virgin female is a black mass visible in the gut on the left side between the 2nd and 3rd abdominal segments. This is called the "meconium" (but only by a very few people!) and is actually larval gut tissue. Within a few hours after emerging, this will be excreted, but until it is excreted it blocks mating, and so is a sure sign of a virgin. The males display thee meconium, too, so its presence is not sufficient to ensure that a fly is a virgin female.

1.5 EXPERIMENTS TO BE DONE

Our experiments will involve mating between genetically defined individuals : "genetic crosses". We will work in pairs; each group will do several sets of crosses.

The crosses are designed to illustrate basic genetic principles, such as dominance, segregation, independent assortment, epistasis, sex linkage, linkage, and recombination. There are several different stocks, and several different crosses that can be made. Which cross we do will be determined by lottery. All the information we need for each cross will be given, but the analysis and interpretation will be up to us.

1.6 SETTING UP CROSSES

When handling the flies each week, two things should be done in each session : (1) setting up the genetic crosses, and (2) setting up new cultures of each true- breeding line ("passaging" each "stock") that we are handling so we have flies to work with ill the future.

When setting up genetic crosses, always we will have to think through what we are to do: What stocks will be crossed? Does it matter which is male and which is female? Do we have all the materials we need to proceed? Bring out stocks one at a time so that we will know what we are working with; it is easy to become confused when we have a table full of sleeping flies. Label a vial with the genetic notations for that stock, the date, and whether the flies from each stock will be male and female.

To passage the flies, it is not necessary to use virgin females. Get a vial of food, place a few grains of yeast into it, label it with the stock name, the date, and the name or initials, and place about a dozen males and a dozen females in it; plug it and put it with the other stocks. One week after setting up a cross, clear the adult flies from the vial by tapping them into a morgue so they aren't around to mate with the F_1 when they emerge. Examine the vial for larvae—if there are not any, the cross has to be repeated.

Two weeks after setting up the cross, we should see new adults in the vials. Tap them into an anesthetizing vial, knock the suckers out, and record their phenotypes. Do these phenotypes match our predictions? Let's hope so! Don't discard these flies after scoring them; use them to set up the $P_1 \times P_1$ cross. It will not always be necessary to use virgin females for the $F_1 \times F_1$ cross (why?). If we are setting up a test cross, note that virgins are necessary. If the purpose of the test cross is to measure recombination frequencies, the F_1 heterozygote should be a female because recombination does not occur in males in Drosophila. Remember, each week, clear the adults out of any vials we set up last week. During the fourth week after setting

up our first cross we will begin collecting data on the F_2 generation. It is best to examine flies that are 1-3 days old, because some phenotypes become less clear-cut with age. Score the phenotypic classes that emerge until we have counted at least 100 flies, or the vial has stopped producing. However, do not count flies beyond the third weeks after setting up the vial, because the progeny of the F_1 generation may start to emerge.

1.7 GENERAL INFORMATION AND FLY HUSBANDRY

The fruit fly *Drosophila melanogasterhas* 3 pairs of autosomal chromosomes and an X and Y chromosome. Each autosome has two arms that are simply referred to as left (L) and right (R). Each chromosome ann is numbered as follows : X (1-20), 2L (21-40), 2R (41-60), 3L (61-80), 3R (81-100), and chromosome 4 (101-102). Each chromosome arm is also numbered by recombination units, thus allowing one to know the expected recombination frequency between two genes located on the same chromosome arm. The chromosomal locations of individual genes are identified either by numerical location or by recombination units.

1.8 NOMENCLATURE USED IN GENETICS

The nomenclature used in *Drosophila* genetics is fairly straightforward .The standard rules of nomenclature is followed to properly and clearly describe the complete genotype of a fly stock.

- Chromosomes are written in order, as follows, with a semi-colon separating each chromosome X/Y; 2; 3; 4
- Genotypes are listed only when a mutation is present and are italicized.
- Recessive mutations are written in lower case (e.g. w for white gene),
- Dominant mutations are capitalized (e.g. B for Bar eye).
- Deficiencies : Df(2L)VA = Deficiency of the left (L) arm of chromosome 2 that includes the gene Venea abnonneis (VA).
- Transpositions : Tp (l; 3) HF308 = Transposition involving the X and 3 rd chromosome.
- Inversions : In (2LR) SMC8 = Inversion of the left (L) and right (R) arm of the 2nd chromosome. (SMC–structural maintenanace chromosome)

- Translocations : T(1; 3) Thl = Translocation between the 1st (X) chromosome and 3rd chromosome commas follow rearrangements and indicate mutations present (Th–Transloction heterozygotes)

  e.g. In(2LR)SM1, al^{2}Cy cn^{2}sp$_2$ = Inversion involving the left (L) and right (R) arms of chromosome 2 with the following mutations present: aristaless (al), Curly (Cy), cinnabar (cn), and speck (sp).

1.9 PROBLEMS OF GENETICS CROSS

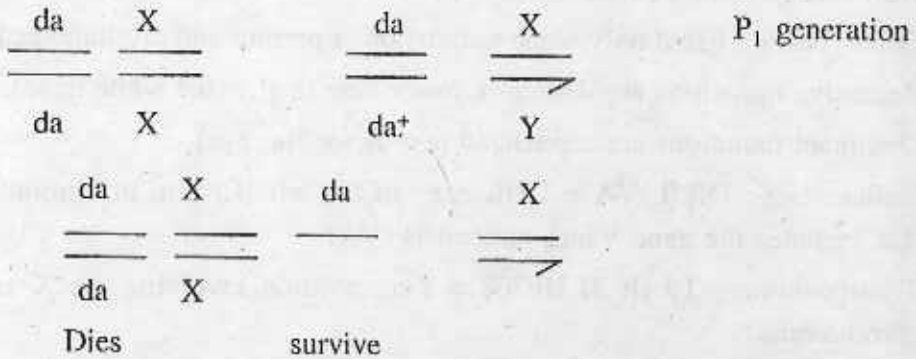
1.9.1 Sample 1

You are provided with a culture vial containing fruit flies. Predict the genotype from the following offspring of the given flies. Test statistically to prove their expected ratio.

Total no flies = 9 Male flies = 9 Female flies =0

Analysis

The observation is that the cross-resulted from the parental progeny yield only male flies. This result indicates that probably a lethality arises in case of female zygote which causes death of all female flies. This may happen if we consider a mutation known as "da" which in homozygous condition causes daughterless progeny. Here probably the female parent was homozygous for the mutation "da" and the male parent mayor may not contain "da" mutation, Daughterless is recessive autosomal mutation located on second chromosome. The probable genotype of the parent is



Statistical Hypothesis

Null Hypothesis : Only male progeny produced (l 00%). Hence the resultant progeny in this case is only 9 male and no females. This is consistent with Null hypothesis. Hence the calculation for chi-square test is not required.

1.9.2 Sample 2

Culture vial containing flies

Total number of flies 20 Curly 14 Normal 6

Analysis

The observation indicates that the appearance of progeny Curly: normal =14 : 6 (approx 2 : 1). In this case probably the parental female & male were heterozygous for the second chromosomal dominant autosomal gene curly. Curly in homozygous condition is lethal.

Cy // Cy$^+$ × Cy // Cy$^+$ Probable genotype of the parent P_1

Cy // Cy Cy // Cy$^+$ Cy // Cy$^+$ Cy$^+$ // Cy$^+$

Dies Curly Curly Normal

Curly: Normal (2: 1)

Statistical analysis

The cross resulted in appearance of progeny curly and normal in the ratio 2 : 1 hence, we have to fill the observed data with 2 : 1 ratio to justify the assumption of the genotype. The evaluation may be made by the χ^2 test for goodness of fit.

Calculation of χ^2

Here the hypothesized ratio for normal : curly is 1 : 2 and tlle formula of χ^2 test is

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Calculation

	Curly	Normal	Total
Observed (o)	14	6	20
Expected (E)	13.45	6.6	20
O – E	0.6	–0.6	
$(O - E)^2$	0.36	0.36	
$\frac{(O-E)^2}{E}$	0.027	0.055	
$\chi^2 = \sum \frac{(O-E)^2}{E}$		0,027 + 0.055 = 0.082	

Degree of freedom 2-1= 1

Interpretation : At 5% level of significance for df value 1, the tabulated X^2 value (0.082) as obtained here was far below the table value at 5% level of significance which represent a P value greater than 0.05. Hence, the deviation between the observed and expected values may be considered insignificant. Therefore, the hypothesized ratio of curly : normal (2 : 1) was accepted at 5% level of significance. In this condition it could be interpreted that the genotype assumed for the curly and normal flies were very much correct.

UNIT 2 ❐ PREPARATION OF POLYTENE CHROMOSOME

Structure

2.1 MATERIAL REQUIRED

1. Live third instar larvae of *Drosophila*
2. Forceps, needles & needle holders
3. Cavity block or watch glass
4. Slide and cover slip.
5. Binocular dissecting microscope
6. Compound microscope
7. Ringers solution [3.25gm NaCl, 0.17gm KCl, 0.06gm $CaCl_2$, 0.10 gm $NaHCO_3$. 0.005gm Na_2HPO_4 in 500ml of Distilled water, pH 7.2
8. Acetoorcein (2%) [2gm orcein dissolved in 50cc warm acetic acid and finally make it volume with another 50 cc acetic acid. Dissolve it completely & filter]
9. Acetomethauol (fixative) [1 : 3 v/v]
10. Lacto aceto orcein (Mounting medium)[in 2% aceto orcein add equal volume of lactic acid]
11. 45% Acetic acid (Destainer)
12. Nail polish

2.2 PROCEDURE

1. Take out third instar larva from *Drosophila* culture bottle and put them in a cavity block containing Ringers solution
2. Place a clean slide on the stage of a dissecting binocular against a dark background & put a few drop of Ringers solution on it.
3. Hold the back portion of the larva with a forceps. Place the point of a needle just behind the mouth hooks
4. Gradually pull the mouth hooks out of the larval body together with the attached structures.
5. Separate the salivary glands from other tissues and transfer to the Ringer's solution & finally transfer it to another slide.

2.3 STAINING AND FIXATION

1. Fix the salivary gland with a few drops of acetomethanol (1:3v/v)
2. Tilt down the fixative from the salivary gland and put 2-3 drops of acetoorcein stain on the fixed tissue. Cover the slide with petridish and leave in this condition for 10 minutes.
3. Wash the stained tissue in 45% acetic acid for 30-40 sec. Tilt the slide and soak the excess acetic acid with a blotting paper.
4. Pour one drop of acetolacto orcein and place one cover glass over the stained tissue and finally squash the stained salivary gland with the help of thumb or back portion of the rubber headed pencil
5. Some of the mounting reagent will ooze out around the coverslip can be blotted by using blotting paper
6. Seal the edge of the slide with nail polish and the preparation is now ready for observation under microscope.

2.4 OBSERVATION

The polytene chromosome have five radiating arms (Fig 5). These are marked as

X, 2R & 2L, 3R & 3L. The chromosome show alternating dark and light bands. All the Centromere of the chromosome are fused to form chromocenter

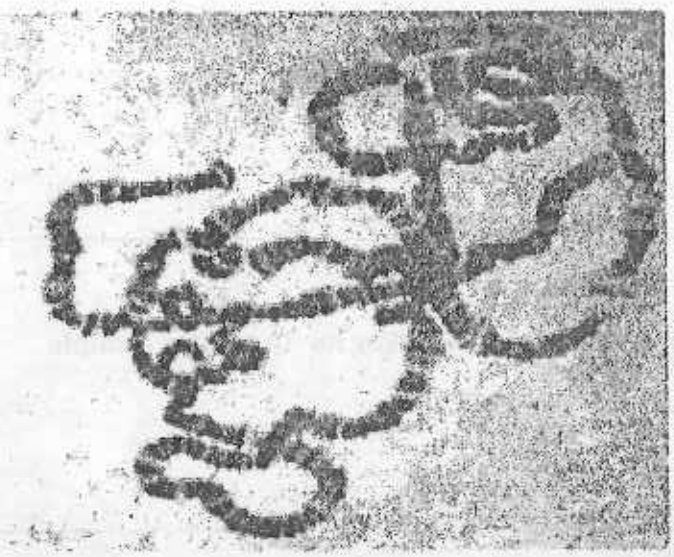


Fig. 5 : Giant polytene chromosome; squash preparation of salivary glands of *Drosophila melanogaster*

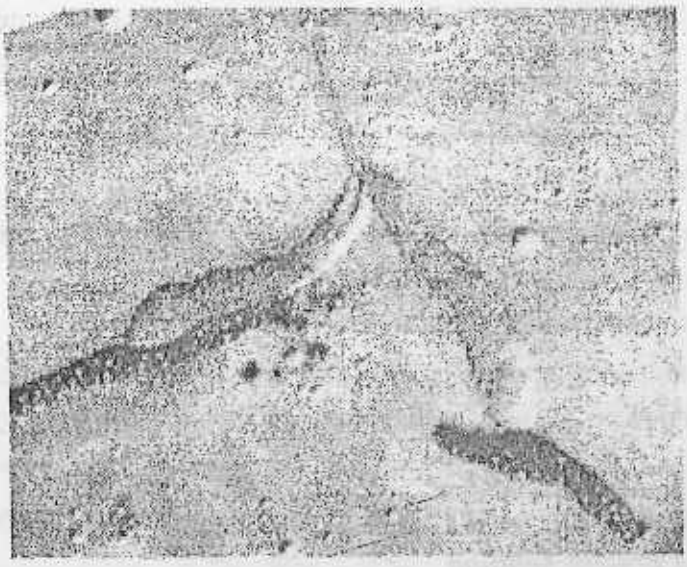


Fig.6 : A pair of dissected salivary gland of *Drosophila sp.*

UNIT 3 ❐ PROTEIN ESTIMATION

Structure

3.1 PRINCIPLE

The method consists of measuring the colour produced by the reaction of peptide bonds with alkaline copper tartrate and between phenolic groups of proteins and the Folin-Ciocalteau reagent. The colour produced has a broad absorbance maximum around 660nm, the wavelength at which this colour is measured.

3.2 REAGENTS

1. ***Folin-Ciocalteau reagent***

Commercially available reagent is freshly diluted (1:1) with equal volume of distilled water before use

2. ***Alkaline copper tartrate solution***

This is prepared fresh by mixing solution A and B in the ratio of 1 : 100

Solution A – 0.3 gm% solution of copper sulphate ($CuSO_4$, $5H_2O$) in 1gm% solution of sodium potassium tartarte (mix in equal volume on the day of use).

Solution B – 2 gm% solution of a sodium carbonate in 0.1N sodium hydroxide.

3. ***Standard protein solution***

This is prepared as a 10-mg/ml solution of Bovine serum albumin

3.3 PROCEDURE

1. 1 ml of protein solution is treated with 5.0 ml of alkaline tartrate solution (Solution 2)and shaken vigorously on a vortex mixer.
2. After exactly 15 min 0.5 ml of the Folin Ciocalteau reagent (solution 1) is added after dilution and the mixture is shaken thoroughly for 30 minutes
3. The optical density of the mixture is measured at 660 nm in a colorimeter,
4. A standard curve is prepared by developing colour in the similar way where solutions containing graded concentration of protein (10 μg to 100 μg).
5. The obtained optical density is then plotted against the protein concentrations determined as above is used to express all values as a function of the protein content.

3.4 ESTIMATION OF KNOWN (STANDARD) PROTEIN

Different concentrations of working solution of BSA were pipetted out into series of test tubes taking at least 3 replica for each concentrations. Then different reagents were mixed step by step as described in the following table :

Conc. of BSA (μg/ml)	*Sample BSA (ml)*	*0.1 N NaOH (ml)*	*Alkaline Copper solution (ml)*	*Folin-Ciocalteu solution (ml)*
0	Blank	1.0	5	0.5
10	0.1	0.9	5	0.5
20	0.2	08	5	0.5
30	0.3	0.7	5	0.5
40	0.4	0.6	5	0.5
50	0.5	0.5	5	0.5
60	0.6	0.4	5	0.5
70	0.7	0.3	5	0.5
80	0.8	0.2	5	0.5
90	0.9	0.1	5	0.5
100	1.0	0	5	0.5

After giving the different concentrations of sample BSA solution in each test tube, 0.1 N NaOH was added so that the total volume of each sample would be 1 ml. After mixing alkaline copper solution and Folin-Ciocalteu solution, the test tubes were shaken well till a bluish colour would appear in the solution. Then the tubes were allowed to incubate at room temperature in dark for at least 30 minutes. The sample contain ing no BSA was taken as the "blank" solution.

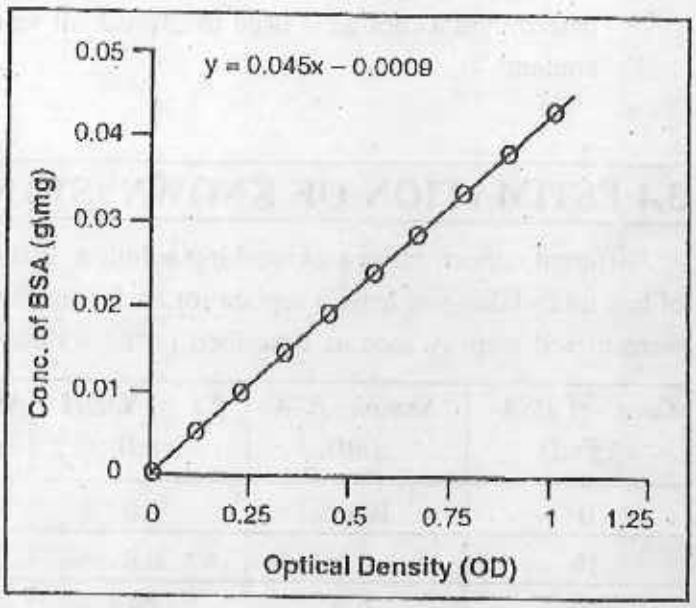
3.5 ESTIMATION OF UNKNOWN PROTEIN

0.1 to 0.5 ml of tissue samples (aliquote) were pipetted out in test tubes keeping at least 3 replica. Then the other steps were followed as described in case of known protein sample.

Reading : The extinction **(Optical Density– OD)** was read at **750 nm** against suitable blank in a spectrophotometer (Double-Beam UV-180 Spectrophotometer, Shimadzu, Japan).

Preparation of standard curve :

At least 3 replicated readings were taken for each concentration of BSA and the mean OD values were plotted against different concentrations of the standard samples prepared (10 µg/ml – 100 µg/ml). A liner curve was obtained which represented the standard curve.



3.6 CALCULATION OF AMOUNT OF PROTEIN FOR UNKNOWN SAMPLE

The concentration (µg/ml) of unknown protein was measured against the standard curve. The amount of protein was calculated as follows :

$$\text{Amount of protein} = \frac{\text{Amount of NaCl} \times \text{Cone. of protein} \times 10}{\text{Sample amount} \times \text{Weight of tissues}} \text{ mg/gm}$$

Sample amount = 0.1-0.5 ml of homogenised tissue solution

UNIT 4 ❐ DETERMINATION OF SPECIFIC ACTIVITY OF ENZYME

Structure

4.0 INTRODUCTION

Enzymes are usually protein that catalyzes a variety of reactions in the biological systems. All the enzymes that are found in living tissue are classified in to major six groups.

A. Oxidoreductase
B. Transferase
C. Hydrolases
D. Lyases
E. Isomerase
F. Ligases

The experiment below describes a few experiments to illustrate the general approach employed for optimizing conditions for assaying activities of enzymes.To study the specific activities of enzyme we select alkaline phosphatase.

4.1 PRINCIPLE

Phosphatase is a broad term used for non-specific phosphomonoestreases, which hydrolyse phosphate ester and thus liberating alcohol derivative of the substrate molecule and inorganic phosphate. Depending on their pH activities this enzymes are of two types, Acid phosphatase (pH 4.0-5.5) and alkaline phosphatase (pH 8-10). For assaying phosphatase enzyme.

Nitrophenyle phosphate can be used as substrate.

4.2 MATERIALS & METHODS

1. Water bath 37°C
2. Colorimeter
3. Glycine NaOH buffer (0.05M, pH 10.5). Dissolved 375 gm glycine in small amount of distilled water then add 42 ml 0.1N NaOH and adjust pH to 10.5
4. NaOH solution (0.085N)
5. $MgCl_2$ solution (10.5mM)
6. ρ nitrophenyle phosphate (35mM). Take 38.8 mg of ρ nitrophenyle phosphate and dissolve it in 5 ml of 0.05 M glycine NaOH buffer, pH 10.5
7. Standard solution of ρ nitrophenyle phosphate (100 mM)
8. Material 5days old germinating seed

4.3 PROCEDURE

1. All the operation must be carried out in cold atmosphere (inside ice bucket). With the help of mortar & pestle grind the germinating seed in presence of chilled glycine NaOH buffer.
2. Centrifuge the homogenate in a refrigerated centrifuge at 10,000 g for 20 min. Decant supernatent and use it as a source of enzyme.
3. Take nine numbered test tubes and add 3.0ml of glycine NaOH buffer, 0.1ml of $MgCl_2$ and 0.3 ml of the enzyme preparation in each tube.
4. Incubate all these tubes in 37°C. After 3 mn start the reaction in seven tubes by adding 0.1 ml ρ nitrophenyle phosphate.
5. Exactly after 5,10,15,20 mn stop the reaction by adding 9.5 ml of 0.085N NaOH.
6. Only one tube receive only P nitrophenyle phosphate and 0.085N NaOH that serves as control and one tube receive 0.1 ml of 0.05M glycine NaOH instead of ρ nitrophenyle phosphate that serves as reagent blank.
7. Adjust colorimeter at 410nm to 100% transmission with the reagent blank and record absorbance of the other tubes.
8. To prepare standard curve take 0-1 ml (0-100mmoles) of ρ nitrophenyle phosphate, Add 3ml of glycine NaOH buffer to all the tubes and make the final volume to 3.5ml with distilled water.
9. Plot a graph of A_{410} vs. μ moles of ρ nitrophenol to obtain a standard curve.
10. From the standard curve determine the amount of ρ nitrophenol present in other tubes.

UNIT 5 ❐ GENOMIC DNA EXTRACTION AND EVALUATION

Structure

5.0 INTRODUCTION

DNA isolation is the basic requirement for any molecular biology work and thus plays a very important role in any molecular biology techniques like PCR, genotyping, sequence analysis etc.

5.1 PRINCIPLE

Blood contains various components, but DNA is present only in the WBC. Firstly all the rest of the components are separated and removed by centrifugation, which is then followed by breaking down the WBC and release of all the various cellular components from the WBC. The proteins are degraded by the enzymatic activity of Proteinase K and DNA purification is facilitated by the treatment of Phenol and chloroform. DNA is alcohol precipated and then is dissolved in the Tris- EDTA (TE) buffer for further analysis.

5.2 REQUIREMENT

- 15 ml and 50 ml plastic tubes
- Lysis buffer :
  320 mM Sucrose
  10 mM Tris- HCl pH 7.5
  5 mM $MgCl_2$
  1 % Triton X-100

- Digestion buffer :
  10 mM Tris-HCI pH 8.0
  110 mM EDTA
  100 mM NaCl
  2% SDS
- Proteinase K (20 mg/ml)
- 1 x TE buffer :
  10 mM Tris-HCI pH 7.5
  10 mM EDTA
- Saturated Phenol
- Chloroform—Isoamyl alcohol (96 : 4)
- Chilled absolute alcohol
- 70% alcohol
- 3M-sodium acetate pH 7.0
- Water bath & centrifuge

5.3 PROCEDURE

1. Take 5 ml blood and add 7ml of lysis buffer to it. Mix it on ice for 2-3 mins.
2. Centrifuge at 1300g for 15 nuns at 4°C.
3. Decant the supernatant
4. Add 10ml of lysis buffer and mix on ice for 2-3 mins.
5. Centrifuge at 1300 g for 15 min at 4°C.
6. Decant the supernatant and add 4ml lysis buffer to it
7. Mix on ice for few mins and then centrifuge at 1300g for 15 min at 4°C
8. Throw away the lysis buffer and add 1200ml digestion buffer
9. Add 20ml Proteinase-K (20mg/ml) and then incubate in water bath at 56°C over night or at least 3-4 hours.
10. Centrifuge for 2 mins to get the precipitate in the bottom of the tube.
11. Add 600 ml phenol and 600 ml chloroform —isoamyl alcohol mixture.
12. Mix gently for about 2 min and centrifuges at 1500g for 5min at 4°C.
13. Remove the supernatant and repeat the above step.
14. Remove the supernatant and add 1200ml chloroform- iso-amylalcohol
15. Mix it gently and centrifuge at 1500g for 5 min at 4°C.

16. Remove the supernatant and add it to a tube containing 10 ml absolute alcohol and 400 ml of 3M sodium acetate pH 7.0.
17. Mix by hand inversion and centrifuge at 8000g for 20 min at 4°C.
18. Remove the alcohol carefully, add 70% alcohol and centrifuge at 8000g for 15 nuns at 4°C.
19. Remove the alcohol and air dry
20. Dissolve in 200ml 1 × TE buffer pH 7.5 overnight
21. Take the photometric readings at 260 nm and 280 nm and check the purity of the sample. Also calculate the amount of DNA present.
22. It is also further subjected to gel electrophoresis for determining the size of the fragments.

5.4 RESULTS AND INTERPRETATION

- For quantitative and qualitative estimation of the DNA, reading should be taken at wavelengths of 260nm and 280nm.
- A solution containing 50 mg/ml of double stranded DNA has an absorbance of 1 at 260nm (A_{260} = 1= 50mg/ml of double stranded DNA)
- The ratio between the reading at 260 nm and 280nm (OD_{260}/OD_{280}) provides an estimate of the purity of the nucleic acid. Pure preparations of DNA have OD_{260}/OD_{280} value 1.8. If there is contamination with protein or phenol, the OD_{260}/OD_{280} will be significantly less than the given above.

UNIT 6 ❐ ISOLATION OF DNA FROM GOAT LIVER

Structure

6.0 INTRODUCTION

The most common method of DNA isolation is phenol extraction. The fundamental aim of phenol extraction is the deproteinization of an aqueous solution containing the desired nucleic acids. In simple terms the phenol reagent is mixed with the sample under conditions, which favours the dissociation of proteins from nucleic acids.

6.1 SAMPLE COLLECTION

Liver from freshly sacrificed goat should be collected and placed within ice box.

6.2 MATERIAL REQUIRED

1. Centrifuge (10,000 rpm)
2. Tissue homogeniser
3. Autopipette
4. Test tubes
5. Vortex
6. Pasteur pipette

7. Cheesecloth
8. DNA spooling glass rod
9. Incubator
10. Chilled Ethanol, Phenol, isoamyl alcohol

6.3 REAGENT REQUIRED

- Buffer A : Sucrose 0.25M, $CaCl_2$ 0.001IM, Tris 0.05M homozenising buffer
- Buffer B : NaCl 0.15M, Na EDTA0.1M DNA extraction buffer
- SDS 10% solution
- Phenol (distilled pH 8.0)
- Chloroform
- Na Acetate 3M
- Proteinase K (200 µg/ml)

6.4 METHOD

1. Mince the liver tissue in to fine pieces
2. Homogenize gently in 2 volume homogenizing buffer in presence of ice
3. Filter the homogenate through cheesecloth. Take the filtrate in test tube
4. Centrifuge the filtrate at 4000 rpm for 15 min and discard the supernatant.
5. Take the nuclear pellet and resuspende in 10 volume of DNA extraction buffer.
6. Add SDS (1%) and proteinase K (200mg/ml), incubate at 65° C for 15-30 min.
7. After incubation add equal volume of saturated phenol and vortex very gently.
8. Centrifuge at 10,000 rpm for 10 min
9. Collect the aqueous layer and keep it in a test tube.
10. Add equal volume of phenol chloroform isoamyl alcohol (25 : 24 : 1 v/v) and shake gently for 15 min
11. Centrifuge at 10000 rpm for 10 min
12. Collect upper aqueous layer and repeat the process as in 10.
13. Add sodium acetate 0.3M and chilled ethanol
14. DNA will precipitate like a thread

15. Spool out DNA and keep it in micro centrifuge tube in deionized water or suitable buffer medium

6.5 REASONS FOR USING CHEMICALS FOR DNA ISOLATION

The following are the reasons for using chemicals for DNA isolation.

Sucrose–rupturing of cell membrane due to change in osmotic concentration

TRIS–Mgl Acetate- maintain ionic strength

SDS–detergent causes rupturing protein from nucleic acid

Phenol–Precipitate protein. Saturated phenol cause dehydration of DNA by -extracting Water

Chloroform–surface denaturation of protein remove lipid separate two layers aqueous and organic layer

Isoamyl alcohol–Prevent esterification of lipid

EDTA–Chelating agent of divalent cataion like Mg^{++} necessary for inactivation of DNAase

ProteinaseK–cuts two peptide bonds and remove proteins

Chilled ethanol–removes bound water from DNA results precipitation

UNIT 7 ❐ COLORIMETRIC ESTIMATION OF DNA

Structure

7.0 INTRODUCTION

This is the general reaction given by deoxypentoses. The deoxyriboses of DNA in the presence of acid is converted to w hydroxylevulinic aldehyde which reacts with diphenylemaine to form a blue coloured complex with absorbance maxima at 600 mm. Compounds such as furfuryl alcohol and arabinol, which can be converted in to w hydroxylevulinic aldehyde will also give this reaction .. In DNA since only deoxyribose of purine uucleotides is released, the value obtained represents only half of the total deoxyribose in the sample. The reactions leading to the formation of the coloured complex are as follows

$$\text{2-deoxy-D-ribose} \xrightarrow{HClO_4} \omega \text{ hydroxylevulinic aldehyde}$$

$$\text{w hydroxylevulinic aldehyde} \longrightarrow \text{Diphenylamine (Blue colour complex)}$$

7.1 MATERILAS AND REAGENTS

1. **Saturated DNA solution** : Dissolve calf thymus DNA (100mg/ml) in 1N $HClO_4$ by heating at 70°C for 15 min. Make different dilutions of this stock solution ranging from 20-100mg DNA/ml using 0.5 $HClO_4$
2. **1.6% (w/v) acetaldehyde :** Prepare by dissolving lml ice cold acetaldehyde in 50 ml distilled water
3. **Diphenylamine solution :** Dissolve 1.5g Diphenylamine in 100ml glacial acetic acid and 1.5 ml concentration H_2SO_4
4. **Diphenylamine reagent :** Prepare by mixing 0.5ml 1.6% of acetaldehyde and 100ml diphenylamine solutions. This solution must he prepared fresh.
5. Colorimeter or spectrophotometer

7.2 PROCEDURE

1. Take 2.0 ml sample from which DNA has to be estimated in a test tube.
2. In another set of test tubes, pipette 2.0ml of standard DNA solution of different dilutions. In one of the test tubes take 2.0ml of 0.5 N $HClO_4$ as reagent blank.
3 Add 4.0ml diphenyleamine reagent (reagent no 4) to all the tubes, mix the contents properly and keep at room temperature in dark for 16-18hr or overnight. Altematively keep the tubes in boiling water for 10 min and cool them under running tap water.
4. Record the absorbance at 600nm in colorimeter.
5. Draw a standard curve of A_{600} vs DNA concentration. Form absorbance of the sample determines the amount of DNA in it. Express the results as mg of DNA/ gm of tissue.

UNIT 8 ❐ COLORIMETRIC ESTIMATION OF RNA

Structure

8.0 INTRODUCTION

This is the general method for estimation of pentoses. Acid hydrolysis of RNA releases ribose, which in the presence of strong acid undergoes dehydration to yield furfural. Orcinol in the presence of ferric chloride as a catalyst reacts with furfural producing a green coloured compound with absorbance maxima at 665nm. DNA gives a limited positive reaction with orcinol test. The reactions leading to the formation of a green coloured complex are as follows.

$$\text{Ribose} \xrightarrow{\text{Acid}} \text{Furfural} \xrightarrow{\text{Orcinol}} \text{Green coloured compound}$$

8.1 MATERIALS AND REAGENTS

1. Boiling water bath
2. 5% $HClO_4$
3. **Standard RNA solution :** Dissolve yeast RNA (500mgRNA/ml) with 5% $HClO_4$ Make different dilution to obtain solutions containing 100-500mg RNA/ml with 5% $HClO_4$
4. **Orcinol reagent :** Dissolve 100mg of ferric chloride ($FeCl_3$ $6H_2O$) in 100 ml of concentrated HCl and then add 3.5 ml of 6% solution of orcinol prepared in alcohol
5. Colorimeter or spectrophotometer

মানুষের জ্ঞান ও ভাবকে বইয়ের মধ্যে সঞ্চিত করিবার যে একটা প্রচুর সুবিধা আছে, সে কথা কেহই অস্বীকার করিতে পারে না। কিন্তু সেই সুবিধার দ্বারা মনের স্বাভাবিক শক্তিকে একেবারে আচ্ছন্ন করিয়া ফেলিলে বুদ্ধিকে বাবু করিয়া তোলা হয়।

— রবীন্দ্রনাথ ঠাকুর

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— Subhas Chandra Bose

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