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.

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

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.