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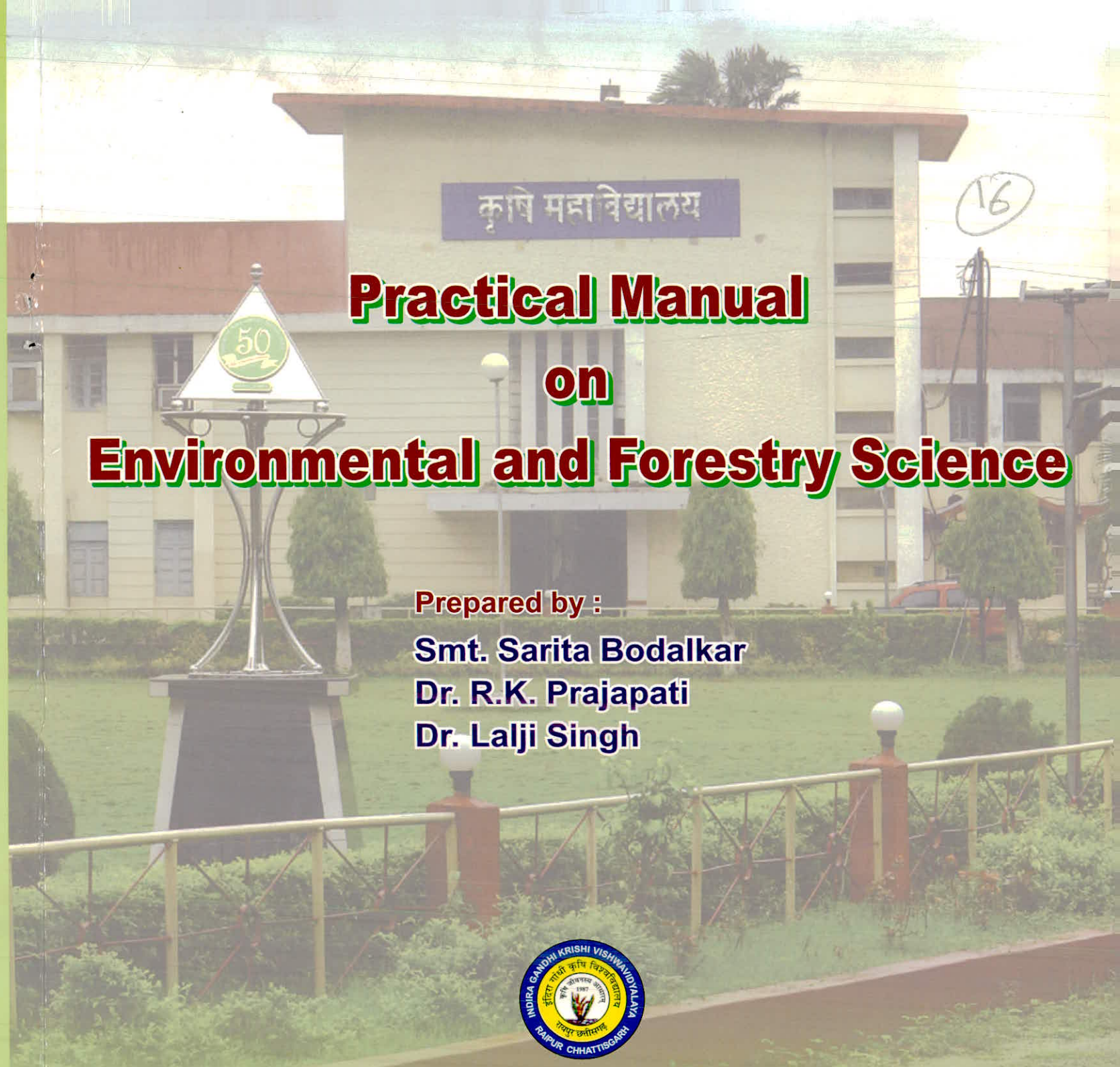

Practical Manual
on
Environmental and Forestry Science

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This is to certify that Shri./Ku. …… Enrolment No./ID No……..…. has completed the practicals of Course No. ……. as per the syllabus of B.Sc. (Ag.) …….. year…….. semester in the respective lab/field of College.

Date: **Course Teacher**

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Experiment No. 1

Title : Processing of effluent samples
Objective : Collection, processing and storage of effluent samples

Introduction

Water pollution is mainly caused by two sources such as point and non-point. This depends on how the pollution enters the water. Point sources are sources of pollution situated at one location, often a specific outlet (discharge) pipe. Example is effluent from factories and wastewater treatment plants. The meaning of the term 'effluent' is "flowing forth out of'. As per the Clean water Act of 1977, effluent is a discharge from a point source, and the legislation specifies allowable quantities of pollutants. The discharges are regulated under section 402 of the act, and the standards must be met before they are released into surface waters. Water sampling is an important factor for evaluation of its quality and it includes two aspects namely (a) when and where to sample (b) how to transfer the samples with minimal changes to the place of analysis.

Aim

To collect a portion of material small enough in volume to be transported conveniently and handled in the laboratory still accurately representing the material being sampled.

Materials required

Samplers, sample containers, cleaning agents, preservatives, tags, labels.

Samplers

Surface samples are collected in a jug/bucket/bottle. For collecting subsurface samples water samplers are commonly used. Different water samplers namely Ruttner water sampler, Von Dorn water sampler, Thermos-flask water sampler, pump samplers *etc.* are available.

Sample containers

There are two basic choices in containers *viz.*, glass or plastic. In both the cases a tight fitting screw cap top is essential. Adsorption or ion exchange on the glass surface invalidates the use of glass bottles for storage of water in which heavy metals are to be estimated. As compared to glass bottles, plastic bottles are inexpensive and less fragile with less ion exchange problem. Among the plastics, high density linear polythene may

be used. Containers made of fluorinated polymers such as polytetrafluoroethylene (PTFE) can be preferred for sampling organics.

Cleaning

Plastic bottles including Teflon may contain impurities which will result in sample contamination. The method of cleaning depends on the use to which the bottles will be put. In general bottles should be cleaned with detergent, rinsed thoroughly with tap water and finally with distilled or de-ionized water. A non ionic detergent used in hot water is the best when heavy metals are to be analyzed. Presoaking of these in 1:1 HNO3 or 1:1 HCl is always advisable.

Collection of samples

As there are random variations in both analytical procedures and constituents of the effluent, a single sample may be insufficient to get reliable data. The number and distance between sampling stations depend on the physical size of the study area, variability or gradients in the processes, which control the distribution of the investigated parameter. For most physical and chemical analyses, a 2 litre sample is sufficient.

Types of Samples

Grab or catch sample: The sample collected at a particular time and place which represent only the composition at that time and place. When a source is known to be constant in composition over a considerable period of time, then such a source may be represented well by single grab samples.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes, which may vary from as little as *5* min to as long as 1 h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space *i.e.,* from location to location rather than time, collect samples from appropriate locations that will meet the objectives of the study (for example, upstream and downstream from a point source etc). Take every possible precaution to obtain a representative sample.

Composite sample: It refers to the mixture of grab samples collected at the same sampling point at different times. A composite sample representing a 24 hours period is

considered standard for most determinations. For determining components or characteristics subject to significant unavoidable changes on storage, composite samples should not be used.

Integrated sample: For certain purposes, the information needed is provided best by analyzing the mixtures of grab samples collected from different points simultaneously or as nearly so as possible. Such mixtures sometimes are called integrated samples.

Sampling Methods

Manual sampling: Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs. It requires trained field technicians and is often necessary for regulatory and research investigations for which critical appraisal of field conditions and complex sample collection techniques are essential. It is required in case of waters containing oil and grease.

Automatic sampling: Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling, and are used increasingly. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts or that can be contaminated (e.g., from phthalate esters) by contact with them. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential incompatibility of plastic components. Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

Sorbent sampling: Use of solid sorbents, particularly membrane-type disks, is becoming more frequent. These methods offer advantages of rapid, inexpensive sampling if the analytes of interest can be adsorbed and desorbed efficiently and the water matrix is free of particulates that plug the sorbent.

Preservation Chemical preservatives should be used only when they are shown not to interfere with the analysis being made (Table 1). For analysis of cations such as Al, Cd, Cr, Cu, Fe, Pb, Mn, Ag and Zn the sample should be collected in a separate clean bottle and acidified with HNO_3 to a pH below 2.0 to minimize precipitation and adsorption of these to container walls. Mercuric chloride @50 mg per litre of sample can be used as

preservative to check biological activity. Addition of 2 or 3 drops of toluene is also an option to prevent bacterial activity.

Field log book

This should include purpose of sampling, location of sampling point, name and address of the field contact, procedure of material being sampled and address. Suspected sample composition including concentrations, number and volume of samples taken, description of sampling point and sampling method, date and time of collection, collector's identification number and sample distribution with transport particulars. Protect the log book and keep it in safe place.

Chain-of-custody record

Properly designed and executed chain-of-custody forms will ensure sample integrity from collection to data reporting. The process of tracing the possession and handling of the sample from the time of collection through analysis and final disposition is referred to as "chain-of-custody" and is required to demonstrate control of samples particularly, when the data are to be used for regulation or litigation or logical interpretations. This should accompany each sample or groups of samples. The record includes sample number, signature of the collector, date, time, and address of collection, sample type, signatures or persons involved in the chain of possession and inclusive of the dates of possession.

Storage of samples

After examining the information accompanying the sample and verification regarding any tampering collection, the sample should be stored in a secured place. For most of the physical and chemical analysis some holding time for sample is permissible. Parameters which need immediate estimation include parameters like pH, free CO2, alkalinity, dissolved oxygen and sulphide. Most of the samples may be stored at $4^{o}C$.

Sample seals and labels

Gummed paper labels or tags are generally adequate. Labels should include name of the collector, date, hour, exact location, water temperature, water level, post sampling handling and steam flow. Labels should be filled in before or at the time of sampling with water proof ink.

Sample delivery to the laboratory

This should be done as soon as practicable to the sample custodian.

Precautions in collection and preservation of samples

- It is always desirable to rinse the water sampler or sampling container thoroughly with the water to be collected by it.
- Hot water samples collected under pressure should be cooled while they are still under pressure.
- Well water should be pumped sufficiently to ensure that sample collected represents ground water source.
- Surface scum should be avoided while sampling.
- Excessive turbulence causes potential loss of volatile constituents and toxic vapours, hence areas of excessive turbulence are to be avoided.
- In case of organic determinations sample should be filled full in the bottle. Samples if shipped an air space of about one percent is to be left.
- If preservatives are used, add them to the sample bottle initially so that all portions of the composite are preserved as soon as collected.
- Dissolved oxygen if to be estimated by Winkler's method should immediately be fixed by adding manganous sulphate and alkaline potassium iodide solutions.

Table Summary of sampling and handling requirements

S No.	Test to be Conducted	container+	Sample type!	Minimum sample size – ml	Preservation procedur	Maximum storage time
1.	Acidity	P,G(B)	g	100	Refrigerated*	24 hours
2.	Alkalinity	P,G	g	200	Refrigerated	24 hours
3.	BOD	P,G	g, c	1000	Refrigerated	6 / 48 hours
4.	COD	P,G	g, c	100	Analyze as soon as possible or add H_2SO_4 to bring pH to < 2	7 days / 28 Days
5.	Chlorine Residue	P,G	g	500	Refrigerated and to be analyzed Immediately	0.5 hours
6.	Chlorophyll	P,G	g	500	30 days in dark	30 day
7.	Hardness	P,G	g	100	Add HNO_3 to bring pH to < 2	6 month
8.	Metals	P(A),G(A)	g, c	-	For dissolved metals filter immediately and HNO_3 to bring pH to < 2	6 month
9.	pH	P,G	g	-	Analyze immediately	0.5 hours
10.	Turbidity	P,G	g, c	-	Analyze same day	24 hours
11.	Solids	P,G	g,c	-	Refrigerated	7 day
12.	Dissolved Oxygen	G, BOD bottle	g	300	Analyze immediately	0.5 hours
13.	Oil and grease	G,wide-mouth calibrated	g	1000	Add HCl or H2SO4 to pH <2, refrigerate	28 day

* Refrigerated means sample is stored at 4oC in dark
+P = Plastic, G = Glass, P (A), G (A) = rinsed with 1+1 HNO_3
. G (B) =glass, borosilicate
! g—grab; c---composite

Experiment No. 2

Title : Estimation of Dissolved Oxygen (DO)
Objective : To measure the dissolved oxygen (DO) in effluent samples by Azide Modification of Winkler's method

Requirements: H_2SO_4, starch indicator, NaN_3, $MnSO_4$, sodium thiosulphate.

Theory: The presence of dissolved oxygen is essential to maintain the higher forms of biological life and to keep proper balance of various populations thus making the water bodies healthy. The chemical and biochemical processes undergoing in a water body are largely dependent upon the presence of oxygen. The main sources of dissolved oxygen are from the atmosphere and the photosynthetic processes of the green plants. Estimation of dissolved oxygen (DO) is a key test in water pollution and waste treatment process control. The solubility of oxygen in water depends upon the partial pressure of oxygen in the air, temperature of water and mineral content of water. Azide modification of Winkler's method is followed to measure the dissolved oxygen. This method for the determination of DO depends on the formation of a white precipitate of manganous hydroxide in a glass stoppered bottle completely filled with the water tinder examination. The oxygen dissolved in the water is rapidly combined with the manganous hydroxide, forming a mixture of higher oxides(brown colour compound), which on acidification, in the presence of an iodide, releases iodine in a quantity chemically equivalent to the oxygen content of the water sample. The liberated iodine is then titrated with a standard solution of sodium thiosulphate. The end point is detected by using a starch indicator.

The chemical reactions involved are as follows:

$MnSO_4 + 2KOH = Mn(OH)_2 + K_2SO_4$

$2Mn(OH)_2 + O_2 = 2MnO(OH)_2$

(Manganic basic oxide)

$MnO(OH)_2 + 2H_2SO_4 = Mn(SO_4)_2 + 3H_2O$

(Manganic sulphate)

$Mn(SO_4)_2 + 2KI = MnSO_4 + K_2SO_4 + I_2$

(Iodine)

$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$

Reagents:

1. *Manganous sulphate solution* — Dissolve 480 g of $MnSO_4.4H_2O$ or 400 g of $MnSO_4.2H_2O$ or 364 g of $MnSO_4.H_2O$ in distilled water and make the volume to 1 liter.
2. Cone. H_2SO_4 acid (sp. gr. 1.83 —1.84).
3. *Alkaline iodide — Sodium azide solution (NaN_3)* — Winkler's reagent- (a) Dissolve 500g NaOH or (700 g KOH) and 135g Nal (or 150 g KI) in water and dilute to one liter. (b) Dissolve 10g NaN_3 in 40 ml of distilled water. Mix the two solutions (a) and (b).
4. *Starch indicator*— Prepare aqueous solution of starch by putting 5 g of starch powder in 800 ml boiling water with stirring. Dilute it to one liter. Boil it for few minutes and leave for setting overnight.
5. *Sodium thiosuiphate solution (0.025 N)* — Dissolve 24.82 g of $Na_2S_2O_35H_2O$ in a preboiled distilled water and make the volume to one liter. Add a pellet of NaOH or 0.4 g of Borax as a stabilizer. This is 0.1 N stock solutions. Dilute it to 4 times to prepare 0.025 N solution (Dilute 50 ml to 1000 ml). Store it in brown glass bottle. OR, Dissolve 6.205 g of sodium thiosulphate in 1 liter of distilled water. Add 0.25 g of NaOH for preservation.

Procedure :

Collection of water samples — Collect the samples in narrow mouth glass stoppered BOD bottles of 300 ml capacity with tapered glass stoppers and flared mouths. Take special precautions to avoid dissolution of atmospheric oxygen. Allow to overflow the bottle two or three times its volume and replace the stopper by avoiding the air bubbles.

Addition of reagents — Add 2 ml of $MnSO_4$ solution followed by 2 ml of alkaline iodide solution to fix the DO. Use separate pipettes for each solution. Introduce the reagents by keeping the tip of the pipettes well below the water surface in the bottle. Mix it thoroughly by inverting the bottle several times so as to distribute the precipitate uniformly throughout the bottle. A white to brownish color precipitate is formed on introduction of the reagents. If the titration is to be prolonged for few days, the sample can be stored at this stage with the precipitate. As the precipitate settles readily, the dissolved oxygen in the upper part of the bottle will be completely absorbed unless the

shaking period is suitably prolonged. With 2 ml of MnSO4 solution, a shorten period of shaking (20-25 seconds) is sufficient.

Acidification - Acidify with 2 ml of conc. H_2SO_4 by allowing the acid to run down the neck of the bottle. To avoid the reduction of manganic salts by organic materials, it is desirable that sample be shaken as soon as possible after the addition of acid when organic substances are present. Precipitate will dissolve leaving a clear solution. The liberated iodine diffuses slowly. It should be uniformly distributed throughout the bottle before withdrawing a part of the sample for titration.

Titration — Take 101.4 ml of sample in a conical flask and titrate against sodium thiosulphate solution until the straw yellow colour appears. Add one or two drops of starch solution. Blue colour develops. Continue titration with sodium thiosulphate to first disappearance of blue colour. Note down the amount of thiosulphate used for titration.

Calculation:

$$\text{DO(mg/l)} = \frac{\text{Volume of thiosulphate solution used x Normality of thio solution (0.025 N) x 1000}}{\text{Volume of water sample taken (101.4)}}$$

$$\text{or DO (mg/l)} = \text{Volume of thiosulphate used x 2}$$

Note — The presence of certain oxidizing and reducing materials may effectively interfere with the determination of oxygen by converting iodide ions to iodine. The azide modification removes the interference of such substances especially nitrite. It is destroyed by sodium azide (NaN.).

Experiment No. 3

Title : Estimation of Chemical Oxygen Demand (COD) of Water

Objective : To Analyse the chemical oxygen demand (COD) in effluent samples.

Requirements : Potassium dichromate, ferrous ammonium sulphate, ferroin indicator, H_2SO_4, $HgSO_4$, Ag_2S0_4.

Theory: Chemical oxygen demand (COD) is the measure of oxygen consumed during the oxidation of the oxidizable organic matter by a strong oxidizing agent. The determinations of COD values are of great importance where BOD values cannot be determined accurately due to the presence of toxins and other such unfavorable conditions for growth of microorganisms. The COD test gives no indication of whether or not the waste is degradable biologically and nor does it indicate the rate at which biological oxidation would proceed. Despite all these limitations, the COD test continues to remain a very important parameter in management and design of treatment plants because of its rapidity in determination. COD values are taken as basis for calculation of the efficiency of the treatment plants and also figure in the standards for discharging industrial/domestic effluents in various kinds of waters.

Potassium dichromate in the presence of sulphuric acid is generally used as an oxidizing agent in determination of COD. The known volume of water sample is refluxed with known volume of potassium dichromate and cone. sulphuric acid in the presence of mercuric sulphate to neutralise the effect of chlorides, and silver sulphate (catalyst). The remaining amount of potassium dichroinate after completing reflux is titrated against ferrous ammonium sulphate using ferroin as an indicator. The amount of $K_2Cr_2O_7$ used is proportional to the oxidizable organic matter present in the sample. The reaction is as follows:

$K_2Cr_2O_7 + 4H_2SO_4\ K_2SO_4 + Cr2\ (SO_4)_3\ 4H_20 + 30$

Reagents:

1. *0.25 N Potassium dichromate solution* - Dissolve 12.259 g of dried $K_2Cr_2O_7$ (AR grade) in distilled water to make 1 liter of solution.

2. *0.025 N Potassium dichromate solution* — Dilute 0.25 N $K_2Cr_2O_7$ 10 times (100 — 1000 ml).

3. *0.1 N Ferrous ammonium sulphate* — Dissolve 39.2 g of Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ in water. Add 20 ml cone H_2SO_4 and make the volume to 1 liter. Standardize this solution with $K_2Cr_2O_7$. For standardization, dilute 10 ml $K_2Cr_2O_7$ to 100 ml, add 30 ml cone. H_2SO_4 and titrate with ferrous ammonium sulphate using ferroin as an indicator.

4. *0.01 N Ferrous ammonium sulphate* — Dilute 0.1 N ferrous ammonium sulphate to 10 times. (100 — 1000 ml).

5. *Ferroin indicator* — Dissolve 1.485 g of 1,10 phenonthroline and 0.695 g of ferrous sulphate ($FeSO_4.7H_2O$) in distilled water to make 100 ml of solution.

6. *Sulphuric acid* (cone. H_2SO_4 — sp. gr. 1.84).

7. *Mercuric sulphate* — $HgSO_4$, solid.

8. Silver sulphate — Ag_2SO_4, solid.

Procedure: Take 20 ml sample in a COD flask. If the sample is expected to have COD more than 50 mg/I, add 10 ml of 0.25 N potassium dichromate solutions. In case the COD is expected below 50 mg/I, add 10 ml of 0.025 N $K_2Cr_2O_7$. Add a pinch of Ag_2SO_4 and $HgSO_4$. If the sample contains chlorides in higher amount, $HgSO_4$ is added in the ratio of 10:1 to the chlorides. Add 30 ml of cone. sulphuric acid. Mix reflux mixture thoroughly before applying heat. Reflux atleast for 2 h on a water bath or a hot plate. Remove the flasks, cool and add distilled water and make the final volume to 140 ml. Add 2-3 drops of ferroin indicator. Mix thoroughly. Titrate with 0.1 N ferrous ammonium sulphate (with 0.01 N ferrous ammonium sulphate if 0.025 N $K_2Cr_2O_7$ has been used). Take as the end point of the titration the first sharp colour change from blue green to reddish brown. The blue green may reappear. Run a blank with distilled water using same quantity of the chemicals.

Calculation:

$$COD = \frac{(A - B)\ X\ N\ X\ 8\ X\ 1000}{ml\ sample}$$

(mgO_2/l)

Where, A = ml of ferrous ammonium sulphate used for the blank

B = ml of ferrous ammonium sulphate used for the sample water

N = Normality of ferrous ammonium sulphate

Note — Take extreme care in case of low COD samples. A small trace of organic matter in glassware may contribute a significant error. If the sample contains more than 2000 mg/I of chlorides, COD cannot be determined accurately.

Experiment No. 4

Title : Estimation of Biological Oxygen Demand (BOD) of Water

Objective : To analyse the biochemical oxygen demand (BOD) in effluent samples.

Requirements: Phosphate buffer solution, $MgSO_4$, $CaCl_2$, $MnSO_3$, $Na_2S_2O_1$, NaN_3, cone. H_2SO_4, $FeCI_3$.

Theory: Polluted waters are turbid, unpleasant for drinking, bathing purposes. They are generally harmful and disease carrier. Micro-organisms like bacteria breakdown carbohydrates and use the energy thus released, but the aquatic system is depleted of its oxygen content in this process. This technique is employed to determine the relative oxygen requirements of waste waters, effluents and polluted waters. An estimate of the oxygen consumed in the unit volume of water over a period of time is called Biochemical Oxygen Demand (BOD). Thus, it is the amount of molecular oxygen required for an average basis, the demand for oxygen is proportional to the amount of organic waste to be degraded aerobically. Hence, BOD approximates the amount of oxidizable organic matter present in the solution. The BOD values are very useful in process design and loading calculations as well as the measure of treatment plant efficiency and operation. The BOD test is also useful in stream pollution control management and in evaluating the self purification capacities of streams.

BOD in general gives a qualitative index of organic substances which are degraded quickly in a short period of time. BOD values should not be used as equivalent to the organic load regardless of the presence of non degradable organic matter, presence of toxins and local changes in population of microorganisms. BOO test should be restricted to only suitable wastes in management of the treatment plants. The organic pollutants are highly oxygen demanding. Carbon is the most important factor. Oxygen is used for degradation of carbon, which is measured by standard test called BOD 5 days test. The amount of dissolved oxygen required for oxidation over 5 days is measured, and the result is expressed in mg of oxygen per liter, Thus, DO is the basis of BOD test.

Reagents:

1. *Phosphate buffer solution* — Dissolve 8.5 g KH_2PO_4 or 21.7 g K_2HPO_4 or 33.4 g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl in 500 ml distilled water and dilute to one liter. The pH of this buffer solution should be 7.2. If it is not so, discard the buffer solution and prepare again. Discard reagent if there is any sign of biological growth in the stock bottle.
2. *Magnesium sulphate solution* — Dissolve 22.5 g $MgSO_4.7H_2O$ in distilled water and dilute to one liter.
3. *Calcium chloride solution* — Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to one liter.
4. *Ferric chloride solution* — Dissolve 0.25 g $FeCl_3$, $6H_2O$ in distilled water and dilute to 1liter.
5. *Manganous sulphate solution* — Dissolve 480 g $MnSO_4$. $4H_2O$ or 400 g $MnSO_4.2H_2O$ or 364 g $MnSO_4.H_2O$ in distilled water and make the volume to one liter.
6. *Alkaline iodide* — Sodium azide solution (NaN_3)-. Winker's reagent (a) Dissolve 500 g of NaOH (or 700 g of KOH) and 135 g Nal (or 150 g KI) in distilled water and dilute to one liter. (b) Dissolve 10 g of NaN_3 in 40 ml of distilled water. Mix the two solutions (a) and (b).
7. *0.025 N Sodium thiosulphate solutions* — Dissolve 24.82 g of $Na_2S_2O_3$. $5H_2O$ in a preboiled distilled water and make the volume to one liter. Add a pellet of NaOH or 0.4 g of borax as a stabilizer. This is 0.1 N stock solution. Dilute it to four times to prepare 0.025 N solution (Dilute 250 ml to 1000 ml) and store in brown glass bottle. OR, Dissolve 6.205 g of sodium thiosulphate in 1 liter distilled water. Add 0.25 g of NaOH for preservation.
8. Cone H_2SO_4, acid(sp. gr. 1.83 — 1.84).

Procedure : Take 10 ml water sample. Add 250 ml distilled water to it. To this add 1 ml each of phosphate buffer (to maintain pH), $MgSO_4$, $CaCL_3$, $FeCl_3$, (to provide nutrition to microbes) and make the volume to one liter. Fill 250 ml of above water solution in two BOD bottles. Incubate one bottle in BOD incubator for 5 days at 20°C in dark. Use other

bottle for initial DO readings as done in experiment 13. At the end of five days incubation period, measure the amount of dissolved oxygen in the incubated bottle.

Calculation:

Volume (ml) of $Na_2S_2O_3$ used in initial bottle sample = a

Volume (ml) of $Na_2S_2O_3$ used in incubated bottle sample b

1 ml of 0.025 N solution = 2 mg/I DO

DO in initial sample (mg/I) a x 2

DO in incubated sample (mg/I) = b x 2 =

$$\text{BOD (mg/I)} = \frac{D_1 - D_2}{P}$$

Where, P = decimal fraction of sample used (0.0j. in this case).

Note — The microbial populations are capable of oxidizing the effluent. Where such population of microbes is already present the sample need not be seeded.

When sample contains a little or no microbial population due to high temperature, pH or chlorination, dilution water should be seeded. The purpose of seeding is to introduce into sample microbial population capable of oxidizing the effluent. Hence in such cases BOD can be calculated as below:

$$\text{BOD} = \frac{(D_1 - D_2) - (B_1 - B_2)}{P} X\ f$$

Where,

D_1= DO of diluted sample taken immediately (mg/I)

D_2 = DO of diluted sample after 5 days incubation (mg/I)

B_1 = DO of seed control before incubation (mg/I)

B_2= DO of seed control after 5 days incubation (mg/I)

P = decimal fraction of sample used

f = Ratio of seed in sample to seed in control = (% seed in D1 I % seed in D2)

Experiment No.5

Title : Species Abundance
Objective : To estimate the species abundance of plants community

Introduction

In nature, organisms belonging to several species can be observed in any given area growing in association with each other. An assemblage of all the interacting population of different species existing in a geographical area is known as a community. It is a complex network of plants, animals and micro organisms. In a community, organisms share the same habit growing in an uniform environment. Communities consists of populations, each of which is a group of interbreeding organisms belonging to the same species. These different species of organisms will impart a structure of community having different populations of organisms. Thus each community is characterized by its growth forms, species diversity, dominance of some species, survival trend *etc.* of nature. Identification of the species which is most populous over the other species and estimating the populations of different species of organisms in that particular area is the estimation of species abundance.

Aim

To study the vegetation by point frame method and determining the frequency of each species present.

Requirements

Point frame apparatus, graph sheet

Procedure

- The apparatus with 10 pins put at a place is one sampling unit. Put the apparatus in random at a number of places (20 or more) in the field.
- Note down each time the various species hit by one or more of the 10 pins and thus, pin is one sampling unit.
- Distribute the various frequencies among five frequency classes and find out the % value in each frequency class.

Observations

Name of the plant.	Plants at the following Pin number of the frame										Total no. of individual plants	Frequency %	Density	Abundance
	1	2	3	4	5	6	7	8	9	10				
A														
B														
C														

Calculations

(i) ***Frequency:*** It is number of sampling unit (as %) in which a particular species occurs.

$$\% \text{ Frequency} = \frac{\text{Number of sampling units in which the species occurred}}{\text{Total number of sampling units studied}}$$

After determining the percentage frequency of each species, various species are distributed among Raunkier's five frequency classes depending upon their frequency values.

Frequency %	Frequency class
0 to 20	**A**
21 to 40	**B**
41 to 60	**C**
61 to 80	**D**
81 to 100	**E**

(ii) ***Density***: It represents the numerical strength of a species in the community. The number of individuals of the species in any unit area is its density.

Density gives an idea of competition.

$$\text{Density} = \frac{\text{Total number of individuals of the species in all the sampling units}}{\text{Total number of sampling units studies}}$$

The value obtained is then expressed as number of individuals per unit area.

(iii) ***Abundance***: This is the number of individuals of any species per sampling unit of occurrence.

$$\text{Abundance} = \frac{\text{Total number of individuals of species in all the sampling units}}{\text{Number of sampling units in which the species occurred.}}$$

Experiment No. 6

Title : Transpiration and Water Balance in Plants
Objective : Study of Transpiration and Water Balance in Plants

Introduction

Plant responses to water deficits are often measured under greenhouse or other controlled environmental conditions. Such studies aid in characterization of fundamental physiological responses of plants to drought stress or to identify genetic variability in the genotypes. The identified species could be used either in breeding programmes or in further phenotypic studies.

Aim

To estimate the water requirement of a given genotype / crop and arrive at a balance sheet for the dry matter produced.

Principle

Gravimetric approach makes it possible to maintain precise level of water requirement or stress at whole plant level. Water stress can be imposed more slowly akin to the natural environment if total plant available water is increased either by using large containers. In such studies the rate at which water stress develops or growth increase depends on the environmental conditions (radiation load, air temperature, humidity) which may vary depending on the water use rates of individual plants. A more consistent treatment that synchronizes well with the experimental units or pots can be achieved by using smaller pots by determining water loss from each pot gravimetrically on a regular basis and replacing part of the transpired water to control the rate of soil dry down. The approach is referred to as gravimetric approach for drought induction studies or assessment of genetic variability and their characterization.

Requirements:

Battery containers or plastic pots, garden soil (soil: sand: manure in the ratio of 2:1:1), mobile weighing device, seed or plant material.

Procedure

- Take the empty pot as used for stress imposition experiments
- Fill the pots with soil

- Weigh the pot along with soil and deduct the empty pot weight to obtain the dry soil weight
- Flood the pot with water to saturate soil and leave it for 24 hours to drain the excess water and consider it as at 100% field capacity (FC)
- Take the pot weight along with pot and deduct the empty pot weight to get the 100% FC soil weight. The dry soil weight is subtracted from the 100% FC soil weight to get the amount of water required to maintain 100% FC.
- Sow the seeds of the crop under irrigation in the pots. Two seedling may be maintained in each pot and watered regularly to maintain moisture level at 100% FC in a poly house.
- After establishment of the seedlings maintain soil at 60% FC *i.e.*, mild stress in pots.
- Take continuous observations at weekly intervals from six leaf stage.
- At each interval the observations include: record of the amount of water added to maintain 60% FC; pull out the plant and take the dry weight of the entire plant or its component parts.
- Equate the amount of water added and the dry weight increase to know the water balance in plants.

Maintenance of 60% FC is calculated as follows:

100% FC, measured by Keens method = X ml of water

$$60\ \%\ FC = \frac{60\ \% \times X \text{ ml of water}}{100\%}$$

For example the amount of water required to maintain 100% FC = X ml = 200 ml

$$\text{The amount of water required to maintain 60\% FC} = \frac{60\% \times 200 \text{ ml}}{100\ \%} = 120 \text{ ml}$$

Observations and Calculations

Pot no (1)	Date (2)	Weight of pot + air dry soil (g) (3)	Amount of water added to bring to 60% FC (ml) (4)	Total Weight of the pot along with plant and soil at 60%FC (g) (5)	Weight of the plant (g) (6)= (5) - (3)+ (4)	Remarks
1						
2						
3						

Discussion

Variation among the genotypes or between crop plants for water requirement could be quantified over a period of time. The genotype which takes little amount of water and puts forth growth or reaches a particular growth stage is considered as a water saver or otherwise water spender. Based on the computation of water requirement and dry matter produced, water balance is arrived at for a genotype. Maintenance of adequate amount of plants / pots would aid in reducing the error and arriving at a water balance sheet for the particular genotype under consideration.

Experiment No. 7

Title : Preparation of Herbarium
Objective : Preparation of Plants Herbarium

Collect the plant sample of 12 inches in size including leaves, flower, and fruit attached with the twig. Press in the news paper for 7 to 15 days. Collected and pressed plants will be properly checked every alternate day and within 2 to 4 days the news paper will be changed so that the collected sample will be properly dried. After completely drying the sample, paste the sample in Herbarium sheet with the help of fevicol or cello transparent tap properly. Now the following information will be required to write on the one corner of the sheet.

1. Local name of the plant
   (Indicate Tree, Shrub or Herb) Large medium or small size
2. Scientific name of the plant
3. Name of family.
4. Place of collection.
5. Date of collection.
6. Uses.
7. Collected by.

   Each student will prepare 50 sheets of wild cultivated/ domestic plant available in the area.

Experiment No. 8

Title : Collection of seeds
Objectives : To demonstrate the seeds collections

Seeds will be collected from fruit which are mature or fully ripped, and collected seeds will be dried till the moisture content remain 5% in the seed.Then clean and clear seed will be properly dried and packed in the plastic bags/ pouch or container in moisture free condition in cool and dry place. Before storage the proper leveling will be done so that the authenticity of the seed will be ensured. Containers should comprised of following information.

1. Name of the plant (Local)
2. Scientific name
3. Name of family
4. Date of collection
5. Place of collection
6. Locality
7. Village
8. Type Orthodox or Recalcitrant
9. Shape---------- Size----------dry weight----------
10. Longevity of seed: - Date------Month-------Year----------
11. Use before date / Expiry date
12. Collected by:-
13. Uses:-

The above mentioned information will be written properly and specimens will be prepared accordingly each student should collect 25 forest tree seed.

Seed collection time:

The collection time of seed is different for different species. The same species in different place may have different seed collection time which is strongly influenced by the climatic conditions.

Maturity of Seed:-

The seed from fruit should be extracted at the time of maturity. The fruit skin should become yellow and seed should be red or brown. The outer skin of the seed will be fully ripened. But seed should be collected before its dispersal from the fruit

Methods of seed Collection:-

1. Ground collection of naturally fallen seed
2. Collection of seed by lopping branches or from currently felled trees.
3. Collection of seed from standing tree

Seed extraction:

The seeds should be separated from the mature fruit as early as possible.

Drying:

The collected/extracted seeds should be dried in direct sunlight/ shade.However, it depends on species to species. After drying the seed should be stored in cool dry place.

Experiment No. 9

Title : Identifications of trees
Objectives : Collection and identification of timber, fuel, minor timber wood and

NTFP species:-

Visit to nearest saw mill and natural forest area, while visiting the saw mill collect the wood sample of 4 inch long 2 inch wide and 1 inch thick in size. Wood sample should be fully dried with smooth surface in the entire portion. However, visiting the forest area the character of the tree should be noted as bark characteristics, branching pattern, crown size, leaf shape & size and leaf characteristics along with the fruit and flower characteristics. The following characteristics as mentioned below should be written to identify the wood sample which will determine the use of wood specifically.

1 Local name of the tree/shrub

2 Scientific name

3 Name of family:-

4 Category of wood:-

5 Characteristics of the wood

6 Color----------Texture----------Grain----------Fiber length----------
Durability of wood----------Nail holding capacity----------lignin content----------

7 Other important characters:-

8 Major and other use of wood:-

9 Collected by:-

10 Date of collection:- date ----Month-----Year--------

11 Place of collection:- Area---------- Locality----------

Experiment No. 10

Title : Visit of Forest and Plantations Area
Objectives : To demonstrate the concept of natural forest area, plantations, nursery for identification of tree species:-

1 Natural forest stand

Student visits will be arranged at nearest natural forest area, commercial plantation sites and nurseries to know the different characters of the species in order to identify the tree, shrubs and annuals species. The vegetation and its composition (tree shrubs and herbs) will be shown to the students accordingly with common names, scientific names and their uses for wood, timber, fuel, fodder, non timber forest product species. Like tree height bark characters, branching pattern, flowers, inflorescence type, color variation of the species Leaf shape, type, size and character will be taught. Fruit shape, size, color, seed characters, wood quality and their different uses will be taught.

Experiment No. 11

Title : Plantations

Objectives : To demonstrate the Forest tree plantations

The difference between natural forest and commercial plantation (mixed and monoculture plantation) will be taught to the student so that they will be aware of these terminology properly and their different uses will be known to them as per their different uses as per the need like manmade forest, natural forest and commercial plantation for different uses for industries and day to day need of the man.

Commercial tree plantations:-

Plantations of teak, khamhar, eucalyptus, bamboo fuel wood species raised by forest department /private farmers/industries will be shown as monocarps or mixed plantation for different purposes and their use accordingly

Industrial Plantation:-

Plantations of bamboo, teak, soft wood species for paper preparation resin/gum extraction Important NTFP product tree plantation raised as mono crops or mixed tree crop for only commercial uses will be also shown so that they will be aquainted of those species which are having high commercial value.

Minor timber tree species plantations:-

Plantation as mono crop or mixed crop with only minor timber species raised by forest department or private/ industrial plantation will be shown accordingly with their objective and uses.

Experiment No.12

Title : **Non Timber Forest Product**

Objectives : **To demonstrate the concept of non timber forest product species plantations:-**

To create the awareness among the students about harad, baheda, aonla, chironjee, tendu patta, sal, bamboo, sahtoot, tusser silk host trees like terminalia species, palash tree for Lac cultivation etc. The major difference between different types of plantation will be taught as per the objectives and their uses and the identification character of the species will be necessarily taught so that they will be able to know the Identification characters of particular species.

The difference between different types of plantation will be taught to the student as per their uses by the human beings. In all the plantation and natural forest stand the species identification will be taught to the students.

Identification of species:

The tree, shrubs, herbs, grasses and annual plants causes will be identified and the method of their identification will be taught to the students in the natural forest and plantation raised by the forest department, University experimental fields.

Experiment No.13

Title : **Characteristics of tree species**
Objectives : **To demonstrate the characteristics of timber, fuel wood, ornamental tree and fodder tree species:-**

Characteristics of the timber species:

1. Timber wood species is highly durable and long lasting wood character.
2. Safe from insect pest like termite, boror and fungus
3. Better fiber and grain alignment, color
4. Free from knots good mail holding capacity
5. Clean and long bole and less branches

Fire wood species: -

1. Fast growing species, high coppicing power
2. High calorific value
3. Less smoke and easily available local species

Fodder species:-

1. Fast growing palatable leaf for animal
2. high crude protein and fiber
3. Rich in essential minerals and calcium
4. Leguminous species preferred

Ornamental species:-

1. Beautiful flowering species
2. Straight and clean bole
3. Suitable architecture for avenue plantation
4. Evergreen species
5. Fast growing species. shade providing crown size larger than other species
6. Non toxic and non poisonous of leaf/flower

Experiment No. 14

Title : Forest Nursery

Objectives : To demonstrate the layout of forest nursery:-

While selecting the area for nursery the soil of that area should be fertile with good slope for drainage of water. It should be nearest to road side.

Nursery area should be divided in to plots for keeping FYM, sand, pots and other raw material. Nursery store pump house and office will be developed. Water availability in the whole year must be looked before finalization of nursery establishment. A well inspection path and 30 meter road should be developed in the nursery area to lift the plants from bed by the four wheelers like truck/tractor trolley etc.

Selection of Site for Nursery: -

The well fertile land area is the right choice for development of nursery. The following things shouldl be kept in mind.

1) Land 2) Soil 3) Water 4) Distance from plantation site

5) Shade

Practical 9:-

Type of Nurseries:-

1) On the basis of use and time

(a) Temporary nursery

(b) Permanent nursery

2) On the basis of irrigation facilities

(a) Un irrigated nursery

(b) Irrigated nursery

Nursery planning:

Before raising of plant one should must know that how much seedling are required for the plantation of particular species. The area required to grow the certain species number of seedlings required can be calculated using following formula:

$$\text{Area required} = \frac{18 \times 1.2 \quad A \times B}{C}$$

18 = Land required for one bed in (SQ. meter)

1.2 = 20% Excess plants to be grown as the real quantity needed

A = Plantation area in (hectare)

B = The number of plants required in one hectare

C = The number of polyethylene bags needed in one bed

In 10 x 1 meter bed 1000 polyethylene bags can be easily kept, size of the polythene bag must be 15 x 23cm which is filled with the soil, sand and FYM.

Size of nursery bed:

The size of standard nursery bed should be 10 meter long one meter wide and between two bed there should be inspection path which is 45cm to 50cm for inspection and other cultural operation can easily be conducted by labour.

Types of nursery bed:

The nursery beds are classified on the basis of their structure

1) Sunken bed - 10 cm deep from the surface of the nursery

2) Raised bed - It is 15cm up from the surface of the nursery

3) Flat bed -These beds are prepared in the same level as of the nursery area

4) Housing bed -

These are basically prepared to keep the polyethylene bags with raised planted seedlings. These bed should be prepared on the basis of size of the polyethylene bags so that the polyethylene bags can be kept properly in line but while keeping they may not fell down. These beds are also prepared at lower level of nursery surface.

Nursery beds on the basis of use and objectives:

1) Mother bed
2) Transplanting bed
3) Housing bed
4) Direct sowing bed

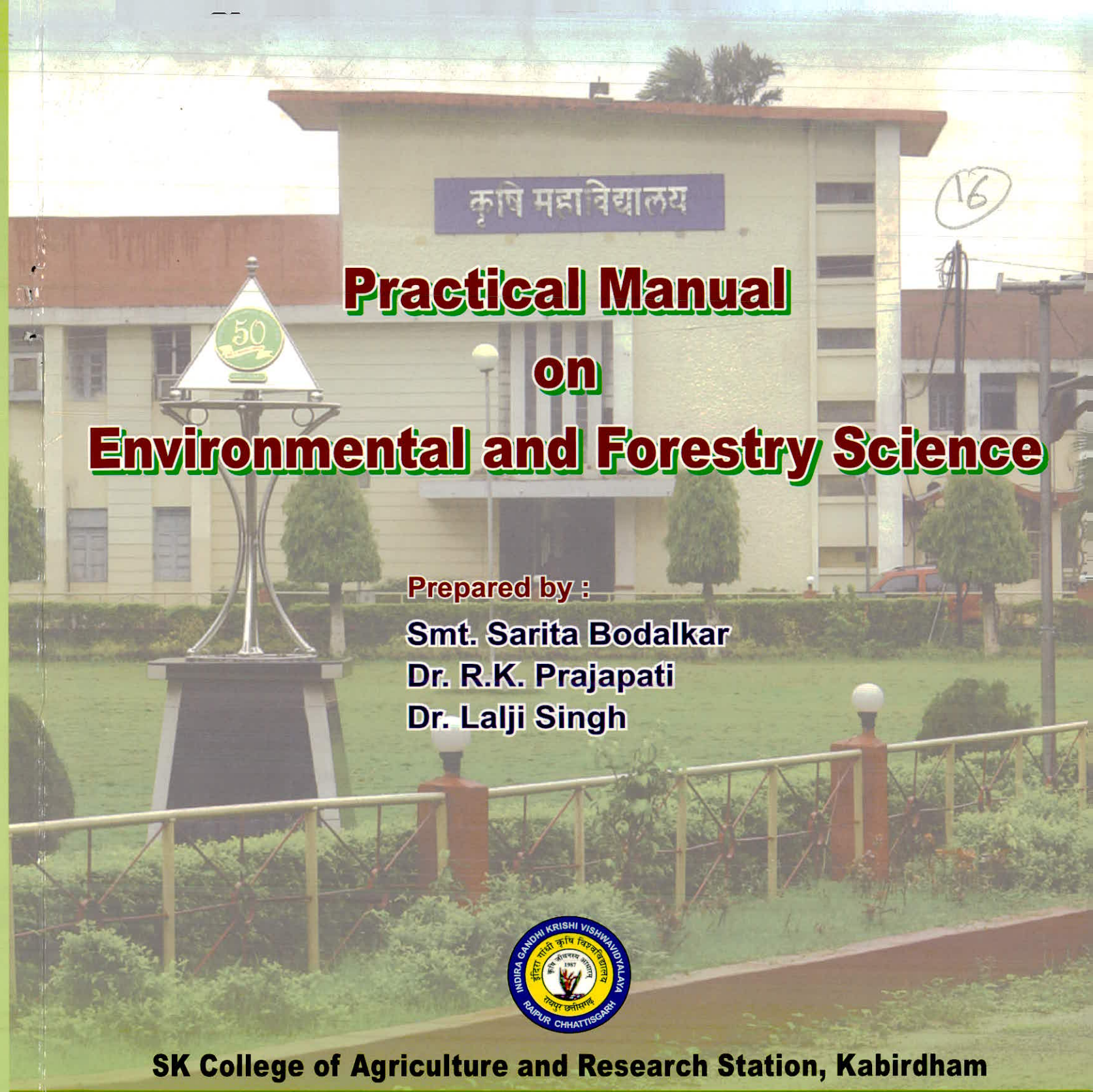
Potting mixture:-

The potting mixtures contain 50% soil, 25% sand and 25% FYM decomposed. These mixture mixed thoroughly and filled in the polyethylene bag, earthen pot or plastic containers as per requirement.

Raising of seedling in the nursery:-

First the viable and better quality seeds will be separated from saline water solution and the heavy seeds or submerged seeds will be taken for the germination purpose. After selection of quality seeds the seeds treatment with the help of Carbandazim or Diaethane M- 45, 4-5 gm powder per kg of seeds will be taken in pot and mix few drops of water so that the powder stick to seed coat. After that these seeds will be dried in shade and on next day these seeds will be sown in properly prepared seed beds. The treated seeds will be shown in line, line to line distance will be kept 10 cm or seeds can be sown through broadcasting methods also in the nursery bed. After sowing of seeds in nursery bed, time to time the moisture availability should be maintained and as and when watering needed the irrigation should be provided through water cane or flood irrigation. The young seedlings should be properly watched and if there is any symptoms of disease or insect the insecticide or pesticide should be used to control the disease or insect damage to the seedlings. When the seedling become 6 to 10 inch long then it can be used for plantation purpose.

Chhattisgarh Samvad



Practical Manual on Environmental and Forestry Science

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