# CHAPTER 3:

# MATERIALS AND METHODS

### 3. Overview

This chapter contains a description of the systematic procedures followed to fulfill the objectives of this research such as the selection of the site, physicochemical analyses of spoil samples (pH, bulk density, organic carbon etc.), biological aspects covered (soil enzymes, plant growth). This also includes the planning and experimental design adopted to carry on the research. A flow chart of the design is given in Fig.3.1.

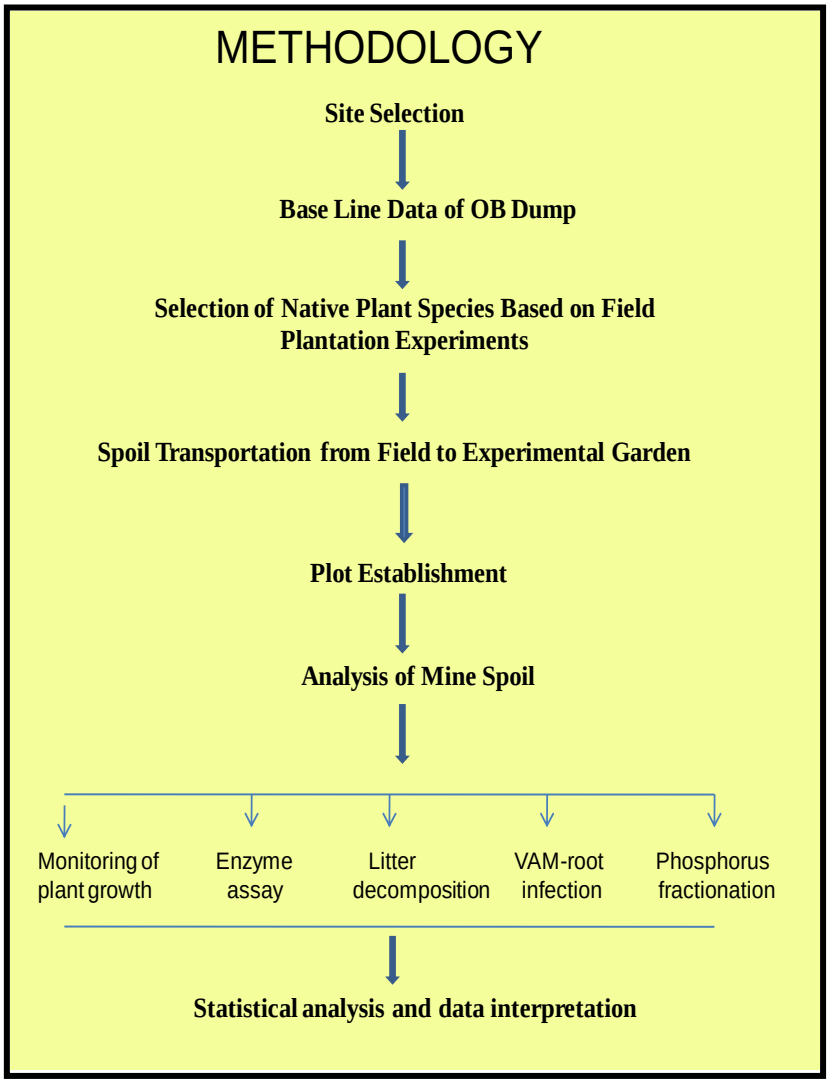


**Fig 3.1 Schematic diagram of the experimental protocol**

### 3.1. Study area: The Jharia coalfields

The Jharia mines have huge deposits of coal operated by private firms since 1896. Since the nationalization of a coal mine in 1971, Bharat Coking Coal Ltd. (BCCL) became the sole operators as underground mining. However, after 1973 BCCL decided to shift to opencast mining. Jharia is situated in between latitude 23˚ 35' N to 23˚ 55' N and longitude 86˚ 05' E to 86˚ 30' E. The coalfield is spread over an area of 456 $km^2$ approximately, extending in east-west direction (Chandra et al., 2001). The major coal-bearing formation in Jharia Coalfield is the Barakar Formation of Early Permian age, forming a sickle-shaped outline in the northern part of the coalfield (Figure 3.2), is severely fire-affected. Barakar Formation consisting of fluviatile deposits is the lowermost member in the Jharia coalfield. This is important formation containing coal seams and covers an area of about 210 sq km. The sediments of this formation maintain erosional contacts with the underlying Talchir rocks near the basin periphery and towards depth, the contact appears normal (Sengupta, 1980). The Barakar Formation comprises of coarse-grained sandstones, conglomerates, shales, carbonaceous shales, silt-stones, fireclays and coal seams. The Barakar sandstones show undecomposed feldspars suggesting unstable source-rock area and rapidly subsiding conditions. The mine-soil is fresh and contains approximately 80% sand, 11% clay and a low concentration of nutrients.

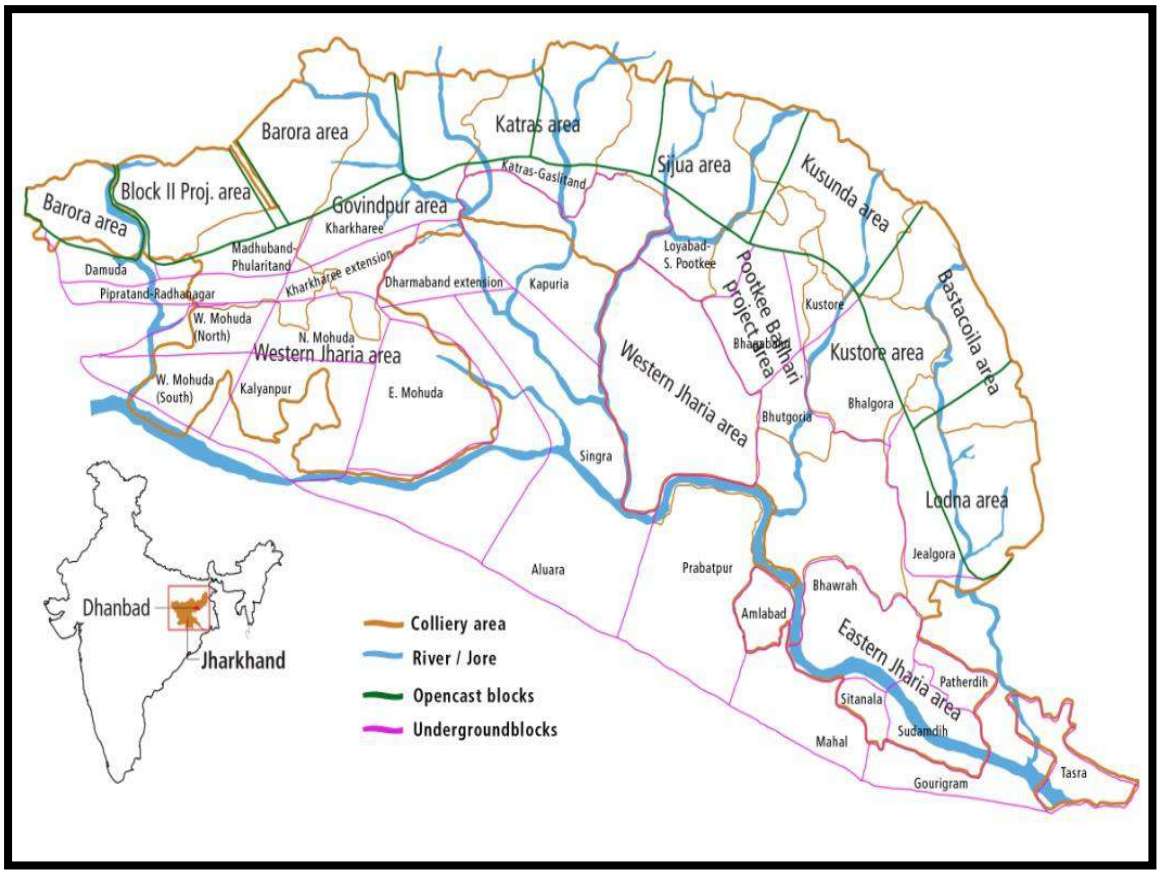


**Fig 3.2** Location of Jharia Coalfields

### 3.1.1. Climate of Jharia

Climatically the Jharia coalfield is of tropical monsoon nature with its hot summer and cold dry winter. The average annual temperature is 26.2 and receives annually 900-1300 mm of rainfall. The data in Fig 1.2 show the climatic parameters from the year 2000 to 2012.

### 3.1.2. Soil of Jharia

Different land use systems reflect wide variation in soil characteristics. In the Jharia coalfields, generally, the entire coalfield has an inclination towards coarse texture but the soils of open cast mining areas are relatively stonier than other areas. Chemically the soil of the coalfield may be regarded as slightly acidic with normal condition of electrical

conductivity and total dissolved salts. Among the concentration of different heavy metals, iron, chromium, vanadium, is reported to be very high whereas other heavy metals are found to be under the critical limit. The soil quality is degraded (Shrestha and Lal, 2008; Mukhopadhyay and Maiti, 2014).

Rare earth elements (REE) have been found in the coalfields of Jharia (Masto et al., 2011) such as La, Pr, Nd, Sm, Eu, Tb, Dy, Ho, Tm, Lu, Ho and Sc in open cast mine sites are found to be higher as compared to the control soils. The average total content of REEs in opencast coal mine soil was the highest (222 mg/kg), followed by control (203.1 mg/kg), coal washery-1 (192.3 mg/kg) coal burning (169.4 mg/kg), and coal washery-2 (162.5 mg/kg). Yttrium (Y) has significantly contributed for the higher observed under control; the level of Y in other soils is depleted up to 60%. These soils are developed from granite/ genesis parent material. Miao et al. (2008) found that soil developed from the granite parent material has the highest REEs followed by basalt and red siltstone. REE concentrations of A horizon (surface soils) were higher than that of the subsoils, which is probably due to the biological effect in topsoil (Wei et al., 2001; Tyler, 2004).

### 3.1.3. Natural vegetation of Jharia

Vegetation cover in the Jharia coalfield area has been found to be predominantly of five classes:

1.Dense Forest

2.Open Forest

3.Scrubs

4.Plantation on Over Burden (OB) Dumps / Backfilled area, and

5.Social Forestry

BCCL has carried out significant plantation on OB dumps as well as backfilled areas during the period for maintaining the ecological balance of the area. The plantation on the OB dumps and backfilled areas are estimated to be 10.53 sq km, i.e., 2.68% of the coalfield area. There has been significant variation in the land use under the vegetation classes within the area as shown below in Table 3.1.

The tree species consist of fast-growing deciduous types, planted by Forest department and detailed composition is given in Table 3.2. The ground vegetation was found totally dried up, only a few natural growth of *Lantana camara* L., *Leonotis nepetifolia* (L.) R. Br., *Eupatorium odoratum* L., *Hyptis suavelons* Griseb. and *Pennisetum pedicellatum* Trin. were observed. Out of the tree species, three species, namely *Cassia siamea*, *Gmelina arborea* and *Acacia auriculiformis* constituted approximately 70% of the tree population. Some accidental tree species like *Ficus retusa* L. and *Ficus religiosa* L. are also seen.

### *3.2. Site selection*

The site was selected as per the suggestion of Bharat Coking Coal Limited (BCCL). It was suggested as the dump was new, bare and would not be disturbed for further opencast mining activities for about next five coming years. The study area was in Bastacola Mines, Jharia Coalfield, Jharkhand, India (Plate 3.1). The stony nature of the dumps and the cracks which develop in the dumps are depicted in Plates 3.2 and 3.3 respectively. The crack development shows the risk of dump collapsing which is a common accident in coalfields. For the baseline data collection of the dump, samples of spoil were collected. The material which was used in this study, is described in Table. 3.3.

**Table 3.3. Materials: list of materials used is enlisted**

| S.N. | Materials | Description |
|---|---|---|
| 1 | OB Dump spoil | Collected from Bastacola colliery. The dump was new devoid of any vegetation |
| 2 | Agriculture soil | Collected from an agriculture field lying adjacent to the dump |
| 3 | Cow dung manure | Collected from nursery of forest department, Dhanbad, Jharkhand |
| 4 | VAM | Vesicular Arbuscular Mychorhhizae applied as tablets purchased from KCP Sugar and Industries, Vuyyuru, Andhra Pradesh. |
| 5 | Plastic sheets | to line the plots for the avoidance of mixing of soil with the underlying garden soil |
| 6 | Plant species | Bamboo (*Dendrocalamus strictus*: Poaceae), Peepal (*Ficus religiosa*: Moraceae), Neem (*Azardiracta indica*: Meliaceae), Ashok (*Saracca indica*: Fabaceae), Amla (*Emblica officinalis*: Phyllanthaceae). |
| 7 | Net Cloth | To make litter bags and also cover the plots so that external litter does not enter the plots |

**Plate 3.1** Dump site in Bastacola

**Plate 3.2** Loose rocks and heterogeneous organization

**Plate 3.3** Cracks on the dumps showing the risk of collapsing due absence of vegetation

### 3.2.2. Selection of plant species based on field plantation experiment

Though the dump was new but some weeds namely *Blumea axillaris* (Lam.) DC., *Tridax procumbens* (L.) L, *Anisomeles indica* (L.) Kuntze., already grew on it, as depicted in Plate 3.4. The species were suggested by Forest Dept. of Dhanbad and two months, old saplings of perennial plants were procured from the forest nursery. Ten species were selected for the field trial to find out stress tolerant plant species namely:

Khair *(Acacia catechu* (L.f.) Willd.: Fabaceae), Gamhar (*Gmelina arborea* Roxb.: Verbanaceae), Bamboo (*Dendrocalamus stictus* (Roxb.) Nees: Poaceae), Peepal (*Ficus religiosa* L.: Moraceae), Neem (*Azadirachta indica* A. Juss.: Melieceae), Ashok (*Saraca asoca* (Roxb.) Willd. : Fabaceae), Karanj (*Pongamia glabra* Vent. : Fabaceae), Amla (*Phyllanthus emblica* L. : Phyllanthaceae), Teak (*Tectona grandis* L.f. : Lamiaceae), Sal (*Shorea robusta* Gaertn. : Dipterocarpaceae). Out of ten species, five plant species were found suitable for plot experiment in ESE Garden, namely Bamboo (*Dendrocalamus strictus* (Roxb.) Nees.: Poaceae), Peepal (*Ficus religiosa* L.: Moraceae), Neem (*Azadirachta indica* A. Juss.: Meliaceae), Ashok (*Saraca asoca* (Roxb.) Willd.: Fabaceae), Amla (*Phyllanthus emblica* L. : Phyllanthaceae). Preparations for field trial are shown in plates 3.5. Plants were planted randomly as shown in plate 3.6, for the experimental determination of the survival of these species. The growth of some plants during this experiment is depicted in plate 3.7.

**a.** Blumea axillaris (Lam.) DC. (Asteraceae) and Tridax procumbens (L.) L. (Asteraceae)

**b.** Anisomeles indica (L.) Kuntze (Lamiaceae)

**c.** Lantana camara L. (Family : Verbenaceae)

**Plate 3.4 Weeds growing on the dump site (a, b, c)**

**Plate 3.5 Preparation for the field trial of the plant species to check the survival rate**

**Plate 3.6 Planting of the selected plant species for the experiment on the site**

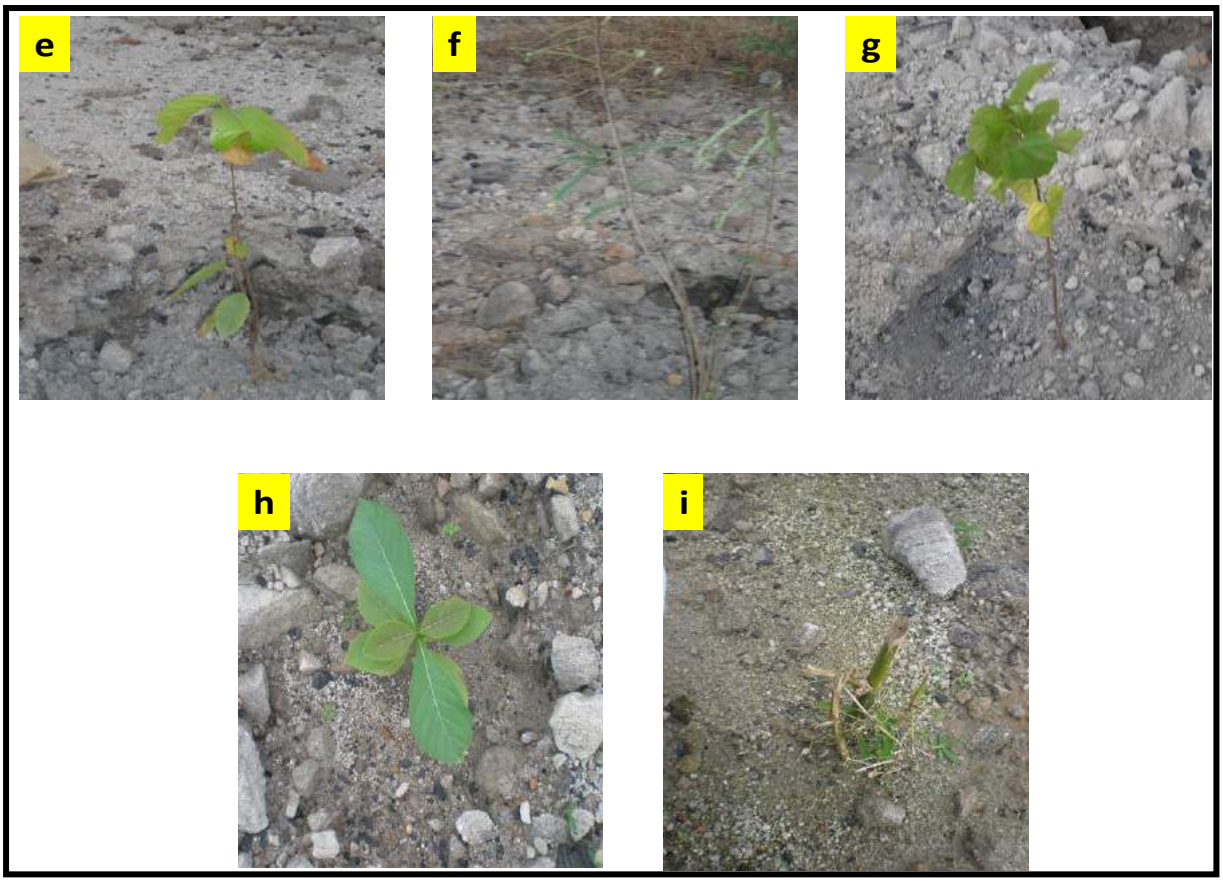


**Plate 3.7 e, f, g, h, i, showing the growth of some plant during the pilot experiment**

**Plate 3.8 Sampling of overburden spoil**

*Dendrocalamus strictus* is a deciduous densely tufted bamboo; flowering cycle varies from 25-45 years. *Azadirachta indica* is a native to India, grows in tropical and semi-tropical regions and has wonderful medicinal uses. *Phyllanthus emblica* is a deciduous tree known for its edible fruit. *Saraca asoca* is considered sacred throughout India. It is found in the foothills of central and eastern Himalayas, almost all over the northern plains of India as well as on the west coast of Bombay. *Ficus religiosa* (Peepal) is of great medicinal value. It is a sacred plant, worshipped in India. Its leaves serve as a wonderful laxative as well as a tonic for the body. It is especially useful for patients suffering from jaundice.

### *3.3. Preparation of plot*

The overburden dump material was collected from Bastacola OCP, Jharia. The baseline data about the physicochemical properties of the site was generated by collecting the overburden material. As the overburden dump materials consisted of rocks, boulders and

hard materials, the overburden samples were collected by hand shovels, manually operated split tube coring tool as well as rammers (Plate 3.8). Then it was transported to the Experimental Garden of provided by BCCL for plot experiments in Dhanbad (Plate.3.9). The agricultural soils were also collected from agricultural plot just beside the dump site in Bastacola region. The manure, used in this experiment was cow dung manure and VAM tablets (*Glomus sp.*) were obtained from KCP Sugar & Industries Corporation Pvt. Ltd., Vuyyuru, Andhra Pradesh.

**Plate 3.9 The over burden material was carried from the site to the garden. For each plot, plastic sheets were laid to avoid mixing of OB material with the garden soil. Plots were covered with a net so that unnecessary fertility enhancement does not occur because of litter fall from the well-established trees in the garden.**

Three sets of control and three sets of each treatment plots (5 x 5 $m^2$) were established and maintained at Experimental Garden from April 2014, making the total no. of plots to be twelve. In these plots, OB materials filled up to 1m depth and the volume of each plot was 25$m^3$. The composition of control and three types of treatment plots used in the experiment are given in Table 3.4. Randomized Block Design (RBD) was followed.

**Table 3.4. Detail of the control and treatment plots**

| Plot Name | Abbreviation | Remarks |
|---|---|---|
| Over Burden Control | OBC | Mine spoil, seedlings of five plant species, no amendments |
| Over Burden + VAM | OBV | Mine spoil, seedlings of five plant species, treatment with Vesicular-Arbuscular Mycorrhizae (VAM) |
| Over Burden +VAM+ Agricultural Soil | OBS | Mine spoil, seedlings of five plant species, treatment with Vesicular-Arbuscular Mycorrhizae (VAM) and agriculture soil to mine spoil (1:4) |
| Over Burden + VAM + Cow dung Manure | OBM | Mine spoil, seedlings of five plant species, treatment with Vesicular-Arbuscular Mycorrhizae (VAM) and manure to mine spoil (1:4) |

The above-selected plant species qualifying the on-site survival experiment were planted randomly with a spacing of 1 meter. The number of individual plant in each plot was constant; treatment was varied. After the successful establishment of the plants in the plots, rhizosphere soil of each plant from the experimental plots was collected carefully, without harming the roots. These soil samples were analyzed in the laboratory for the

above mentioned physicochemical and biological properties from time to time. Along with the physicochemical properties, the enzymatic activity of the soil, namely catalase and dehydrogenase were also evaluated. The plots were covered with a net to stop the entry of litter from external sources (plate 3.10.). This was done to avoid nutrient enrichment in the soil in plots from external tree litter fall.

### 3.4.Methodology

This section deals with the procedure used to collect samples from the study area and experimental plots. Processing of the samples is described as well. This also includes the laboratory experiments involving various chemicals, instruments, glassware and apparatus.

#### *3.4.1. Baseline data of the OB dump*

After collection of the OB samples, the physicochemical parameters were analyzed to get the baseline information about the soil. This was done to know the characteristics of the OB materials so that restoration could be planned accordingly. For proper analyses of the OB dumps samples, the following procedures were followed in the laboratory:

##### *3.4.1.1. Sample preparation*

The sample preparation devices mainly consisted of pestle and mortar, different sieves, spatula, plastic papers etc. The important steps taken for the samples are as follows (Maiti, 2003):

- Drying of samples: Soil samples were air-dried by spreading them out in shallow trays and plastic sheets at the room temperature. Up to 7 days room-drying was carried out.

Air-dried, sieved soil samples were stored for subsequent analysis. For some parameters, fresh samples were used, while for others, the soil sample was stored after proper drying. The drying temperature for soil is very crucial, as the nature of many constituents is highly sensitive to temperature. High-temperature drying generally affects the exchangeable characteristics of clay and organic colloids, thus avoided.

- Sieving: The air-dried soil was weighed and then sieved through a 2 mm sieve. If some clods were formed, they were crushed by mortar and pestle in a soft circular motion so that the rocks did not get crushed but the soil particles adhering to the pebbles got separated.

### *3.5.The soil physicochemical analyses*

After the samples were properly dried and sieved the physicochemical analyses were performed as listed in table 3.5 and detailed description is followed.

**Table 3.5. Methods used for various physicochemical estimation**

| S.N. | Parameters | Methods | Chemicals Used | Instrument and manufacturer |
|---|---|---|---|---|
| 1. | pH (1:2.5; Soil : Water W/V) | pH Meter Method (Jackson, 1973) | Buffer of pH 4, 7, 9.2 | pH Meter, (Thermo Scientific) |
| 2. | Electrical Conductivity(1:2.5; Soil : Water W/V) | Conductivity Meter Method | Potassium Chloride | Conductivity Meter, Thermo Scientific |
| 3. | Bulk Density | Gravimetric Method (Jackson, 1973) | | Weighing Balance, Sartorius |
| 4. | Water Holding Capacity | Gravimetric Method (Jackson, 1973) | | Weighing Balance, Sartorius |
| 5. | Moisture Content | Gravimetric Method (Jackson, 1973) | | Weighing Balance, Sartorius |
| 6. | Organic Carbon | Rapid Dichromate Oxidation Technique (Walkey & Black, 1934) | Potassium Dichromate, Diphenylamine Indicator, Ortho-Phosphoric Acid, Ferrous Ammonium Sulphate, Sulphuric Acid | |
| 7. | Available Nitrogen | Alkaline Permanganate Method (Subbiah & Asija, 1956) | Hydrochloric Acid, Sulphuric Acid, Cupric Sulphate Pentahydrate, Sodium Carbonate, Selenium Powder, Potassium Sulphate, Sodium Hydroxide, Boric Acid, Methyl Red, Bromocresol Green, Ethyl Alcohol | |
| 8. | Available Phosphorus | Bray And Kurtz, 1945 | Ammonium Fluoride, Hydrochloric Acid, Ammonium Molybdate, Stannous Chloride, Potassium Dihydrogem Phosphate | UV-VIS Spectrophotometer, Techcomp |
| 9. | Exchangeable Calcium and Magnesium | EDTA Trimetric Method (Jackson, 1973) | EDTA, Eriochrome Black Tea, Murexide, Calcium Carbonate | |
| 10. | Exchangeable Sodium and Potassium | Knudsen Et Al., 1982; Mclean And Watson, 1985 | Ammonium Acetate | Flame Photometer, Systronics |
| 11. | Cation Exchange Capacity | Laukulich, 1981 | Ammonium Acetate, Sodium Acetate, Ethanol | Flame Photometer, Systronics |
| 12. | Phosphorus Fractionation | Ruttenberg, 1992 | Ferric Chloride, Magnesium Chloride, Hydrochloric Acid | |
| 13. | Heavy Metal Analyses | Atomic Absorption Spectroscopy | Nitric Acid | Atomic Absorption Spectrophotometer, Avanta |
| 14. | DTPA Extractable Metals | Atomic Absorption Spectroscopy | Diethylene Triamine Pentaacetic Acid, Calcium Chloride, Hydrochloric Acid | Atomic Absorption Spectrophotometer, Avanta |
| 15. | Enzyme Assay | Casida et al. (1964), Jonson and Temple (1964) | Triphenyl Tetrazolium Chloride, Potassium Dichromate, Hydrogen Peroxide, Sulpuric Acid | UV-VIS Spectrophotometer, Techcopm |
| 16. | Litter Decomposition (Bag Method) | Gilbert and Bocock, 1960 | | Hot Air Oven, Weighing Balance, Sartorius |
| 17. | Nutrient Release From Plant Litter | Jackson, 1973 | | |
| 18. | Plant Growth Analyses | Hunt, 1978; Pedraza and Williams-Linera, 2003 | | Hot Air Oven, Weighing Balance, Sartorius |
| 19. | Mineralogical Analyses | X-Ray Diffraction | | |
| 20. | Statistical Analyses | Mean, Standard Deviation, Correlation, ANOVA | | |

### *3.5.1. pH (Soil to water ratio of 1:2.5)*

Soil pH is normally measured in soil/water slurry in the ratio of 1:1, 1:2 or 1:2.5 (w/v). However, pH measurement is usually recommended at 1:2.5 ratio to maintain the longevity of pH electrode. For pH measurement at 1:2.5 (w/v), 20 g of soil in a 100-mL beaker was taken and 50 mL of distilled water was added. The suspension was stirred at regular intervals for 30 minutes and pH was recorded. The suspension must be stirred well just before electrode was immersed.

### *3.5.2. Electrical conductivity*

20 g of the soil sample was taken 150 mL conical flask and 50 mL of distilled water was added to it, then shaken for 1 hour in a mechanical shaker and allowed to stand. The conductivity of the supernatant liquid was measured with the help of a conductivity meter. Time allotted for the measurement was kept constant for each sample.

### *3.5.3. Bulk Density*

The bulk density of soil was measured by taking an undisturbed block of soil by soil core. The soil was dried at $105^{O}C$ for 12 hours and weighed. The exact volume of soil was determined by measuring the cylinder volume. It is expressed as g/cc.

$$\text{Bulk density} = \frac{\text{Weight of oven} - \text{dried soil}}{\text{Volume of soil core (cm3)}}$$

### *3.5.4. Water holding capacity (WHC)*

Filter paper was placed in the keen-box of an appropriate dimension so as to cover the whole perforated bottom of the box. The weight of the box along with the filter paper (W1) was recorded. The crushed sample (dried in an oven at $105^{O}C$) was transferred to

the keen-box and weighed (W2). The box was placed in a Petri dish of 10-cm diameter containing distilled water and kept overnight, so that water entered the box and saturated the soil. In the next day, the soil box was taken out from the water, wiped to remove the adhering water to the container and then finally weighed (W3).

Calculation

Weight of empty box + filter paper = W1

Weight of Box + filter paper with soil = W2

Weight of the soil = W2 -W1

Weight of water absorbed = W3

$$\text{WHC (\%)} = \frac{(W3 - W2)}{(W2 - W1)} \text{ x } 100$$

### *3.5.5. Moisture Content*

For the measurement of natural moisture content, the approximate mass of soil sample was 100 g. The soil sample was put immediately in the moisture can and closed to prevent moisture loss by evaporation. The can containing the moist soil was brought to the weighing balance and weighed immediately. The soil was oven dried at 105-110 °C for approximately 24 hours. Drying in the oven was continued until the soil sample attained a constant weight. Then the cans were put in desiccators for further cooling. Finally, the samples in cans were weighed after cooling. The calculation was done as follows:

Weight of the empty moist can = m1

Weight of moisture can + moist soil = m2

Weight of moisture can + oven dried soil = m3

Therefore,

$$\text{Moisture content (\%)} = \frac{\text{Loss of moisture } (m2 - m3)}{\text{Weight of oven dried sample } (m3 - m1)} \text{ x } 100$$

### *3.5.6. Organic carbon*

The organic carbon was estimated as per the Walkley and Black (1934) by rapid dichromate oxidation techniques. The oven-dried soil was ground and completely passed through 0.2-mm sieve, and 0.50-g sample was placed at the bottom of the dry 500-mL conical flask. 10mL of 1N potassium dichromate was added to the conical flask and the flask was swirled gently to mix the soil in the dichromate solution. 20 mL of concentrated $H_2SO_4$ (containing 1.25% $Ag_2SO_4$) was added very carefully with a measuring cylinder and swirled two to three times. The flask was allowed to stand for 30 min. 200 mL of distilled water and 10 mL of orthophosphoric acid were added to get a sharper end point of the titration. 1 mL of diphenylamine indicator (approx. 10 drops) was added. The indicator was added just prior to titration to avoid deactivation by adsorption onto clay surfaces. The contents are titrated with FAS solution until the colour changed from blue-violet to green. Simultaneously, a blank was run without soil.

Oxidisable organic carbon (%) = [10 (B-T) x 0.003 x100] / (B x wt. of soil)

where B = volume (mL) of ferrous ammonium sulphate required for blank titration

T = volume of ferrous ammonium sulphate needed for soil sample

Wt. of soil in g (1 L of 1N $K_2Cr_2O_7$ will be equal to 12/4 g carbon = 3 g carbon or 1 mL of 1N $K_2Cr_2O_7$ will be equal to 3 g C x $10^{-3}$ = 0.003 g C

To convert easily oxidisable organic carbon to total carbon, divide by 0.77 or multiply by 1.334.

Total organic carbon (%) = oxidisable organic carbon (%) x 1.334

Organic matter (%) = total organic carbon (%) x 1.724

### *3.5.7. Available nitrogen*

Easily Mineralizable Nitrogen of soil was estimated by Alkaline Permanganate Method given by Subbiah and Asija (1956) as referred in table 3.3. For this, 20 g of soil sample was taken in a 800-mL Kjeldahl flask along with 20 mL of water, 100 mL 0.32% $KMnO_4$, 100 mL 2.5% NaOH solutions. The frothing during boiling was prevented by adding a few glass beads. The content was distilled in a Kjeldahl assembly at a steady rate, and the liberated ammonia gas was collected in a 250-mL conical flask containing 20 mL of boric acid (mixed indicator solution). It was assured that the lower open end of the condenser was dipped into the boric acid solution. With the absorption of ammonia gas in the boric acid, the pinkish colour boric acid solution turned green. Nearly 100 mL of distillate was collected in about 30-min time and titrated with 0.02N $H_2SO_4$. The blank correction (without soil) was made for the final calculation:

Available N (ppm) = $[(A-B) \times N \times 14 \times 10^3] / W$

Where,

A = volume of $H_2SO_4$ consumed for blank, mL

B = volume of $H_2SO_4$ consumed for sample, mL

N = normality of $H_2SO_4$ acid

W = wt. of the soil sample

in % = nitrogen in ppm / 10,000

in kg/ha = % of nitrogen x 22,500 (bulk density = 1.5 $g/cm^3$)

### *3.5.8. Available phosphorous*

This method is based on the principle that, in an acidic molybdate solution containing orthophosphate ions, a phosphomolybdate complex are formed that can be reduced by

$SnCl_2$ to a Molybdenum blue colour. 5 g of soil and 50 mL of the Bray's reagent were taken in a 100-mL conical flask and shaken for 5 minutes. The mixture was filtered through Whatman filter paper no. 42 paper. After the supernatant was collected, the colour was developed by adding 5 mL of filtrate and to which 5 mL of Dickman and Bray's Reagent was also added with constant swirling. The neck of the flask was washed down and the content was diluted to about 22 mL. To that 1 mL of diluted $SnCl_2$ was added and volume was made up to mark. The intensity of the blue colour was measured (using 660 nm filter) just after 10 min, and the concentration of phosphorus was determined from the standard curve in spectrophotometer.

Calculation:

The available P in kg/ha can be calculated using the formula:

$$\text{Bray's P (kg/ha)} = \frac{R \times 50 \times 1 \times 2.24}{5 \times 5}$$

= µg of P x 4.48

where, R = µg of phosphorus in aliquot (obtained from standard curve).

### *3.5.9. Exchangeable calcium and magnesium*

2.5 g of less than 2-mm soil was taken in a 250-mL Erlenmeyer flask. 100 mL of 1N HCl was added and heated till boiling. The flask was removed from the heat, and the content was transferred to a funnel lined with Whatman no.1 filter paper. The filtrate was collected in a 250-mL volumetric flask, and the soil was washed with four 15-mL portions of 0.1 N HCl. After the filtrate had cooled, volume was diluted and mixed thoroughly. Ca and Mg was determined by EDTA titrimetry methods. Exchangeable Ca and Mg was determined in $NH_4OAc$ extracts of soils by titration with EDTA. Both Ca and Mg was titrated at pH 10 using Eriochrome black T (EBT) as an indicator. Calcium was estimated

separately by adding NaOH buffer and murexide indicator. Then the difference in total ions and Ca was subtracted to get Mg ion concentration.

### *3.5.10. Exchangeable potassium and sodium*

5 g air-dried soil (less than 2-mm) was shaken with 25 mL of neutral normal ammonium acetate solution for 5 min and filtered immediately through a dry filter paper (Whatman no.1). First few mL of the filtrate was rejected. Potassium and Sodium concentration in the extract was determined by flame photometer. The standard for **Potassium (K) and Sodium (Na)** were strandrad certified material of 1000 ppm. The calculation is as follows:

Available Potassium (in ppm) = (R x V) / W = (R x100 mL of extract) / 10 gm of soil

= R x 10

where,

R = K content of soil extract from standard curve, mg/l;

V = Volume of the soil extract (mL)- in the present case 100 mL was taken as final volume of extract for calculation;

W = Weight of air-dried samples taken for extraction in g; 10 g was taken as final volume of extract for calculation;

K meq/L = K mg/L x 0.02558

K mg/L = K meq/L x 39.10

The same calculation was done for sodium estimation.

### *3.5.11. Cation exchange capacity (CEC)*

25 mL of 1N sodium acetate solution was added to the soil sample in a tube and shaken for 5 min. Then the tubes were centrifuged at 2,000 rpm approximately for 5 min or until

the supernatant liquid was clear. The supernatant was carefully discarded without draining the soil. The whole process was repeated three times more. Then the above process was repeated with 95% ethanol instead of sodium acetate. Again the supernatant was carefully discarded. The third step was performed in the same way as above but with ammonium acetate. By this process, ammonium ions replaced sodium ions, which were then collected as a supernatant liquid. The supernatant was diluted up to 100 mL in a volumetric flask and sodium concentration was determined by flame photometer. Standards used were strandrad certified material of 1000 ppm.

CEC in meq/100g = ($Na^+$ in extractant in meq/L x10) / Wt. of soil in g

### *3.5.12. Phosphorus fractionation*

For this study, the chemical speciation of P was carried out by the wet chemical sequential extraction scheme (SEDEX) for determining five sedimentary P reservoirs (Ruttenberg, 1992): (Step-I) exchangeable and loosely sorbed-P; (Step-II) Fe(III)-bound-P; (Step-III) authigenic-P [authigenic carbonate fluorapatite (CFAP) and biogenic apatite plus CaCO3- bound-P]; (Step-IV) detrital inorganic-P [detrital apatite of igneous or metamorphic origin (FAP)] and (Step-V) organic-P (Fig 3.9). All phosphate concentrations were determined colorimetrically by the phosphomolybdate blue method (Bray and Kurtz, 1945). In step II, P after first reacting it with 1% v/v $FeCl_3$ (Lucotte and D'Anglejan, 1985) following the procedure of Watanabe and Olsen (1962). The $FeCl_3$ treatment is necessary to obviate the interference of citrate with reduction of the molybdate complex. This scheme is better than other methods for the following reasons: first, it is a means of chemical separation of CFAP from AFP of igneous or metamorphic origin; secondly, the use of a $MgCl_2$ wash following extraction steps stops secondary re-

adsorption onto the residual sedimentary matrix; and thirdly, each extraction step has been standardized individually to ensure the selectivity and specificity of the extractants.

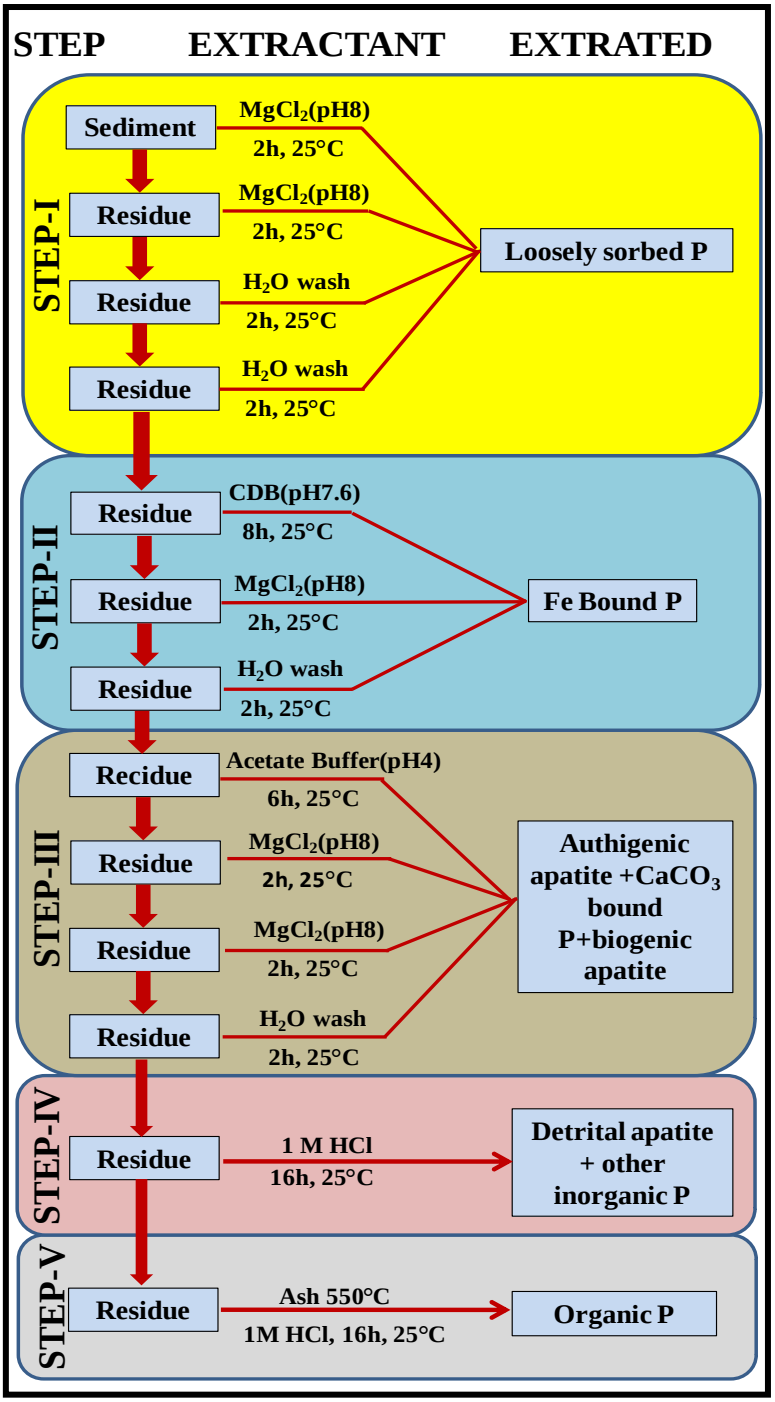


**Fig. 3.9** SEDEX scheme for different P fraction (Ruttenberg, 1992)

### *3.5.13. Determination of plant-available Pb, Zn, Cu, Fe, Mn, Ni and Cd (DTPA-Extractable)*

This procedure has been widely used on a large range of soil types since initially reported by Lindsay and Norvell, (1978). 10 g of air-dried soil was taken in a 100-mL conical flask with 20-mL DTPA extracting solution and shaken for 2 hours. After exactly 2 hours of shaking, the suspension was filtered through Whatman filter no. 42. The time of shaking is crucial because extraction is not complete before 2 hours. Pb, Cu, Zn, Mn, Ni, Cr, Cd, and Fe were determined in AAS. Standard of strandrad certified material of 1000 ppm were used.

The concentration of DTPA-extractable elements are as follows (in ppm):

$$\frac{20 \times (\text{ppm of elements in sample solution} - \text{ppm in blank solution})}{(\text{Weight of air dried sample (g)})}$$

### *3.5.14. Enzyme assay*

The enzymes were studied to identify the microbial activity which in-turn depends on the quality and quantity of root exudates. The concept was that for each plant's rhisozpheric soil the microbes were the same but the roots exudates for each plant differed. Hence those plants would be preferred which have more enzymatic activity at their rhisosphere so that soil restoration can be promoted quickly. There are various soil enzymes but in the present study, two vital enzymes which give the overall picture of the soil has been taken into account, namely, catalase and dehydrogenase.

Catalase activity was measured by back-titrating residual $H_2O_2$ with $KMnO_4$ (Johnson and Temple, 1964; Roberge, 1978). Two grams of soil samples were added to 40 mL distilled water with 5 mL of 0.3% hydrogen peroxide solution. The mixture was shaken

for 20 min and then 5 mL of 1.5 mol/L $H_2SO_4$ were added. Afterward the solution was filtered and titrated using 0.02 mol/L $KMnO_4$. Two grams of soil samples were added to 40 mL distilled water with 5 mL of 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 5 mL of 1.5 mol/L $H_2SO_4$ were added. Afterward the solution was filtered and titrated using 0.02 mol/L $KMnO_4$. The reacted amount of 0.02 mol/L $KMnO_4$, calculated per gram of dry soil (Minczewski and Marczenko, 1973), was used to express the activity of catalase.

Catalase (oxidoreductase) is a tetrameric heme-containing enzyme that catalyzes decomposition of $H_2O_2$ into the water and molecular oxygen (Nelson and Cox, 2000).
Catalase: $H_2O_2 \rightarrow H_2O + O_2$

Dehydrogenase activity was determined with triphenyl tetrazolium chloride (TTC) according to Casida et al. (1964). The enzyme activities were expressed as nmol triphenyl tetrazolium formazan (TPF) $g^{-1}$ oven dry soil $min^{-1}$ in the case of dehydrogenase.

### *3.5.15. Determination of litter decomposition by litter bag method*

The litter decomposition was carried out by litter bag technique as proposed by Gilbert and Bocock (1960), by preparing respective litter bags of different plant species as depicted in the plate. 3.11. The litter bags had a mesh size of 1 mm x1mm to allow unhindered entry of microbes and small soil fauna. The initial weight of litter was taken as 10 g for each plant species and packed in litter bags and buried under the treatment and control mine spoil plots in December 2009. After each month, one set of five bags was taken out from treatment and control plots and brushed carefully to remove the soil particles. To evaluate the nutrient release pattern, nutrients remaining in the decomposing litter were estimated by the equation (Bockheim et al., 1991):

% Nutrient remaining = (C/Co) x (D/Do) x 100

where,

C = Concentration of nutrient element in decomposing litter at the time of sampling

Co = Concentration of nutrient element at the beginning of the study

DM = Mass of dry matter of litter at the time of sampling

DMo = Initial mass of dry matter of litter kept for decomposition

The decay rate coefficient ($k_d$) of the decomposing litter of different species for the entire period was calculated through the negative exponential decay model (Olson, 1963):

This represents the total amount of nutrient present in the litterbag and was calculated for each sample at each sampling period.

$X/X_o = e^{-kt}$

Where X = dry weight of litter remaining at end of the study period (time t)

X = original dry weight of litter

e = base of the natural logarithm

k = decay rate coefficient

Further, the time required for attaining half-life ($t_{50}$) and 99% weight loss ($t_{99}$) was calculated by the following formulae given by Olson (1963):

$t_{50} = 0.693/k$

$t_{99} = 5/k$

**Plate 3.10** Net cover over the plots to stop the entry of external litter

**Plate.3.11** litter bag

### *3.5.16. Nutrient release from plant litter*

The litter samples were ground and passed through a sieve of opening 1mm for chemical analysis. After each month one set of five bags were taken out from treatment and control plots, brushed carefully to remove the spoil material. The litter was then dried at 80°C till constant weight was attained. The N concentration was determined by Kjeldhal method

(Jackson, 1973) using conc. $H_2SO_4$ and digestion mixture ($CuSO_4$, $K_2SO_4$, and Selenium powder) for digestion followed by distillation with NaOH in Kjeldhal assembly. The P and K analysis were carried out by digesting 1 gm litter samples in a ternary mixture of acids ($HNO_3$-$H_2SO_4$-$HClO_4$) in the ratio 10:1:4 (Jackson, 1973). P was analyzed by colorimetry (Jackson, 1973) while K was estimated in the flame photometer. The experiments were done to know which plant litter could release more nutrients and at what rate so that such plants could be recommended for fast recovery of soil fertility.

### *3.5.17. Plant growth analyses*

Plant biomass was estimated on a dry weight basis at 70ºC (Jackson, 1973). The relative growth rate (RGR) in height (RGRH) and circumference (RGRC) were estimated annually using the formula (Hunt, 1978):

$$RGR = \frac{(\ln h2 - \ln h1)}{t2 - t1}$$

Where h is height or circumference at an initial time (t1) and final time (t2) in months. To compare experimental plantation of various treatment and control plots, annual increment (I) in height and circumference was calculated per species using the following formula (Pedraza and Williams-Linera, 2003).

$$I = \frac{(h1 - h2)}{t}$$

Where, h1 and h2 are heights or circumferences at times 1 and 2 and t is the total time in years.

### *3.5.18. Mineralogical analysis*

X-ray diffraction (XRD) offers a means for measuring the distance between repeated planes in a crystal structure, such as the basal spacing of clay minerals (Shroff and Shah,

2002). X-ray diffraction (PW1710) analysis was performed on a Philips powder diffractometer employing Cu *K*a radiation (40 kV, 20mA) in the range 2θ = 3-700 at a goniometer rate of 2θ = 30/min to analyze mineral compositions. This test was done at Dept. of Geology, Jadavpur University, Kolkata, West Bengal, India.

### *3.5.19. VAM- Root infection study*

The plants after one year of infection were taken for the study. The plants were uprooted and dried at 60$^{o}$ C for 48 Hours and transported to KCP sugar and Industries where the infection study was done. The roots were softened with 10% KOH and stained with Trypan Blue and observed under a microscope for Hypahe, Arbuscules and Vesicles in the roots. The percentage of root length colonization was calculated using the Biermann and Linderman (1981) method (frequency distribution method). The roots were observed under Olympus microscope.

## *3.6. Statistical analyses*

Statistical Package for the Social Sciences (SPSS)-16 was used for statistical analyses like analysis of variance better known as ANOVA and post hoc test like Duncan's Multiple Range Test (DMRT). Other statistical calculations were done in Microsoft Excel and Origin.