

## **Chapter – III**

*Materials and Methods*

### 3. MATERIALS AND METHODS

#### 3.1 A Brief Physiographic Information on Study Area (Terai Agroclimatic Zone)

Soils of West Bengal has been divided into six agroclimatic zones, viz. Hill, Terai, New Alluvium, Old Alluvium, Red and laterite and Coastal- Saline.

The word *terai* (*terai*) in Hindi means moist, indicating thereby that the soils have a wet regime and high water table conditions for most part of the year. Terai soils are foot-hill soils and extend in strips of varying widths at the foot of the Himalayas in Jammu & Kashmir, Uttar Pradesh, Bihar and West Bengal.

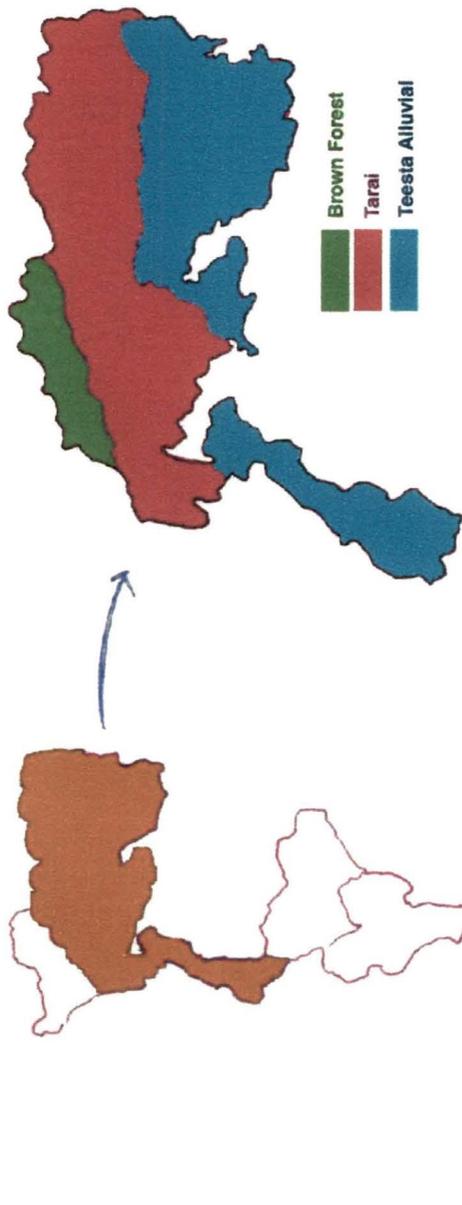
The *terai* zone of West Bengal is comprised of the geographical area under the entire Cooch Behar and Jalpaiguri district, Siliguri sub-division of Darjeeling and Islampur sub-division of Uttar Dinajpur district. The zone lies in between  $26^{\circ}12'$  and  $26^{\circ} 56'$  North latitude and  $88^{\circ} 7'$  and  $89^{\circ} 53'$  Eastern longitude.

Based on the old concept of soil classification, soils of *Terai* zone of West Bengal can be broadly divided into three classes viz Teesta Alluvium, Terai and Brown Forest (Fig: 1).

##### 3.1.1 Teesta Alluvium

Since the principal river in northern districts is Teesta, the soils formed on alluvial deposits carried down by it along with other rivers and its or their tributaries are categorised under the group. The area occupied by these soils is found to be the highest (5.68 lakh ha) among other soil types present in this zone covering the whole of Cooch Behar district and Islampur sub-division of Uttar Dinajpur district.

According to new system of soil classification, the major great groups found in the form of association in Teesta alluvium are Dystricchepts and Haplaquepts belonging to the order Inceptisol and Udifluvents and Ustorthents of Entisol order.



**Fig 1. Map of Terai agro-climatic region of West Bengal showing its soil groups**

### 3.1.2 Terai soils

*Terai* soils are found in a narrow strip of land at the foot of the Himalayas and is extended towards the south in the Siliguri sub-division of Darjeeling and Jalpaiguri District. The area occupied by this soils group is about 2.80 lakh ha only. These soils are developed primarily by the deposition of various types of soil and rock materials brought down by the hilly rivers like Teesta, Mahananda, Torsa, Jaldhaka and their numerous tributaries.

According to modern soil classification system, the groups, which are found to be associated in these soils are Dystrochrepts and Haplaquepts of order Inceptisol and Udifluvents of order Entisol.

### 3.1.3 Brown Forest Soil

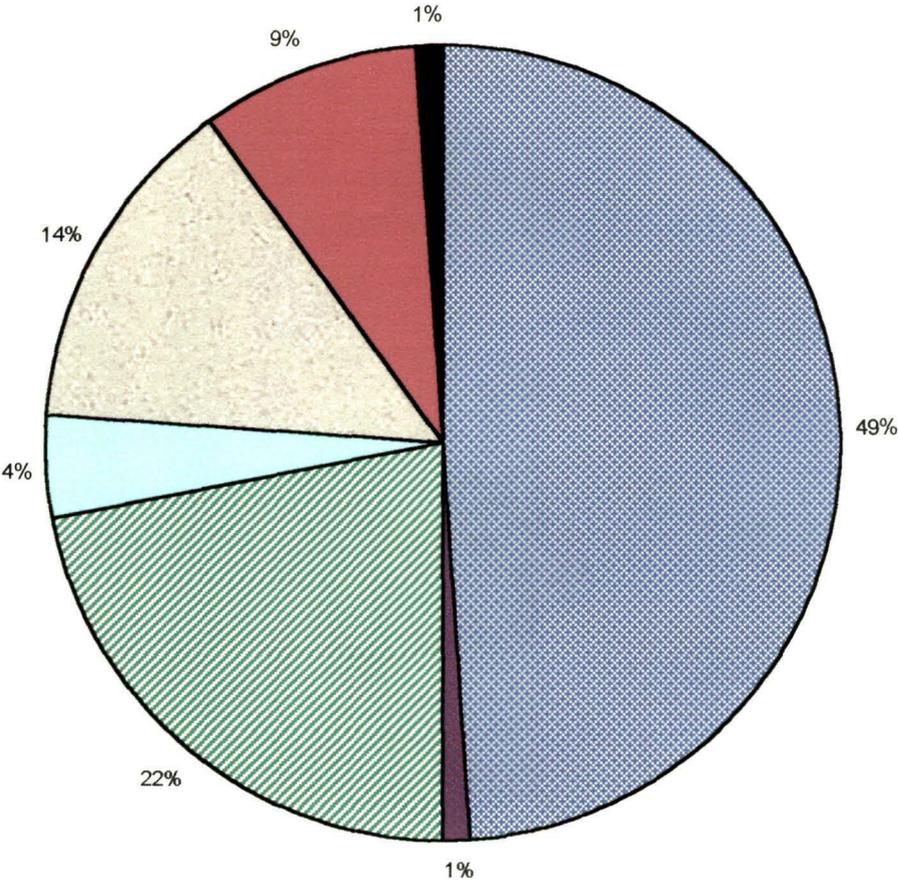
Though these soils spread over the northern fringe of Jalpaiguri district and all areas other than Siliguri sub-division of Darjeeling district, but from agroclimatic point of view only the area lying under Jalpaiguri district is included under *Terai* zone and the remaining part belonging to the Hill zone. Most of the area is either covered by forest or tea plantation.

According to modern system of classification, the great groups associated with these soils are found to be namely Dystrochrepts of Inceptisol and Udifluvents and Udorthents of Entisol order.

## 3.2 Land use Pattern of Terai Zone of West Bengal

Fig: 2 show the utilisation pattern of land in four *terai* districts. The net sown area of this zone is noted to be nearly 50 per cent, while 14% area is under forest cover. Distribution of geographical area also demonstrates that orchards, plantation and miscellaneous trees taken together accounts for 9% of area of the *Terai* districts and 22% land is being utilised for nonagricultural purposes.

**Fig: 2 Land use Patterns of Terai districts of West Bengal**



Net Sown	Fallow
Non Agril	Baren
Forest	Orchard
Cultivable waste	

### 3.3 Materials Used and their Analysis

#### 3.3.1 Soil Sample

##### (A) Collection and Processing of samples

The surface (0-15 cm) soil samples were collected from the different locations of four land use pattern namely tea garden, orchard, cultivated land and forest under *Terai* zone of West Bengal as mentioned in Table 6.

##### (B) Analysis of Soil Sample (methods)

###### (a) Physico-chemical Properties of the soil sample

The following methods were followed for the determination of the important physico-chemical properties of the experimental soils.

###### (i) Mechanical Analysis (Percentage of clay, Sand and Silt)

Mechanical analysis was carried out by Bouyoucos Hydrometer method (Day, 1965).

###### (ii) Bulk density, Particle density, Maximum Water Holding Capacity, Percentage pore space

These soil parameters were determined by Keen and Rackzowsky's method (Piper, 1966).

###### (iii) pH

pH of the soil samples was determined by using soil suspension in water in the ratio of 1:2.5 ( Jackson, 1973 ) and a glass electrode pH meter ( Model : Systronics,335).

###### (iv) Electrical Conductivity ( E.C.)

E.C. of the soil suspensions (soil : water :: 1: 2.5 ) was determined at the room temperature by using a direct reading conductivity meter (Model : Systronics, 303 ).

###### (v) Organic carbon

Organic carbon of soil samples were determined following the method of Walkley and Black (Jackson,1973 ).

###### (vi) Cation-Exchange Capacity (CEC)

The CEC of the soils were determined by leaching soils with 1N NaOAc (pH 8.2) and subsequently displacing the adsorbed Na<sup>+</sup> by 1N

$\text{NH}_4\text{OAc}$  (pH 7) and finally estimating Na released in the extract by a flame photometers (Barthakur and Barua, 1997).

(vii) **Available Nitrogen**

The available nitrogen was determined by alkaline potassium permanganate method (Subbiah and Asija, 1956).

(viii) **Exchangeable calcium ( $\text{Ca}^{2+}$ ) and Magnesium ( $\text{Mg}^{2+}$ )**

Exchangeable  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  was determined by Versene method (Black, 1965)

(ix) **Available Potassium (K)**

Available Potassium of the soil was determined by a flame photometer after extracting the soil with neutral 1N  $\text{NH}_4\text{OAc}$  solution.

(x) **Anion Exchange Capacity (AEC)**

AEC of the soil was determined as per the method outlined by Barthakur and Barua (1997). The soil was first leached with a solution of TEA-  $\text{BaCl}_2$  (pH8.1), followed by calcium saturation and then treated with standard phosphoric acid solution. The remaining phosphorus was determined by a spectrophotometer.

(xi) **Free Iron Oxide ( $\text{Fe}_2\text{O}_3$ )**

Free iron oxide of the soil was determined using citrate buffer- Sodium dithionite extractant and O-phenanthroline (O-phen) indicator as outlined by Hesse (1971).

(xii) **Total Acidity**

Total acidity of the soil sample was determined by extracting with 1N  $\text{NaOAc}$  solution and then titrating with 0.1 N  $\text{NaOH}$  solution using phenolphthalein as indicator (Kappen, 1934).

(xiii) **Exchange Acidity**

Exchange acidity was determined solution and finally titrating with 0.1N  $\text{NaOH}$  solution using phenolphthalein as indicator.

(xiv) **Exchangeable Aluminium (  $Al^{3+}$  )**

After estimating exchange acidity (Mc Lean, 1965), exchangeable aluminium ( $Al^{3+}$ ) of the soil was determined from the same solution titrating against 0.1 N HCl after adding NaF (4%) solution.

(xv) **Extractable Acidity**

Extractable acidity (extractable  $Al^{3+}$ ) of the soil was determined by extracting the soil with 1N  $NH_4OAc$  (pH 4.8) solution and treating the extracted solution with a colour developing reagent aluminium (Hesse,1971)

(xvi) **Total Potential Acidity**

Total potential acidity of the soil sample was determined by extracting the soil with  $BaCl_2$ -TEA (pH 8.2)buffer solution by Peech's method (Black,1965) .

(xvii) **pH- Dependent Acidity**

pH dependent acidity of the soil sample was evaluated from the difference between extractable acidity and exchange acidity.

(xviii) **Non-Exchangeable Acidity**

Non-exchangeable acidity of the soil was computed as the difference between total potential acidity and exchange acidity.

**(b) Forms of Soil P**

**i) Total Phosphorus**

Suitable weight (0.4 g) of soil sample was taken in a conical flask and suitable amount of  $HNO_3$  was added and heated to oxidise the sample to remove organic matter and then the sample was soaked overnight with 10ml  $HClO_4$ . Subsequently the samples digested on a hot plate, the digested material was filtered through Whatman No.1 filter paper to get rid of silica (Olsen and Dean, 1965 ) and made to a known volume.

Phosphorus content of the material was determined colorimetrically as per the procedure outlined by Watanabe and Olsen (1965) using ascorbic acid.

**i) Total organic Phosphorus**

Total organic phosphorus of the soil samples was determined following the method adopted by Mehta *et al.* (1954).

**ii) Fractionation of Inorganic Phosphorus**

Soil inorganic Phosphorus was fractionated as per the procedure outlined by Chang and Jackson. (1957), modified by Peterson and Corey (1966) (Table-4).

**(c) Available Soil Phosphorus**

Available phosphorus of the soil samples were extracted by the following five different methods and P content in the extracts were determined colorimetrically as per the procedure outlined by Watanabe and Olsen (1965) using ascorbic acid (Table 5)

**(d) Adsorption- Desorption Behaviour of Soil P****i) Phosphorus Adsorption**

3g soils was equilibrated in centrifuge tube for 6 days with 30ml of 0.01M  $\text{CaCl}_2$  solution containing 5,10,15,20,30,50 and 100 mg  $\text{PL}^{-1}$  as  $\text{KH}_2\text{PO}_4$  with one drop of toluene to inhibit microbial activity. The soil was shaken for 30 minutes twice daily using a reciprocal mechanical shaker. At the end of requisite incubation period at 25°C, the contents of the tubes were centrifuged at 5000 RPM for 20 minutes and P concentration in the supernatant solution was determined by the ascorbic acid method (Olsen and Sommers, 1982). Sorbed P was calculated as the difference between added P and equilibrium P concentration.

**ii) Phosphorus desorption**

The soil residues of the adsorption studies were used for quantifying desorption. The samples first washed with alcohol to make it free from soluble P and washed

**Table 4. Fractionation of Soil inorganic phosphorus by the modified procedure of Chang and Jackson and after Peterson and Corey**

Soil: Place 1g of soil in a 100cm<sup>3</sup> polypropylene centrifuge tube, add 50 cm<sup>3</sup> of 1M NH<sub>4</sub>Cl solution, shake for 30 minutes and centrifuge.

Solution: Determine saloid bound- P	Soil : Add 50 cm <sup>3</sup> of 0.5M NH <sub>4</sub> F solution made to pH 8.2 with NH <sub>4</sub> OH, shake for 1 hour and centrifuge.			
	Solution : Filter through activated carbon if necessary and determine Aluminium bound – P (Al-P)	Soil : Wash twice with 25cm <sup>3</sup> portions of saturated NaCl solution, centrifuging each time to recover soil. Discard washing. Add 50 cm <sup>3</sup> of 0.1M NaOH solution, shake for 17 hours and centrifuge.		
		Solution: Add 5 drops of conc. H <sub>2</sub> SO <sub>4</sub> and centrifuge.	Soil: Wash twice with saturated NaCl solution and discard washing. Suspend soil in 25 cm <sup>3</sup> of 0.3 M Na citrate solution, add 1g of Na dithionite and shake for 15 minutes. Heat to 80°C, dilute to 50 cm <sup>3</sup> , shake for 5 minutes and centrifuge.	
	Solution : Filter through 0.5g activated carbon and determine Iron bound-P (Fe-P)	Residue: Discard	Combined solution and Washings: To 3 cm <sup>3</sup> add 1.5 cm <sup>3</sup> of 0.25 M KMnO <sub>4</sub> to oxidize excess dithionite and Citrate. Allow to stand 2 minutes and determine Reluctant soluble-P (RS-P)	Soil : Wash twice with saturated NaCl solution and add washings to previous solution. To soil add 50 cm <sup>3</sup> of 0.1M NaOH solution, shake for 1 hour and centrifuge.
				olution : etermine ccluded-P (OCCI-P)

**Table 5. Details of five extractants used for estimating available P in this study**

Methods	Composition of the (Extractant)	Soil : Extractant	Shaking time (min)	References
a) Olsen's method	0.5 M NaHCO <sub>3</sub> (pH 8.5)	1 : 20	30	Olsen <i>et al.</i> , 1954
b) Bray's 1 method	0.03 N NH <sub>4</sub> F + 0.025N HCl (pH 2.5)	1 : 10	5	Bray and Kurtz, 1945
c) Bray's 2 method	0.03 N NH <sub>4</sub> F + 0.1N HCl (pH 1.0)	1 : 20	2/3	Bray and Kurtz, 1948
d) Mehlich's method	0.05N HCl + 0.025 N H <sub>2</sub> SO <sub>4</sub> (pH 1)	1 : 4	5	Nelson <i>et al.</i> , 1953
e) AB-DTPA Method	1M NH <sub>4</sub> HCO <sub>3</sub> + 0.05 M DTPA (pH 7.6)	1 : 2	15	Sultanpour and Schwab, 1977

**Table 6. Sites selected for sampling from different land use patterns of terai agroclimatic zone of West Bengal.**

Land use Pattern	Site of collection	No of samples collected	P.S./Sub Division	District
Tea Garden	Subhasini Tea Estate	5	Hasimara	Jalpaiguri
	Mathura Tea Estate	5	Alipurduar	Jalpaiguri
	Cooch Behar Tea Estate	5	Falakata	Do
Orchard (pine apple)	Jagannathpur	10	Bidhannagar	Darjeeling
	Mohitnagar	5	Jalpaiguri Sadar	Jalpaiguri
Cultivated land	District Seed farm	3	Cooch Behar	Cooch Behar
	Contral Tobacco Research Institute. Research Station	3	Dinhata	Do
	Barakodali Farm	3	Tufanganj	Do
	Bidhan Chandra Krishi Viswavidyalaya, North Bengal campus	4	Pundibari	Do
	Madhupur	2	Pundibari	Do
Forest	Patlakhaoa Forest	5	Do	Do
	South Khayerbari Forest	5	Madarihat	Jalpaiguri
	Chilapata Forest	5	Alipurduar	Do

soils were shaken with 0.5 M  $\text{NaHCO}_3$  ( pH 8.5 ) for 2 hours. The suspensions were centrifuged at 5000 RPM for 20 minutes and filtered through Whatman No-1 filter paper. The samples were subjected to two successive desorptions. Phosphorus in the clear solution was determined by ascorbic acid method.

### 3.3.2 Plant Samples

#### A. Collection and Processing of Sample

Wheat (cv.HB 1553) seedlings were grown in Neubauer seedling technique in a Neubauer ( 11cm × 7 cm ) containing 100g soil and 50g nutrient free sand. Wheat plants (both root and shoot) were harvested after 17days. Plant samples were washed, shade-dried to remove excess moisture and then kept in a oven at 60°C for eight hours. The dried samples were weighed for dry matter yield and ground in a Willey Mill. The ground samples were stored in paper bags for determination of P concentration in root and shoot.

#### B. Analysis of plant Samples

A suitable weight (0.2g) of plant sample was taken in conical flasks and soaked overnight with 10ml of triacid mixture of  $\text{HNO}_3$  :  $\text{H}_2\text{SO}_4$ :  $\text{HClO}_4$  in 10:4:1 (volume basis) ratio. Subsequently the samples were digested on a hot plate. The digested material was filtered through Whatman No-1 filter paper and made to a known volume. The phosphorus content of the material was determined colorimetrically by Vanadomolybdate yellow colour method ( Piper,1966)

## 3.4. Statistical Analysis

The data on different forms of soil P, available P extracted by five soil test methods and P adsorbed in soils of four land use patterns were analysed by using one way ANOVA. The coefficients of variation (CV) in different P estimates as well as physico-chemical parameters of soils under different land uses of terai agroclimatic zone were calculated using the formula:

$$\text{CV} = \frac{\sigma_{n-1}}{\text{Sample Mean}} \times 100$$

Where  $\sigma_{n-1}$  was the Standard Deviation (S.D) of samples. For measuring the goodness of fit of the adsorption data to various adsorption isotherms and testing of linearity between / among various P estimates and soil properties or plant parameters, the coefficient of correlation (r) was used. Stepwise regression analysis was done using MICROSTAT Computer programme. Here, no restriction was imposed i.e. the independent variables entered into the regression models competitively according to their relative contribution towards the dependent variable. The variables included in the stepwise regression equations were significant at 5% probability level.