CHAPTER 2
STUDY AREA AND METHODOLOGY

This chapter gives a brief description of the study area and describes the basis of sampling site selection, their partition into categories, environmental situation of the sites, and their GPS locations. Details of collection of soil samples, their preliminary treatment and preservation for analysis are also described. The soil physicochemical parameters selected for analysis, their importance in soil chemistry and in carbon sequestration studies, and their experimental determination form the section in methodology.

2.1 The study area
2.1.1 General description of the study area

Located 47 km east of Guwahati, the capital of Assam, at the western part of Morigaon District, Assam (India), the study area covers Jagiroad and adjoining areas and is close to the eastern part of Kamrup (metro) and northwestern part of Karbi Anglong districts. The area is largely agricultural in nature as is also reflected in the land-use pattern of Morigaon district (Statistical Handbook of Assam, 2006), shown below.

<table>
<thead>
<tr>
<th>Land use type</th>
<th>Area in hectares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>17,626</td>
</tr>
<tr>
<td>Total cropping area</td>
<td>124,352</td>
</tr>
<tr>
<td>Multiple cropping area</td>
<td>31,930</td>
</tr>
<tr>
<td>Area under non-agricultural use</td>
<td>21,310</td>
</tr>
<tr>
<td>Non sown area</td>
<td>13,780</td>
</tr>
</tbody>
</table>

The location of the study area is shown in Fig. 2.1.
2.1.2 Geomorphologic features of the study area

The general topographical features of the study area suggest mostly a flat surface. The low lying areas include the wetlands, marshy lands, various stagnant water bodies like ponds and the interconnected natural shallow channels with meandering movements of the water flows. The younger and older alluvium of this region is of quaternary age imparting various textures to the soil.
The ground elevation of the region varies from maximum 53 m to minimum 43 m above the mean sea level. The floodplain surrounded by low mountains at the southern side and the mighty Brahmaputra at the north has very low slope. This along with siltation and variations in the stream velocity has resulted in meandering water channels supporting the flood attenuation process.

2.1.3 Historical background of the area

In earlier times, Jagiroad was known as ‘Nakhola’ and was ruled by the Lalung Kings. This locality was once ruled by the mighty king. In a battle with the Kacharies, once he was defeated and escaped from the battlefield through the Kalang river and took shelter at a place in the northern part of this area. Since the king stayed awake in the locality, the area was named as ‘Jagi’ (Assamese term meaning awake). During the British rule, the railway department faced problems in despatching parcels, etc. due to Nakhola and Noakhali (presently in Bangladesh), which were in the same railway route. For this reason, the then government changed the name from Nakhola to Jagiroad (the road approaching Jagi), and this name soon became popular.

2.1.4 Nagaon Paper Mill as a source of effluents and solid wastes

The present study was carried out with reference to a huge Pulp and Paper Mill at Jagiroad. This mill uses bamboo as the raw material for production of pulp with a Kraft pulping technique. The installed capacity of the Mill is 300 tons per day with production of approximately 270 tons per day of pulp. 3-4 tons of bamboo is required for the production of one ton of pulp. The sludge generated by a pulp and paper mill using Kraft pulping process is about 98 kg/ton of pulp (Bajpai 2010) and the total amount of sludge production is approximately 26.5 tons/day. This study makes an attempt to arrive at a conclusion whether the pollutant accumulation potential or health of the soil near the Paper Mill is exhausted or not and to predict long term effluent treatment potential of the soil.

The paper making process can be subdivided into three steps, i.e. conversion of raw Materials into pulp, conversion of pulp into paper and recovery of chemicals. Pulp is made from the chipped and screened bamboo chips by treating with the white liquor (Na₂SO₄ + Na₂CO₃) at 1600°C for two hours under elevated pressure. In this process,
lignin is separated from the cellulose and hemicellulose. After screening and washing the pulp, it is subjected to chlorine bleaching process. During bleaching, HCl is generated lowering the pH of the effluent to 2.0 – 2.5. Afterwards during alkali extraction and hypochlorite treatment, lignin and chlorolignin are released.

During the process of conversion of pulp into paper, a variety of chemicals like rosin, alum, clay, dye, etc. are utilised to get hydrophilic cellulose. These chemicals are likely to be associated with the effluent. The hydrophilic cellulose is converted to paper sheets through a series of mechanical operations. The sheets are finally treated in the finishing house to give finished products.

A general view of the paper mill and its effluent treatment operations is presented in Fig. 2.2.

In the chemical recovery stage, heat treatment of lignin, resin and non-cellulosic plant materials releases considerable amounts of the gases, CO₂ and CO to the atmosphere. In the causticising plant, the effluent attains basic nature due to the formation of Ca(OH)₂ and NaOH as a result of the following reactions:

\[
\begin{align*}
\text{CaO + H₂O} & \implies \text{Ca(OH)₂}. \\
\text{Na₂CO₃ + Ca(OH)₂} & \iff 2 \text{NaOH + CaCO₃}.
\end{align*}
\]

The effluent is subjected to a number of physical processes such as sedimentation, coagulation, flocculation and floatation. Biological treatment is based on an activated sludge method. During this process, nitrogen fertilisers are added to the effluent as the food for the microorganisms. Causticising plant releases large volume of waste lime while a large amount of coal fly ash remains as residue after the boiler operations. A general view of waste lime and coal ash dumping sites are presented in Fig. 2.3.

Both pulping and paper making processes generate a large volume of effluents with unutilised or discarded chemicals including black liquor, plant extracts, organic halides, persistent organic pollutants (POP) along with high pH washings and inorganic chlorine compounds. (Pokhrel and Viraraghavan, 2004).
Fig. 2.2. Views of (A) Nagaon Paper Mill along the Asian Highway 1, and (B) the Effluent Treatment and Disposal operations
Fig. 2.3. Views of (C) Waste Lime dumping site and (D) Coal Ash dumping site
2.1.5 Environmental situations in the study areas

Areas with likely to be affected by the Paper Mill operations

The areas covered by the villages, Tegheria, Ghunusa, Nakholagrant, Dayan, Palighuri and Chakumaku, on the northern periphery of the paper mill receive treated and untreated effluents of the mill and are likely to be most severely affected. The inhabitants of the villages are mostly dependent on agriculture (mainly paddy cultivation) for their livelihood. The improperly released effluents and the haphazard disposal of solid wastes over large low lying areas are the main concerns for the villagers and are likely to affect the soil physicochemical properties. It was observed that the paddy productivity of the paper mill effluent released paddy fields decreased from 2300 - 3000 kg ha\(^{-1}\) to 615- 925 kg ha\(^{-1}\) after the commissioning of the Nagaon Paper Mill (Baruah and Das, 2000). The lowering in crop productivity is an indication of lower soil carbon sequestration through agroforestry.

Areas likely to be less affected by the Paper Mill operations

The natural forests in the southeast direction and the wetlands and the paddy cultivation areas in the northwest direction from the paper mill are likely to be less affected by the paper mill operations as effluent and solid waste discharge is not a major activity in these areas. Three different types of soil are in use for paddy cultivation in the area, namely, (i) soil affected by flood, (ii) soil without irrigation facilities and fertilizer, and (iii) soil with the use of modern fertilizers as well as irrigation facilities. Irrigation is mainly dependent on water drawn from tubewells. The tillage practices applied in the paddy cultivation is conventional in nature (Fig. 2.4).

Two types of residential land-use are covered in this work and the sampling sites are at a distance of 5-7 km from the paper mill in the western direction. These sites experience urban and rural residential impacts and are not likely to be affected by the paper mill effluents and solid wastes.
Fig. 2.4. Conventional tillage practices applied for paddy cultivation
2.1.6 General climate of the study area

The study area belongs to the tropical rainforest climate region, subjected to a humid subtropical climate. The experienced climate is moderate and humid (73% relative humidity). The average rainfall in the area is 1530 mm with the wet season extending from June to September. The temperatures are moderate with the mean maximum summer temperature of 34°C and the mean minimum winter temperature of 9°C.

The changes in climatic conditions perturb the soil-water system and prevent the soil pore water from attaining equilibrium conditions. This also affects the soil carbon dynamics and consequently the soil nutrient and trace metal availability. The climatological and anthropogenic activities are always associated with the dry-wet cycles. The following three distinct climatic seasons can be observed in the study area:

(a) hot dry (April to June; A, A2, A3),
(b) hot wet (July to September; B, B2, B3) and
(c) cold dry (November to February; C, C2, C3).

2.2 Soil sampling and analysis

2.2.1 Selection of sampling sites

Keeping in view the basic requirement of the present study, a survey was carried out for the selection of the sampling sites. Some of the sites were to be selected from the effluent discharged and solid waste dumped areas of the pulp and paper industry. After survey, one site each was selected in (i) the waste lime dumped area and (ii) the area used earlier for coal ash dumping.

Five other sites were selected on the basis of their likelihood of being affected by the discharge of the paper mill effluents as follows:

(i) one site from the area where the sludge from the Effluent Treatment Plant (ETP) is dumped,
(ii) another site near the area where the treated effluent from the ETP is released through an underground drain,
(iii) three other sites at distances of 50, 100 and 500 m from locations that receive a continuous flow of treated effluents from the ETP through an open earthen channel,

(iv) seven other sites on consideration of different land use patterns and from areas which are not receiving paper mill effluents or solid wastes directly.

Table 2.1 (a and b) shows the 14 sampling sites in two groups, (a) those sites likely to have been affected by the Paper Mill operations, and (b) the other sites not likely to have been affected by the same. The table also shows the GPS coordinates and a brief description of the environmental situation in each site. Fig. 2.5 (a, b, c) shows the general view of the sampling sites (E1 to E7) likely to have been affected by the paper mill operations and Fig. 2.6 (a, b, c) of the sampling sites (S1 to S7) not likely to have been affected by the paper mill.

2.2.2 Sampling frequency

The soil samples were collected in three seasons, viz.,

(i) hot dry summer (April to June),
(ii) wet hot summer (July to September) and
(iii) cold dry winter (November to February)

to take into account the impact of change in climate as well as the land use pattern on carbon storage and decomposition in soil. The samples were collected in each season for the 3-year period of 2012 – 2014. Another batch was collected on monthly basis during 2014.
Table 2.1(a). The sampling sites likely to have been affected by the Paper Mill effluents and solid waste dumping

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil type</th>
<th>GPS Location</th>
<th>Description of the site</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Waste lime dumping area</td>
<td>N 26°07’43.0&quot; E 92°14’05.6&quot;</td>
<td>Lime waste discarded and dumped by Nagaon Paper Mill from its Caustic Plant on the outskirts of the township</td>
</tr>
<tr>
<td>E2</td>
<td>Waste coal dumping area</td>
<td>N 26°07’38.5&quot; E 92°14’30.7&quot;</td>
<td>Waste burnt coal from the thermal power plant is dumped at this site (near the coal dump)</td>
</tr>
<tr>
<td>E3</td>
<td>Land receiving effluents and sediment dumping</td>
<td>N 26°07’50.8&quot; E 92°12’41.9&quot;</td>
<td>The lagoon of the Effluent Treatment Plant is cleaned every two/three years and the sediments from this operation are dumped in this area, close to the slum area. It also received treated effluent during the rainy season through overflow of the discharge channel.</td>
</tr>
<tr>
<td>E4</td>
<td>Land receiving effluent</td>
<td>N 26°08’07.1&quot; E 92°12’31.4&quot;</td>
<td>Untreated and partially treated wastewater from the Paper Mill is discharged in this area near the natural channel of Elenga Beel.</td>
</tr>
<tr>
<td>E5</td>
<td>Land receiving effluent</td>
<td>N 26°07’52.6&quot; E 92°12’40.8&quot;</td>
<td>Receiving untreated and treated effluents (50 m across the site 3) adjacent to the slum area.</td>
</tr>
<tr>
<td>E6</td>
<td>Land receiving effluent</td>
<td>N 26°07’53.5&quot; E 92°12’40.9&quot;</td>
<td>Receiving untreated and treated effluents (100 m across the site 3) behind a culvert adjacent to the paddy cultivation area</td>
</tr>
<tr>
<td>E7</td>
<td>Land receiving effluent</td>
<td>N 26°07’57.4&quot; E 92°12’35.5&quot;</td>
<td>Receiving untreated and treated effluents (500 m across the site 3) in paddy rice cultivation area.</td>
</tr>
</tbody>
</table>
Fig. 2.5 (a). Sampling sites E1, E2 and E3.
Fig. 2.5 (b). Sampling sites E4 and E5.
Fig. 2.5 (c). Sampling sites E6 and E7.
Table 2.1(b). The sampling sites not likely to have been affected by the Paper Mill effluents and solid waste dumping.

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil type</th>
<th>GPS Location</th>
<th>Brief description of the site</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Virgin Forest Land</td>
<td>N 26°05'57.2&quot; E 92°19'14.3&quot;</td>
<td>Foothills of Karbi Hill range, Nellie, Morigaon District, Assam. Forest maintained by Department of Forests, Govt. of Assam. Not harvested for last 60 years. Suffers break out of fire during dry seasons.</td>
</tr>
<tr>
<td>S2</td>
<td>Natural Wetland</td>
<td>N 26°11'49.6&quot; E 92°11'53.2&quot;</td>
<td>Natural body receives overflown water from vast paddy cultivated area and remains covered under water for about eight months a year and moist throughout the year.</td>
</tr>
<tr>
<td>S3</td>
<td>Land Affected by Flood and used for paddy cultivation</td>
<td>N 26°13'48.8&quot; E 92°10'38.8&quot;</td>
<td>Dighaldal area in Morigaon District of Assam, flooded by water from the river Brahmaputra during the rainy seasons.</td>
</tr>
<tr>
<td>S4</td>
<td>Land under paddy cultivation</td>
<td>N 26°12'32.8&quot; E 92°12'58.4&quot;</td>
<td>Soil irrigated by shallow tube well. Fertilizer applied on the basis of experience without soil test. Regular conventional tillage during the cold dry season for several generations.</td>
</tr>
<tr>
<td>S5</td>
<td>Land under paddy cultivation</td>
<td>N 26°12'46.9&quot; E 92°10'52.6&quot;</td>
<td>Conventional tillage practices during the rainy season for several generations. No irrigation. No fertilizer application.</td>
</tr>
<tr>
<td>S6</td>
<td>Residential Land</td>
<td>N 26°07'21.0&quot; E 92°11'53.0&quot;</td>
<td>Urban area developed for last thirty years. No tillage activity on the soil.</td>
</tr>
<tr>
<td>S7</td>
<td>Residential Land</td>
<td>N 26°07'25.1&quot; E 92°11'28.5&quot;</td>
<td>Rural area, no water stagnation Tillage activity very rare.</td>
</tr>
</tbody>
</table>
Fig. 2.6 (a). Sampling sites S1 and S2
Fig. 2.6 (b). Sampling sites S3 and S4.
2.2.3 Soil sample collection and pre-treatment

From each site, three soil samples were collected from an area of 1 m² (one from the centre and two from two diagonally opposite corners) up to 20 cm depth (average conventional tillage depth) using a 5 cm corer. Each of the three samples was air dried in a shade, pebbles, roots etc. were removed, and after further drying, ground to a fine powder and passed through a 2 mm sieve. The three samples obtained from a single site were then homogenized to obtain a composite sample that was representative of the specific soil type for the particular site.

The soil samples were stored in a desiccator at room temperature till laboratory analysis was completed.

2.3 Soil sample analysis

2.3.1 Selection of soil physicochemical parameters

In line with the objectives of the work, the following parameters were selected for the assessment of impact on soil organic carbon dynamics:

(a) Physical parameters
- Particle size analysis and assignment of textural class
- Bulk density
- Particle density and porosity
- Moisture content
- Water holding capacity
- Saturated hydraulic conductivity
- Soil aggregate analysis.

(b) Chemical parameters
- Soil pH
- Electrical conductivity (EC)
- Buffering capacity
- Cation exchange capacity (CEC)
- Base saturation
- Soil organic carbon (SOC)
• Total soil nitrogen (TSN)
• Carbon to nitrogen ratio (C/N)
• Available phosphorous,
• Extractable potassium,
• Retention capacity of soil for major soil cations and
• Determination of soil trace metals Co, Cr, Cu, Mn, Ni, Pb and Zn.

(c) **Mineralogical parameters**
• Soil mineralogical analysis with X-ray Fluorescence Spectrophotometry (XRF)
• Soil clay mineralogy with X-ray diffraction (XRD) measurements
• FT-IR spectrometry to confirm the presence of minerals as found by XRD measurements.

### 2.3.2 Particle size analysis and assignment of textural class

Soil particle size analysis and determination of soil texture is a primary requisite in almost all cases of soil analysis. Many soil properties and processes are related directly or indirectly to the soil texture. These include water holding capacity, amount of plant available water, extent of soil compaction and ease of root growth, air and water movements into and through the soil, cation exchange capacity etc.

**Laboratory determination**

Hydrometer method is a routine method for the soil particle size analysis. This method is based on the principle of dispersion and sedimentation of the soil particles of various sizes in soil-water suspension. Settling time of the dispersed particles is governed by Stokes law. The hydrometer is used to determine soil suspension density. The 40 second reading of the hydrometer gives the value of silt + clay, since larger sand particles settle during this period. The reading at the end of two hours provides the estimation of clay. The record of soil suspension temperature at the time of insertion of the hydrometer is used for the temperature correction of the hydrometer readings.

The soil was sieved with a 2 mm sieve and an amount (approx. 50 g) was accurately weighed and mixed with 50 ml of 6 % H₂O₂ to decompose the soil organic matter, the mixture was kept overnight and the excess H₂O₂ was removed by boiling. The solution
was mixed with 100 ml of 5% sodium hexametaphosphate, stirred and transferred to a 1 L measuring cylinder, where it was agitated with a plunger and the hydrometer readings were taken at specific time intervals (4 min and 2h) to determine clay, silt and sand contents (Sarkar and Haldar, 2010).

Calculation
The air dry weight of the soil sample is first converted to the oven dry weight, by dividing the former with the moisture correction factor (Bashour and Sayegh, 2007).

a) Oven dry soil weight (g) = \( \frac{\text{Weight of air dry soil (g)}}{\text{Moisture correction factor}} \)

The moisture correction factor (mcf) is obtained from the following relation:

\[
\text{mcf} = \frac{100 + \text{moisture content of air dry soil}}{100}
\]

b) Temperature correction is done by adding 0.4 to the hydrometer reading for each degree above 20°C and subtracting in a similar manner if the temperature is below 20°C.

c) Amounts of sand, clay and silt are determined as follows:
- Sand (in g) = Soil oven dry weight – corrected 4 min reading.
- Clay (in g) = corrected 2 h reading
- Silt (in g) = corrected 4 min reading – corrected 2 h reading.

d) Percentage composition of sand, clay and silt are determined as follows:

\[
\text{Sand \%} = \frac{\text{Sand in g}}{\text{Oven dry soil weight}} \times 100
\]

\[
\text{Silt \%} = \frac{\text{Silt in g}}{\text{Oven dry soil weight}} \times 100
\]

\[
\text{Clay \%} = \frac{\text{Clay in g}}{\text{Oven dry soil weight}} \times 100
\]
Assignment of textural class

Soil textural classification is based on the presence of relative proportions of sand, silt and clay in a particular type of soil. The classification is obtained by plotting the composition in an equilateral triangle containing twelve soil textural classes (ISSS classification, Fig. 2.7) as shown below (Bashour and Sayegh, 2007):

![Soil Textural Triangle (ISSS classification)](image)

Fig. 2.7. Soil Textural Triangle (ISSS classification)

2.3.3 Bulk Density

The soil bulk density is the ratio of oven dry soil particles to the soil bulk volume (including soil pore volumes). It can provide information like soil structure and available void space. It is frequently used in the calculation of soil porosity and in the conversion of weight based calculations to volume based calculations and vice versa.

Since most of the soil sampling points suffer from soil upper horizon disturbances, soil bulk density was determined by repacked cube method as follows:

The sieved (2 mm) and air dried soil was repacked in a preweighed dry measuring cylinder to a known volume (50 cm³), tapping the cylinder on palm with successive soil addition. The bulk density (BD) of the sieved soil was obtained after determining the
weight, moisture correction factor (mcf) and the volume (1 ml = 1 cm³) of the soil sample (Bashour and Sayegh, 2007; Sarkar and Haldar, 2010).

Calculation

\[
BD \, (g \, cm^3) = \frac{\text{Weight of air dry soil (g) / mcf}}{\text{Volume of soil (cm³)}}
\]

2.3.4 Particle density

The particle density (PD) is the ratio of mass of oven dry soil to the soil bulk volume (excluding soil pore space). Its value helps an analyst to predict the presence of mineral particles and the extent of introduction of organic matter to the soil. It is used in the calculation of soil porosity in association with the soil bulk density.

Laboratory determination

For the determination of the particle density in the laboratory, graduated cylinder method was applied. To a 100 ml cylinder, 70 ml water was added. 30 g finely ground soil was introduced using a funnel and allowed to stay for 5 minutes to release the entrapped air in the soil. The displaced volume of the water in the cylinder by the added soil was recorded to determine the particle density (Bashour and Sayegh, 2007) as follows:

Calculation:

\[
PD \, (g \, cm^3) = \frac{\text{Weight of air dry soil (g) / mcf}}{\text{Volume of water displaced (cm³)}}
\]

2.3.5 Soil porosity

Soil porosity (SP) is important to indicate the soil structural conditions, and to determine the usefulness of soil for agroforestry application, since soil porosity can provide some idea about root penetration into soil. Based on the value of soil porosity, it is possible to predict the capacity of the soil to exchange atmospheric gases like oxygen and carbon dioxide and the capacity to store water.
Laboratory determination

In the laboratory, SP is determined from the soil bulk density and particle density using the relationship (Bashour and Sayegh, 2007) given below:

\[
SP(\%) = 1 - \frac{\text{Bulk Density (BD)}}{\text{Particle Density (PD)}} \times 100
\]

### 2.3.6 Moisture Content

Soil moisture content is the trapped water in soil matrix. Due to the property of absorbing moisture, soil can show its properties even at the extreme conditions. The major role of soil moisture is the regulator of soil temperature. Increasing soil moisture content contributes to lower soil temperature in two ways, water absorbs heat and during evaporation, the absorbed heat in soil is remitted to the atmosphere.

Laboratory determination

Soil moisture content was determined by direct or gravimetric method. It was calculated on the basis of ratio of mass of water present to the mass of the soil sample after it has been removed from it, by drying in a hot air oven until constant weight. Moisture content is expressed as percentage composition on mass basis (Reeuwijk, 2002).

Calculation

\[
\text{Moisture (\%)} = \frac{(W_1 - W_2) \times 100}{W_2}
\]

\(W_1\) = Air dry soil weight before drying.
\(W_2\) = Oven dry soil weight.

### 2.3.7 Water Holding Capacity

The amount of water that can be retained by unit mass or volume of soil at a time is known as its water holding capacity (WHC). It is directly related to the presence of soil pore space, specific surface area and surface charge density. Increase in soil clay and
soil organic matter content contributes to higher water holding capacity. Water holding capacity is utilised to determine the extent of plant available water, transportation of nutrients and microorganisms and the soil aerobic conditions.

Laboratory determination

The water holding capacity (WHC) was determined by filling up a 6.0 cm PVC cylinder (fitted with a lid and a perforated base) with a known amount of the soil, which was immersed in water with the lid tightly held, maintaining a lower water level than the soil level, and was allowed to saturate for 2 h. The excess water was allowed to drain out and the WHC in % was obtained from the difference in weight of the air-dried soil and the water-saturated soil (Wilke, 2005).

Calculation

\[
\text{WHC (\%)} = \frac{M_s - M_o}{M_o - M_e} \times 100
\]

\(M_s\) is the mass of PVC cylinder containing water saturated soil.
\(M_o\) is the mass of PVC cylinder containing air dry soil.
\(M_e\) is the mass of empty PVC cylinder.

2.3.8 Saturated Hydraulic Conductivity

This parameter is directly related to the soil internal drainage property. It depends on the soil texture and the distribution of soil pores. Soil with higher saturated hydraulic conductivity suffers lesser slaking and runoff and supports stability of soil matrix to act against erosion.

Laboratory determination

The saturated hydraulic conductivity (\(K_{sat}\)) was obtained by constant head permeameter method based on Mariotte syphon principle (Sarkar and Haldar, 2010) in which a permeameter of known cross sectional area was filled with sieved soil up to 2.5 cm height, saturated with water overnight and water was allowed to flow through the soil keeping a constant water head and without disturbing the top layers. From the
values of cross sectional area, depth of water over soil, volume of water discharged in a
time interval, $K_{sat}$ was calculated (Sarkar and Haldar, 2010).

Calculation

$$K_{sat} \text{ (cm min}^{-1} \text{)} = \frac{Q}{A.t} \times \frac{L}{L+H}$$

t = Collection time of discharge (in minutes).
Q = volume of collected water (cm$^3$)
L = Length of soil column (cm)
H = Depth of water above soil surface (cm).
A= Cross section of permeameter (cm$^2$).

2.3.9 Soil Aggregate Analysis

It is suggested that soil aggregates or peds are formed due to the cumulative
contributions of cohesive forces like cationic bridging, organic matter- clay complexes,
through water molecule bonding and due to the cementing action of CaCO$_3$, Fe and Al
oxides/hydroxides. The soil aggregates are able to physically protect SOC and
contributes to the soil carbon sequestration.

Laboratory determination

Soil Aggregate analysis was done following the wet-sieving technique of Yoder 1936
with the help of a nest of six sieves ($5$, $2$, $1$, $0.5$, $0.25$ and $0.1$ mm). Soil aggregates ($>5.0$ – $8.0$ mm) were spread on the top sieve and soaked with salt free water twice at the
interval of five minutes. The nest of sieves were then submerged under water and
oscillated at 35 cycles/ minute with a stroke length of 3.8 cm keeping the soil on the
uppermost sieve under water throughout the process. The aggregates on each sieve was
collected, oven-dried and weighed. The aggregates were treated with 30% (vol/vol)
H$_2$O$_2$ and $0.1$ N HCl and passed through the same sieve and the weights of
nonaggregated primary particles were taken after oven drying. Soil aggregate stability
was expressed in terms of percentage as well as mean weight diameter (MWD) in mm,
defined as the sum of the product of the mean diameter and the weight of the sample at
the particular diameter, of each size fraction (Baruah and Borthakur 1997).
Calculation: \[ \text{MWD} = \sum_{i=1}^{n} X_i W_i \]

\( X_i = \) Mean diameter of a given fraction.
\( W_i = \) Weight proportion of a given fraction of soil aggregates.
\( n = 6 \) (number of size fractions, i.e. 5 to 0.1 mm).

The percent aggregation is calculated as follows

\[ \% \text{ aggregation (of a particular mean diameter)} = \frac{(W_1 - W_2) \times 100}{g} \]

\( W_1 = \) Sieve retained particle weight before dispersion.
\( W_2 = \) Sieve retained particle weight after dispersion.
\( g = \) Oven dry weight total of soil taken for analysis.

2.3.10 Soil pH

The naturally available soil cations may be acidic (\( \text{Al}^{3+}, \text{H}^+, \text{NH}_4^+ \)) or basic (\( \text{Ca}^{2+}, \text{Mg}^{2+}, \text{K}^+, \text{Na}^+ \)) in nature. Since, their nature is opposite, net balance of the cations reflect the overall acid-base character of the soil. Soil pH is a measure of the presence of active hydrogen ions in soil solution and can act as a predictor of the soil chemical activities. Soil management practices regarding suitable plant selection for a particular location and the soil available nutrient status are only possible after knowing the soil pH.

Laboratory determination

The pH of the soil samples was determined in 1:5 soil: water suspensions (Elico pH-meter, LI120). Prior to determination of the pH, the pH meter was standardised with buffers of pH 4 and 8.

2.3.11 Electrical conductivity

Electrical conductivity (EC) is an ion induced process. In soil, the ions originate from the soluble salts. Higher EC of soil may indicate its saline nature. The soil samples with higher values of EC should be checked for its sodium absorption ratio (SAR), since
SAR is a better indicator of soil salinity. SAR is defined as the ratio between the concentration of Na\(^+\) ion to the square root of total concentrations of Ca\(^{2+}\) and Mg\(^{2+}\).

Laboratory determination

The electrical conductivity (EC) of the soil samples was determined in 1:5 soil: water suspensions (Elico conductivity meter, CM180) with a conductivity cell of cell constant 1.0. A blank was run with 0.01 N KCl solutions.

2.3.12 Buffering capacity

Soil clay and humus fractions together act as a buffer resisting changes in soil pH in an acidic soil. Soil with higher cation exchange capacity imparts higher buffering capacity. If the buffering capacity is high, then the soil requires addition of more lime to bring the pH to the acceptable level.

Laboratory determination

To 10 g of air dry soil, 50 ml distilled water is added, stirred and allowed to stand for an hour. The pH of the suspension is determined. To this suspension, a 5 ml increment of already standardised 0.01 N NaOH solution is added and the pH is recorded after each addition. A graph is plotted with volume of added base as the abscissa and the corresponding pH as the vertex. From the pH-titration curve, the pH inflexion point is determined. At the inflexion point, all the soil acids are neutralised and due to the addition of further base, sudden hike in pH is observed.

Calculation

\[
\text{Buffering Capacity (soil) cmol kg}^{-1} = N \times V \times \frac{100}{W}
\]

where N is the normality of NaOH, V is the titre value of NaOH and W is the weight of soil taken in g.

2.3.13 Cation Exchange Capacity and Base Saturation

Soil clay and organic matter contain surface negative charges, which are balanced by cations occupying the available exchangeable sites. It is known that the higher the
valence state of the cations, the greater is its efficiency to be adsorbed by clay and soil organic matter.

The capacity of the soil to retain exchangeable cations like \( \text{Ca}^{2+} \), \( \text{Mg}^{2+} \), \( \text{K}^{+} \), \( \text{Na}^{+} \), \( \text{NH}_4^{+} \), \( \text{Al}^{3+} \), and \( \text{H}^{+} \) is known as its cation exchange capacity (CEC). CEC was obtained by simple summation of all ion concentrations in cmol kg\(^{-1}\). Base saturation of soil is the ratio of CEC due to the basic cations to the total effective CEC (Baruah and Borthakur, 1997; Pansu and Gautheryou, 2006).

The CEC determination by ion concentration summation method is carried out with the preparation of extracts using two solvents as shown below:

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Ions to be determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Acetate</td>
<td>( \text{Ca}^{2+}, \text{Mg}^{2+}, \text{Na}^{+} ) and ( \text{K}^{+} )</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>( \text{H}^{+}, \text{Al}^{3+} ) and ( \text{NH}_4^{+} )</td>
</tr>
</tbody>
</table>

Preparation of ammonium acetate extract

10 g of soil is taken in a stoppered reagent bottle and 100 ml of 1 M ammonium acetate at pH 7 is added to it. The bottle is shaken for some time and is allowed to stay overnight. The mixture is filtered with Whatman 42 filter paper. To remove organic matter, 50 ml of the filtrate is taken in a beaker, concentrated to about 10 ml on a hot plate. To it, 1 ml of aqua regia is added for organic matter decomposition and the mixture is evaporated to dryness. The residue is extracted with distilled water and the volume is made up to 50 ml. This extract is used for the determination of \( \text{Ca}^{2+}, \text{Mg}^{2+}, \text{Na}^{+} \) and \( \text{K}^{+} \) as given below:

Estimation of \( \text{Ca}^{2+}, \text{Mg}^{2+} \)

Total amount of calcium and magnesium is estimated taking 10 ml of the ammonium acetate extract. 50 ml of distilled water and 5 ml of NH\(_4\)Cl - NH\(_4\)OH buffer are added followed by a pinch of Eriochrome black T indicator. The solution acquires a wine red colour. It is titrated with 0.01M EDTA solution till the colour changes to blue. Let B be the titre value.

For estimation of calcium alone, 10 ml of the extract, 50 ml of distilled water and 5 ml of 10% NaOH are mixed together and a pinch of murexide indicator is added, when
the solution acquires pink colour. It is titrated with 0.01M EDTA solution till a dark purple colouration is reached that indicates the end point. Let A be the titre value.

Calculation

\[
\text{Ca}^{2+}, \text{cmol kg}^{-1} (\text{soil}) = \frac{V}{V_1} \times \frac{100}{W} \\
\text{Mg}^{2+}, \text{cmol kg}^{-1} (\text{soil}) = (B - A) \times \frac{V}{V_1} \times \frac{100}{W}
\]

where

V is the total volume of the extract prepared
V1 is the volume of the aliquot used for estimation
W is the weight of soil taken for extract preparation
0.01 is the molarity or normality of the used EDTA for estimation.
A and (B – A) are the titre values for calcium and magnesium respectively.

\textit{Estimation Na}^{+}, K^{+}

A part of the ammonium acetate extract is subjected to flame photometric determination of Na\(^+\) and K\(^+\) (Elico Model CL 361). The result is obtained in mg/L which is converted to mg kg\(^{-1}\) using the following relationship

\[
\text{Na}^{+} \text{ or K}^{+} (\text{mg kg}^{-1}) = \frac{\text{Flame photometer reading in mg L}^{-1} \times \text{total volume of extract}}{\text{Oven dry weight of soil}}
\]

The concentration in mg kg\(^{-1}\) is converted to cmol kg\(^{-1}\) by dividing the value with (Equivalent weight of Na or K \times 100). The result of Na\(^+\) or K\(^+\) in mg kg\(^{-1}\) is also used as the exchangeable sodium or potassium value for the particular soil sample in the study of NPK status of the soil.

\textit{Preparation of KCl extract}

40 g of soil is placed in a 250 ml stoppered reagent bottle and to it 100 ml of 1N KCl solution is added. The mixture is shaken in a shaking machine for 10 minutes and the suspension is filtered through Whatman 42 filter paper. During this process the
following exchange processes take place resulting in the liberation of $H^+$, $Al^{3+}$ and $NH_4^+$ ions into the solution from the soil.

\[
\begin{align*}
\text{Soil} - H + KCl & \rightarrow \text{Soil} - K + HCl & (1) \\
\text{Soil} - Al + KCl & \rightarrow \text{Soil} - K + AlCl_3 & (2) \\
\text{Soil} - H + KCl & \rightarrow \text{Soil} - K + NH_4Cl & (3)
\end{align*}
\]

The filtrate obtained in this process is used to determine the soil exchange acidity, exchangeable aluminium and the ammonium ion concentrations as described below:

*Estimation of $H^+$ and $Al^{3+}$*

The soil exchange acidity and exchangeable aluminium content together is termed as the exchangeable acidity (EA). HCl obtained in equation (1) along with AlCl$_3$ obtained in equation (2) liberates HCl through the process AlCl$_3$ + H$_2$O $\rightarrow$ Al (OH)$_3$ + HCl when titrated against standard NaOH solution giving exchange acidity. 20 ml of the KCl extract is taken in a titration flask and is heated to the boiling temperature to drive out dissolved CO$_2$ if any. It is allowed to cool and a drop of phenolphthalein indicator is added and is titrated against standard NaOH solution till pink colour appears. The chemical changes occurring during this process are $\text{HCl} + \text{NaOH} \rightarrow \text{NaCl} + \text{H}_2\text{O}$.

Calculation

\[
\text{Exchange Acidity (soil) cmol kg}^{-1} = N \times V \times \frac{E_v}{A_v} \times \frac{100}{W}
\]

where $N$ is the normality of NaOH, $V$ is the titre value of NaOH, $E_v$ is the total volume of the extract, $A_v$ is the volume of aliquot taken for titration and $W$ is the weight of soil taken in g.

To the resultant solution, after determination of exchange acidity, 5 ml freshly prepared 4% NaF solution is added and is allowed to stand for 5 minutes. The liberated NaOH in this reaction is titrated against the standard HCl to the point of just disappearance of pink colour. The chemical changes during this process can be summarised as

\[
\text{Al (OH)$_3$} + 6 \text{NaF} \rightarrow 3 \text{NaOH} + \text{Na}_3\text{AlF}_6
\]
The liberated NaOH is titrated against standard HCl to obtain the measure of exchangeable aluminium as shown below:

\[
\text{Exchangeable Aluminium (soil) cmol kg}^{-1} = N \times V \times \frac{E_v}{A_v} \times \frac{100}{W}
\]

where \(N\) is the normality of HCl, \(V\) is the titre value of HCl, \(E_v\) is the total volume of the extract, \(A_v\) is the volume of aliquot taken for titration, \(W\) is the weight of soil taken in g.

Estimation of \(NH_4^+\)

To 5 ml of the KCl extract, 5 ml of a solution mixture (containing 6.8 g sodium salicylate, 5 g sodium citrate, 5 g sodium tartrate and 0.025 g sodium nitroprusside in 100 ml of distilled water) is added, mixed well and is allowed to stand for 15 minutes. To this, 5 ml of a solution containing (10% NaOH and NaOCl) is added and is allowed to stand for 1 h to develop brilliant green colour (Anderson and Ingram, 1993). The developed colour is subjected to UV- Vis spectrophotometric determination (Shimadzu, UV- 1800) at 665 nm. Finally, the concentration of \(NH_4^+\) ions is obtained from standard curve prepared using standard ammonium chloride solution.

Calculation

\[
NH_4^+ \text{ mg kg}^{-1} = \frac{C_{NH_4^+} \times F_v}{T_v} \times \frac{V}{W}
\]

where \(C_{NH_4^+}\) is the concentration of \(NH_4^+\) in mg L\(^{-1}\), \(F_v\) is the total volume used in colour development, \(T_v\) is the volume of extract used for colour development, \(V\) is the total volume of extract prepared, \(W\) is the oven dry weight of soil taken for analysis. The mg kg\(^{-1}\) value obtained was divided by 1800 to convert it to cmol kg\(^{-1}\).

The effective CEC and the basic CEC are obtained as follows:

(i) Effective CEC = EA + Sum of concentrations of major cations \((Ca^{2+}, Mg^{2+}, K^+, Na^+ , NH_4^+)\) in cmol kg\(^{-1}\)

(ii) Basic CEC = Sum of concentrations of basic cations \((Ca^{2+}, Mg^{2+}, K^+, Na^+)\) in cmol kg\(^{-1}\)
**Base Saturation**

Base saturation is the measure of contribution of basic cations present in soil in ratio to the total cation exchange capacity. It provides the information of soil fertility status. It is expressed as percentage and obtained by calculation from the obtained results for cation exchange capacity.

Calculation

\[
\text{Base Saturation} \% = \frac{\text{Basic CEC}}{\text{Effective CEC}} \times 100.
\]

**2.3.14 Soil Organic Carbon**

Soil organic carbon (SOC) originates from soil organic matter (SOM). The plant and animal residues at various stages of decomposition constitute the SOM. Various soil microorganisms, insects, worms etc. utilize the SOM as their nutrient source. The refusals or the residues, after decomposition are able to act as the source of plant nutrients. The higher SOM content in soil is supported by plants, no or low tillage activity, poor soil drainage and fine soil texture. Presence of adequate amount soil organic matter contributes to improve the physical nature of soil through soil aggregation and pore space. The improved soil physical condition contributes to higher water holding capacity, soil infiltration rate and decreased erodibility. The soil pores can improve soil tilth and support soil fertility status (Daniels and Haering, 2006).

Laboratory determination

The soil organic carbon (SOC) was measured by the modified Walkley-Black method in which the soil carbon was oxidized with excess potassium dichromate and sulphuric acid followed by titration with Fe(II) using diphenylamine indicator. The titre value was subtracted from that of a blank titration without soil and the SOC was obtained from the amount of potassium dichromate consumed in oxidation. To minimize the interference of iron etc. and to obtain a sharp endpoint, silver sulphate, phosphoric acid and sodium fluoride were used in the titration mixture.
Calculation

\[
\text{% easily oxidisable carbon} = \frac{(B-T) \times M \text{ of Fe}^{2+} \times 12 \times 100}{g \text{ of oven dry soil} \times 4000}
\]

B = Blank titre value of Fe\(^{2+}\) in ml.
T = Titre value of Fe\(^{2+}\) in ml for soil.

\[
\text{% Total carbon} = \text{% Easily oxidisable carbon} \times 1.30
\]

The soil organic carbon stock of each sample was obtained from the equation (1)

\[
C_{\text{Stock (min)}} = C_{\text{con}} \times BD \times d \times CF_{st}
\]  (1)

where \(C_{\text{Stock (min)}}\) is the carbon stock in mineral soil in t ha\(^{-1}\), \(C_{\text{con}}\) is the concentration of soil organic carbon in percentage, \(d\) is the thickness of soil layer in m, \(BD\) is the bulk density in kg dm\(^{-3}\) and \(CF_{st}\) is the correction factor for stoniness with \(CF_{st} = 100 - (\% \text{ stones})/100\) (Dinca et al, 2012).

2.3.15 Total Soil Nitrogen

The soil nitrogen exists either in the form of NH\(_4^+\) or NO\(_3^-\). Nitrogen is incorporated to the soil either naturally through SOM mineralisation or artificially through the application of compost or manure. Nitrogen is the most essential plant nutrient.

Laboratory determination

Total soil nitrogen (TSN) was determined by Kjeldahl method (Hasse, 1994) in which the soil sample was digested with a catalyst mixture (K\(_2\)SO\(_4\) : CuSO\(_4\) : Se powder in 100 : 10 : 1 ratio) and sulphuric acid. The digested soil was treated with 10 M NaOH, distilled immediately and the distillate was collected in 2 % boric acid solution with methyl red and bromocresol green indicators. A blank distillation without soil was also carried out. The TSN was obtained by titration with 0.1 N H\(_2\)SO\(_4\).
Calculation

\[
TSN\ (\text{mg/kg}) = \frac{(S-B) \times N \times 14 \times 1000}{W}
\]

(S-B) = Difference in volume of H\textsubscript{2}SO\textsubscript{4} used in titration with soil and blank.
N = Normality of H\textsubscript{2}SO\textsubscript{4} used in the titration.
W = Oven dry weight of soil

2.3.16 Carbon to Nitrogen ratio

The soil nitrogen is present either in mineralized form or immobilized form. N-mineralization is the process of conversion of soil organic nitrogen to inorganic form (e.g. NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{-}). Again, N-immobilization is the process of conversion of inorganic form of nitrogen into organic form (e.g. amino acids or proteins).

The proportion of C (%) to the corresponding N (%) for a kind of soil is known as its carbon to nitrogen ratio. This ratio is helpful to determine the existing nitrogen and consequently, to assess the favourable conditions for soil carbon sequestration. This can be summarised as follows:

When 

<table>
<thead>
<tr>
<th>C: N</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 30:1</td>
<td>nitrogen immobilization is favoured.</td>
</tr>
<tr>
<td>≥ 20:1 ≥ 30:1</td>
<td>both immobilization and mineralization are equal.</td>
</tr>
<tr>
<td>&lt; 20:1</td>
<td>rapid nitrogen mineralization is favoured.</td>
</tr>
</tbody>
</table>

Thus, C:N ratio of greater than 30:1 favours carbon sequestration in soil. When C: N ratio is < 20:1, N-mineralization occurs at the cost of SOC resulting in the liberation of CO\textsubscript{2} to the atmosphere, acting against soil carbon sequestration.

Laboratory determination

In the laboratory, soil organic carbon and total soil nitrogen were determined separately and converted to percentages. The results were computed to obtain carbon to nitrogen ratio.
2.3.17 Available Phosphorous

Soil phosphorous may be in organic form associated with the SOM and in inorganic form closely associated with the soil minerals. Moist and warm soil with proper aeration at pH 6.5 is found to optimise the release of phosphorous from its bound state to be available for the plants. Plants are able to utilise the stored energy obtained from photosynthesis through the available absorbed phosphorous content. Hence, soil phosphorous deficiency, in agroforestry system is reflected through poor fruit and seed yield, because its deficiency imparts growth impairment of plants.

Laboratory determination

Available phosphorous (Av. P) in soil was determined by Olsen’s method. To 5 g of soil, 50 ml of 0.5 M NaHCO₃ and a pinch of activated carbon were added, shaken mechanically for half an hour and filtered. To 10 ml of the filtrate, 5 ml of ammonium molybdate and 1 ml of SnCl₂ working solution were added. The available P was determined with a UV-Visible spectrophotometer (Shimadzu, UV- 1800) at 660 nm immediately, (Baruah and Borthakur, 1997).

Calculation

\[ P \text{ mg kg}^{-1} = \frac{C_{P} \times F_{v}}{T_{v}} \times \frac{V}{W} \]

\(C_{P}\) is the concentration of P in mg L⁻¹.
\(F_{v}\) is the total volume used in colour development.
\(T_{v}\) is the volume of extract used for colour development.
\(V\) is the total volume of extract prepared.
\(W\) is the oven dry weight of soil taken for analysis.

2.3.18 Retention capacity for major cations in soil

The major cations act as the source of plant nutrients in soil. The effectiveness of retention of the major cations in soil is of special interest in agroforestry system because of its importance in the productivity in the cropping conditions. The macro-nutrient
retention capacity of soil acts as a balance between the retention of input nutrients and its subsequent loses in the process of leaching. This capacity mainly depends on the soil texture, presence of organic carbon, cation exchange capacity, base saturation and pH of the soil. It is known that the higher the valence of the cation, the greater is the efficiency of its adsorption on soil surface (Al > Ca > Mg > K > Na).

Laboratory determination

In the laboratory, the major cation retention capacity of a particular soil is determined by column adsorption technique. For the determination, four 10 cm long columns of finely ground soil are prepared and through each column, separately, 1M solution of CaCl₂, MgCl₂, NaCl and KCl is passed. The differences in concentration before and after adsorption are determined titrimetrically for Ca and Mg; flame photometrically for Na and K (Elico Model CL 361). The differences in the concentrations before and after adsorption are the measures of the adsorbed amounts of the major cations.

Calculation

\[
\text{Retained cations (C}_{\text{ret}}) \text{ in cmol kg}^{-1} \text{ (soil)} = \frac{(C_r - C_l)}{m} \times \frac{10 \, v}{M}
\]

where (Cₙ - Cᵓ) is the difference in concentration before and after retention of a cation in mg L⁻¹, m is the oven dry soil weight in g, v is the charge of the cation (1 for Na and K; 2 for Ca and Mg) and M is the atomic mass of the cation in g.

2.3.19 Determination of Soil Trace Metals (Co, Cr, Cu, Mn, Ni, Pb and Zn)

Soil trace metal analysis has a key role in arriving at a comparison with the natural background levels and in assessing the extent of weathering processes governing the association and dissociation of trace metals to soil (Wilson et al., 2008, Bini et al., 2011). The present work incorporates monitoring of Cr, Co, Cu, Mn, Ni, Pb, and Zn in tropical agricultural soil and soil affected by pulp and paper mill effluents and solid wastes with a view to establish their ecological hazards. The potent source of the trace metals in soil constitutes both the free and bound states. The bound metals might
become bioavailable under appropriate unfavourable soil conditions. This work aims to determine the regional averages of the trace metals in the soil and to establish their correlation with soil physicochemical parameters.

Laboratory determination:

Atomic absorption spectrophotometer was used for the determination of soil trace metal concentration. Hollow cathode lamp (HCL) filled with argon or neon and having the filament of the particular metal is used to provide matching radiation of the studied metal. Such radiations are suitably absorbed by the atomised metal atoms to give the characteristic spectra of the metal. The detector matches the spectra to the calibrated standard to provide the metal concentration.

To determine the total amounts of trace metals present in the soil samples, approximately 1 g of soil was digested with tri-acid mixture (concentrated hydrochloric, nitric and sulphuric acids in 1:2:4 proportions), the volume was made up to 100 ml and the trace metals were analysed in an Atomic Absorption Spectrophotometer (PerkinElmer AAAnalyst 200) using air acetylene flame. Before the analysis, for each metal, the spectrophotometer was separately calibrated using at least three standard solutions.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength(nm)</th>
<th>Slit Width (nm)</th>
<th>Flame Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>240.73</td>
<td>1.8/1.35</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Cu</td>
<td>324.75</td>
<td>2.7/0.8</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Cr</td>
<td>357.87</td>
<td>2.7/0.8</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Mn</td>
<td>279.48</td>
<td>1.8/0.6</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Ni</td>
<td>232</td>
<td>1.8/1.35</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Pb</td>
<td>283.31</td>
<td>2.7/1.05</td>
<td>Air-Acetylene</td>
</tr>
<tr>
<td>Zn</td>
<td>213.86</td>
<td>2.7/1.8</td>
<td>Air-Acetylene</td>
</tr>
</tbody>
</table>

Calculations

The determined concentrations are converted to mg kg⁻¹ using the formula:

\[
\text{Trace metal (mg kg}^{-1}) = \frac{\text{AAS reading in mg L}^{-1} \times \text{volume of extract}}{\text{Oven dry weight of soil}}
\]
2.4 XRF analysis

In X-ray fluorescent (XRF) analysis, the emergent X-rays knock out the penultimate electrons from particular electron cells of atoms. The energy of an electron in an atom is its unique property. The scattered x-rays have energies characteristic of the electronic transitions and their measurement makes it possible to determine the chemical composition of a material.

The soil chemical composition is determined with respect to the major and minor element oxides. The oxides include SiO₂, Al₂O₃, Fe₂O₃, MnO, MgO, CaO, Na₂O, K₂O, TiO₂, and P₂O₅. The XRF analysis is done with a Philips PW 1480 (Rh anode and LiF 200 catalyst analyser; SAIF, Gauhati University).

Sample preparation

0.7 g of finely ground soil is mixed with flux (a mixture of 35.3 % Li-Borate and 64.7 % Li-metaborate) in a platinum crucible. The crucible is heated in a furnace at 1050°C for 1 h. The molten mass when solidified is converted to amorphous glass like bead, which is subjected to XRF study.

2.5. XRD and FT-IR analysis

XRD powder diffraction and FT-IR spectroscopy are used as complementary techniques for characterisation of the clay minerals present in soil.

The clay minerals possess crystalline structures. Under the influence of various cationic environments along with variable compositions of retained water, the crystal geometry is usually modified. Such situations impart very complex chemical compositions to the soil clay minerals and X-Ray diffraction (XRD) has been found to be very useful in identifying these minerals from their distinctive diffraction patterns. The powder XRD gives absorption bands against 2θ (θ, the diffraction angle) from which the d- spacings could be obtained and by comparing with standard values, the clay minerals could be identified. Even if XRD has advantages, it cannot be considered as the sole and concrete technique to identify all the soil clay minerals. Frequently, the presence of amorphous materials like allophones or Si- clusters in soil make the identification complicated. The amorphous materials do not possess regular symmetry
and give broad peaks in the XRD pattern, sometimes masking the peaks due to the clay minerals imposing limitations in their identification.

As a supporting method for the characterisation of soil clay minerals, IR had been used since a long period. Infrared spectroscopy is a non-destructive device and is a preferred spectroscopic tool for soil clay characterisation. In this method, a continuous beam of IR- radiation is allowed to fall on the soil clay sample under investigation. Under the influence of the irradiated IR beam, the sample absorbs some of it and transmits the rest, yielding a characteristic spectrum of the sample under, which is often regarded as the fingerprint of the material. These results in combination with those obtained from the XRD, make it possible to identify the clay minerals with reasonable accuracy.

Separation of clay fraction from soil

To approximately 25 g of a soil sample, 200 ml of sodium acetate buffer (prepared by mixing 270 ml glacial acetic acid and 92 g sodium acetate in 1 L distilled water) (pH 5) is added in a beaker and is digested for 30 min with stirring in a near boiling water bath. The mixture is rapidly filtered through Whatman no 1 filter paper. Now the soil is carbonate free. This is transferred to a 500 ml beaker and 5 ml 30 % H₂O₂ is added (to remove SOM) and the beaker is kept covered till effervescence stops. The sample is mixed with an additional 5 ml 30 % H₂O₂ and is heated for a few minutes. Another 10 ml of 30% H₂O₂ is added and the mixture is allowed to settle overnight. It is then digested in a water bath for 2 h to remove the peroxide. To remove released cations, the soil suspension is washed over filter paper with 1 N sodium acetate solution. To this suspension, 80 ml of citrate-bicarbonate buffer (prepared by dissolving 88 g sodium citrate and 10.5 g sodium bicarbonate in 1 L distilled water) is added along with 3.0 g of sodium dithionite (Na₂S₂O₄) in three successions (each 1 g) and is heated on a water bath at around 80°C for 20 min with intermittent stirring. During the process, iron present is removed in the form of FeS precipitate. After separating the precipitate, the solution is treated with 20 ml of 1 N NaCl solution, centrifuged, and the supernatant is rejected (to remove amorphous material). The residue is washed with 95 % methanol to remove the bulk of NaCl. The suspension is transferred to a 1000 ml measuring cylinder and the volume is made up to 1000 ml with the addition of distilled water. The cylinder
is vigorously agitated and is allowed to stand for 6 h. Before the completion of 6 h, the suspension is drawn out with a pipette by inserting the same at least 10 cm below the top level. The collected suspension is centrifuged to obtain the clay fractions of the soil. The clay fraction (< 2 μm) is washed with deionized water and centrifuged, rejecting the supernatant and collecting the separated solid, which is freeze dried overnight to remove any excess moisture within the sample (Baruah and Barhakur, 1997). The separated soil clay fraction is subjected to X-ray powder diffraction and FT-IR measurements.

FT-IR Measurement

Approximately 1.5 mg of the sample and 80 mg of spectroscopic grade KBr are weighed out and ground in an agate mortar until thoroughly homogenized. The mixture is pressed into a disc and is analysed for IR spectra (IR Affinity-1 FTIR spectrophotometer, Shimadzu; range 4000 to 400 cm\(^{-1}\); 4 cm\(^{-1}\) resolution).

XRD measurement

Powder XRD patterns are obtained with a Phillips Analytical X-ray spectrometer (PW 1710, SAIF, Gauhati University) using Cu K-alpha radiations at 40 kV, 30 mA and Ni filter with continuous scan. Time per step is 0.5 s; step size 0.02 and data analysis range is 5.01 to 29.93 degrees (2θ) with 1247 points in each case. The diffractograms were analysed and compared with the existing database for soil clay mineralogical characterization.

2.6 Statistical analysis

Statistical analysis of experimental data is the tool for organising and simplifying data that enables to estimate whether the analysis is under control or change has occurred. The importance of application of the statistical tools in soil and environmental science has been described by several authors (Webster and Oliver, 1990; Chandler and Scoll, 2011).

In the present study, summary statistics are done with MS Excel for obtaining minimum, maximum, mean, median and standard deviation of the data.

The maximum and the minimum values describe the range of spread of the results. The mean (or average) is the arithmetic average of the set of data. Median signifies the
middle value of a series irrespective of the arrangement of the data series. Standard deviation indicates the spread (dispersion) of data around the mean. Alternately, the standard deviation also indicates the square root of variance (sum of the squared deviation from the mean which are averaged).

The comparison of the data is obtained by multiple bar diagrams, correlation analysis and various other indices to signify the various correlations and trends of interactions of mutually interrelated parameters.

Correlation of any two parameters is found by observing how one parameter varies with the other simultaneously. Variations of both the parameters in the same direction are designed as positive and in opposite direction are called negative correlations.

The quantity R, called the linear correlation coefficient, measures the strength and the direction of a linear relationship between two variables, X and Y. The formula for computing R is:

\[
R = \frac{N \sum XY - (\sum X)(\sum Y)}{\sqrt{[N \sum X^2 - (\sum X)^2][N \sum Y^2 - (\sum Y)^2]}}
\]

where n is the number of pairs of data of X and Y. The value of R is \(-1 < r < +1\). The + and − signs are used for positive linear correlation and negative linear correlation, respectively. These are defined as

(i) Positive correlation: If X and Y have strong positive linear correlation, R is close to +1. An R value of exactly +1 indicates a perfect positive fit. Positive values indicate a relationship between X and Y variables such that as values for x increase, values for y also increase.

(ii) Negative correlation: If X and Y have a strong negative linear correlation, R is close to -1. An R value of exactly -1 indicates a perfect negative fit. Negative values indicate a relationship between X and Y such that as values for X increase, values for Y decrease.

(iii) No correlation: If there is no linear correlation or a weak linear correlation, r is close to 0. A value near zero means that there is a random, nonlinear relationship between the two variables.
R is a dimensionless quantity; that is, it does not depend on the units employed. A perfect correlation of ± 1 occurs only when the data points all lie exactly on a straight line. If R = +1, the slope of this line is positive. If R = -1, the slope of this line is negative. A correlation greater than 0.8 is generally described as strong, whereas a correlation less than 0.5 are generally described as weak. These values can vary based upon the "type" of data being examined.

The coefficient of determination, R², is useful because it gives the proportion of the variance (fluctuation) of one variable that is predictable from the other variable. It is a measure that allows us to determine how certain one can be in making predictions from a certain model/graph. The coefficient of determination is such that 0 < R² < 1, and denotes the strength of the linear association between the two variables, X and Y. The coefficient of determination represents the percent of the data that is the closest to the line of best fit. For example, if R = 0.922, then R² = 0.850, which means that 85% of the total variation in Y can be explained by the linear relationship between X and Y (as described by the regression equation). The other 15% of the total variation in Y remains unexplained. The coefficient of determination is a measure of how well the regression line represents the data. If the regression line passes exactly through every point on the scatter plot, it would be able to explain all of the variation. The further the line is away from the points, the less it is able to explain.

The correlation can be termed from perfect to very low (or absent) on the following values of R²:

<table>
<thead>
<tr>
<th>Degree</th>
<th>Positive Correlation</th>
<th>Negative Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfect</td>
<td>R² = +1</td>
<td>R² = -1</td>
</tr>
<tr>
<td>High</td>
<td>+ 0.75 ≥ R² ≥ +1</td>
<td>-0.75 ≥ R² ≥ -1</td>
</tr>
<tr>
<td>Moderate</td>
<td>+ 0.25 ≥ R² ≥ +0.75</td>
<td>-0.25 ≥ R² ≥ -0.75</td>
</tr>
<tr>
<td>Low</td>
<td>0 &gt; R² ≥ +0.25</td>
<td>0 &gt; R² ≥ +0.25</td>
</tr>
<tr>
<td>Absence</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
2.7 Pollution Index and Potential Ecological Risk Index

In the study of the soil trace metals, the effectiveness of the obtained concentration is studied on the basis of pollution indices and ecological risk indices. A brief description of these indices can be summarised as follows:

The pollution Index (PI) is obtained from $PI = \frac{C_i}{C_{ref}}$ (where $C_i$ is the mean concentration obtained by averaging values measured and $C_{ref}$ is the reference value for the studied parameter) (Jiang et al., 2014).

The Potential Ecological Risk Index for a single trace metal ($E_i$) and the overall Potential Ecological Risk Index (PERI) are calculated using the expressions:

$$E_r = T_i^1 \times PI;$$
$$PERI = \sum_i E_r^i,$$

where $T_i$ is toxicity response coefficient with a value of 5 for Co, Cu, Pb and Ni; 2 for Cr and 1 for Zn (Hakinson, 1980; Krupadam et al., 2006). The pollution indices may be used to determine the risk levels on the following basis:

<table>
<thead>
<tr>
<th>Pollution Index (PI)</th>
<th>Ecological Risk Index ($E_i$)</th>
<th>Potential Ecological Risk Index (PERI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>Level</td>
<td>$E_r$</td>
</tr>
<tr>
<td>PI &lt; 1</td>
<td>Unpolluted</td>
<td>$E_r &lt; 30$</td>
</tr>
<tr>
<td>1 ≤ PI &lt; 3</td>
<td>Moderate pollution</td>
<td>$30 \leq E_r &lt; 60$</td>
</tr>
<tr>
<td>3 ≤ PI &lt; 6</td>
<td>Considerable pollution</td>
<td>$60 \leq E_r &lt; 120$</td>
</tr>
<tr>
<td>PI ≥ 7</td>
<td>Very High pollution</td>
<td>$E_r \geq 120$</td>
</tr>
</tbody>
</table>