CHAPTER 3
METHODOLOGY

3.1. Introduction

The study was conducted through both (i) Field investigation and (ii) Laboratory investigation. This chapter describes the methodology adapted in the two processes.

3.2. Field investigation

3.2.1. Selection of sampling sites

For determining the locations of the sampling points, the toposheet of the area was enlarged to the requisite scale and traverses were made through the area of the study. The sampling sites for the collection of water and soil were selected within a radius of 25 km from the coal mining area. The locations of the sampling sites for ground water, pond water and river water (10 each) with distance and direction (measured with reference to Coal India Limited's residential complex at Margherita) are given in Table 3.1. The locations of the sampling stations for soil (20 locations) are given in Table 3.2.

3.2.2. Sample collection

Water samples from the different sources were collected in pre-cleaned polythene containers of 5 liter capacity.

Soil samples were collected from the banks of rivers at sites where flow rates were low and sedimentation was more (Sakai et al., 1986; Subramanian et al., 1987). Only surface soil samples were collected from a depth of 0-15 cm. At least 6 samples were collected simultaneously from the same site within a small radius in different directions and these were mixed thoroughly to obtain a composite representative sample. Samples were carried to the laboratory in polythene bags, then dried in a shade and preserved for analysis.

Sampling for water and soil in all the sites was done in the same day starting at 8 in the morning to late afternoon.

3.2.3. Frequency of Sampling

Soil samples were collected for a period of 3 years in two seasons, dry season (Nov-January) and monsoon (wet) season (May-July) and Water samples were collected for a period of 3 years in three seasons namely pre monsoon (January-March), monsoon (May-July) and Post monsoon (Oct-Dec) seasons.
Table 3.1: The sampling stations for water from the coal field area. (Distance and direction are measured with reference to Coal India Limited’s residential complex at Margherita)

<table>
<thead>
<tr>
<th>S.N</th>
<th>Locations</th>
<th>Sources</th>
<th>Distance (km)</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Ground water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>CIL Complex, Srimanta Nagar</td>
<td>Tube well</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>G2</td>
<td>Polo field at D duara’s residence</td>
<td>Tube well</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>G3</td>
<td>At CMPF Office CIL</td>
<td>Tube well</td>
<td>1</td>
<td>W</td>
</tr>
<tr>
<td>G4</td>
<td>Near Sarbajanin Kali Mandir</td>
<td>Dug well</td>
<td>20</td>
<td>E</td>
</tr>
<tr>
<td>G5</td>
<td>Lalpahar Village, Tipong</td>
<td>Dug well</td>
<td>19</td>
<td>E</td>
</tr>
<tr>
<td>G6</td>
<td>No. 2 Tirap Gate</td>
<td>Tube well</td>
<td>18</td>
<td>E</td>
</tr>
<tr>
<td>G7</td>
<td>Lekhapani Shiva Temple, Tinali</td>
<td>Dug well</td>
<td>15</td>
<td>S</td>
</tr>
<tr>
<td>G8</td>
<td>Lido Itakhola near a bridge</td>
<td>Dug well</td>
<td>9</td>
<td>E</td>
</tr>
<tr>
<td>G9</td>
<td>Ledu Bazaar, Barua’s Hotel</td>
<td>Tube well</td>
<td>7</td>
<td>E</td>
</tr>
<tr>
<td>G10</td>
<td>At the Stadium, Tikok Colliery</td>
<td>Tube well</td>
<td>5</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td><strong>Pond water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>Shiva Mandir, Tipong</td>
<td>Pond</td>
<td>20</td>
<td>E</td>
</tr>
<tr>
<td>P2</td>
<td>Lalpami Village, Tipong</td>
<td>Pond</td>
<td>19</td>
<td>S</td>
</tr>
<tr>
<td>P3</td>
<td>No. 2 Tirap Gate</td>
<td>Pond</td>
<td>18</td>
<td>S</td>
</tr>
<tr>
<td>P4</td>
<td>Tirap Colliery, a road side pond</td>
<td>Pond</td>
<td>12</td>
<td>E</td>
</tr>
<tr>
<td>P5</td>
<td>Ledo Itakhula, near a bridge</td>
<td>Pond</td>
<td>9</td>
<td>E</td>
</tr>
<tr>
<td>P6</td>
<td>Ledo</td>
<td>Pond</td>
<td>8</td>
<td>E</td>
</tr>
<tr>
<td>P7</td>
<td>Patkai Stadium</td>
<td>Pond</td>
<td>7</td>
<td>E</td>
</tr>
<tr>
<td>P8</td>
<td>Ledu Bazaar</td>
<td>Pond</td>
<td>6</td>
<td>E</td>
</tr>
<tr>
<td>P9</td>
<td>Borgolai area</td>
<td>Pond</td>
<td>4</td>
<td>E</td>
</tr>
<tr>
<td>P10</td>
<td>Changlang Road, Margherita</td>
<td>Pond</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td><strong>River water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Tipong River, Colliery Gate</td>
<td>River</td>
<td>22</td>
<td>E</td>
</tr>
<tr>
<td>R2</td>
<td>GE Office under airon bridge</td>
<td>Rivulet</td>
<td>16</td>
<td>E</td>
</tr>
<tr>
<td>R3</td>
<td>Lekhapani River, Lekhapani Station</td>
<td>River</td>
<td>15</td>
<td>E</td>
</tr>
<tr>
<td>R4</td>
<td>Lekhapani Station, ASEB office</td>
<td>River</td>
<td>14</td>
<td>E</td>
</tr>
<tr>
<td>R5</td>
<td>Shiva Mandir, Tirap colliery</td>
<td>Rivulet</td>
<td>11</td>
<td>E</td>
</tr>
<tr>
<td>R6</td>
<td>Tirap Colliery near a bridge</td>
<td>Rivulet</td>
<td>10</td>
<td>E</td>
</tr>
<tr>
<td>R7</td>
<td>Tikak Colliery under a bridge</td>
<td>Rivulet</td>
<td>5</td>
<td>E</td>
</tr>
<tr>
<td>R8</td>
<td>New colony near a bridge</td>
<td>River</td>
<td>2</td>
<td>E</td>
</tr>
<tr>
<td>R9</td>
<td>Back side of Borgolai</td>
<td>River</td>
<td>4</td>
<td>E</td>
</tr>
<tr>
<td>R10</td>
<td>Margherita</td>
<td>River</td>
<td>3</td>
<td>W</td>
</tr>
</tbody>
</table>
Table 3.2: The sampling stations for soil from the coal field area. (Distance and direction are measured with reference to Coal India Limited’s residential complex at Margherita)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Locations</th>
<th>Source</th>
<th>Distance (km)</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>CIL Residential Complex</td>
<td>Play ground</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>S2</td>
<td>Polo Field</td>
<td>Play ground</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>S3</td>
<td>Shiva Temple, Tipong</td>
<td>Bank of Rivulet</td>
<td>25</td>
<td>E</td>
</tr>
<tr>
<td>S4</td>
<td>Tipong Colliery’s Entrance</td>
<td>Bank of Rivulet</td>
<td>15</td>
<td>E</td>
</tr>
<tr>
<td>S5</td>
<td>Near Sarbajanin Kali Mandir</td>
<td>Paddy field</td>
<td>20</td>
<td>E</td>
</tr>
<tr>
<td>S6</td>
<td>Lalpahar Village, Tipong</td>
<td>Paddy field</td>
<td>19</td>
<td>E</td>
</tr>
<tr>
<td>S7</td>
<td>Tipong Colliery (tea garden)</td>
<td>Roadside soil</td>
<td>18</td>
<td>E</td>
</tr>
<tr>
<td>S8</td>
<td>Lekhapani</td>
<td>Roadside soil</td>
<td>17</td>
<td>E</td>
</tr>
<tr>
<td>S9</td>
<td>Lekhapani River, near Station</td>
<td>Bank of Tirap River</td>
<td>15</td>
<td>E</td>
</tr>
<tr>
<td>S10</td>
<td>Lekhapani, ASEB Office</td>
<td>Bank of Tirap river</td>
<td>14</td>
<td>E</td>
</tr>
<tr>
<td>S11</td>
<td>Tirap Colliery</td>
<td>Roadside soil</td>
<td>12</td>
<td>E</td>
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<td>S12</td>
<td>Tipong colliery</td>
<td>Roadside soil</td>
<td>11</td>
<td>S</td>
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<td>Ledo Bricks Hearth</td>
<td>Bank of the Rivulet</td>
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<td>E</td>
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<td>S15</td>
<td>Ledo Itakhola</td>
<td>Bank of pond</td>
<td>7</td>
<td>E</td>
</tr>
<tr>
<td>S16</td>
<td>Patkai Stadium</td>
<td>Bank of pond</td>
<td>7.5</td>
<td>E</td>
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<td>S17</td>
<td>Tikak Colliery Entrance</td>
<td>Bank of Rivulet</td>
<td>6</td>
<td>E</td>
</tr>
<tr>
<td>S18</td>
<td>Tikak Colliery (near Bridge)</td>
<td>Road side soil</td>
<td>5</td>
<td>E</td>
</tr>
<tr>
<td>S19</td>
<td>Borgolai area (Thekeratenga)</td>
<td>Bank of River</td>
<td>2</td>
<td>S</td>
</tr>
<tr>
<td>S20</td>
<td>Dihing River</td>
<td>Bank of River</td>
<td>3</td>
<td>W</td>
</tr>
</tbody>
</table>
3.3. Laboratory Investigation

The methods of analysis of water and soil samples are described below. Analysis for all the parameters was done following standard procedure (Jackson, 1967, APHA, 1995; Boruah and Borthakur, 1997). The details of parameters selected and the measurement procedures are given below.

3.3.1. Chemicals and Glassware

High purity reagents obtained from E. Mark (India) were only used. Double distilled deionized water and borosil glassware were used throughout the study.

3.3.2. Analysis of water

The following water quality parameters were chosen for the present work:

(i) pH, Electrical Conductivity (EC), Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solid (TSS),
(ii) Major anions, CO$_3$\(^{2-}\), HCO$_3$\(^{-}\), Cl$^{-}$, F$^{-}$, NO$_3$\(^{-}\), S$^{2-}$, SO$_4$\(^{2-}\), and PO$_4$\(^{3-}\),
(iii) Major cations, Na, K, Ca, Mg, Fe and Al
(iv) Trace metals, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, Sn, V, Zn.

For the determination of trace metals, water samples were digested with 5 ml of HNO$_3$ acid to release the metals associated with suspended matter and colloidal organic matter and to protect the water samples from change in composition and deterioration from aging due to various interactions. In the digestion procedure, 500 ml of water sample was evaporated slowly at 50 - 60°C to 50 ml achieving 10 times pre-concentration.

(i) pH and Electrical Conductivity

\textit{pH}. The pH was determined directly with the help of a digital pH meter (Model LT-120, Elico, India). The Instrument was calibrated for each set of measurements with standard buffer solution having pH 7.

\textit{Electrical Conductivity}. Electrical Conductivity of water samples was determined directly with the help of a digital conductivity bridge (Elico, India CM180) using a conductivity cell of cell constant 1.0 after thorough calibration with standard KCl solution.
(ii) **Total Solid (TS), Total Dissolved Solid (TDS), Total Suspended Solid (TSS)**

TS, TDS and TSS were determined by the simple evaporation technique. To determine TS, 100 ml of water sample was taken in a pre-weighed 250 ml beaker. Water was then evaporated to dryness and the weight of the beaker is taken. The difference between this weight and that of the empty dry beaker is the measure of TS in 100 ml water sample.

TDS was also determined as above but taking 100 ml of filtered water sample. TSS is obtained by subtracting the TDS value from TS.

(iii) **Major anions** (CO$_3^{2-}$, HCO$_3^-$, Cl, F, NO$_3^-$, S$^{2-}$, SO$_4^{2-}$, and PO$_4^{3-}$)

**Carbonate and bicarbonate.** Carbonate (CO$_3^{2-}$) and bicarbonate (HCO$_3^-$) are formed in water due to the dissolution of CO$_2$ at higher pH (carbonate) and at lower pH (bicarbonate). Beside this, bicarbonate may enter the water bodies due to the dissolution of limestone and dolomites, weathering of primary and secondary silicate minerals and organic activities (Garrels and Mackenzie, 1971).

Carbonate and bicarbonate are determined titrimetrically using H$_2$SO$_4$ (0.01N) as the titrant in presence of phenolphthalein indicator for CO$_3^{2-}$ and in presence of methyl orange indicator for HCO$_3^-$.  

\[
\text{CO}_3^{2-} + \text{H}_2\text{SO}_4 = \text{HSO}_4^- + \text{HCO}_3^- \]
\[
2\text{HCO}_3^- + \text{H}_2\text{SO}_4 = \text{SO}_4^{2-} + 2\text{CO}_2 + \text{H}_2\text{O} 
\]

Phenolphthalein gives colour as long as CO$_3^{2-}$ remains in solution. When all the CO$_3^{2-}$ is converted to HCO$_3^-$, it will be discharged. HCO$_3^-$ can be titrated to neutrality with methyl orange as indicator.

50 ml water sample was taken in a flask; 3 drops of phenolphthalein indicator were added and then titrated with H$_2$SO$_4$ until the pink colour just disappeared. This end point corresponded to the neutralization of the CO$_3^{2-}$. To this solution, 2 drops of methyl orange indicator was added and titrated against H$_2$SO$_4$ until the colour of the solution turned red. This reading corresponded to the neutralization of both CO$_3^{2-}$ and HCO$_3^-$.  

CO$_3^{2-}$ (mg/L) = (Vol. of H$_2$SO$_4 × 0.01 ×$ Eq.W. of CO$_3^{2-}$) $× 1000/ \text{ml of water sample}$.

HCO$_3^-$ (mg/L) = (Vol. of H$_2$SO$_4 × 0.01 ×$ Eq.W. of HCO3$^-)$ $× 1000/ \text{ml of water sample}$.

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Chloride. Chloride is a mobile ion and commonly occurs as soluble salts such as NaCl, CaCl₂ and MgCl₂. Argentometric titration method is used to determine the Cl⁻ content in water samples. This method is based on the formation of slightly soluble white precipitate of AgCl due to the addition of AgNO₃ solution. AgNO₃ reacts with chloride to form AgCl. At the end point, when all the chlorides get precipitated, free silver ions react with chromate to form silver chromate (Ag₂CrO₄) of reddish brown colour.

\[
\begin{align*}
\text{Cl}^- + \text{Ag}^+ & = \text{AgCl} \\
\text{Cl}^- + \text{AgNO}_3 & = \text{AgCl} + \text{NO}_3^- \\
\text{K}_2\text{CrO}_4 + 2\text{AgNO}_3 & = \text{Ag}_2\text{CrO}_4 + 2\text{KNO}_3
\end{align*}
\]

50 ml of water sample was taken for the determination. To it, 5 drops of K₂CrO₄ indicator was added. It was titrated with 0.02 N AgNO₃ solutions (with stirring) till the first reddish brown tinge appeared. The concentration of Cl⁻ is calculated using the following formula,

\[
\text{Cl}^- (\text{mg/L}) = \left( \frac{\text{ml} \times \text{N of AgNO}_3}{\text{ml of water sample}} \right) \times 35.5 \times 1000
\]

Fluoride: Fluoride reacts with the coloured complex of zirconyl acid and SPADNS reagent (Sodium-2-(Parasulphophenylazo)-1, 8-dihydroxy-3, 6 naphthalene disulphonate) forming colorless [ZrF₅]²⁻ and releasing the dye. This reaction, which can be followed conveniently by colorimetric measurement of the dye, is the basis of fluoride estimation. The absorbance measurements were done at 570 nm with a cell of width 1 cm using a UV spectrophotometer (Hitachi 3210). Fluoride concentration was read directly by operating the instrument in photometry mode calibrating against a standard and a blank.

Standard curve for Fluoride was prepared in the range of 0 to 1.4 mg F/L. If the absorbance falls beyond the range of the standard curve, diluted water samples were used.

Nitrate: It is determined by spectrophotometric method using a Spectrophotometer (Hitachi 3210). Measurement of UV absorption at 220 nm enables rapid determination of NO₃⁻ ion. Dissolved organic matter also absorbs at 220 and 275 nm, but NO₃⁻ ion does not absorb at 275 nm. The second measurement made at 275 nm is thus used to correct NO₃⁻ ion value.
Here water samples were prepared by adding 1 N HCl in 25:1 ratio. The absorbance against D/W as blank was taken and the reading were taken at 2, 3 wavelength program at W1 = 220 nm (NO₃⁻ + Organic matter) and W2 = 275 nm (Organic matter)

Now, NO₃⁻ (mg/L) = W1 - 2W2.

0.7218 g of KNO₃ was dissolved in distilled water and diluted to 1 L to get a solution of concentration, 100 mg/L, which was taken as the stock solution. Standard solutions containing 10, 8, 6, 4, 2 mg of NO₃⁻ per liter are prepared by dilution with double distilled water. For the determination of nitrate, 25 ml of water sample was taken in a conical flask, to which 1 ml of 1 N HCl was added. After thorough mixing, the absorbance was read at 220 and 275 nm. The procedure was repeated with the standard solution.

**Sulphide.** Determination of sulphide is important in the coal mining area to know the coal quality and indirectly the rate of conversion of sulphide to sulphate. In water, S²⁻ is naturally present due to the sulphide minerals. In acidic water, it may be easily converted to SO₄²⁻.

The Sulphide content in water samples was determined by iodometric titration method (APHA 1998). 50 ml of water sample was taken in a flask. 5 ml of 0.025N iodine solution was added to this solution followed by 5 ml of distilled water and 5 ml of 6N HCl. It was then titrated with 0.025N sodium thiosulphate solution using starch as indicator. Amount of sulphide was calculated using the following formula:

\[ S²⁻ (mg/L) = 16000 \times \left[ \frac{(A \times B) - (C \times D)}{\text{Volume of water (50 ml)}} \right] \]

where
- \( A \) = ml of iodine solution.
- \( B \) = normality of iodine solution (0.025N).
- \( C \) = ml of NaS₂O₃ solution required for titration.
- \( D \) = normality of NaS₂O₃ solution (0.025N).

**Sulphate.** Determination of sulphate is necessary to know the salinity of water. Sulphate occurs in appreciable quantity in all natural water, especially high in arid and semi arid regions where natural water in general has high salt content. At low pH, sulphide minerals may be oxidized to sulphate by the action of chemolithotrophic type of bacteria.
To estimate sulphate content, turbidimetric method was used (APHA, 1998). 20 ml of a filtered water sample was taken along with 2 ml of conditioning reagent (75g of NaCl + 30 ml of concentrated HCl + 100 ml of 95% ethyl alcohol and 300 ml distilled water mixed together) was added. During stirring, 2 g of BaCl₂ crystals was added and again stirred for one minute. Then, exactly after 4 minutes, absorbance of the BaSO₄ and water suspension was recorded at 420 nm using UV Visible spectrophotometry. The concentration of sulphate in water was calculated from a calibration curve prepared by following the same procedure with standard sodium sulphate solution at 5 different concentrations in equal range. The calculation was

\[ \text{SO}_4^{2-} (\text{mg/L}) = \frac{\text{Absorbance} \times \text{Factor (from standard curve)}}{\text{ml of water}}. \]

**Phosphate.** Phosphate in water samples was determined using Dickman and Brady (1940) method. 50 ml of water sample was taken in conical flask to which 2 ml of ammonium molybdate solution was added followed by 5 drops of stannous chloride solution. A blue colour developed. After 5 minutes but before 12 minutes of addition of all the reagents, the absorbance of the solution was read using UV-visible spectrophotometer (Hitachi 3210) at 690 nm. The concentration of the phosphate is determined with the help of a standard curve obtained with standard potassium hydrogen orthophosphate solution at five different concentrations of equivalent ranges from 0.0 to 10 mg/L.

(iv) **Major cations, Na, K, Ca, Mg, Fe and Al**

**Sodium (Na) and Potassium (K).** Na and K are two exchangeable cations always present in water. High Na content in water can adversely affect the aquatic life forms and may be responsible for the deficiencies of elements such as K, Zn, Cu and Mn (Levitt, 1980). In water samples, Na and K were determined directly using flame photometry (Elico CL 361 flame photometer) after proper calibration with standard NaCl and KCl solution.

**Calcium and magnesium.** Calcium (Ca) and Magnesium (Mg) are two exchangeable cations present in water. Ca occurs in all the natural water in great abundance. Likewise Mg also occurs in all natural water but generally somewhat in low concentration than calcium. It has been observed that in water having low pH, Mg is predominant over Ca. Ca⁺² and Mg⁺² ions are considered to be the two ions with similar behavior and this is true
in many phenomena of the hydrosphere. Their source has been in the rock from which it gets leached.

Ca and Mg in water were determined by complexometric titration method, first introduced by Schwartzenbach et al. (1946), using EDTA solution. EDTA forms complex with different metals depending upon pH. The EDTA salt used here is disodium salt of EDTA (Na₂H₂Y₂H₂O), where Y is the tetravalent anion of EDTA.

\[
\begin{align*}
\text{Ca}^{2+} + \text{H}_2\text{Y}^{2-} &= \text{CaY}^{2-} + 2\text{H}^+ \\
\text{Mg}^{2+} + \text{H}_2\text{Y}^{2-} &= \text{MgY}^{2-} + 2\text{H}^+
\end{align*}
\]

50 ml of water sample was taken in a flask and 1 ml of buffer solution (NH₄Cl and NH₄OH) was added to make the pH of the solution about 10. 100 mg of Eriochrome Black T indicator was added to this solution. The wine red-coloured solution is then titrated with 0.01 N EDTA solutions until the colour changed to blue. This reading (B ml) of EDTA corresponded to the value of total Ca and Mg. Again another 50 ml sample was taken and 2 ml of 10% NaOH solution was added to make the pH of the solution 11. To this, 100 mg murexide indicator was added. The pink coloured solution is then titrated with 0.01 N EDTA solutions till the colour changed to dark purple. This reading (A ml) of EDTA corresponds to the value of Ca.

Calculation

\[
\begin{align*}
\text{Ca (mg/L)} &= A \text{ ml of EDTA} \times 1000 \times 0.4008 / \text{ml of sample} \\
\text{Mg (mg/L)} &= (B-A) \text{ ml of EDTA} \times 1000 \times 0.2431 / \text{ml of sample}
\end{align*}
\]

Aluminium (Al) and Iron (Fe). Al is a non-toxic element (Dubey, 1985) at trace level, but higher levels are toxic. Similarly, Fe at high levels is toxic to plants and animals. Both Al and Fe are determined with Atomic absorption spectroscopy (Varian SpectrAA220) at different experimental conditions (Table 3.3) using standard solutions.

(v) Trace metals, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Sn, V, Zn.

When water is of low pH, more of the heavy metals go into solution. To determine the concentration of heavy metals and trace elements, water samples were digested as follows:
<table>
<thead>
<tr>
<th>Elements</th>
<th>Wave Length (nm)</th>
<th>Slit Width (nm)</th>
<th>Operating Range</th>
<th>Lamp Current (mA)</th>
<th>Flame type</th>
<th>Fuel gas flow rate (L/min)</th>
<th>Airflow Rate (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>193.7</td>
<td>0.5</td>
<td>3-150</td>
<td>10</td>
<td>*</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Al</td>
<td>309.3</td>
<td>0.5</td>
<td>0.3-250</td>
<td>10</td>
<td>C_2H_2-N_2O</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.5</td>
<td>0.02-3.00</td>
<td>4</td>
<td>Air-C_2H_2</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Co</td>
<td>240.7</td>
<td>0.2</td>
<td>0.05-15.0</td>
<td>7</td>
<td>Air-C_2H_2</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.2</td>
<td>0.06-15.0</td>
<td>7</td>
<td>Air-C_2H_2</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>0.5</td>
<td>0.03-10.0</td>
<td>4</td>
<td>Air-C_2H_2</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.2</td>
<td>0.06-15.0</td>
<td>5</td>
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<tr>
<td>Hg</td>
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<td>2-400</td>
<td>4</td>
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<td>Mn</td>
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<td>0.2</td>
<td>0.02-5.0</td>
<td>5</td>
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<td>3.5</td>
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<td>3.5</td>
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<tr>
<td>Pb</td>
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<td>0.1-30.0</td>
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<td>3.5</td>
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<tr>
<td>Se</td>
<td>196.0</td>
<td>1.0</td>
<td>5-250</td>
<td>10</td>
<td>*</td>
<td>1</td>
<td>--</td>
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<tr>
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<td>1-200</td>
<td>7</td>
<td>C_2H_2-N_2O</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>V</td>
<td>--</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>C_2H_2-N_2O</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>1.0</td>
<td>0.01-2.0</td>
<td>5</td>
<td>Air-C_2H_2</td>
<td>1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Hydride generation with Electro thermal temperature controller, ‡ the operating working range is in mg/L except for As, Hg and Se where the range is in μg/L.
500 ml of water sample was taken in a beaker and acidified with 5 ml of concentrated 
HNO₃. The sample was evaporated to almost dryness and the volume was made up to 50 
ml with distilled water. This solution was used to determine the elements with Atomic 
absorption spectroscopy (Varian spectrAA220).

3.3.3. Analysis of soil

The following physico-chemical and other parameters were determined in the soil 
samples:

(i) pH, Electrical Conductivity(EC), Soil Texture, Bulk Density, Particle 
Density, Porosity, Hydraulic Conductivity, Water Holding Capacity, 
Moisture Content, Organic Carbon, Nitrogen Content, Available 
Phosphorous

(ii) Major anions CO₃²⁻, HCO₃⁻, Cl⁻, SO₄²⁻,

(iii) Major cations like Na, K, Ca, Mg

(iv) Fe and Al and Trace metals, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, Sn, V, Zn

(v) Soil composition with respect to major and minor oxides, with the help of 
XRF measurement,

(vi) Identification of the soil clay fractions with XRD and IR measurements, and

(vii) Chemical partitioning of the trace metals in Soil into different fractions.

Preliminary treatment. Soil samples after collection were brought to the laboratory in 
sterile polythene bags, big lumps were broken down and undesirable matter like stones, 
roots, leaves, grasses, etc., were removed. The dried samples were sieved through 2 mm 
sieve and were used for analysis of the physico-chemical parameters and metal contents 
(Pinta 1975). The equipments used were calibrated by using standard solutions made from 
analytical grade chemicals in double distilled water.

(i) Physical properties of soil

pH. The pH is the negative logarithm of the H⁺ ion activity in the soil-water system. Water 
or soil become acidic when the basic cation Ca²⁺, Mg²⁺, Na⁺, K⁺ are replaced by H⁺ ion 
due to the leaching effect of rain. The exchange mechanism according to Townsend (1973) 
is as follows:

\[
\text{Soil} - \text{Na}^+ + \text{H}^+ \rightleftharpoons \text{OH}^- \leftrightarrow \text{H}^+ + \text{Na}^+ \rightleftharpoons \text{OH}^-
\]
In this study, pH of the soil samples was determined in 1:5 soil/water suspensions using a digital pH meter (Elico India LI 127) after thorough calibration with standard buffer solutions.

**Electrical conductivity.** Salinity or the concentration of the dissolved salts of water or soil is most conveniently measured by electrical conductivity. Electrical conductivity of water or soil is mainly due to the presence of charged solute and to a lesser extent in the nature of the solute and associated temperature.

Electrical conductivity of soil samples was determined using a conductivity meter (Elico India CM180) by dipping the conductivity cell of cell constant 1.0 in 1:5 soil/water suspensions after thorough calibration with standard KCl solution.

**Soil texture.** Determination of soil texture helps to characterizing the water and nutrient retention and transmission properties of soil in evaluating structural conditions and dispersibility. It also provides information about ion absorption and the exchange behaviour of soil (Tamhane et al., 1964). Soil texture was determined using hydrometer method where relative percentage of clay, silt and loam were calculated. 50 g of air dried soil was taken in a 500 ml beaker and was treated with 50-60 ml of 6% H₂O₂. The beaker was covered with a watch glass and was placed on a water bath at about 700°C until the organic matter was oxidized. The beaker was allowed to cool. The above process was repeated at least three times and finally the beaker was put on the water bath again for about two hours to remove the excess H₂O₂. About 400 ml of distilled water and 100 ml of calgon (sodium hexametaphosphate) solution was added to it. The suspension was stirred with an electric stirrer for about 10 minutes. The suspension was then transferred into a settling cylinder and the volume was made up to one liter with distilled water. The mixture was shaken vigorously back and forth for 1 minute by placing a rubber stopper over the mouth of the cylinder. Time was recorded immediately. The hydrometer was inserted into the suspension. The first hydrometer reading was taken after 4 minutes. The temperature of the suspension was recorded. The hydrometer was inserted into the suspension. The first hydrometer reading was taken after 4 minutes. The temperature of the suspension was recorded. The hydrometer was removed carefully and washed with distilled water. The hydrometer was calibrated at 19.45°C. The suspension was allowed to remain undisturbed for two hours and the hydrometer reading was taken by reinserting the hydrometer into the suspension.

The sand, silt and clay percentages were then calculated from the following expressions:
Sand % = 100-P4
Silt % = P4 – P120
Clay % = P120

\[
\frac{(R_4 \pm r) \times 100}{W}, \quad \frac{(R_{120} \pm r) \times 100}{W}
\]

where

\( R_4 \) = hydrometer reading at 4 min, \( R_{120} \) = hydrometer reading at 120 min

\( r \) = temperature correction = \( \pm (t-19.45) \times 0.2 \)

\( W \) = Oven dry weight of soil sample

\( t \) = temperature in °C at time of measurement.

If the working temperature is more than 19.45°C, the temperature correction \( r \), is positive and if it is less than 19.45°C, \( r \) is negative.

**Bulk density**: Bulk density determines the volume of pore space of the soil and the degree of compactness. Soil having high proportion of pore space has lower bulk density than those having low proportion of pore space. It is also an indicator of aeration status of soil and it provides information on the environment availability to the many soil microorganisms which live within the soil.

Soil bulk density \( (\rho_b) \) is defined as the oven dry weight of soil per unit of its bulk volume. It is expressed in the unit of g cm\(^{-3}\). The bulk density was determined in the laboratory using “tube core method” (Baruah and Borthakur, 1997) and calculated applying the formula given by Chopra and Konwar (1986) as follows:

\[
\text{Bulk density (} \rho_b \text{), g cm}^{-3} = \frac{W_2 - W_1}{V}
\]

Where

\( W_1 \) = Weight of the empty cylindrical tube
\( W_2 \) = Weight of the tube packed with oven dry soil at 105°C
\( V \) = Volume water that fills the tube fully.

**Particle density**. Particle density (PD) gives information about several soil parameters like porosity, bulk density and void ratio. PD is used to calculate the settling velocity of particles of different sizes during particle size analysis.
Particle density of soil samples was determined using “Pycnometer method” (Summer 2000). According to this method, a clean, dry Pycnometer was filled up with distilled water. The stopper of the Pycnometer was replaced and after wiping out the surface of the Pycnometer, the weight was taken. 10 g of oven dry soil sample was then introduced into the Pycnometer and the remaining area is then filled up with distilled water and the stopper was inserted. Water on the surface of the Pycnometer was wiped out, dried and weight was taken again. The Particle density $\rho_s$ is calculated using the following formula:

$$\rho_s = \frac{WS}{WW} = \frac{WS}{(WPW + WS - WPSW)} \text{ g cm}^{-3}$$

Here,
- $WPW = \text{Weight of Pycnometer filled with water.}$
- $WPSW = \text{Weight of Pycnometer filled with water and soil.}$
- $WS = \text{Weight of dry soil (10g)}$

Weight of water, $WW = WPW + WS - WPSW$

**Porosity.** Porosity is defined as the amount of space occupied by air and water inside the soil particles. The porosity of the soil samples are calculated using the formula-

$$\text{Porosity} = 1 - \frac{\text{Bulk Density}}{\text{Particle density}}$$

**Hydraulic Conductivity.** Hydraulic Conductivity is the measure of internal drainage capacity of the soil. It is an important parameter for land classification and to determine whether the soil is fit for agriculture or not.

Hydraulic Conductivity may be calculated using Darcey’s law. According to this law, the flow rate of a liquid through a soil column increases with an increased height of water above the bottom of the soil column and decreases with an increased depth of soil. The flow rate constant is called Hydraulic Conductivity.

To measure Hydraulic Conductivity, a soil core of 15.4 cm height was taken inside an aluminium cylindrical tube which was supported by a filter paper. This arrangement was placed on a funnel clamped to a rack. Water is allowed to pass through the core with an aspirator bottle maintaining a constant height of 2.5 cm above the core. Water flowing down the core was collected in a beaker in interval of 30 minutes. The Hydraulic Conductivity was calculated using the following formula
Hydraulic Conductivity (K), cm/min = QL/HAT

where,

- Q = Quantity of collected water in cm³
- A = Cross sectional area of the inside of the tube in cm²
- L = Length of the soil core in cm
- H = Total height of water column (i.e. core height + water head) in cm
- T = Time of flow in minutes

**Water holding Capacity.** Water Holding Capacity (WHC) is an essential parameter to determine the soil quality. It is a property related to the maximum water availability in soil, the soil strength, the rate of movement of water in soil and the plant response to water.

Water holding capacity of soil samples was determined by using a circular brass box of known weight (a). The perforated bottom plate of the box was supported on a Whatman No. 1 filter paper. Approximately 10 g of the soil was added to the brass box and weighed (b). The box with the soil was kept in a Petri dish containing water, so that about one fourth of the box remained under water. The box was kept overnight. Next day the box was removed and the excess water was allowed to drain off. When there was no more water dripping from the bottom of the box, it was weighed again (C). The weight (m) of a moist Whatman No. 1 filter paper was also measured. The water holding capacity was calculated applying the following formula-

\[
\text{Water holding capacity \%} = \frac{C - (b + m) \times 100}{(b-a)}
\]

**Moisture content:** The air space inside the soil particles is normally filled up with some moisture. The moisture content can be calculated following the same set of experiment as bulk density using the formula stated below-

\[
\% \text{ of water content} = \frac{(W2 - W3) - W1}{(W2 - W1)}
\]

where

- W1 = Weight of the empty cylindrical tube
- W2 = Weight of the soil + W1
W3 = Weight of the tube packed with oven dry soil at 105oC.

**Organic Carbon.** Organic matter is determined as organic carbon which is the sum total of organic compounds present in soil, excluding the undecayed plant and animal tissues, their partial decomposition products and the soil biomass (Stevens, 1982).

To determine organic carbon (%) of the soil samples, titrimetric method of Walkley and Black (1974) was used. 1 g of the soil sample was taken in a 500 ml conical flask. 10 ml of 1 N potassium dichromate solution was added to it and shaken. To it, 20 ml of concentrated sulphuric acid (containing 1.25% Ag$_2$SO$_4$) was added and the flask was swirled 3 times. The flask was allowed to stand for 30 minutes for the reaction to complete. 200 ml of distilled water was added to the flask to dilute the suspension followed by 10 ml of 85% H$_3$PO$_4$ and 1 ml of diphenylamine indicator. The solution was then titrated with 0.5 N ferrous ammonium sulphate till the colour changes from violet through blue to green. A blank titration without soil was carried out in the same way. The calculation was as shown below:

- Weight of the sample = W (1.0 g)
- Volume of 0.5 N Fe (NH$_4$)$_2$(SO$_4$)$_2$ solution used = B ml (for the blank titration)
- Volume of 0.5 N Fe (NH$_4$)$_2$(SO$_4$)$_2$ solution used = S ml (for the sample titration)
- Volume of the 1 N K$_2$Cr$_2$O$_7$ used for oxidation of C = 0.5 x (B-S) ml
- [1 ml of 1 N K$_2$Cr$_2$O$_7$ (=1 meq) = 0.003 g of org C]

Walkley averaged a 77% recovery of organic C by this method. Thus, a correction factor of 100/77 = 1.3 is to be used.

\[
\text{% of organic C in the soil (uncorrected)} = 0.5 \times (B-S) \times 1 \text{N} \times 0.003 \times \left(\frac{100}{W}\right) = Q
\]
\[
\text{% of org. C in the soil (corrected)} = Q \times 1.3 = R
\]

**Total Nitrogen:** Total nitrogen of soil samples is useful in determining the C/N ratio of soil which gives an indication of the process of transformation of organic nitrogen to available nitrogen like ammonical nitrogen and nitrate nitrogen. Total nitrogen is merely an indicator of the soil potential for the element but not the measure in which it becomes available to the plant.

Total nitrogen in soil samples was estimated by micro Kjeldahl method (Jackson, 1967). In this method, 10 g of soil sample was taken in a 300 ml Kjeldahl flask and was mixed
with 25 ml of distilled water to make a suspension. 20 g of the digestion catalyst mixture (20 g copper sulphate, 3 g mercuric oxide, 1 g selenium powder were mixed together and 1 g of this was mixed with 20 g of sodium sulphate to obtain the catalyst mixture) and 35 ml of concentrated H$_2$SO$_4$ were added to the suspension and were mixed by gentle swirling motion. The content was heated at low temperature for about 10-30 minutes until the frothing stops. The heat was then raised and the flask was cooled and the supernatant liquid was transferred to 100 ml volumetric flask. The residue was washed several times with distilled water and after each washing the supernatant liquid was transferred to the flask.

25 ml of the above solution was taken in a round bottom flask of the micro Kjeldahl apparatus. 25 ml of 40% NaOH solution and some pieces of Zn were added to the flask and swirled gently. A 500 ml conical flask containing 25 ml of boric acid and mixed indicator (Mixed indicator was prepared by mixing alcoholic solutions of bromocresol green (0.5%) and methyl red (0.1 %) in 2:1 ratio. 5 ml of this solution is added to 100 ml of 4% boric acid solution) was placed below the condenser. Distillation was commenced and about 100 ml of distillate was collected. This was then titrated with 0.1 N HCL until the colour changes from blue to light pink. A blank titration without soil was also carried out in the same way using other chemicals. Total nitrogen was calculated using the formula

$$
\%N = (a - b) \times N \times 1.4 \times \frac{V}{(v \times S)}
$$

where

- $a = \text{ml of HCL acid required for sample titration.}$
- $b = \text{ml of HCL acid required for blank titration.}$
- $N = \text{Normality of acid solution}$
- $V = \text{ml of total solution after digestion (= 100 ml)}$
- $v = \text{ml of digested solution taken for distillation (25ml).}$
- $S = \text{weight of the soil taken (10 g)}$

**Available Phosphorous:** Phosphorus is an essential nutrient element needed for plant growth. Soil and water used for irrigation should contain phosphate but the drinking water should be free from phosphate.

A suspension of 1: 20 soil/H$_2$SO$_4$ (0.002N) was made, which was shaken for half an hour and was filtered through Whatman No.50 filter paper to get a clear solution. 50 ml of the clear solution was taken in a conical flask and phosphate was estimated as in the case of water.
The available phosphorous is given by

\[ P \text{ mg/kg} = \frac{\text{mg P/L in soil extracted} \times V}{S \times v} \]

Where \( V = \) total volume of the soil extract prepared (200 ml)
\( S = \) wt. of soil taken in grams
\( v = \) volume of the aliquot taken for analysis (50 ml)

(ii) Major anions \( \text{CO}_3^{2-}, \text{HCO}_3^-, \text{Cl}^-, \text{SO}_4^{2-} \)

**Bicarbonate and carbonate:** A suspension of 1:5 soil/water was made, with 10 gm of soil and 50 ml of water. Shaken thoroughly and filtered. 25 ml of the filtrate was taken and both \( \text{CO}_3^{2-} \) and \( \text{HCO}_3^- \) were estimated as in the case of water with the following formula for calculation:

\[ \text{CO}_3^{2-} \text{ (mg/kg)} = (\text{Vol.} \times 0.01 \text{ N}) \times 50/10 \times 30/25 \times 1000 \]
\[ \text{HCO}_3^- \text{ (mg/kg)} = (\text{Vol.} \times 0.01 \text{ N}) \times 50/10 \times 61/25 \times 1000 \]

**Chloride:** The content of chloride in soil samples was determined in a 1:5 soil/water suspension. The suspension was shaken for half an hour and filtered. 50 ml of the filtrate was taken and chloride was determined argentometrically as in water samples. The calculation is done using the following formula:

\[ \text{Cl}^- \text{ (mg/Kg)} = (\text{ml} \times \text{N of AgNO}_3) \times 35.5 \times 1000 \times 5 / \text{ml of soil solution.} \]

**Sulphate:** In soil, bacterial reduction usually converts sulphate to sulphides in the presence of organic matter (Doner and Lynn 1989) and therefore, the sulphate measured in a soil sample may not be the actual content. For the purpose of measurement, 1:5 soil/water suspensions was shaken vigorously, allowed to settle and then, filtered through a Whatman No. 44 filter paper and 50 ml of the filtrate was taken for sulphate estimation following the turbidimetric method as in case of water. The calculation was done as follows:

\[ \text{SO}_4^{2-} \text{ (mg/kg)} = [\text{SO}_4^{2-} \text{ (mg/L) in soil solution}] \times 5 \]
(iii) Major cations Ca, Mg, Na and K

**Calcium and Magnesium.** Ammonium Acetate extract of a soil sample was prepared to determine Ca and Mg. 25 g of soil sample was treated with 50 ml of 1.0 N CH$_3$COONH$_4$ (pH 7) solution and kept overnight. The suspension is filtered (Whatman no 40) and the volume was made up to 250 ml with distilled water. 50 ml of the above extract was taken and evaporated almost to dryness to eliminate the inference by organic matter. The residue was dissolved in aquaregia and the solution was again evaporated almost to dryness. The residue was dissolved in distilled water to make up the volume to 50 ml. Taking 25 ml of the extract, Ca and Mg were determined as in water by EDTA titrimetric method. The calculation was as shown below:

Calcium $\% = \frac{A \times V \times 400.8}{V \times S \times 10000}$

Magnesium $\% = \frac{(B-A) \times V \times 400.8}{V \times S \times 1.645 \times 10000}$

where,

A = Volume of EDTA used for Ca determination.
B = Volume of EDTA used for (Ca + Mg) determination.
V = Total volume of soil extract prepared (250 ml).
S = Weight of soil sample taken (25 g).
V = Volume of soil extract titrated (25 ml).

Values can be converted to mg/ kg multiplying the % value with 10000.

**Sodium (Na) and Potassium (K).** Na and K are two other exchangeable cations present in soil. Na and K were determined in the NH$_4$-acetate extract by a flame-photometric method as in water and the concentration was calculated using the following formula-

Sodium $\% = \frac{Na \text{ (mg/L) of soil extract} \times V}{S \times 10000}$

Potassium $\% = \frac{K \text{ (mg/L) of soil extract} \times V}{S \times 10000}$

Where,

V = Total volume of soil extract prepared.
S = Weight of the soil sample taken.

Values can be converted to mg/ kg multiplying the % value with 10000.
For the determination of the metals and the trace elements in soil, the soil was sieved through a 80 mesh sieve and 1 g of sieved soil was digested with 35 ml of acid mixture (4 parts of concentrated H₂SO₄, 2 parts of concentrated HCl and 1 part of HNO₃). The mixture was evaporated till the white fumes no longer evolved. The residue was dissolved with 9:1 H₂O: HCl solution and the volume were made up to 50 ml (Pinta 1975).

All the elements were determined with Atomic absorption spectroscopy (Varian SpectrAA220) at different experimental conditions (Table 3.3) and the calculation was done using the following formula:

\[
\text{Metal concentration, mg/kg} = P \times Q \times R / W
\]

Where
- \( P \) = Concentration of metal in digested sample
- \( Q \) = Final volume of the digested solution, ml.
- \( R \) = Dilution ratio.
- \( W \) = Weight of the sample.

(v) Major and minor oxides

The percentage composition of major and minor oxides (SiO₂, Al₂O₃, Fe₂O₃, MnO, TiO₂, K₂O, Na₂O, MgO, CaO and P₂O₅) of the soil samples was measured with the help of a wavelength dispersive (WD) type X-ray fluorescence spectrometer (XRFS) (Philips model PW 1480 with Au-Cu dual anode system) at University Science Instrumentation Centre, Gauhati University, by applying pressed pellet technique, using standard samples. For multi elemental analysis of geochemical samples, it has now become a proven technique and has been widely used as a rapid and accurate analysis method (Chen, 1985; Xu et al, 1987). Analysis of a group of elemental oxides in a single trial is possible in XRF (Ashry, 1994).

The Loss on ignition (LOI), which is a measure of the total volatile matter present in the soil, was also calculated from incineration (approximately at 1100°C) of 1 g of the samples.

For sample preparation, the method given by Thompson et al (1996) is used. The powdered soil sample is sieved with a 200 mesh sieve. It is thoroughly mixed with 0.5 g boric acid. The mixture is uniformly spread over the top surface of the boric acid base in the metal cup of a deformable aluminium metal cup filled with boric acid as base. It is then
pressed by a cylindrical die of 40 mm diameter by mean of a hydraulic press (AIMIL Model 315) applying pressure in the range of 125 KN to 175 KN for about 5 minutes. A pellet of circular size having 40 mm diameter with smooth surface is obtained. This pellet is placed in a sample holder of the XRF instrument and used to determine the major and minor Oxides.

**Identification of clay fraction of the soil samples with the help of XRD technique:**

Clay fraction of the soil samples were identified with the help of XRD [Phillips X-ray spectrometer (PW 1710) using Cu anode] technique. The scanning range was from 8.0 to 59.9 0(2θ) in the continuous scan mode. Type of minerals was identified using standard technique (Jackson 1975, Imam 1994, Moore and Reynolds, Jr.1989).

**Extraction of Clay part from the soil samples:**

25 gm of soil sample is shaken with sodium acetate acetic acid buffer (pH= 5.0) solution for 30 minutes. It is then centrifuged and 50 ml of acetone was added. It is again centrifuged and the sample is transferred to a 500 ml beaker to which 5 ml of 30 % H2O2 solution is added. The beaker is then heated on a hot plate till the effervescence ceased. The process is repeated for three times.10 ml of more 30 % H2O2 solution is added and the sample is left overnight. Next day, the suspension was digested for 2 hours on a hot water bath, washed with sodium acetate solution (IN) and centrifuged. To the residue 40 ml of 0.3 M sodium citrate and 0.125 M sodium bicarbonate (prepared by dissolving 88 g of sodium citrate and 10.5 g of sodium bicarbonate in 1 liter distilled water) is added and is placed on a water bath at a temperature of 70- 80°C. To this mixture 1.0 gm of sodium dithionate (Na$_2$S$_2$O$_4$) is added with constant stirring. The suspension was centrifuged and washed with 95% methanol. To the residue 100 ml of distilled water is added and the suspension is transferred to a 1000 ml measuring cylinder and the volume is made 1000 ml with distilled water. The suspension is shaken vigorously and allowed to stand for about 6 hours. The top 10 cm of the suspension was collected at intervals and centrifuged to obtain the clay fraction.

**IR measurements**

The Infrared measurement is the complementary technique to XRD. The IR spectra are recorded with a Perkin-Elmer FTIR spectrometer (Model Spectrum RXI. range 4400-4500
Metal Partitioning

Metal speciation has become very important in environmental studies due to the close relation between toxicity and speciation (Smiles, 1983).

For this study, soil samples from 6 different sampling stations were collected from the coal mining area in dry season in the month of November. The locations of the sampling stations are shown in Table 3.4.

Samples were collected from the depth of 0-10 cm in polythene bags and were sealed until analysis. In laboratory, these samples were completely air dried, made homogeneous by using a mortar and sieved with 2 mm sieve. The final samples were used for analysis of various physico-chemical parameters and the total metal content. Some amount of soil was sieved with 149μm sieve and was used for the determination of major oxides of soil samples and speciation studies.

Table 3.4: Location of soil samples for fractionation study

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Location</th>
<th>Source</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Srimanta Nagar, Mergerita</td>
<td>Play ground</td>
<td>0 Km</td>
</tr>
<tr>
<td>S2</td>
<td>Shiva Temple, Tipong Colliery</td>
<td>Bank of the Rivulet</td>
<td>25 Km E</td>
</tr>
<tr>
<td>S3</td>
<td>Tipong Colliery's Entrance</td>
<td>Bank of the Rivulet</td>
<td>15 Km E</td>
</tr>
<tr>
<td>S4</td>
<td>Tikak Colliery Entrance</td>
<td>Bank of the Rivulet</td>
<td>6 Km E</td>
</tr>
<tr>
<td>S5</td>
<td>Borgolai area (Thekeratenga)</td>
<td>Bank of the River</td>
<td>2 Km S</td>
</tr>
<tr>
<td>S6</td>
<td>Dihing River</td>
<td>Bank of the River</td>
<td>3 Km W</td>
</tr>
</tbody>
</table>

Several methods are in use to determine the metal speciation of soil and sediment (Akcay et al, 2003., Chao, 1972; Clevenger, T.E, 1990., Das et al, 1995; Howard and Vanderbrink, 1999; Kersten and Forstner, 1991; Lopez-Sanchez et al, 1993; Sposito et al., 1982; Tessier et al, 1979., Ure et al, 1993., Welte et al., 1983). However limitations of the procedures have also been addressed (Jonanneau et al., 1983; Kheboian and Bauer., 1987; Rouret et al., 1989). For this study, Tessier et al, (1979) extraction procedure modified by
Akcay et al., (2003) is used. Here, five steps sequential leaching procedure is used to determine the trace metal species in various environmental conditions.

**Fraction 1 (F1)**

One gm of finely divided soil samples having size $\leq 149 \mu m$ was extracted with 20 ml of MgCl$_2$ (1.0 M) buffered at pH 7.0 with continuous shaking for 1 hr at 300 K. This gave the exchangeable fraction (F1).

**Fraction 2 (F2)**

The residue from (F1) was leached for 5 hrs at 300K with 20 ml of NaOAc (1 M) adjusted to pH 5.0 with acetic acid (HOAc). This gave the concentration of metal bound to carbonates (F2).

**Fraction 3 (F3)**

The residue from the second (F2) fraction was extracted with 20 ml of NH$_2$OH.HCl (0.04 M) dissolved in 25% of (v/v) HOAc at 369 ± 6K with occasional agitation and the time needed for complete dissolution of free iron-oxides. This fraction gave the concentration of trace metal species bound to Fe-Mn oxides (F3).

**Fraction 4 (F4)**

The residue after the third (F3) fraction was mixed with 3 ml of 0.02 M HNO$_3$ and 5 ml of 30% (v/v) H$_2$O$_2$, adjusted to pH 2.0 with HNO$_3$. The mixture is heated to 358 ± 4 K for 2 hr with intermittent agitation. Then again 3.0 ml of 30% (v/v) H$_2$O$_2$ adjusted to pH 2.0 with HNO$_3$ is added and the sample is heated again to 358 ± 4 K for 3 hrs with intermittent agitation. After cooling, 5 ml of NH$_4$OAc (3.2 M) in 20% (v/v) HNO$_3$ is added and the sample is diluted to 20 ml and agitated continuously for 30 min. This step gave the trace metal concentration bound to organic matter (F4).

**Fraction 5 (F5)**

The residue after the forth (F4) fraction was digested with an acid mixture of HNO$_3$ /HF /HClO$_4$ /HCl mixed in the ratio of 4:1:1:1 for 3 hrs in a water bath. On complete dissolution, the mixture was diluted to 100 ml with DDW. This step gave the residual metals, which are of detrial and Lattice Origin (F5).
The above extractions were carried out in 25 ml polypropylene centrifuge tubes for minimizing losses of solid materials. After the extraction of each step, the mixtures were centrifuged at 5000 rpm (Remi R 24 Research centrifuge) for an hour. The supernatant was removed with a pipette and was used to find the metal concentration. The residue was then washed with 8 ml of (maximum) double distilled water and was centrifuged again to obtain the solid phase for the next steps, by removing the supernatant liquid.

The estimation of all the trace metal was done by using AAS spectrophotometer (Varian SpectrAA 220) by applying different conditions (Table 3.2) for different metals. All the reagents used in this study were Merck analytical grade or extra pure quality and all the water used were double distilled water. Standard working solution of the elements analyzed was prepared from the corresponding 1000 mg/l E Merck solution supplied with the instrument AAS. All standard reagent solutions were stored in polythene bottle.