

Chapter II

Methods and materials

2.0 Introduction

Ledo colliery is one of the most active collieries among all other collieries namely Tirap, Tikak, Tipong, and Baragolai in Northeastern coalfield, Margherita, Assam (India). Ledo colliery is the first colliery of Assam which was started in the 1882 by Assam Railways and Trading Company (incorporated by British in 1881). The open cast mining in Ledo colliery has been adopted by Northeastern coalfield limited (NECL) by extracting coal in this region at a stripping rate of 1:14 (this is the ratio of the coal produced to waste generated). As a result of which hills of mine waste has been created. Due to the production of high sulphur coal, acid mine drainage from the Ledo colliery contaminates the mining and nearby area. Preliminary tests conducted by the Centre for Science and Environment (CSE) have reported that the heavy metals like Cd, Cr, Pb, Hg and As are present in the acidic water discharges from Ledo colliery and also in the stream water near the colliery. The acidic water which is called as the 'gas-pani' (gas water) by the local people ultimately flows towards nearby rivers and ravines. When this water comes into contact with the paddy fields, it completely degrades the soil fertility as observed during our field visit.

2.1 Study area

The different types of samples for study were collected from different locations in and around the Ledo colliery. Tirap colliery is in north direction of Ledo colliery at a distance of about 1.3 km. The mining process adopted in both collieries is open cast mining. To compare the environmental issues in both collieries through different physico-chemical parameters, the Tirap colliery has been taken under consideration in some aspects of our study. The approximate area of Ledo colliery along with nearby Tirap colliery is shown by Figure 2.1. Different samples points around the Ledo colliery are given in Table 2.1.

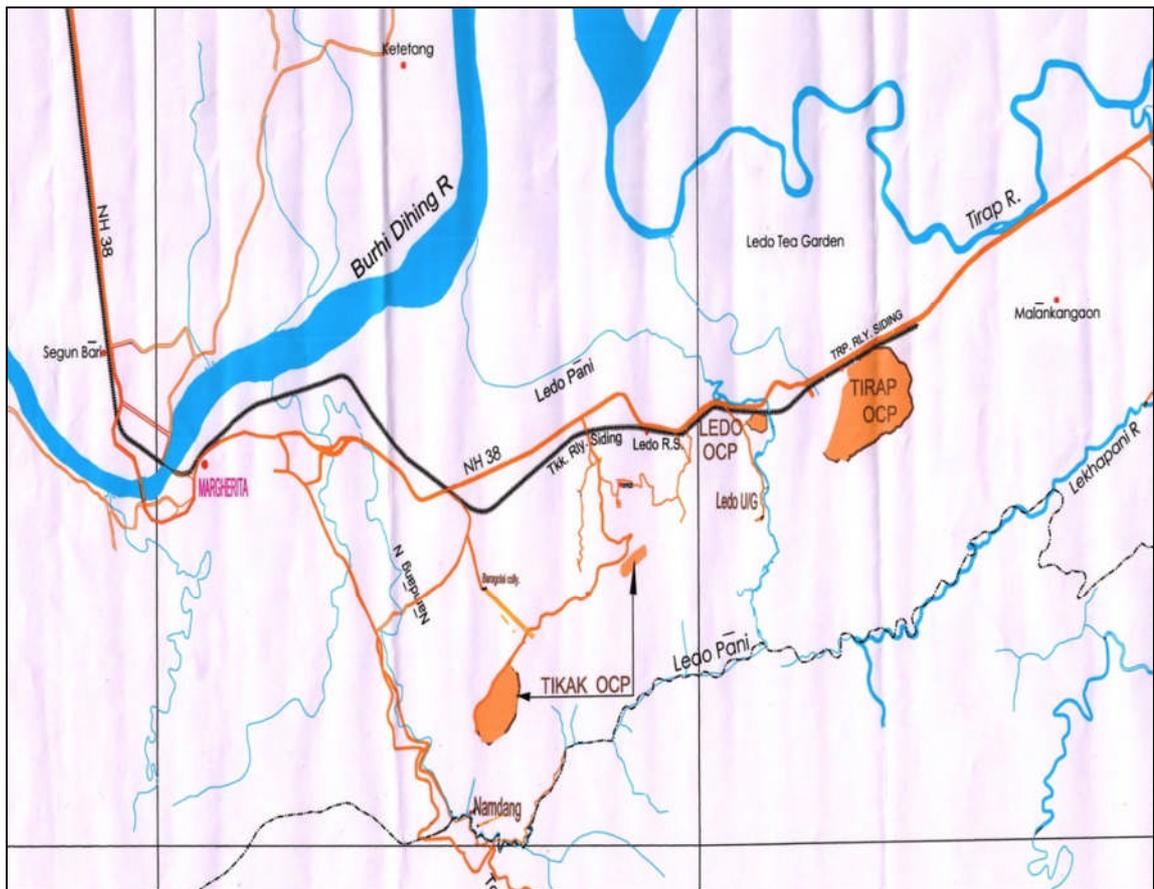


Figure 2.1. Showing the location of Ledo colliery under study

Table 2.1: Different sample points around Ledo colliery

Sample code	Sample type
LC-20A	Ledo coal, 20ft
LC-60A	Ledo coal, 60ft
TC-20A	Tirap coal 20ft
TC-60A	Tirap coal 60ft
LOB-15A	Overburden from Ledo OCP, 20ft
LOB-15B	Overburden from Ledo OCP, 60ft
TOB-15A	Overburden from Tirap OCP, 20ft
TOB-15B	Overburden from Ledo OCP, 60ft
LS-15A	Soil from Ledo OCP (from mine source)
LS-15B	Soil from paddy field near Ledo OCP
TS-15A	Soil from Tirap OCP
TS-15B	Soil from Tirap OCP (at a distance of 1km from mine source)
LSE-15A	Sediment from Ledo OCP (from mine source)
LSE-15B	Sediment from Ledo OCP at a distance of 0.5 km
LW-15A	AMD water from Ledo OCP (from mine source)
LW-15B	AMD water at a 0.5 km distance from Ledo OCP
LW-15C	Seepage Ledo OCP (water at a distance of 0.5 km from mine source)
LW-15D	Seepage Ledo OCP (water at a distance of 1 km from mine source)
LW-15E	Ledopani(at a distance of 2km from Ledo OCP)
LW-15F	Kachanalla(at a distance of 5km from Ledo OCP)
TW-15A	AMD water from Tirap OCP (from mine source)
TW-15B	AMD water at a 1 km distance from Tirap OCP
TW-15C	Seepage Tirap OCP (water at a distance of 0.5 km from mine source)
TW-15D	Seepage Tirap OCP (water at a distance of 1.5 km from mine source)

The representative coal and other environmental samples were collected within the 5 km radius from the Ledo colliery of Northeastern coalfield (27°15'N - 27°25'N and 95°40'E - 95°50'E). The standard methods e.g. ASTM (American Society for Testing and Materials, ASTM D8006-16, ASTM E1727-16), IS (Indian Standards, Indian Standards, IS: 436) BIS (The Bureau of Indian Standards, BIS IS 436-1-1: 1964), ISO (International Organization for Standardization, ISO 5667-3:2003(E), ISO 18512:2007(E)), APHA (The American Public Health Association, 2001) were used for the collection of the samples. The sample points were chosen in all the directions (north, south, west and east) of Ledo colliery to assess the impact of mining on surrounding environment. The collected samples were of different types including coal, AMD water, seepage water, stream water (from Ledo pani, Kachanalla, and Tirap River), sediments and soils from and around Ledo and Tirap collieries. Some of the representative sampling points are shown in the Figure 2.2.



Figure 2.2: Sampling sites of fresh mining [A], AMD water [B], Seepage water [C], and sediment with $\text{Fe}(\text{OH})_3$ stain[D] in Ledo colliery

The other samples were collected from OB dumps, water samples from seepage, nearby streams; soil samples from the mining source and nearby areas within 5 km radius and preserved for respective analyses (Figure 2.3).



Figure 2.3: Sampling sites of OB dump [E], Ledopani [F], Kachanallah [G], and seepage water in Ledo colliery in monsoon season [H]

2.2 Collection of different types of samples

2.2.1 Collection of coal, OB, soil, and sediments

The coal, OB, soil, and sediment samples were collected twice in a year during monsoon and non-monsoon seasons to examine the variation of physico-chemical

parameters of coal, OB, soil/sediments and mine water with different seasons. The coal and OB samples were collected from mine sites. Soil and sediment samples were collected randomly near the mining site and from mine affected areas within 5 km radius by using standard ASTM (ASTM E1727–16) method. During collection of the samples, the sample number, date of collection and a key description about the sample were written with marker pen (permanent ink) on each bag, and on a label attached to the tie on the bag. The collected samples were air dried; ground into 36 BS (0.420 mm) and/or 72 BS (0.211mm) mesh sizes and kept in air tight plastic containers for relevant analysis.

2.2.2 Collection of mine water/mine affected water samples

The mine water and mine affected water samples were collected twice a year in two seasons, monsoon and non-monsoon seasons by using standard ASTM (ASTM D8006-16) method. The area of sampling was so selected that it replicates the effect of the mining operations taking place in the area. Water samples were collected from the mine source and from the nearby areas in laboratory standard polyethylene bottles (capacity 10 liters). The water bodies from where the samples collected were around the mining area and connected to the mining operations directly or indirectly. The collected samples were preserved as per standard methods and used for further analytical methods.

2.3 Analysis of mine water/ mine affected water

2.3.1 Physico-chemical analyses of mine water / mine affected water

Water is one of the most important components to the environment and it sustains life system on the earth. Proper quality of water is described by its physical and chemical characteristics. Human beings as well as other life systems depend on water for their survival. Thus, assessment of quality of water is particularly important for proper identification of the associated hazards. The physico-chemical parameters like pH, electrical conductivity (EC), total dissolved solid (TDS) were analyzed by using a EUTECH PC700 pH/EC/TDS meter.

2.3.2 Chemical analyses of mine water / mine affected water

Different chemical analyses like inductively coupled plasma-optical emission spectrometry (ICP-OES), ion-chromatography (IC) were done for complete characterization of mine water and other water samples from nearby water resources.

2.3.2.1 ICP-OES analyses of mine water / mine affected water

ICP-OES analytical techniques for mine water /water samples were carried out by using an inductively coupled plasma optical spectrometer [SCIEX Perkin Elmer Elan DRC II (Canada)] for identification and determination of concentrations of various elements present in mine water in dissolved form. In this process 0.2 g from a reference material for digestion, was used. Wet digestion of samples was performed using 5 mL mixtures of acids: HNO₃:HClO₄ in a 3:1 ratio. Each sample was heated up to 180° C for 3.0 hrs (for this purpose the sample should be transparent) on the heating digestion block. After digestion, the sample was allowed to cool and filtered into a 25 mL volumetric flask by using Whatmann filter paper and the volume was further made up to a mark with de-ionised water. The digested samples were then put into the instrument for analysis. The calibration curves for elements were plotted from mixtures of high-purity element standard solutions (Merck, Germany) in a 4% (v/v) HNO₃ matrix.

2.3.2.2 Ion-chromatographic analyses of mine water/mine affected water

Ion-chromatographic analyses of collected water samples were carried out for determination of concentrations and presence of various cations including Na⁺, K⁺, Mg²⁺, Ca²⁺, NH₄⁺, Li⁺ ions and anions like F⁻, Cl⁻, Br⁻, SO₄²⁻, NO₃⁻ ions. Ion-Chromatographic analyses were done after immediate collection of the samples to avoid any dissociation. Before starting the analysis, the mine samples were filtered by using 0.22 mm Millipore filter paper. A part of the flittered sample was stabilized with pure HNO₃ (in 1:1 ratio) (for the analysis of cations) and kept in PET bottles at 4°C. For the detection of F⁻, Cl⁻, and Br⁻ ions no preservation was needed. The anions were analysed using a 4 mm 9 250 mm Metrosep A Supp 5 analytical column with the chemical suppression sulphuric acid and Metrosep RP2 3.5 guard column. Similarly, the cations were analysed using a 4 mm 9 150 mm Melrose C-4 analytical column and Melrose RP2 3.5 guard column. The pH of the samples was adjusted so that it should

not be less than 3.5-4. In case of pH less than 3, the pH of the samples were adjusted by diluting with different dilution factor (DF).

Calculation of dilution factor (DF):

$$DF = V_f / V_i$$

Where, DF is dilution factor

$$V_f = \text{Final volume, } V_i = \text{Initial volume}$$

The samples were then filtered by 0.22 μ m Millipore filter paper to remove any solid materials. Then the respective ions were analyzed by using Metrosep A Supp-5 250/4 analytical column and Metrosep RP2 Guard/ 3.5 guard column in Ion-Chromatography system (882 Compact Ion-Chromatography, manufactured by Metrohm AG).

2.4 Analytical characterizations of coal, OB, soil, and sediment

2.4.1 Chemical analyses of coal, OB, soil, and sediment

The proximate were carried out with a Proximate Analyzer (Model: TGA 701; Leco Corporation, USA) by following the ASTM method (ASTM D3172-13). The ultimate analysis of carbon, hydrogen, and nitrogen were determined by using an Elemental Analyzer (Model: Perkin-Elmer 2400). The amount of total sulphur present in coal was determined by 'Sulphur Analyzer' (Leco Corporation, USA) following ASTM methods (ASTM D5373-16; ASTM D4239-14e2).

2.4.2 Determination of total and forms of sulphur in coal

Sulphur is present in coal in three forms namely pyritic, sulphate and organic sulphur. After determining the weight percent of total sulphur, these forms were determined manually by using standard ASTM method [(ASTM D2492-02(2012)]. Sulphate and pyritic sulphur were determined by several stepwise reactions and organic sulphur was determined by subtracting the amounts of sulphate and pyritic sulphur from the total. As the amount of sulphur associated with overburden, soil and sediment was comparatively low, thus the forms of sulphur could not be calculated for these samples manually. The overall method for the determination of forms of sulphur is shown in the form of flowchart (Figure 2.4.1 and Figure 2.4.2).

2.4.2.1 Determination of sulphate sulphur

In this method, 5g of finely ground raw coal sample was taken and different steps were carried out according to the Figure 2.4.1. The last part of the process was a simple gravimetric estimation of sulphate from barium sulphate precipitate from which the weight percentage of sulphate sulphur was calculated by simple calculation.

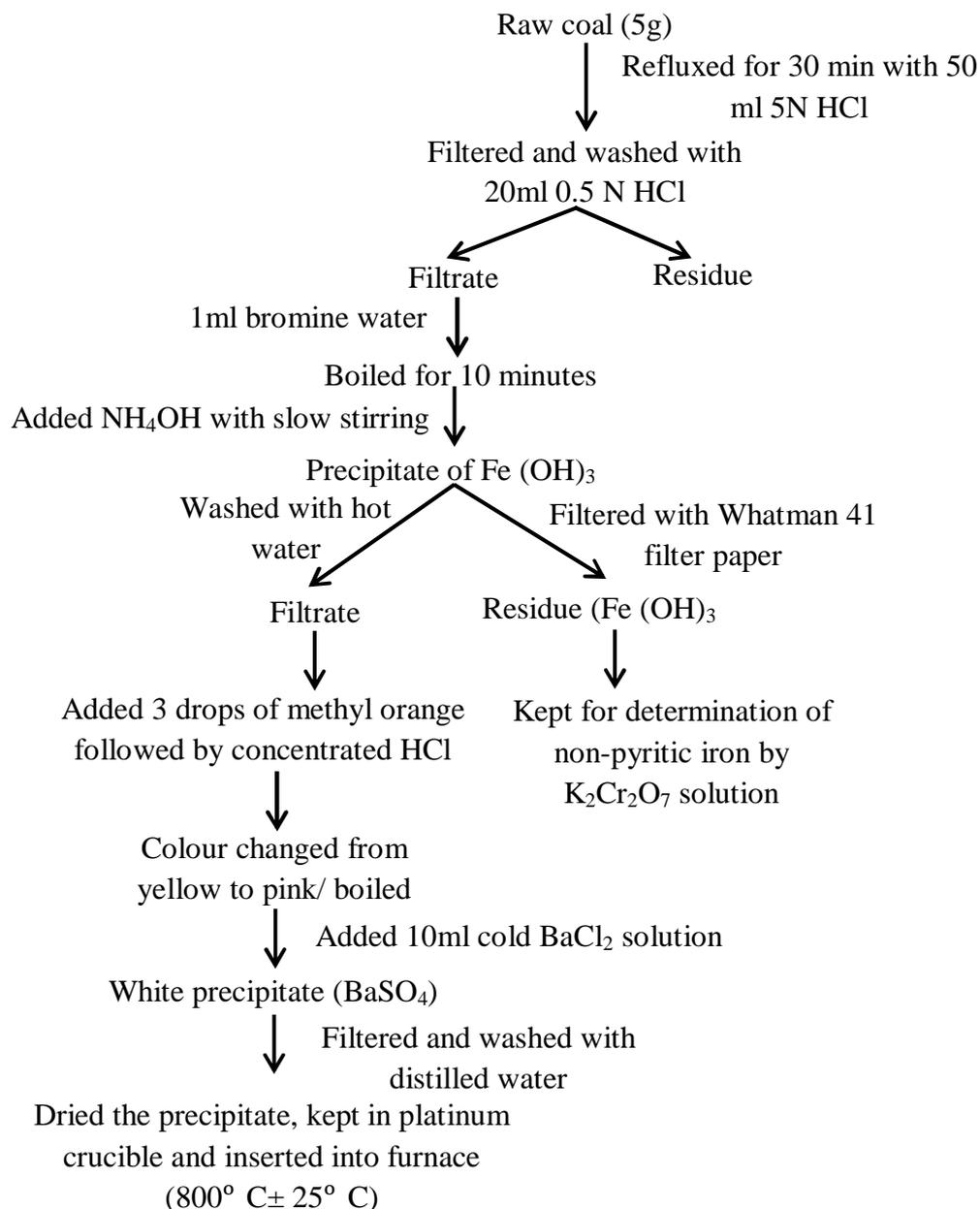


Figure 2.4.1: Flowchart for determination of sulphate sulphur [ASTM D2492-02(2012)]

Before insertion into furnace, the empty crucible was weighed. After complete burning of the filter paper along with the precipitate, the weight of the crucible was taken. The weight of the BaSO₄ was obtained by the difference. The percentage of sulphate sulphur was obtained by using following calculations:

Here,

Weight of coal taken = W₁

Weight of the empty crucible = X g

Weight of the crucible with precipitate = Y g

Weight of the BaSO₄ precipitate = Y-X g =W₂ g

Weight of BaSO₄ in blank test = W₃

The value of W₃ is taken as 0.005 g

Atomic mass of Sulphur = M_S (32)

Molecular mass of BaSO₄ = M_{BaSO4} (233.33)

$$\text{Percentage of sulphate sulphur} = \frac{W_2 - W_3}{W_1} \times \frac{M_S}{M_{BaSO4}} \times 100$$

$$\text{Or, Percentage of sulphate Sulphur} = \frac{W_2 - W_3}{W_1} \times 13.74$$

2.4.2.2 Determination of pyritic sulphur

The finely ground raw coal (1g) is refluxed with dilute nitric acid to bring the pyritic iron into solution. The amount of iron present in the coal was then determined by titration. The percentage of pyritic sulphur in the coal sample was calculated from this pyritic and non-pyritic iron concentrations, assuming the Fe, S stoichiometry of FeS₂ is 1:2 in pyrite. The last step of this determination is the volumetric estimation of iron against the standard K₂Cr₂O₇ solution. From the estimated iron, the percentage of pyritic sulphur was calculated by an indirect method. The different steps involved in the determination is shown in the form of flowchart (Figure 2.4.2)

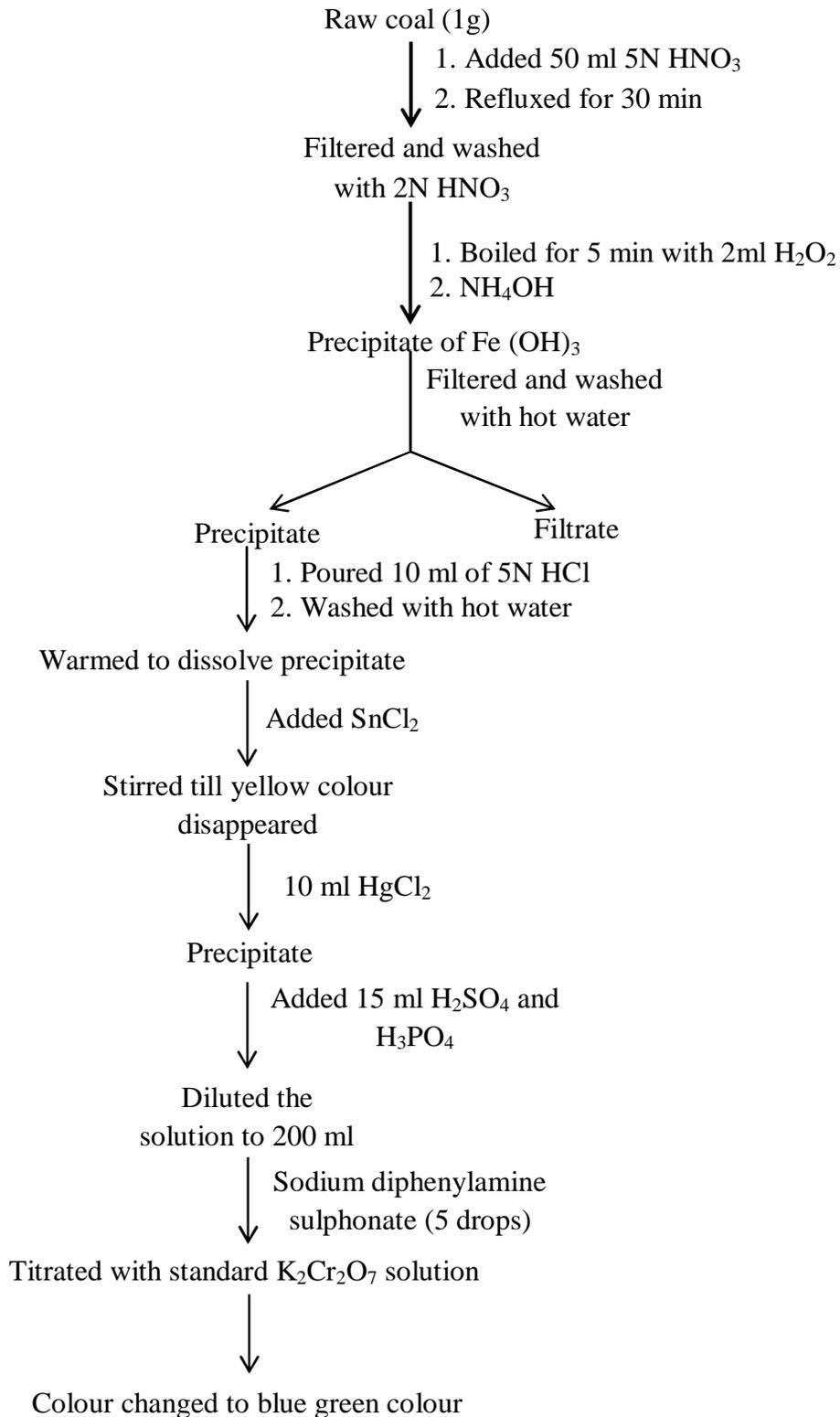


Figure 2.4.2: Flowchart for determination of pyritic sulphur [(ASTM D2492-02(2012))]

2.4.2.3 Calculation for pyritic sulphur:

Here,

V_1 = Volume of $K_2Cr_2O_7$ solution for pyritic iron

V_2 = Volume of $K_2Cr_2O_7$ solution for non- pyritic iron

W_1 = Weight of coal for taken for pyritic sulphur

W_2 = Weight of coal for taken for sulphate sulphur (non-pyritic iron)

Thus, percentage of pyritic sulphur:

$$\frac{V_1}{W_1} - \frac{V_2}{W_2} \times 2[S] \times \frac{6C}{1000}$$

$$\text{Or, } \frac{V_1}{W_1} - \frac{V_2}{W_2} \times 0.115$$

2.4.2.4 Determination of organic sulphur

Organic sulphur present in a coal was determined from sulphate and pyritic sulphur present in a coal by applying the following formula.

$$*S_{\text{organic}} = S_{\text{Total}} - (S_{\text{sulphate}} + S_{\text{pyritic}}).$$

*All types of sulphur are expressed in w %.

2.4.3 Proximate analyses of coal, OB, soil, and sediment

The proximate analyses of coal, soil, sediment and OB samples were carried out with a Proximate Analyzer (Model: TGA 701; Leco Corporation, USA) by following the ASTM method (ASTM D3172-13).

2.4.4 Determination of carbon, hydrogen and nitrogen in coal, OB, soil, and sediment

The presence of carbon (C), hydrogen (H) and nitrogen (N) in coal, OB, soil, and sediment was determined by using an Elemental Analyzer (Model: Perkin-Elmer 2400).

2.4.5 X-ray diffraction (XRD) analyses of coal, OB, soil, and sediment

The mineral identification and their composition in coal, OB, soil and sediment samples were identified by X-ray diffraction (XRD) technique. The analysis was carried out by X-ray diffractometer (Rigaku, ULTIMA IV) and the diffraction data of

the samples were obtained with starting angle set as 2.00° and the stopping angle at 75.00° with a step in angle 0.05°, with a scanning rate of 1° per minute and the target used was a Cu-K α ($\lambda=1.7902\text{\AA}$) target. The library database 'Rigaku PDXL 1.2.0.1' was used for identification of peaks and observed in diffractogram.

2.4.6 Fourier transform spectroscopic (FTIR) analysis

The Fourier transform infrared spectroscopy (FTIR) analyses of the respective samples were carried out by using Perkin-Elmer System 2000, model 640B apparatus with KBr pellet. The detector used was deuterated triglycine sulphate (DTGS). The number of total scans was 50 with a spectral resolution of 4 cm^{-1} during the period of recording of the spectra. By applying this analytical technique, types of organic groups including sulphur containing groups and their binding pattern in coal and other solid samples were detected.

2.4.7 Raman spectroscopic analyses of coal, OB, soil, and sediment

This analysis is used to identify the Raman character of the natural organic matter in carbon- containing compounds like coal and relate the Raman bands to the structural behaviour of the amorphous carbons. The basis of these studies was the assignment of the G (graphitic, $\sim 1580 \text{ cm}^{-1}$) band to crystalline graphite and the other band, called D band (disorder, various from 1100 to 1500 cm^{-1}) to identify the structural disorder in the graphitic structure.

2.4.8 Mössbauer spectroscopic analyses of coal, OB, soil, and sediment

The ^{57}Fe Mössbauer spectra of coal, overburden and soil/sediment samples were analyzed in order to identify different iron containing minerals with their relative abundance present in coal, OB, soil and sediment samples of mining and nearby areas. A Halder Mössbauer spectrometer capable of operating in conventional constant acceleration mode, using a proportional counter, filled to 2 atm. with Xe -gas and a ^{57}Co γ -ray source was used for the Mössbauer analyses. The spectrometer was calibrated using a Fe as the reference and a 50 mCi ^{57}Co source, plated into an Rh-foil, was used to produce the γ -rays. The samples were analysed at room temperature and data collected in a multi-channel analyzer (MCA) to obtain a spectrum of count rate against source velocity. A least-squares fitting program was used and by

superimposing Lorentzian line shapes, the isomer shift, quadrupole splitting and/or hyperfine magnetic field of each constituent was determined with reference to the centroid of the spectrum of a standard α -Fe foil at room temperature. The amount of each constituent present in a sample was determined from the areas under the relevant peaks.

2.4.9 Field emission scanning electron microscopy-energy dispersed spectroscopic (FESEM- EDS) analyses of coal, OB, soil, and sediment

The mineralogical identification and structural of the collected coal and coal mine reject solid samples were performed by Field Emission Scanning Electron Microscope (FE-SEM, Model: FEI QuantaTM650 FEG), integrated with an Oxford Inca 400 energy dispersive X-ray spectrometer.

2.4.10 High resolution transmission electron microscopy-energy dispersed spectroscopic (HRTEM - EDS) analyses of coal, and OB samples

The binding pattern of different minerals associated with coal, and overburden and their nano-mineralogical characterization were carried out with a high-resolution transmission electron microscopy with energy dispersive X-ray spectroscopy (HRTEM-EDS; Joel JEM-2100, resolution: 1.9 Å – 1.4 Å, accelerating voltage: 60-200 KV in 50V steps).

2.4.11 Atomic absorption spectroscopic (AAS) analyses of coal, OB, soil, and sediment

Atomic absorption spectrometry (AAS), flame atomic absorption spectrometry (FAAS), and graphite furnace atomic absorption spectrometry (GFAAS) analytical methods were used to detect different elements. The elemental analyses including Ca, Mg, Cd, Cr, Zn, Cu, Co, Hg, Fe, Mn, Ni present in coal, OB, soil and sediment samples of Ledo colliery were done with the help of an atomic absorption spectrophotometer (AAAnalyst-700, Perkin Elmer). In this technique, a specific amount (0.20-0.25 g) of sample was taken and 9ml of HNO₃ (65%) was added in case of coal sample and 7ml of HNO₃ (65%) was added in case of other samples (soil and sediment). After this, 2ml of HF (50%) was added to the mixture and digested in Teflon vials at a temperature range between 85° C to 100° C for 120 minutes. For Hg,

the digestion was carried out at low temperature as at high temperature Hg was lost due to volatilization (Mohammed et al., 2017). The digested samples were then filtered with Whatmann 42 filter paper and volume were made upto 25ml by adding distilled water and used for relevant elemental analyses.

2.4.12 Aqueous leaching of coal and overburden samples

The aqueous leaching process of coal and overburden samples of Ledo and Tirap collieries were carried out in an electronic leaching apparatus (VELP, Scintifica JLT6) (Figure 2.4.3) by mixing with distilled water followed by stirring the mixture for 1hr, 3hrs, 5hrs and 8hrs at a stirring rate of 250 rpm. This experimental process was done for comparative study of physico-chemical and elemental characteristics of collected fresh mine waters and aqueous leachates of coal and OB samples.



Figure 2.4.3: Leaching apparatus used for stirring of experimental samples

2.4.13 Study of cytotoxicity assays for acid mine drainage water

The cytotoxicity study for AMD water were performed to assess the impact of acid mine water on different cells including normal rat muscle, human carcinoma like pancreatic, lung and liver cells by studying the percentage of cell viability with concentrations of AMD and other mine affected water samples.

Some of the specific methods of our study are also incorporated in respective chapters.

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