



*Chapter 3*

**MATERIALS AND METHODS**

### **3.1 Description of study area**

The study was conducted around two oilfields, Borholla and Nambar of Assam-Arakan Basin located in Jorhat and Golaghat district, respectively of upper Assam categorized as category-I basin. This sedimentary Basin is a shelf–slope–basinal system, of which the shelf part extends over the Brahmaputra valley and the Dhansiri valley. Both the oilfields are operated and regulated by Oil and Natural Gas Corporation (ONGC) Limited. The study area and the sampling points are shown in fig. 3.1.

#### **3.1.1 Borholla oilfield**

Borholla oilfield is situated in Jorhat district of upper Assam geographically positioned around 26°41.335' N and 93°66.635' E, with an operational area of approximately 300 acres, which had been generating crude oil at an estimated capacity of two million tonnes per annum since 1972 [1]. In Borholla oilfield, oil reserves occur in structurally controlled subtle trap fractured granitic basement rock of Precambrian age. The production from this reservoir peaked in 1988-89 at the rate of 159TPD (water cut 3.34%) with 04 producers. Consequently rising trend of water cut in producing wells and relocation of wells to the other layers resulted in gradual decline of production [2].

#### **3.1.2 Nambar oilfield**

Nambar oilfield is situated in Golaghat district of upper Assam geographically positioned around 26°02.373' N and 93°49.234' E, productive from the year 1999, covering an area of 26 km<sup>2</sup>. Nambar oilfield is located at southeastern part of the Dhansiri Valley lie in a belt bordering the Naga thrust, where oil accumulations in the Bokabil Formation (Middle Miocene) occur in structural combination.

No new drilling activity is going on for past about 10 years in both of the oilfields oilfield.

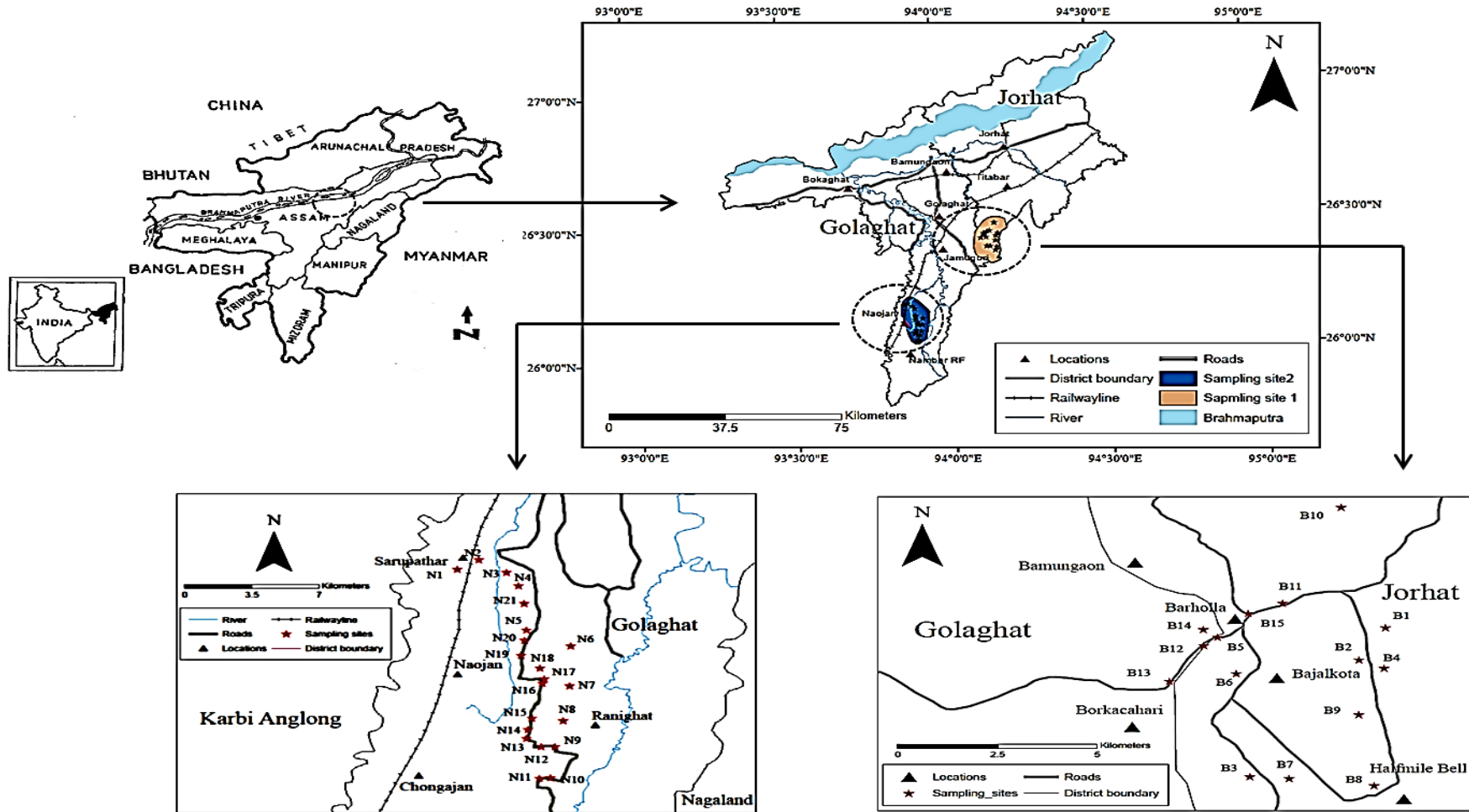


Fig. 3.1 The study area (map prepared by integrating different thematic layers in GIS environment). Sampling locations in Borholla (B1-B15) and in Nambar (N1-N21).

### **3.2 Geology of the study area**

The study area is located at a slight elevation from the present day floodplains and its major portion is occupied by young alluvial plain [3]. The basement of the study area is formed by the Pre-Cambrian metamorphic rock complexes. The Pre-Cambrian rocks comprise of quartzites and phyllites, which confined in a small area, but granitic gneisses are dispersed along the north and south bank of Brahmaputra basin [4]. The subsurface of these areas are mostly shale, sandstone, limestone and conglomerate.

The Assam-Arakan Basin is confined to the shelf zone, which is structurally less complex and tectonically less disturbed compared to the thrust zones to the southeast. The tertiary sediments in the shelf zone of the basin are considerably thick, ranging from 3.6 to more than 7 km, and include shallow marine Paleogene and continental Neogene sediments overlying a granitic basement.

### **3.3 Climate of the study area**

The study area experiences the predominant influence of the southwest “Tropical Monsoon Climate” [5]. The monsoon period lasts from June to September, have been reported to get highest rainfall. The annual average rainfall of the study area varies between 160 and 430 cm. The average temperature of the study area varies from 4° to 19°C during the winter and 26° to 37°C during the summer accompanied by high humidity. The pre-monsoon period lasts from March to May, which receives about 20-30% of total rainfall, maximum temperature remains above 35°C at this time. The monsoon season, from June to September receives 65-80% of total annual rainfall. The period after monsoon is dry and the post-monsoon period starts. Rainfall becomes low; about 3-6% of total annual average rainfall during this period.

### **3.4 Site Description**

Depending upon the land use pattern we have selected 15 sites around Borholla oilfield (B1-B15) and 21 sites around Nambar oilfield (N1-N15). Descriptions of the sampling sites along with their geographical co-ordinates are given in table 3.1(a) and 3.1(b).

**Table 3.1(a) Description of sampling sites around Borholla oilfield.**

Sl. No.	Site name	Latitude	Longitude	Altitude	Land use type
<b>Site B1</b>	Inside GGS	26°26.944' N	94°10.43'E	168 m	Group Gathering Station where crude oil are stored
<b>Site B2</b>	Gorajan	26°26.385' N	94°09.816'E	115 m	Residential area
<b>Site B3</b>	MK 3 Field	26°24.168'N	94°08.222'E	109 m	Oil drilling area*
<b>Site B4</b>	BR#6	26°26.341'N	94°09.996'E	105 m	Oil drilling area*
<b>Site B5</b>	Borholla Town	26°26.877' N	94°07.901'E	98 m	Commercial place with hotels & shops lined along the main road
<b>Site B6</b>	Negheri	26°26.648' N	94°07.165'E	91 m	Residential area
<b>Site B7</b>	Bojalkota Mini Primary School	26°24.099' N	94°08.807'E	89 m	Roadside area
<b>Site B8</b>	Bekajan-6	26°23.905' N	94°10.075'E	119 m	Residential area
<b>Site B9</b>	Balijan Miching Gaon II	26°25.281' N	94°09.962'E	106 m	Residential area
<b>Site B10</b>	Rajabahor Namghar	26°29.287' N	94°09.909'E	92 m	Borholla main road opposite to paddy fields
<b>Site B11</b>	Gorajan Tiniali	26°27.484' N	94°08.910'E	99 m	Junction of three roads
<b>Site B12</b>	Borholla Town	26°26.723' N	94°07.690'E	90 m	Borholla main market, commercial place with some residences
<b>Site B13</b>	Lonpuria	26°26.063' N	94°07.132'E	76 m	Residential area
<b>Site B14</b>	Nangal Gaon, Phukanchuk	26°27.035' N	94°07.699'E	76 m	Residential area
<b>Site B15</b>	A.T Road	26°27.317' N	94°08.393'E	87 m	On the main road & close to Borholla town. On the other side of the road, there are large tea gardens

\*No drilling activities are going now-a-days.

Table 3.1(b) Description of sampling sites around Nambar oilfield.

Sl. No.	Sites	Latitude	Longitude	Altitude	Land use type
Site N1	Sorupathar, near Railgate	26°11.238'N	93°51.339'E	104m	Located near a railway track
Site N2	Sorupathar main town	26°11.454'N	93°52.145'E	110m	This site is in town area and placed in the main road with a high traffic density
Site N3	Bimala prasad chaliha Primary School	26°11.135'N	93°53.049'E	114m	Road side area
Site N4	3 No. Sarupathar Primary School	26°10.426'N	93°53.276'E	116m	Road side area
Site N5	Sukia pathar 2no. Sarupathar	26°09.022'N	93°53.354'E	121m	Roadside area
Site N6	East Sarupathar gaon panchayat, uriamghat	26°08.221'N	93°54.554'E	124m	Roadside area
Site N7	Mazgaon	26°06.536'N	93°54.485'E	108m	Rural residential area
Site N8	1no. Pukhuripara, Dhupguri	26°05.351'N	93°54.311'E	115m	Roadside area
Site N9	1no. Salupathar Tiniali	26°04.373'N	93°54.130'E	116m	Junction of three roads
Site N10	Haldhibari	26°03.277'N	93°54.00'E	119m	Residential area
Site N11	Nambar GGS	26°03.277'N	93°53.401'E	123m	Group Gathering Station where crude oil are stored
Site N12	Chalukpathar, near namghar	26°04.384'N	93°53.473'E	111m	Residential area
Site N13	Sonal gaon	26°04.586'N	93°53.208'E	112m	Residential area
Site N14	KHAV Well, Drilling Point	26°05.182'N	93°53.240'E	112m	Oil drilling area*
Site N15	Village road, KHAV Well, Drilling Point	26°05.433'N	93°53.337'E	117m	Nearby drilling area
Site N16	Joypur	26°07.005'N	93°53.573'E	121m	Residential area
Site N17	Joypur gyanodoy L P School, Joypur Dhupguri	26°07.102'N	93°54.02'E	117m	Roadside area
Site N18	1 no.Joyapothar Madhaympur	26°07.351'N	93°53.569'E	113m	Market place
Site N19	Lukti pathar	26°08.056'N	93°53.225'E	117m	Rural residential area
Site N20	Lakshmipathar	26°08.401'N	93°53.307'E	108m	Roadside area
Site N21	2no. Gondhokori chariali	26°10.022'N	93°53.348'E	109m	Busy commercial place

\*No drilling activities are going now-a-days.

### **3.5 Sampling**

Soil and water samples were collected for three successive seasons viz. pre-monsoon (April), monsoon (July) and post-monsoon (October) for two years in 2011 and 2013 around Borholla oilfield and for one year (2013) around Nambar oilfield. Description of sampling locations along with their land use type and major environmental features were recorded at the time of sampling. The geographic co-ordinates of sampling locations were recorded by a GPS set (Garmin GPS Map 76CSX).

#### **3.5.1 Soil sampling**

Soil samples were taken from surface soil (0-5 cm) layer by using a stainless steel scoop and packed in aluminum foil. In laboratory, the soil samples were air dried in dark at room temperature. Composite samples were taken, which were thoroughly mixed by coning and quartering. Pebbles, twigs, plant debris, plant material were removed and crushed the soil by using mortar and pestle and sieved (<2 mm). Finally, the samples were stored at refrigerator (4<sup>0</sup>C) in laboratory till analysis.

#### **3.5.2 Water sampling**

Water samples were collected from both tube well and hand pump in amber colour glass bottle to prevent photochemical degradation. The glass bottles were pre-washed with acetone and dried in oven to avoid contamination. Samples were brought to laboratory within 24 hours of sampling and preserved at 4<sup>0</sup>C until further analysis.

### **3.6 Chemicals and standards for PAHs analysis**

PAHs calibration standard mixture of 16 USEPA PAHs was procured from Sigma Aldrich (Germany; Catalog No. 36979). The concentrations of individual PAHs in the standard mixture were different, which are given in Table 3.2. Internal standard mixture containing naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub> and chrysene-d<sub>12</sub> was procured from Supelco, Bellefonte, USA (Catalog No. 48902). Solvents used while preparation and analysis of PAHs viz. acetone, dichloromethane, n-hexane, cyclohexane, acetonitrile etc. were of HPLC grade. High purity deionized water available from the Milli-Q system was used for analysis.

### **3.7 Preparations of silica gel and sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) for column chromatography**

Silica gel (100-200 mesh) (Merck) was used for column chromatography. The silica was activated by heating at 130<sup>0</sup> for 16 hours and subsequently deactivated with 10% water prior loading into the column. Sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>; granular, anhydrous) was used for capping the silica gel column. To avoid contamination the Na<sub>2</sub>SO<sub>4</sub> was also purified by heating at 400<sup>0</sup>C for 4 hours in muffle furnace. After treatment both of them were kept in a desiccator to avoid moisture till use.

### **3.8 Cleaning of glassware**

The PAHs are abundant in the environment due to which, maximum care was taken to reduce its contamination. Glassware were thoroughly cleaned with detergent and chromic acid (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>+ conc. H<sub>2</sub>SO<sub>4</sub>) followed by washing with tap water and deionized water and at last rinsed with acetone. The washed glassware were kept in oven to dry. After drying and cooling, glassware were sealed with aluminum foil and stored in a clean environment to avoid accumulation of dust or other contaminants until use. Aluminum foil was also pretreated at 600<sup>0</sup>C prior to use to avert contamination.

## **3.9 Polycyclic Aromatic Hydrocarbon Determination**

### **3.9.1 PAHs extraction**

#### ***3.9.1.1 Extraction of PAHs from soil samples***

Extraction of PAHs from soil samples was done by ultrasonic agitation [6]. Prior to extraction all the samples were spiked with a 50 microliter of known concentration of internal standard (50 ppm). An aliquot of soil sample (approximately 10 g) was ultrasonicated in 50 ml of dichloromethane (DCM) for a period of 30 minutes at 20 kHz in a sonication bath (Bandelin Sonorex) for three times. The triplicate extracts were combined, allowed to settle for a few minutes, and then filtered. Following this, the filtrate was concentrated to approximately 1-2 ml, using a rotary evaporator (Hahnvapor, model: HS 2000-NS, Korea) and the solvent was exchanged to cyclohexane and kept ready for clean-up.



### **3.9.1.2 Extraction of PAHs from water samples**

Extraction of PAHs from water samples were performed by liquid-liquid extraction (LLE) method as described in the APHA [7]. A 50 microliter internal standard of known concentration (50 ppm) was added before the extraction procedure start. One liter of water sample was poured into a 2 liter separating funnel, to which 100 ml of n-hexane and DCM (1:1, v/v) was added and shake vigorously for 30 minutes. Then allow to separate the organic phase from water phase in the separating funnel and the water phase was drained into a one liter measuring cylinder. The organic phase was collected into an amber bottle. Every sample was considered for three repeated extractions and the triplicate extract were united and concentrated to about 2 ml in a rotary evaporator. The extract was exchanged to cyclohexane for next step i.e. clean up.

### **3.9.2 Clean up**

Both water and soil sample extracts were cleaned up by using the USEPA method 3630C [8]. The concentrated samples were purified and fractionated by using glass column (10 mm internal diameter) packed with slurry of activated silica gel (130<sup>0</sup> for 16 hours) of 100-200 mesh (merck) in DCM. Anhydrous Na<sub>2</sub>SO<sub>4</sub> was added on top of the column and pre-eluted with 40 ml of pentane with an elution rate of 2ml min<sup>-1</sup>, which was discarded later on. The concentrated sample extract was then loaded onto the column in 4ml of cyclohexane and eluted with 25ml of pentane and discarded. Finally, the column was eluted with 25 ml of DCM/pentane (2:3) (v/v) ratio and the eluent having desired compounds were concentrated to 0.5- 1.0 ml by rotary evaporator. The solvent was exchanged with acetonitrile and the volume was adjusted to 1 ml for HPLC analysis.

### **3.9.3 Analysis of PAHs**

Sample analysis for PAHs was carried out using a High Performance Liquid Chromatography (HPLC) system (Waters) equipped with a UV- detector (model 2489, Waters) at a wavelength of 254 nm [9]. The method of operation was gradient type with a combination of acetonitrile and deionized filtered water as the mobile phase (50% acetonitrile held for 5 min; linear gradient to 100% acetonitrile in 5–20 min; 100% acetonitrile held till 28 min and linear gradient to 50% acetonitrile from 28

to 32 min; flow rate: 1 ml/min) and a stationary phase made up of silica gel loaded in Waters PAH C<sub>18</sub> column (4.6mm×250mm, 5µm-particle size). Chromatogram of external standard containing 16 PAHs mixture along with their retention time is shown in fig. 3.2.

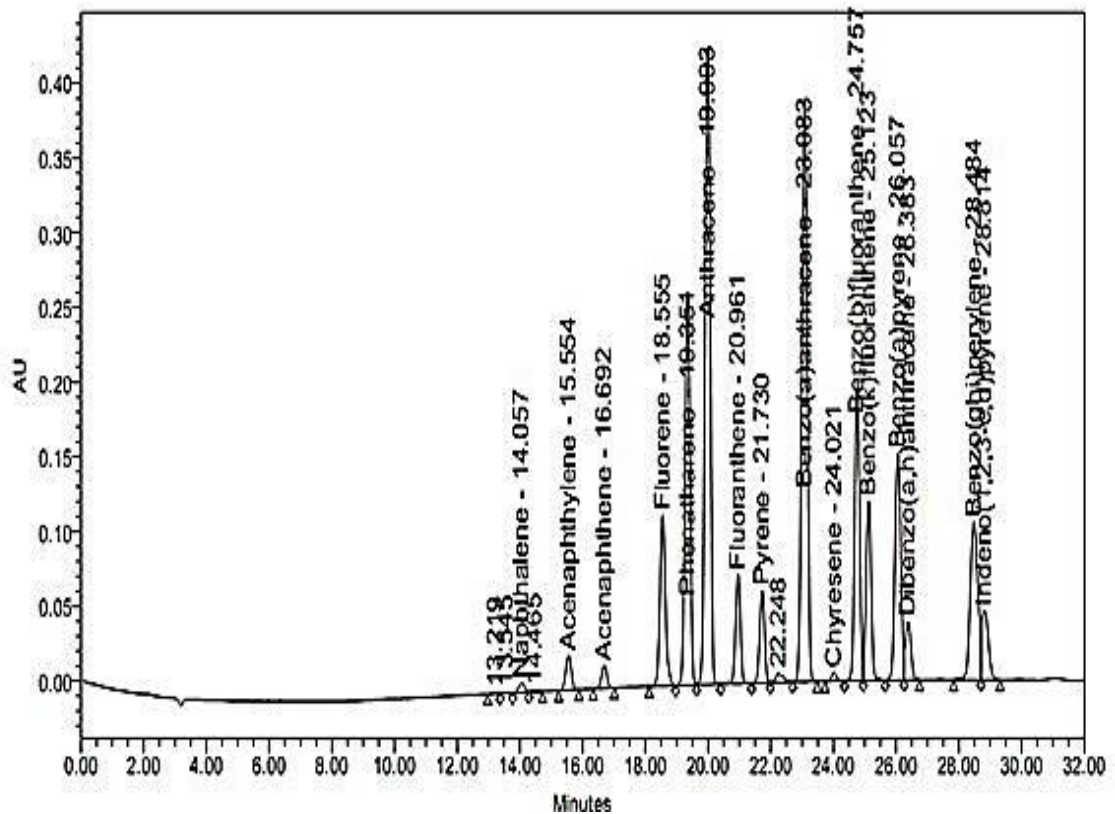


Fig. 3.2 HPLC-UV chromatogram of external standard containing 16 PAHs mixture along with their retention time.

### 3.9.4 Quantification of PAHs

The identification and quantification of 16 PAHs compounds were based upon both the external and internal standard calibration. Estimation of the compound concentrations using internal standard is considered to be more validate than that of external standard. Concentration of each compound was calculated using the peak area response of both sample and standard and Relative Response Factors (RRF). A

known amount of internal standards were added to each sample and calibration standards before analysis. The RRF was calculated as per the following formula:

$$RRF = \frac{As \times Cis}{Ais \times Cs}$$

Where,

As = Peak area (or height) of the analyte.

Ais = Peak area (or height) of the internal standard.

Cs = Concentration of the analyte.

Cis = Concentration of the internal standard.

The mean RRF value must be within  $\leq 20\%$  relative standard deviation (RSD) for each analyte, or averaged across all analytes [10], can be used for determination of the PAHs compounds concentration in the samples by using the formula given below.

***For aqueous samples:***

$$\text{Concentration} = \frac{(As)(Cis)(D)}{(Ais)(RRF)(Vs)}$$

Where,

As = Area (or height) of the peak for the analyte in the sample.

Ais = Area (or height) of the peak for the internal standard.

Cis = Concentration of the internal standard in the concentrated sample.

D = Dilution factor [10].

RRF = Mean response factor.

Vs = Volume of the aqueous sample extracted.

***For non-aqueous samples:***

$$\text{Concentration} = \frac{(As)(Cis)(D)}{(Ais)(RF)(Ws)}$$

Ws = Weight of sample extracted.

Retention time, Maximum % RSD and LOD value of 16 PAHs compounds are given in table 3.2.

**Table 3.2 Retention time, Maximum % RSD, LOD value and External Standard Concentration of 16 PAHs compounds.**

PAHs	Retention time	Maximum % RSD	LOD (ng ml <sup>-1</sup> )	External Standard Concentration (µg ml <sup>-1</sup> )
Nap	14.05	10.42	33.11	10.2
Any	15.55	3.55	8.25	9.2
Ane	16.69	17.45	5.98	9.7
Flu	18.55	6.90	8.01	8.3
Phe	19.35	9.09	5.56	9.9
Ant	19.99	4.16	3.58	10.7
Fla	20.96	4.37	23.25	9.5
Pyr	21.73	10.02	1.75	10
BaA	23.08	3.99	4.13	10.5
Chr	24.02	15.54	28.78	9.7
BbF	24.75	5.68	5.12	9.6
BkF	25.12	7.58	5.23	9.5
BaP	26.05	5.74	6.79	9.4
DaA	26.38	17.57	8.11	9.7
BgP	28.48	16.04	3.01	8.0
IcdP	28.81	16.44	4.51	8.9

### 3.10 Analysis of total organic carbon (TOC)

#### 3.10.1 Organic carbon determination in soil samples

**Reagents used:** Potassium dichromate solution, sulphuric acid, phosphoric acid, ferrous ammonium sulphate, diphenylamine indicator.

Total organic carbon (TOC) in surface soil sample was determined by Walkley and Black Method [11]. In this method combination of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) is used to digest the organic matter present in the soil and the remaining unutilized dichromate is titrated with ferrous ammonium sulphate. Soil sample of 0.5g was weighted in a 250 ml conical flask, to which 2.5 ml 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution and 5 ml conc. H<sub>2</sub>SO<sub>4</sub> were added and kept it to react for 30 minutes. As the reaction completed, the contents were diluted with 50 ml of distilled water and added

2.5 ml phosphoric acid followed by 1 ml of diphenylamine indicator. The sample mixture was titrated with 0.4 N ferrous ammonium sulphate  $[\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2]$  till the color changed to brilliant green. A blank was run with same quantity of the chemicals but without soil. About 60-90% of the total organic carbon is recovered by this method. The % organic carbon was calculated by using the following formula,

$$\text{a) \% C} = \frac{3.951}{g \times (1 - \frac{T}{S})}$$

$$\text{b) \% Organic carbon} = \frac{\% \text{ C}}{1.724}$$

Where, g= Weight of sample (g)

S= ml Ferrous solution with blank titration.

T= ml Ferrous solution with sample titration.

### 3.10.2 Organic carbon determination in water samples

The organic carbon of the water sample was estimated in TOC analyser (Elementer, Liqui TOC II, Germany).

### 3.11 Determination of black carbon (BC)

Black carbon present in soil samples was quantified by TOC analyzer (Analytik Jena, HT1300) [12] equipped with a Non-dispersive Infrared Detector by thermochemical oxidation method. Sodium carbonate graduated standard solutions were used for calibration. The determinations of BC need the following steps to perform; firstly, oxidation of the organic part of a measured sample was done at 600<sup>0</sup>C in a muffle furnace. Secondly, removed the inorganic part of the sample by washing with 6M HCl till the effervescence stopped and finally oven dried it at 60<sup>0</sup>C.

### 3.12 Determination of Heavy metals

#### 3.12.1 Heavy metal determination in surface soil

For estimation of heavy metals 0.5 g of soil sample was weighted and digested with 12 ml of aqua regia ( $\text{HNO}_3$ :  $\text{HCl}$ ; 3:1) and 4 ml of HF [13]. The 4ml HF was added to

the soil sample and kept overnight to react at room temperature. Following this, aqua regia was added to the sample and digested at 100°C for 8 hrs. After digestion was completed 2 g of boric acid was added to neutralize the HF. The sample was then filtered with syringe filter (pore size 0.45µ) and made up the volume up to 30 ml by adding milli-Q water. Teflon bomb was used for digestion of soil sample. All the samples were analysed in ICP-OES (Inductive coupled plasma- optical emission spectroscopy; Perkin Elmer Optima 2100DV). The quantitative analysis was executed with graduated calibration method by using 5 standards of 1.5 µg ml<sup>-1</sup>, 2.5 µg ml<sup>-1</sup>, 5 µg ml<sup>-1</sup>, 10 µg ml<sup>-1</sup> and 20 µg ml<sup>-1</sup>.

### 3.12.2 Heavy metal determination in groundwater

For the determination of heavy metals in the groundwater samples, the nitric acid digestion method [14] was adopted. The water samples were immediately acidified with HNO<sub>3</sub> to bring the pH below 2 to avoid the precipitation of the metals.

A schematic illustration of sample preparation and analysis for PAHs and heavy metals is given in fig. 3.3

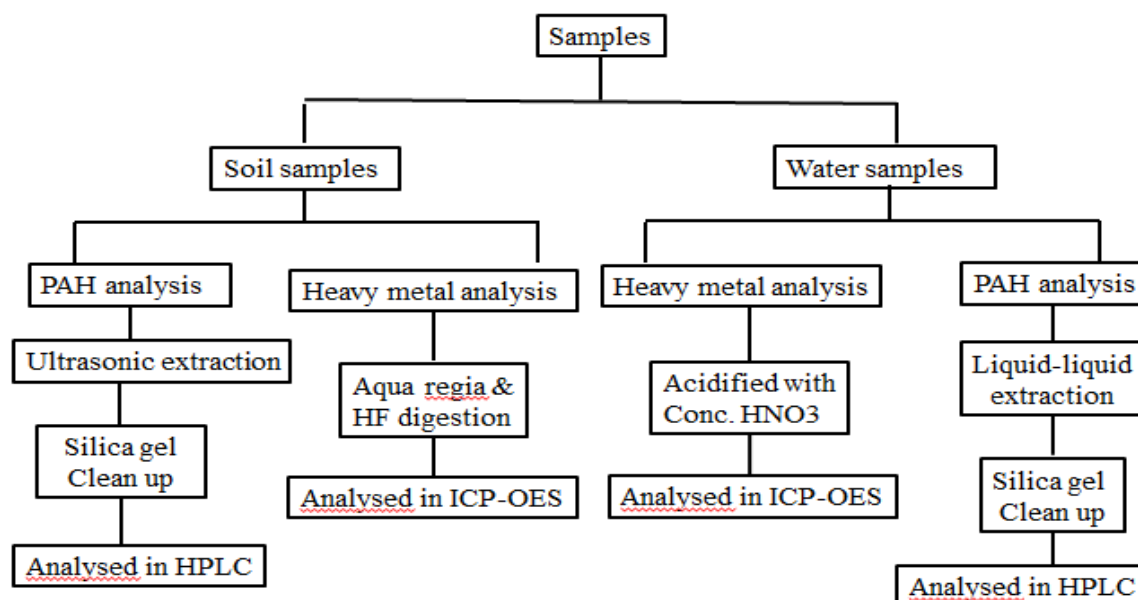


Fig. 3.3 A schematic illustration of sample preparation and analysis for PAHs and heavy metals.

### 3.13 Determination of major cations and anions

#### 3.13.1 Determination of major cations and anions in water samples

All the major cations and anions in water samples were analysed as per standard procedure given in APHA [7].

##### 3.13.1.1 Chloride ( $Cl^-$ )

**Reagents used:** Silver nitrate, potassium chromate.

Chloride was analysed by titrating a solution of 25ml water sample and 1ml of potassium chromate ( $K_2Cr_2O_7$ ) against silver nitrate ( $AgNO_3$ ) solution.  $AgNO_3$  reacts with  $Cl^-$  to form very slightly soluble white precipitate of silver chloride ( $AgCl$ ) at the end point when all the  $Cl^-$  get precipitated; free silver ions react with chromate to form silver chromate of reddish brown colour.  $Cl^-$  concentration was determined by the following formula

$$\text{Chloride, mg/lit} = (\text{ml} \times N) \text{ of } AgNO_3 \times 1000 \times 35.5 / \text{ml sample}$$

##### 3.13.1.2 Sulphate ( $SO_4^{2-}$ ) (Turbidimetric method)

**Reagents used:** Buffer solution,  $BaCl_2$  crystals and standard sulphate solution.

The  $SO_4^{2-}$  concentration of the water sample was estimated by turbidimetric method. About 25ml of water sample was measured in a conical flask to which 1.25 ml of conditioning reagent was added and stirred in a magnetic stirrer. A measured amount of barium chloride ( $BaCl_2$ ) crystals was added during stirring and light absorbance was determined by UV-Visible spectrophotometer (UV-1700 Pharmascope, Germany) at 420 nm.  $SO_4^{2-}$  is precipitated in an acidic medium with  $BaCl_2$  crystals and form barium sulphate ( $BaSO_4$ ). The  $SO_4^{2-}$  concentration was determined by comparison of the reading with a standard curve.

##### 3.13.1.3 Nitrate ( $NO_3^-$ ) (Phenol disulphonic acid method)

**Reagent used:** Phenol disulphonic acid, liquid ammonia 30% and standard nitrate solution.

The concentration of  $NO_3^-$  was determined calorimetrically by UV-visible spectrophotometer (UV-1700 Pharmascope, Germany) at 410 nm. In a 250 ml conical

flask 25 ml of water sample was taken to which an equivalent amount of silver sulphate was added to remove the  $\text{Cl}^-$  present in the sample. The precipitate of silver chloride ( $\text{AgCl}_2$ ) comes on a slight heating, which was filtered and the filtrate was evaporate to dryness. After cooling 1 ml of phenol disulphonic acid was added to dissolve the residue and dilute it to 25 ml following an addition of 3ml of liquid ammonia.  $\text{NO}_3^-$  reacts with phenol disulphonic acid to form a nitro derivative which in alkaline medium develops yellow colour. Finally measured the absorbance and calculated the  $\text{NO}_3^-$  concentration by comparison with standards of five different concentrations.

### **3.13.1.4 Sodium and Potassium ( $\text{Na}^+$ and $\text{K}^+$ ) (Flame photometric)**

Concentrations of  $\text{Na}^+$  and  $\text{K}^+$  in a filtered water sample were determined in a flame photometer (Systronics 128). The fluid under analysis is sprayed into a non-luminous flame as a fine mist. A coloured light is produced due to excitation of the electrons according to the characteristic emission of the element (Na: 589 nm; K: 768 nm). The intensity of the radiation is proportional to the concentration of  $\text{Na}^+$  and  $\text{K}^+$ .

### **3.13.2 Determination of major cations and anions in soil samples**

#### **3.13.2.1 Chloride ( $\text{Cl}^-$ )**

Most of the  $\text{Cl}^-$  are soluble in water and thus it can be measured directly in soil solution [15]. Soil  $\text{Cl}^-$  was determined by titrating soil solution against silver nitrate ( $\text{AgNO}_3$ ) using potassium chromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) as an indicator by the same procedure followed in case of water samples (Argentometric method) [16]. Soil solution were prepared in 1:5 ratio, by adding 25 ml of distilled water in 5g of soil, following shaking in a mechanical shaker (Relitech) for 30 minutes and then filtered the suspension.

#### **3.13.2.2 Sulphate ( $\text{SO}_4^-$ )**

Similar to the  $\text{Cl}^-$ ,  $\text{SO}_4^-$  are also soluble in water and can directly be measured in soil solution by turbidimetric method [17]. Here also soil suspension was made in distilled water in the ratio 1:5 and filter.



### 3.13.2.3 Nitrate ( $\text{NO}_3^-$ )

$\text{NO}_3^-$  from the soil was extracted by using copper sulphate solution ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) by using 12.5g of soil sample and 62.5 ml of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . To this soil extract 0.1g of calcium hydroxide [ $\text{Ca}(\text{OH})_2$ ] and 0.25 g of  $\text{MgCO}_3$  was added for clarifying and decolorizing and also to precipitate Cu and Ag [15]. Finally the extract was filtered and determined the  $\text{NO}_3^-$  content by using phenol disulphonic acid method as described for the water sample [18].

### 3.13.2.4 Sodium and Potassium ( $\text{Na}^+$ and $\text{K}^+$ )

For analysis of  $\text{Na}^+$  and  $\text{K}^+$  in soil, soil samples were digested by using tri-acid digestion method [13], filtered and make the volume upto 30ml and analysed in flame photometer (Systronics 128).

## 3.14 Back trajectory analysis

Air mass trajectories were built to trace the pathway of long range transport of particulates to the region. Back trajectories were computed from the data archive of Global Data Assimilation System model using National Oceanic Atmospheric Administration (NOAA) Air Resource Laboratory's (ARL) Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model available at [www.arl.noaa.gov/HYSPLIT.php](http://www.arl.noaa.gov/HYSPLIT.php). Temperature profiles are computed with the vertical temperature data, obtained from the archives of the Department of Atmospheric Science, University of Wyoming available at <http://weather.uwyo.edu/upperair/sounding.html> [19]. Vertical temperature data of the station nearest to our study area were used.

## 3.15 Quality control

### 3.15.1 Recovery efficiency of PAHs

Triplicate sample analyses were done to achieve right precision and accuracy of measurements. The percentage recovery was carried out by analyzing the samples spiked with 50 ppm of PAHs internal standard and the recovery ranges are given in

table 3.3. Procedural blank runs were performed intermittently to prevent contamination.

**Table 3.3 Recovery ranges of PAHs for water and soil samples.**

<b>Compounds</b>	<b>Recoveries for water samples</b>	<b>Recoveries for soil samples</b>
Naphthalene –d <sub>8</sub>	80.2±19.6	102±8
Acenaphthene-d <sub>10</sub>	73.3±6.64	60.8±39
Phenanthrene-d <sub>10</sub>	60.1±15.6	54.9±3
Chrysene-d <sub>12</sub>	74.7±16.0	95.1±13

### **3.15.2 Recovery efficiency of Heavy metals**

The % recovery of the samples were carried out by injecting a known concentration of standard reference material (SRM, NIST USA). For water sample (SRM 1640a) and for soil sample (SRM 8704) SRM was used to calculate the recovery of the heavy metal concentration in water and soil respectively. The recovery % of various metals in soil varies from 80-120% and in water samples it varied from 79.43-125.84%.

### **3.15.3 Limit of detection (LODs) of PAHs**

The limit of detection of the HPLC system was determined by analyzing standard of very low concentration (0.5ppm) for 5 times. The LOD value ranged from 1.75 to 33.11 ng ml<sup>-1</sup> for the 16 analyzed PAHs (Table 3.2) .The LOD was calculated as per the following

$$\text{LOD}=3.3*(\sigma/S)$$

Where,  $\sigma$  = The standard deviation of the response using analyte concentration.

S = The slope of the calibration curve.

### **3.15.4 Limit of detection (LODs) of Heavy metals**

LOD values for heavy metals were taken from Standard Operation Procedure, Soil and Plant Analysis Laboratory [20] which is given in table 3.4.

**Table 3.4 LOD values for heavy metals.**

Name	Abbreviation	LOD ( $\mu\text{g g}^{-1}$ )
Cadmium	Cd	0.004
Chromium	Cr	0.001
Copper	Cu	0.005
Iron	Fe	0.001
Manganese	Mn	0.0003
Nickel	Ni	0.004
Lead	Pb	0.02
Vanadium	V	0.004
Zinc	Zn	0.001
Calcium	Ca	0.01
Manganese	Mg	0.007
Sodium	Na	0.006
Potassium	K	0.03

### 3.16 Statistical Analysis

All the statistical analysis was done using SPSS 16 (Statistical Package for Social Sciences). For all statistical analysis the data below detection limit (BDL) was substituted by half of LOD value. Analysis of variance (ANOVA), correlation analysis and principle component analysis with multiple linear regressions (PCA-MLR) were the statistical techniques used in the study. PCA was carried out using varimax rotation with keiser normalization factors and PCs with eigen values >1 were extracted. MLR was performed stepwise using the PCA factor scores as independent variables, and z-scores of  $\Sigma 16$  PAHs as the dependent variables.

### 3.17 Preparation of Maps

The software Arc GIS 9.1 was used for preparing the study area maps.

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