CHAPTER 3

Materials and methods

3.1.   Study area

3.1.1. The Brahmaputra Flood Plains (BFP)

The Brahmaputra Flood Plains is formed by the river Brahmaputra and its numerous tributaries. The river Brahmaputra originates in the Tibetan part of China from the Kanglung Kang Glacier which is located near the Kailash range of the Himalayan Mountains. In India, the Brahmaputra valley is bound by Himalayas in the north, Patkai in the east and the lower (Assam) hills and Shilllong plateau in the south [1]. The BFP is said to be of tectonic origin, the valley portion was formed due to compression between the European and the Indian plate which also leads to the formation of the Himalayan Mountains [2-5]. The figure 3.1 shows the sequence of formation of the Brahmaputra valley through tectonic compaction which ultimately lead to the formation of the BFP after numerous episodes of sedimentation and deposition.

3.1.1.1.   Geomorphology and climatic conditions

The Indian part of the BFP covers an area of 195000 km^2, from the Indo-China border to the Indo-Bangladesh border, the Brahmaputra River flows for 916 km in India [1]. The river has a very high sediment discharge load and is ranked 2nd in the world in terms of sediment load [1]. The main types of sediments in the BFP are reported to be undifferentiated alluvium deposited over successive period of sedimentation (Fig. 3.2). The sediment characters were found to vary based on the origins and the characteristics of the northern and the southern tributaries [5]. The larger northern tributaries which are of Himalayan origin were found to have higher sediment discharge, mainly silt [5]. The beds and the banks of the southern tributaries were found to be formed of non alluvial sediments [5].
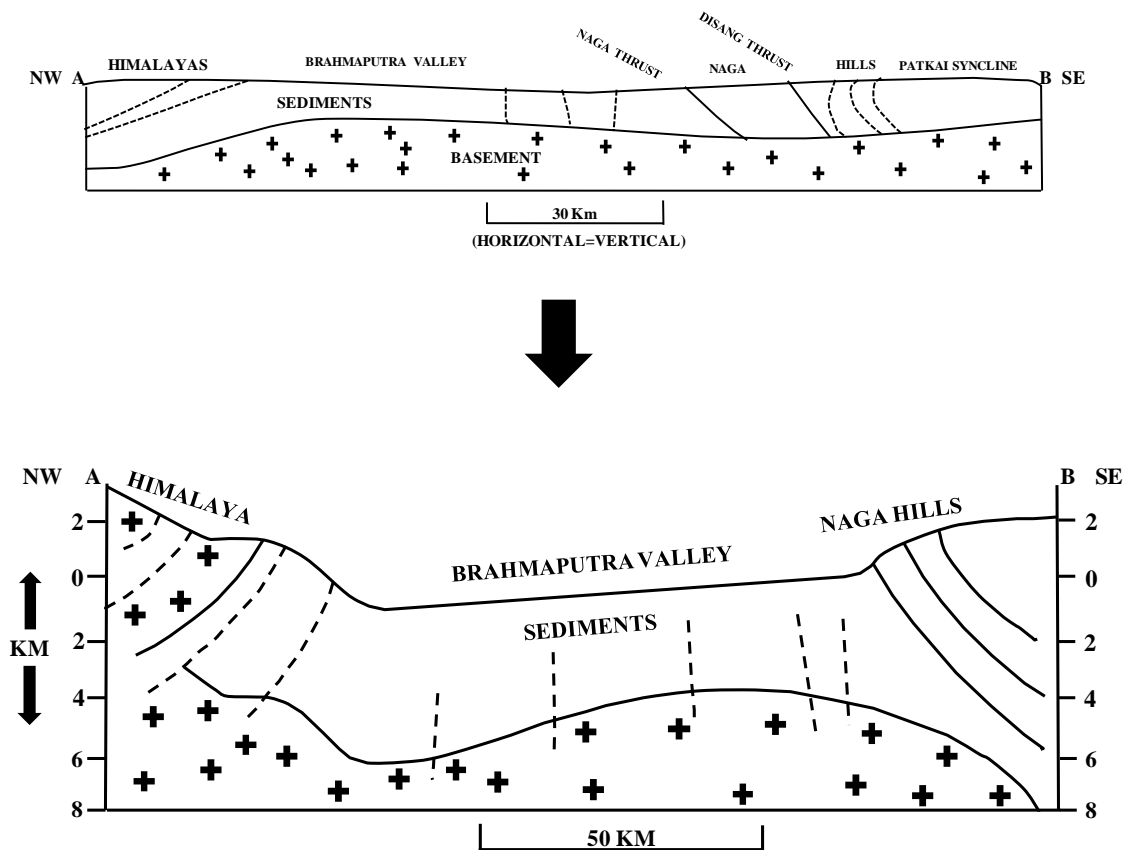
Figure 3.1: Formation of the Brahmaputra Valley through tectonic compaction the Himalayas in the north and the Naga Hills in the south

Two major geological formations have been found to give rise to the unique geology of the BFP: (i). The Himalayan Mountains in the north and (ii). The Shillong Plateau and Mikir Hills in the south [5]. The "Himalayan watershed" of the Brahmaputra has formed out of four topographic units of the Himalayan Mountains: the sub-Himalayas, the Middle Himalayas, the Greater Himalayas and the Trans Himalayas [5]. The sub-Himalayas have been found to form out of Tertiary sandstones and the Middle Himalayas mainly from "Palaeozoic deposits comprising of shales, slates and phyllites and overlain by basaltic rocks" [5, 6]. Granite and gneisses were found to be the dominant components of the Greater Himalayas, while the Tibetan Trans Himalayas are reported to be mainly "Palaeozoic to Eocene sedimentary formations" [5]. The Shillong Plateau and the Mikir Hills have been found to form mainly of gneisses and schists of the Precambrian age.
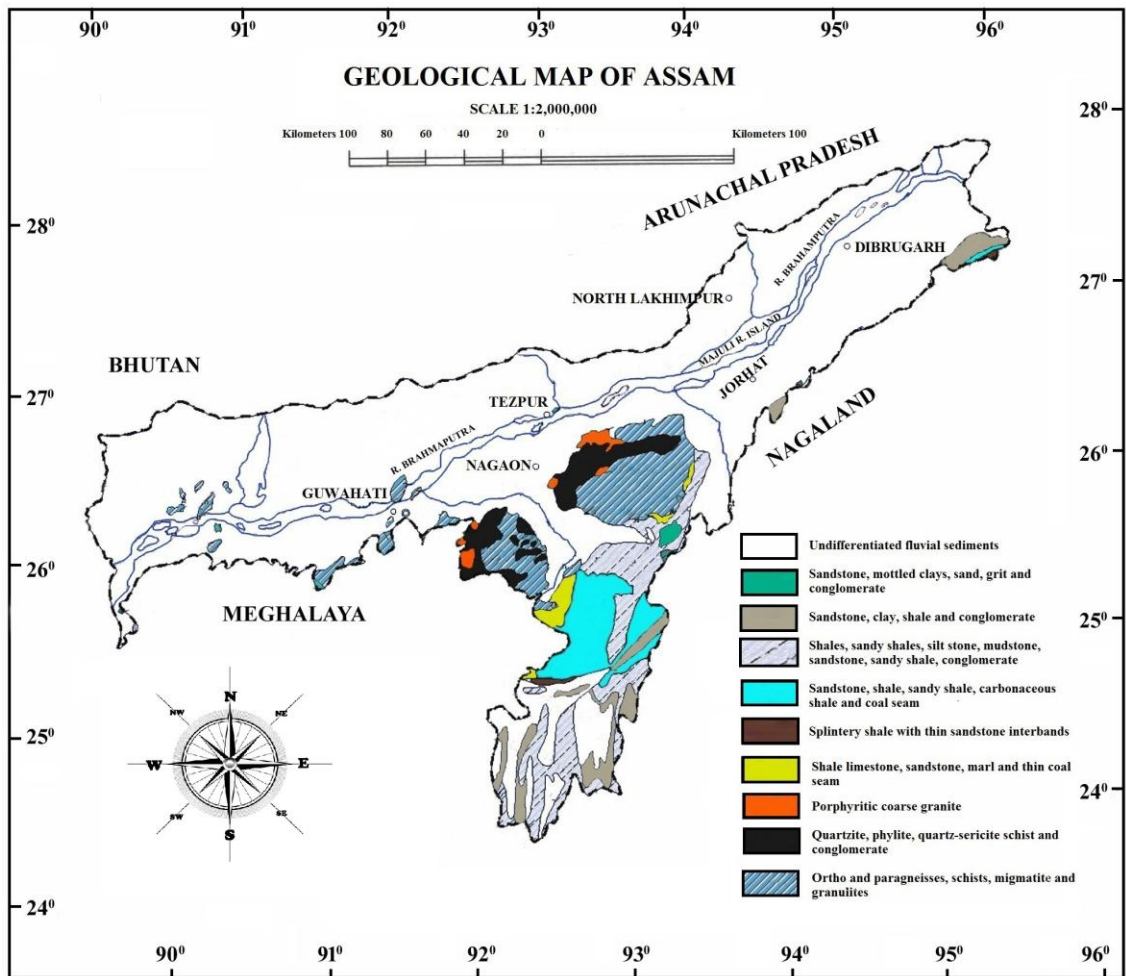
Figure 3.2: Geological base map of Assam modified from Geological Survey of India showing the different geological formations

Jain et al [1] has classified the climate of the region as "Tropical Monsoon Climate". The monsoon months from June to September have been reported to receive the highest rainfall. The climate in the BFP has been divided into four distinct seasons: the three months from March to May have been classified as the summer months; this season receives about 20 to 30% of the total rains and is also called the pre-monsoon season. Maximum temperature reportedly remains above 35°C for most of the summer. The monsoon season from June to September is reported to receive 65 to 80% of the total annual rainfall. However a lot of vagaries can be observed in the distribution of rainfall. Rain shadow zones like the Kapili basin in the Nagaon district of Assam, India were found to get much lower rainfall than the average annual figure. While areas like the

Jiadhal basin were found to receive very high amounts of rainfall, much above the average annual figure. The period immediately after the monsoon is dry and the months of October and November have been called the post-monsoon season. Rainfall is reportedly low; about 3 to 6% of the annual average value. The last season is the winter season, which was found to extend from December to February, the minimum average temperature in winter decreases towards the east. It is about 9°C in the western parts which decreases to about 4°C in the east [5].

3.1.1.2.   Hydrogeology

The river Brahmaputra and its Himalayan tributaries have been reported to flow through and weather a number of different rock types including "Precambrian metamorphics (high-grade schists, gneisses, quartzites, metamorphosed limestones), felsic intrusives, and Paleozoic–Mesozoic sandstones, shales and limestones" [7, 8]. In the sand fractions of the BFP, the "heavy minerals" (specific gravity > 2.5) was found to range from 0.1 to 6.1%. The heavy minerals in sand mostly consist of chlorite, biotite, amphibole and pyroxene [5]. In the clay fractions, clay mica was the dominant mineral component (53–61%), Kaolinite is reported to be another dominant clay mineral which constitute 36–40% of the clays [5]. Chlorite was found to be less abundant and constituted 8 to 10% of the clay fraction in the BFP [5]. The major soil groups in the BFP are reported to be entisol, inceptisol and alfisol. The highest fraction of soil have been reported to belong to inceptisols (45%) followed by entisols (36.6%) and alfisols (12.3%) [5].The soils of the BFP have been classified based on the mode of their formation into two types, (i). Residual and, (ii). Transported [5]. Residual soils were found to form *in situ* from the parent rocks of the Archaean age consisting mainly of gneisses, schists and granites [5], while the transported soils were reported to form by the weathering action on rocks of the Himalayas and the Assam plateau [5]. Spatial distribution of the different types of soils showed that alluvium had formed along the recent river deposits and occurred in the valley region. Older alluvium was found to occur in the piedmont or the uplands region [5]. Repeated sequence of clay, fine sand, coarse sand with cobbles, pebbles and boulders has been detected in borings up to 100 m below the ground level [5]. Drilling of exploratory wells by Mahanta et al. 2015 [9] in BFP (Bongaigaon and Darrang

districts) (Fig. 3.3) revealed the existence of thick clay layers (6-13.5 m) which could act as confining units for the sandy aquifers lying below. The sediment colours which varied from green to gray indicated their relatively young age as opposed to reddish or yellowish colour of older oxidizing sediments. The reddish and the yellowish colour of the older sediments is because of the presence of ferric oxides and hydroxides (FeOOH) (von Bromssen et al. 2007; Mahanta et al. 2015) [9, 10]. The greenish to greyish colours of sediments indicates towards the existence of a highly reducing condition in the BFP.
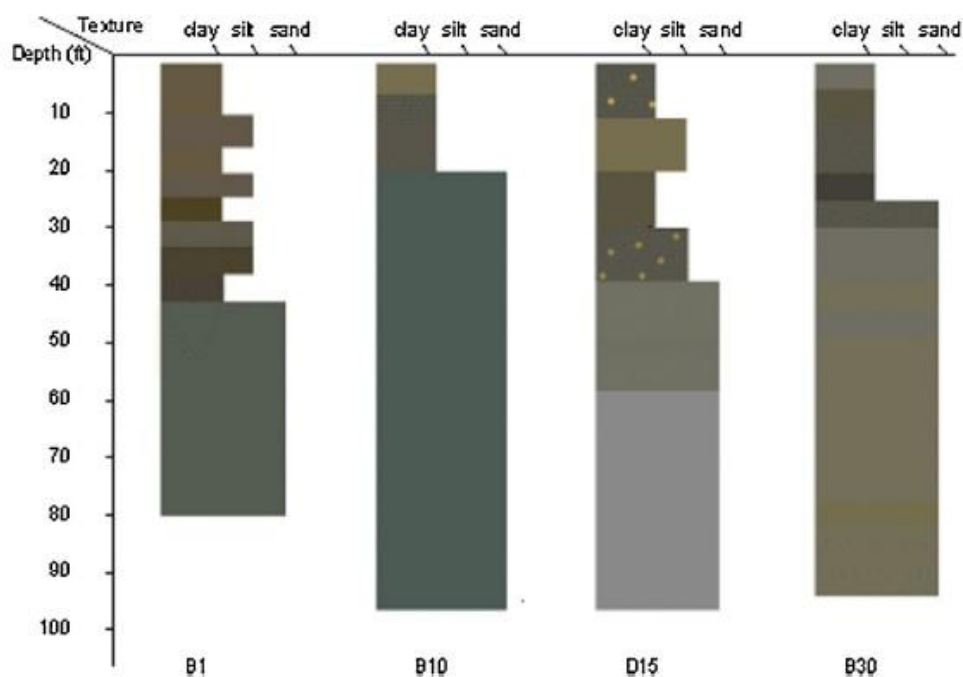

Figure 3.3: Lithologs from [9], shows the succession of different fractions (clay, silt and clay) in sediments from Bongaigaon (B1, B10 and B30) and Darrang (D15) districts.

3.1.2. Jorhat district

The Jorhat district is one of the 27 districts in the state of Assam. It lies on the south bank of the river Brahmaputra (Fig. 3.3) and it is bound by the Naga-Patkai range in the south, Golaghat district in the west and by Wokha and Mokokchung districts of Nagaland in the south-east [11]. The climate of Jorhat district has been classified as

mesothermal wet climate with forest type vegetation [11]. January is reported to be the coldest month in the district with average temperatures around 6ºC, while July and August are recorded to be the hottest months with temperatures exceeding 30ºC [11]. The relative humidity is about 78.8 % and the amount of rainfall increases from south-west to north-east. The average annual rainfall for the last ten years from 1998 to 2007 has been computed to be 1,867.08 mm [11]. Unconsolidated alluvial sediments from the Quaternary age has been found to underlain the entire district, which again has been classified into two types (i). Older and (ii). Younger Alluvium. The older alluvium has been found mainly in the upland areas, the sediments are oxidized and compact in nature here. Younger alluvium on the other hand has been found to occur in the low-lying areas close to the river banks or along their courses [11]. In the southernmost extremity of the district lying adjacent to the Naga Hills, a layer of superficial clay has been detected, and could have possibly originated from the nearby hills which are formed of rocks of Tertiary age [11].
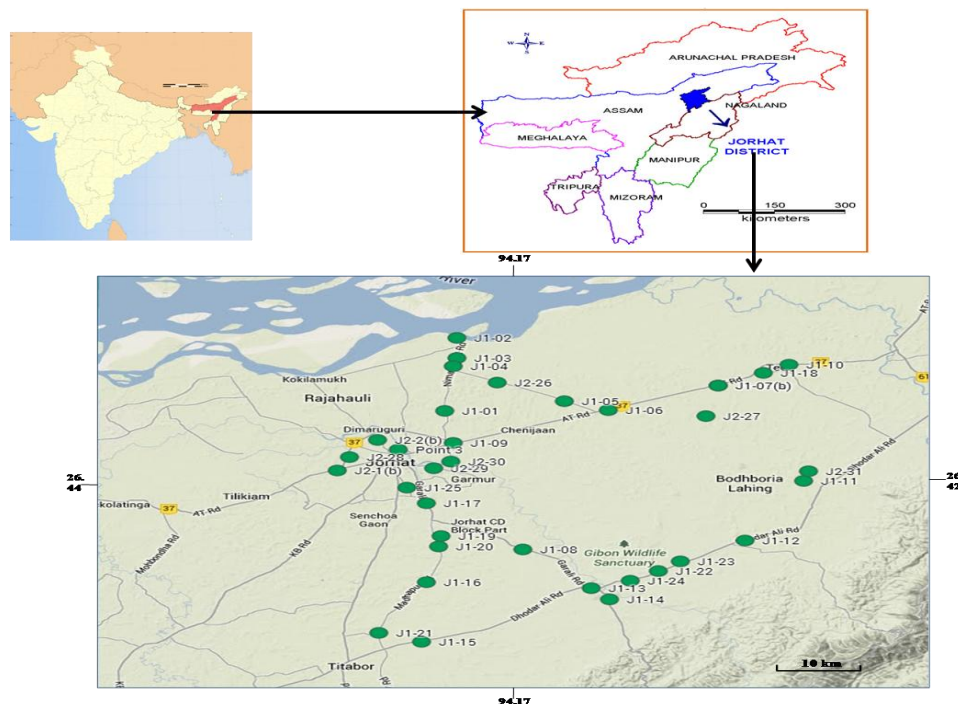

Figure 3.4: Map of the study area, shows Jorhat district as part of Assam and India. The points with the prefix J1 are monsoon samples while those with the prefix J2 depict post-monsoon samples

The district was found to be composed of five geomorphological units: the flood plain of the Brahmaputra River in the north, the central upland area covering younger alluvial formations, the central upland area covering older alluvial formations, the southern undulating hill area running along the Naga-Patkai range covering the piedmont plain and structural hills [11]. The district is drained by major tributaries of the Brahmaputra like the south Dhansiri, Bhogdoi and Kakodonga, all of which originate in the Naga-Patkai range and flow north towards the Brahmaputra River. The district has been found to have both newer alluvial deposits which are light grey to dark grey in colour and have been found to be confined to Brahmaputra River and its tributaries; and older alluvial soil which is sandy loam to silty, the older alluvium is yellowish brown to light brown in colour [11]. The climate and soil of the district have been found to be suitable for growing tea, and thus a number of tea plantations exist in this region. Available phosphorus and low potash has been found to characterise the soils of Jorhat district. The shallow aquifers in the region are mostly of semi-confined nature while deeper aquifers are reported to be either semi-confined or confined in nature [11]. In the central part, three to four prolific aquifer systems have been found to exist up to explored depth of 300 m. Close to the Brahmaputra River five to six aquifer systems with limited thickness have been found to exist up to depths of 400 m [11]. The thickness of the clay beds have been found to increase towards the south attaining maximum thickness in the Titabor region of the district

3.2. Analytical section

The methodology for the sampling and analytical part has been discussed in the following section under each chapter title pertaining to the objectives of the chapter. Standard procedures prescribed in [12] were followed except where separately mentioned. All the reagents used were of analytical reagent grade (A.R) or higher.

*Accuracy of the data was analysed by calculating the charge balance error or CBE which is formulated as:

$$\text{CBE} = \frac{(\text{Tz}^+ - \text{Tz}^-)}{(\text{Tz}^+ + \text{Tz}^-)} \times 100$$

Where Tz$^+$ is the total cationic strength calculated by adding all the cation concentrations in meqL^{-1} and Tz$^-$ is the total anionic strength calculated by adding all the anion concentrations in meqL^{-1}.

3.2.1. Groundwater
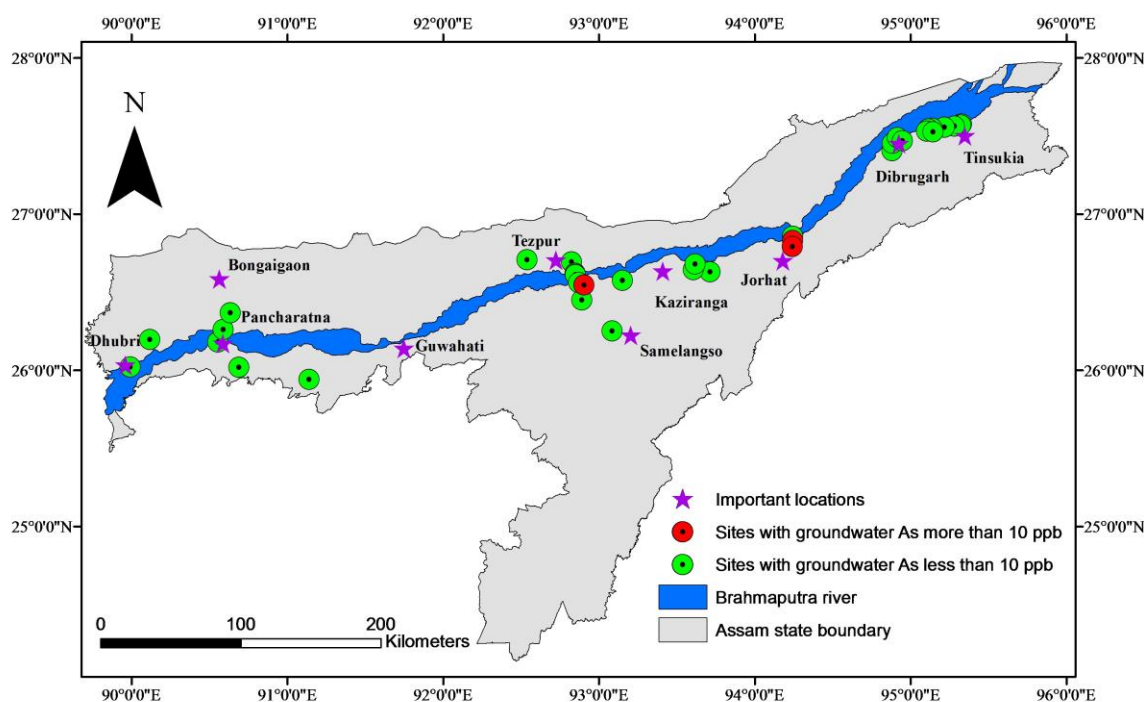
3.2.1.1. Sampling

Figure 3.5: Map of the BFP showing the groundwater samples and the sediment sampling sites

Groundwater samples were collected along the Brahmaputra River from Guijan (Tinsukia district), in the upstream to Dhubri, in the downstream (Fig. 3.5). The groundwater samples were collected strategically by taking into account the distance of the sampling points from the river Brahmaputra and the spatial extent covered. The coordinates of the sampling points were collected using a handheld GPS set (Garmin GPS map 76CSX). All the groundwater samples collected were from tube wells which were pumped for a minimum of 10 minutes to remove the residual water and ensure minimum interference of oxygen. The groundwater samples were collected in

polypropylene bottles (Tarsons) which were washed 2-3 times with the samples prior to storage. Samples for trace metal and cation analysis were filtered (by using 0.45 µ Millipore filters) and acidified with concentrated HNO_3 (Merck A.R grade) to bring down the pH to ≤ 2 for preservation. EC, TDS, pH and ORP were measured *in situ*, and the samples were stored at $4^{O}C$ till further analyses. The same protocols were also followed during the intensive sampling of groundwater in Jorhat district. The Fig. 3.4 shows the location of the groundwater samples in Jorhat district.

3.2.1.2.   *In situ* analysis

pH, EC, ORP and TDS were measured *in situ* using the HANNA HI9128 multi parameter water quality portable meter. The equipment has individual sensors or electrodes for recording the pH, EC, ORP and TDS. The specifications of the equipment have been provided in the table 3.1. The equipment was calibrated everyday prior to the start of sampling by using the HI9828-25 calibration solution provided with the instrument.

Table 3.1: Specification of the sensors in HANNA HI9128 multi parameter water quality portable meter

	HI769828-0	HI769828-1	HI769828-2	HI769828-3
Sensor Type	pH	pH/ORP	DO	EC
Measure Type	pH; mV (pH)	pH; mV (pH); mV	DO% sat; DO conc.	EC; TDS; resistivity; salinity
Measure Range	0.00 to 14.00 ±600.0 mV (pH)	0.00 to 14.00 ±600.0 mV (pH) ±2000.0 mV	0.0 to 500.0 % 0.00 to 50.00 mgL^{-1}	0.000-200.000 $mScm^{-1}$ 0-400000 mgL^{-1} 0 to 1.0000 $M\Omega cm^{-1}$ 0.00 to 70.00 PSU
Maintenance solution	HI 70300 (storage)	HI 70300 (storage)	HI 7042S (refilling)	
Dimensions	100 x 14 Ø mm	100 x 14 Ø mm	101 x 16.5 Ø mm	111 x 14 Ø mm

3.2.1.3.   Cation analysis

The following cations were analysed using standard procedures from [12] for this work.

Sodium: Sodium (Na^+) was analysed by flame photometry (Systronics Flame Photometer 128). The absorption was observed at a wavelength of 589 nm. The standards were prepared from NaCl (Merck A.R grade). Stock Na^+ solution was prepared by dissolving 2.542 g NaCl dried at 140^OC in 1000 mL deionised water to get strength of $1000 mgL^{-1}$. Intermediate Na^+ solution was prepared by diluting the stock solution to 100 mgL^{-1}. The calibration curve was made by preparing standards from 1 to 10 mgL^{-1}.

Potassium: Potassium (K^+) was also analysed by flame photometry (Systronics Flame Photometer 128). For K^+ analysis a wavelength of 767 nm was selected. Standards were prepared from KCl (Merck A.R grade). Stock solution was prepared by dissolving 1.907g KCl dried at 110 ^{O}C in 1000 mL deionised water to get strength of 1000 mgL^{-1}. Intermediate K^+ solution was prepared by diluting the stock solution to 100 mgL^{-1}. The calibration curve was made by preparing standards from 1 to 10 mgL^{-1}.

Calcium and Magnesium: Calcium (Ca) and Magnesium (Mg) were analysed using inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 2100 DV). The ICP standard used for Ca and Mg analysis was obtained from Perkin Elmer (Perkin Elmer Pure Atomic Spectroscopy Standard for Ca and Mg).

3.2.1.4.   Anion analysis:

The anions analysed for this study were HCO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, F^- and reactive Silica. The standard procedures prescribed in [12] were used for anion analysis.

Bicarbonate: The Bicarbonate (HCO_3^-) content was determined by Potentiometric Titration Method [12]. Calibration curve was prepared by using bicarbonate standards ranging from 10-100 mgL^{-1}, which were prepared from $NaHCO_3$ (Merck A.R grade). 50

ml sample were titrated against 0.02 N HCl (Merck A.R grade) and the end point was noted at pH 4.5. A standard graph was plotted between bicarbonate standards and volume of acid consume. The readings of sample were noted down from this graph.

Chloride: Argentometric method [12] was used for measuring Cl^- in the groundwater samples. In this method Cl^- in water samples is determined by titration with standard silver nitrate ($AgNO_3$), using potassium chromate as an indicator. 50 mL of well mixed sample was adjusted to a pH 7.0-8.0 and 1.0 mL $K_2Cr_2O_7$ (Merck A.R grade) was added. The mixture was titrated against standard $AgNO_3$ (Merck A.R grade) solution till $AgCrO_4$ (Merck A.R grade) started to precipitate as a pale red precipitate. For better accuracy deionised water (50 mL) was titrated first for establishing a reagent blank.

Calculation

$$\text{Chloride mgL}^{-1} = \frac{(A - B) \times N \times 35.45 \times 1000}{\text{mL of sample}}$$

Where,

A = mL $AgNO_3$ required for sample

B = mL $AgNO_3$ required for blank, and

N = Normality of $AgNO_3$ used

Sulphate: Sulphate was determined by turbidimetric method [12]. The principle of this method is that SO_4^{2-} is precipitated in an acetic acid medium with barium chloride ($BaCl_2$), forming barium sulphate crystals. Equal volumes of standard and sample (100 mL) were measured in a volumetric flask and 20 of buffer solution [prepared by dissolving 30 gm of $MgCl_2.6H_2O$, 5 gm of sodium acetate (Merck A.R grade), 1 gm potassium nitrate (Merck A.R grade) and 0.111 gm of sodium sulphate (Merck A.R grade) and 20 ml of acetic acid (Merck A.R grade) in 500 ml of deionised water and then the total volume was made up to 1000 ml] was added and mixed with the help of magnetic stirrer. A spoon full of $BaCl_2$ (Merck A.R grade) was added while stirring. The stirring was continued for 60 seconds at a constant speed. 5 minutes after the stirring, absorbance was observed at a wavelength of 420 nm using ultra violet visible spectrophotometer' (Shimadzu, UV-1700). Sulphate concentration was determined by

comparing reading with calibration curve. Calibration curve was prepared from sodium sulphate (Merck A.R grade) between 0 to 40 mgL^{-1} at the interval of 5 mgL^{-1}.

Phosphate: The stannous chloride ($SnCl_2$) [12] method was used for analysing the inorganic phosphorous or PO_4^{3-} in the groundwater samples. 50 mL of clear sample was taken and 2 mL of ammonium molybdate heptahydrate solution [Ammonium molybdate solution was prepared by mixing two solutions, A and B and finally diluting to 1 liter. Solution A was prepared by dissolving 25 g of ammonium molybdate heptahydrate (Merck A.R grade) in 175 mL of deionised water and solution B was made by adding 280 mL of concentrated H_2SO_4 to 400 mL of deionised water] was added followed by 5 mL of stannous chloride solution [The stannous chloride solution was prepared by dissolving 2.5 g stannous chloride (Merck A.R grade) in 100 mL glycerol (Merck A.R grade) while heating on a water bath for rapid dissolution]. A blue precipitate forms due to formation of molybdophosphoric acid. The absorbance of the samples was observed at 690 nm by UV-visible spectrophotometry. The phosphate standards were prepared from potassium hydrogen phosphate (K_2HPO_4, Merck A.R grade). Stock phosphate solution had strength of 50 gL^{-1}. It was diluted to obtain the working standard of strength 2.5 gL^{-1}. The standard curve was prepared from standards of strength 1 mL^{-1} to 5 mL^{-1} at 0.5 mL^{-1} interval.

Nitrate: Nitrate was analysed by using the UV screening method [12] at wavelengths of 220 and 275 nm. The standards were prepared from potassium nitrate (KNO_3 Merck A.R grade). Stock solution had strength of 100 mgL^{-1}; working standard was prepared by diluting the stock 10 times. Calibration curve was prepared from nitrate standards of concentrations 0 to 10 mgL^{-1} at intervals of 2 mgL^{-1}. The absorbance at 275 nm (corresponding to interference from dissolved organic matter) was subtracted twice from the absorbance at 220 nm to get the NO_3^- concentration in the groundwater samples.

Fluoride: Fluoride was analysed by using an ion selective electrode (Thermo Scientific Orion fluoride meter). This is an EPA-approved ISE test procedures for fluoride in drinking water [13, 14]. The F^- standards prepared had a range from 1 to 10 mgL^{-1} and

were made from the standard NaF solution provided with the electrode which had a stock strength of 100 mgL^{-1}. Total ionic strength adjustment buffer (TISAB) was used for maximum effectiveness; TISAB provides a constant background ionic strength by decomplexing F$^-$ ions and masks most other chemical interferences.

Reactive silica: The silica content was determined by the Molybdo Silicate Method [12]. Sodium silicate (Na_2SiO_3, Merck A.R grade) was used to prepare the silica standards ranging from 5 to 20 mgL^{-1}. In 50 mL of sample, 1.0 mL 1 + 1 HCl and 2.0 mL ammonium molybdate reagent [prepared by dissolving 10 g $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ in deionised water by stirring and gentle warming, and diluting to 100mL] were added in rapid succession. The mixture was allowed to stand for 10 minutes after proper mixing and finally 2 mL of oxalic acid was added. The absorbance was read at a wavelength of 650 nm in a UV spectrophotometer (Shimadzu, UV-1700).

3.2.1.5.   Trace metal analysis

The trace elements As and Fe were analysed in atomic absorption spectroscopy (AAS) (Thermoscientific ICE 3000) and ICP-OES respectively. The sodium arsenite ($NaAsO_2$) standard and sodium borohydride ($NaBH_4$) used for As analysis were obtained from Sigma Aldrich (Fluka Analytical). Hollow Cathode Lamps for iCE™ 3000 Series was used in the AAS. For Fe analysis Perkin Elmer Atomic Spectroscopy Standard for Fe was used.

3.2.1.6.   Dissolved Organic Carbon

Dissolved organic carbon (DOC) was measured by the combustion oxidation method [15] in NPOC (non-purgeable organic carbon) mode by using Elementer Liquid TOC II total organic carbon analyzer.

3.2.2. Sediment

3.2.2.1. Sampling

Grab soil and sediment samples were collected from the BFP (Fig. 3.5) for mineralogical analysis, and for sequential extraction of As phases and batch desorption experiment. The soil/sediment samples were collected strategically to cover the extent of the BFP. The samples were taken after scrapping away the first 30 cm of the top layer to remove anthropogenic interference. While collecting the samples a handmade bamboo tool was used; metal tools were not used to prevent contamination. The samples were initially dried at 50^OC and stored at 4^OC in a freezer till further analysis. The location of the soil/sediment sites were marked using a handheld GPS set (Garmin GPS map 76CSX).

3.2.2.2. pH and EC

pH and EC were measured in 1:5 soil-solution mixture using the HANNA HI9128 multi parameter water quality portable meter. The specifications and the calibration details have already been mentioned previously.

3.2.2.3. Total Organic Carbon

Total Organic Carbon, TOC was measured by the combustion oxidation method [15] in NPOC (non-purgeable organic carbon) mode by using a TOC analyzer [Multi C/N 2100 Analyzer (Analytik Jena AG, Germany).

3.2.2.4. Mineralogy and elemental analysis

The mineralogy of collected sediment samples was characterized by X-ray powered diffraction analysis (XRD) (Rigaku miniflex). All the samples were pulverized to powder for analyses. The samples were mount on the 'quartz plate' and the XRD instrument was run. The quartz plate itself has no diffraction peak. Therefore, the diffraction peak coming out is purely from the sample. The samples were routinely run

from 2.0 to 70.0 degree with a step size of 0.02º and applicable time for 4s. Mineral phases were identified by comparing diffraction pattern with the reference standard in the electronic database JCPDS-ICDD. The definition of the intensity peaks was carried out using standard in the electronic archive JCPDS (Joint Committee on Power Diffraction Standards). Detection of the different elements was done by using scanning electron microscope energy dispersive Xray spectroscopy (JEOL, JSM-6390LV, Japan). A constant voltage of 20 KV was applied and a lifetime of 50 seconds was used, the level of magnification used was 500X.

3.2.2.5. Grain size analysis

Grain size analysis was done using the sieving technique. Six sieve sizes were used for this purpose; the details of the sieves are tabulated below (3.2). The sieves were arranged from largest to the smallest size grading and 100 grams sample was poured into the largest sieve. The sieves were then shaken for 10 minutes. The weight of the fraction in each sieve was taken and the percentage share of each fraction was found out by the following formula.

$$\text{Percentage share} = \frac{(\text{weight of the fraction})}{100} \times 100$$

The size fractionation was done by comparing the fractions to the size chart given by [16] (Table 3.2).

Table 3.2: Size of the different grading of sieve sizes used for grain size analysis

Sieve grading	Sieve size (micron)
5	4000
10	2000
35	500
60	250
125	125
230	63

3.2.2.6. Cation exchange capacity

Cation exchange capacity (CEC) is actually the measure of the number of negatively charged sites on soil surfaces that can potentially bind cations like Ca^{2+}, Mg^{2+}, K^+ and Na^+. Clay and organic matter have high CEC density; acidification can have a negative effect on soil CEC. The soil CEC is expressed in meq/100 g of dry soil [17]. Cation exchange capacity was analysed by the method given by [18]. The equation below gives the calculation for CEC. The cations for CEC analysis were analysed by ion chromatography (Metrohm IC model 882 compact IC plus).

$$\text{CEC (Meq/100g)} = \frac{\text{mgL}^{-1}\text{Ca}}{200} + \frac{\text{mgL}^{-1}\text{Mg}}{120} + \frac{\text{mgL}^{-1}\text{K}}{390}$$

3.2.2.7. Sequential extraction of As:

Table 3.3: Outline of the SEP for As fractionation in the soil and sediment samples

Fraction	Extractants	Wash step	Target phase	Possible mechanisms
1	$(NH_4)_2SO_4$ (0.05 M); 4 h shaking, 20°C		Physisorbed As (outer sphere surface complexes), commonly referred to as labile or reactive As [20, 21]	Anion exchange of SO_4 for As
2	$(NH_4)H_2PO_4$ (0.05 M); 16 h shaking, 20°C		Chemisorbed As (inner sphere surface complexes; also labile)	Anion exchange of PO_4 for oxyanion As
3	NH4-oxalate buffer (0.2 M); pH 3.25; 4 h shaking in the dark, 20°C	NH_4-oxalate (0.2 M); pH 3.25; 10 min shaking in the dark	Non labile As associated with amorphous and poorly crystallized Fe (Mn, Al) oxide/oxyhydroxides	Ligand-promoted dissolution
4	NH_4-oxalate buffer (0.2 M); + ascorbic acid (0.1 M)c pH 3.25; 30 min in a water basin at 96 ± 3°C in the light	NH4-oxalate (0.2 M); pH 3.25; 10 min shaking in the dark	Non-labile As associated with well crystallized Fe (Mn, Al) oxide/oxyhydroxides	Reduction of Fe (III) and Al(III)
5	HNO_3/H_2O_2; Microwave digestion		Liberate As associated with residual minerals such as orpiment [21, 22]	Oxidation of sulphides and organic matter

Sequential extraction of As was adopted to identify and extract the "operationally defined" fractions of As in the samples. The method adopted for this experiment is the modified sequential extraction procedure (SEP) given by [19] (Table 3.3). The details of the above experiment have been elaborated in the section below:

Fraction I:
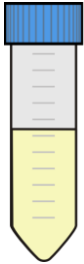
In a 50 ml centrifugation tube (Tarsons) 1 gram of dry soil/sediment sample was taken. 25 mL of 0.05 M $(NH_4)_2SO_4$ was added to the centrifuge tube and the mixture was shaken for 4 hours in a mechanical shaker. After this the mixture was centrifuged (Eltek Multispin TC650D) at 2000 rpm for 15 minutes. The supernatant was separated as extract I, it was acidified with HNO_3 (Merck A.R grade) and stored in a freezer at 4^OC till analysis. The residue that was left was used in the next step.

Fraction II

The residual soil from the 1st step was again poured in a 50 mL centrifugation tube. 25 mL $(NH_4)H_2PO_4$ (Merck A.R grade) of 0.05 M strength was added to the tube and the mixture was shaken for 16 hours at 20^OC. Afterwards the mixture was centrifuged at 2000 rpm for 15 minutes. The residue was used for the next step while the supernatant was marked as extract II and stored as mentioned in the earlier step till analysis.

Fraction III

The residue of the last step was poured in a 50 mL centrifugation tube. NH_4-oxalate buffer (0.2 M) was added to the residue and the pH was adjusted to 3.25 using oxalic acid (Merck A.R. grade), the soil solution ratio was 1:25. The mixture was shaken at 20^OC for 4 hours in the dark and washed with NH_4-oxalate (0.2 M, pH 3.25) in a soil solution ratio of 1:12.5 for 10 minutes. The mixture was centrifuged as in the previous steps and the residue was used in the next step, while the supernatant was marked as extract III and stored till analysis in a manner described in the previous steps.

Fraction IV

The residue of the last step was again poured in a centrifugation tube. NH_4-oxalate buffer (0.2 M) was added to the residue and ascorbic acid (0.1 M) was added to adjust the pH to 3.25. The soil solution ratio was 1:25. The mixture was shaken for 30 minutes in water bath at $96\pm3^\circ C$ in the light. Later NH_4-oxalate (0.2 M, pH 3.25) was used in the wash step with a soil solution ratio of 1:12.5 by shaking for 10 minutes.

Fraction V

The residue of the last step was finally digested in a metal digester (Pelican Kelplus Kelvac) by adding 50 mL solution of 16 N HNO_3 (Merck A.R grade) and 30 % H_2O_2 (Merck A.R grade).

The As content of the individual fractions was analysed by atomic absorption spectrometry (AAS). The accuracy of the procedure was observed by comparing the As content of raw samples and the total As content obtained by adding the As concentrations of each step. The As content of the raw sample were obtained by following the procedure of step 5 directly on the raw samples.

3.2.2.8.   Calculation of partition coefficient (Kd)

Partition coefficient has been described as the ratio of the quantity or amount of contaminant in the soil/sediment (C_T) to the quantity that has been leached out (C_L) [223], the formula being:

$$K_d = \frac{C_T}{C_L}$$

Two different partition coefficients were calculated in our studies, K_{d1} (where the sum of fractions I and II was taken as C_L) and K_{d2} (where the groundwater As level of the corresponding sites was considered C_L), the values were compared with each other and with the other variables by utilizing correlation coefficient to observe the trend.

3.2.2.9.   Batch desorption experiment:

The desorption technique used by [24] has been used in this experiment which is shown below.

Table 3.4: Outline of the batch desorption experiment for the soil and sediment samples

<table>
<tr>
<td colspan="4">Soil pre treatment:

Raw soils: 4 g of raw soil was pulverised and dried at 50°C for leaching

CBD treated soils: Fe (hydr)oxide free soil was prepared by reacting the raw soil (4 g) with 40 mL of 0.3 M Na-citrate + 5 mL 1 M Na-bicarbonate + 1 g Na-dithionite (CBD) in a water-bath at 80°C for 0.5 h [24]. The treatment was repeated three times to ensure complete removal of Fe hydr(oxide).</td>
</tr>
<tr>
<td colspan="4">Spiking: Both the raw and the CBD treated soils were spiked with a solution of known As and F$^-$ concentrations (Na$_2$HAs(V)O$_4$-7H$_2$O–NaF (Himedia chemicals) solution (As 1000 µgL$^-$1, F$^-$ 100 mgL$^-$1; 1:10)</td>
</tr>
<tr>
<td colspan="2">Experiment A (desorption at pH 5)</td>
<td colspan="2">Experiment B (desorption at pH 10)</td>
</tr>
<tr>
<td>Leaching was observed in raw samples for 4 hours</td>
<td>Leaching was observed in CBD treated samples for 4 hours</td>
<td>Leaching was observed in CBD treated samples for 4 hours</td>
<td>Leaching was observed in raw samples for 4 hours</td>
</tr>
<tr>
<td colspan="4" align="center">Arsenic and F$^-$ analysed in AAS and IC respectively</td>
</tr>
</table>

The CBD treatment step was repeated three times to ensure the complete removal of Fe, the extracts from each step were analysed for Fe and As in AAS, where it was revealed that after the first treatment step the level of Fe and As was beyond the detection limit of the instrument.

3.2.3. Computational analysis

3.2.3.1. Graphical interpretation of data

The software Aqqa (version 1.1.1) was used for plotting Piper [26], Durov [27] and Schoeller [28] diagrams, while Excel 2007 was used for preparing Gibbs plots, scatter plots and column diagrams.

3.2.3.2. Statistical analysis

Statistical analysis was performed by using the "statistical package for social sciences 20" (SPSS 20) software. Correlation analysis, principal components analysis (PCA) and hierarchical cluster analysis (HCA) were the statistical techniques used in our studies.

Correlation analysis: Pearson product-moment correlation is the measure of the linear correlation between two variables for example X and Y, the range of dependence or the correlation is between +1 and -1. This technique was developed by Karl Pearson based on an idea by Francis Galton. A correlation coefficient >0.7 is considered to be good, while that between 0.5 and 0.7 is considered to be moderate.

Multivariate statistical analysis: Principal components analysis (PCA) and hierarchical cluster analysis (HCA) are multivariate techniques. In PCA a large set of data can be reduced to unrelated principal components or PCs. The relationship between multiple parameters can be understood by studying the loadings of each of the parameters in the PCs, and the importance of the PCs can be assessed by the percentage variance of each PC; higher the variance more important is the PC [29]. For this study varimax rotation was used [30]. It is one of the orthogonal modes of rotation which assumes that the PCs are not related to each other, also PCs with Eigen values <1 were discarded during PCA. In HCA, relationships between the different parameters are observed based on the similarities and pairings in successive steps [31]. Smaller is the distance between two parameters, more is the similarity, while larger is the distance

between two parameters lesser is the similarity. For this study the Ward's method with squared Euclidian distance was used.

3.2.3.3. Speciation modelling

MINTEQA2 v 3.1 was used to calculate the saturation indices (SI) of selected groundwater species. The redox couple H_3AsO_3/AsO_4 was selected while calculating the SI values. The SI values can be used to assess the potential of different chemical phases to pollute the groundwater. For calculating SI values, the program has built in data bases (THERMO.DBS, TYPE6.DBS, REDOX.DBS, and GASES.DBS). H_3AsO_3/AsO_4 was selected as the redox couple while calculating the SI values for our samples.

3.2.3.4. Preparation of maps: The software ARC GIS 9.1 was used for preparing the maps.

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