CHAPTER 2

Samples and Methodology
2.1. Sample Collection and Treatment

Sampling locations for this study were selected based on variations in parent rock lithology and topography and absence of human perturbations. Sampling was done in areas not affected by human activities, except in the case of the Kundamkuzhy soil profile (APP17). The Kundamkuzhy profile, situated near a road with regular traffic was chosen for sampling to understand the effect of pollution on the magnetic signature of soils by comparing the values of magnetic parameters of these soils with the values of magnetic parameters of soils from other profiles developed in unpolluted regions where the soil forming factors are similar.

Modern soil samples (surface and sub-surface) were collected from locations that were apparently undisturbed or unaffected by anthropogenic activities. Only soil profiles and surface soils developed in situ over stable regions were sampled. Before sampling, the profile surface was gently scraped to expose a fresh surface. Plastic knives were used to avoid contamination from iron and rust. Samples were collected at an interval of 2 cm up to 20 cm depth, at 4 cm interval from 20 to 50 cm depth, at 8 cm interval from 50 to 85 cm depth and at sparser intervals at deeper levels. The soil profile samples and surface samples from Aribail, Miyapadavu and Uliyathadka were collected during May 2007 (pre-monsoon season). The Aribail and Miyapadavu profiles were sampled again at the same depths during September 2007 (post-monsoon season). Soil profile and surface samples from Panjikallu, Shanthinagar, Ramanchira and Thekkilparamba were collected during May 2008 (pre-monsoon season) and those from Devalokam, Narampady, Cherupanathady, Mundott, Karichery and Kundamkuzhy during May 2009 (pre-monsoon season). The samples were collected in pre-labeled polyethylene bags and transported to the laboratory where they were air-dried. The dried samples were gently disaggregated and sieved through an ASTM (American Standard for Testing Materials)
sieve (number 10) to obtain the < 2 mm fraction which was used for all subsequent laboratory measurements.

2.2. pH and Electrical Conductivity (EC)

For pH and EC measurements, 4 g of the < 2 mm size fraction of soil samples was mixed with Milli-Q (Millipore) deionised water in the ratio of 1:5 and stirred thoroughly. After the particles settled down, the supernatant was decanted and used for measurements. An ELICO PE-136 water quality analyzer was used for pH and EC measurements. Measurements were made for 45 samples of surface soils and 125 samples from 3 soil profiles collected during pre- and/or post-monsoon seasons.

2.3 Mineral Magnetic Measurements

Standard techniques were used for sample preparation in the lab (Walden, 1999a). The < 2 mm fraction (about 8-10 g) was filled in small polythene covers which were then tightly packed in 8-cm³ non-magnetic plastic bottles.

A range of magnetic parameters, described below, was determined on the samples (Dearing, 1999, Walden et al., 1998b; Thompson and Oldfield, 1986). These measurements are rapid, inexpensive, non-destructive and sensitive to low concentrations of magnetic minerals. Thirteen soil profiles (378 samples) and 148 surface soil samples were studied.

2.3.1 Magnetic Susceptibility ($\chi_{lf}$)

Magnetic susceptibility was determined at low (0.47 kHz; $\chi_{lf}$) and high (4.7 kHz; $\chi_{hf}$) frequencies using a Bartington Susceptibility Meter (Model MS2) with a dual-frequency sensor (Model MS2B). Measurements were made in range 1 of the instrument as the soils are magnetically strong. The Fe$_3$O$_4$ (1%) standard was used to calibrate the sensor. Two “air” measurements each were made before and after the sample
measurement. The air measurements were subtracted from the sample measurements to take care of the drift of the sensor during the course of measurements.

Frequency-dependent susceptibility was calculated from the difference in low- and high-frequency susceptibility values (Dearing, 1999). Mass-specific frequency-dependent susceptibility ($\chi_{fd}$) is given by

$$\chi_{fd} = \chi_{lf} - \chi_{hf}.$$ 

Percentage frequency-dependent susceptibility ($\chi_{fd\%}$) is given by

$$\chi_{fd\%} = \left[\frac{\chi_{lf} - \chi_{hf}}{\chi_{lf}}\right] \times 100$$

2.3.2 AnhystereticRemanent Magnetization (ARM)

Anhysteretic remanent magnetisation (ARM) was induced in the samples using a Molspin AF demagnetiser (with an ARM attachment) set with a peak alternating field (AF) of 100 mT and a DC biasing field of 0.04 mT (comparable to the intensity of the geomagnetic field). The ARM induced was measured on a Molspin spinner fluxgate magnetometer and calculated using the equation,

$$\frac{\text{Total combined vector } (10^{-3} \text{ Am}^{-1}) \times 1.29 \left(10^{-5} \text{ Am}^2 \text{ kg}^{-1}\right)}{\text{Sample weight (g)}}$$

The parameter value was converted into susceptibility of ARM ($\chi_{ARM}$) by dividing it by the size of the biasing field ($0.04 \text{ mT} = 31.84 \text{ Am}^{-1}$; Walden, 1999b).

2.3.3 Isothermal Remanent Magnetisation (IRM) and Saturation Isothermal Remanent Magnetisation (SIRM)

Isothermal remanent magnetisation (IRM) was induced in the samples at different field strengths (20, 60, 100, 300, 500, 600 and 1000 mT) using a Molspin pulse magnetiser. The isothermal remanence induced at 1T field (the maximum field attainable
in the Environmental Magnetism Laboratory at Mangalore University) was considered as Saturation isothermal remanent magnetisation (SIRM). The remanence acquired was measured using the Molspin spinner fluxgate magnetometer.

2.3.4 Inter-parametric Ratios

Inter-parametric ratios like S-ratio (the ratio between $\text{IRM}_{300\text{mT}}$ and SIRM), $\chi_{\text{ARM}}/\chi_{\text{lf}}$, $\chi_{\text{ARM}}/\text{SIRM}$ and $\text{SIRM}/\chi_{\text{lf}}$ were calculated to determine the magnetic mineralogy and grain size (Walden, 1999b). The S-ratio used in this study is the forward field S-ratio (Kruvier and Passier, 2001; Heslop, 2009) and not the classical back field S-ratio proposed by Stober and Thompson (1979).

Table 2.1 gives the details of the magnetic parameters and inter-parametric ratios studied, their units, interpretation and the instruments used (after Thompson and Oldfield, 1986; Maher, 1988; Oldfield, 1991, 1994, 2007; Oldfield et al., 2010; Snowball, 1991; Snowball and Thompson, 1990; Dearing et al., 1997).

Table 2.1 Magnetic measurements, their interpretation and instrumentation (after Thompson and Oldfield, 1986; Maher, 1988; Oldfield, 1991).

<table>
<thead>
<tr>
<th>Magnetic measurements and their units in parantheses</th>
<th>Interpretation</th>
<th>Instruments used</th>
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<tr>
<td>Low- and high-frequency magnetic susceptibility</td>
<td>Proportional to the concentration of magnetic minerals.</td>
<td>Bartington susceptibility meter with a dual-frequency sensor.</td>
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<td>$\chi_{\text{lf}}$ and $\chi_{\text{hf}}$ ($10^{-8}$ m$^3$ kg$^{-1}$)</td>
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<tr>
<td>Frequency-dependent magnetic susceptibility</td>
<td>Proportional to the concentration of superparamagnetic grains.</td>
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<tr>
<td>$\chi_{\text{fd}}$ ($10^{-8}$ m$^3$ kg$^{-1}$)</td>
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<td>$= \chi_{\text{lf}} - \chi_{\text{hf}}$</td>
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<tr>
<td><strong>Susceptibility of Anhysteretic Remanent Magnetization (ARM)</strong></td>
<td>Proportional to the concentration of magnetic minerals of stable single domain size range.</td>
<td><strong>Molspin AF-demagnetiser with ARM attachment and fluxgate magnetometer.</strong></td>
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<tr>
<td>$\chi_{ARM} (10^{-5} \text{ m}^3 \text{ kg}^{-1})$</td>
<td>Isothermal Remanent Magnetisation and Saturation Isothermal Remanent Magnetisation (IRM and SIRM) $(10^{-5} \text{ A m}^2 \text{ kg}^{-1})$</td>
<td>Proportional to the concentration of remanance-carrying magnetic minerals.</td>
</tr>
</tbody>
</table>
| **‘Hard’ Isothermal Remanent Magnetisation**  
HIRM = SIRM-IRM$_{300\text{mT}}$ $(10^{-5} \text{ A m}^2 \text{ kg}^{-1})$ | Proportional to the concentration of magnetically ‘hard’ minerals like haematite and goethite. | **Molspin Pulse magnetizer and fluxgate magnetometer.** |
| | | |
| $\chi_{ARM}/\chi_{lf}$ | Indicative of magnetic grain size. A higher ratio value indicates a finer grain size (fine SSD) and *vice versa*. Values of $\chi_{ARM}/\chi_{lf} > 40$ and $\chi_{ARM}/\chi_{fd} > 1000$ are indicative of bacterial magnetite. | |
| $\chi_{ARM}/\chi_{fd}$ | A higher ratio value indicates a finer magnetic grain size and *vice versa*. Very low ratio values indicate a coarse magnetic grain size, probably indicative of anthropogenic sources. | |
| $\chi_{ARM}/\text{SIRM}$ | High ratio values (~ $70 \times 10^{-5} \text{ A m}^{-1}$) are indicative of greigite, which forms under reducing conditions. | |
| SIR/M/\chi_{lf}$ | Indicative of the relative proportions of ferrimagnetic and anti-ferromagnetic minerals (High ratio = A relatively higher proportion of magnetite and *vice versa*). A higher proportion of hematite indicates warm and oxidizing conditions. | |
2.4 Citrate-Bicarbonate-Dithionite (CBD) Treatment

Citrate-bicarbonate-dithionite treatment was carried out on 20 soil samples according to the standard procedure of Mehra and Jackson (1960). Approximately 4 g of the soil sample was taken in a plastic centrifuge tube and oven-dried at 35 °C. Forty ml of 0.3 M sodium citrate solution was added to the sample followed by 5 ml of sodium bicarbonate solution. The sample mixture was heated in a Rotekwater bath. One g of sodium dithionite was added to the sample once the temperature reached 75 °C. It was heated at a constant temperature for 15 minutes with intermittent stirring every 5 minutes. The sample mixture was centrifuged for 10 min at ~ 4400 rpm using a research centrifuge (ELTEK TC-4100) and the supernatant decanted. The soil samples were rinsed with sodium citrate and the resultant liquid was added to the original supernatant and stored in plastic bottles. The solid residue was dried overnight at 35 °C and its low-and high-frequency magnetic susceptibility measurements were carried.

The CBD treatment was carried out four times in succession and the total reaction time was around one hour. Low- and high-frequency magnetic susceptibility (at 0.47 kHz and 4.7 kHz) of the samples was measured before and after the CBD treatment. The difference between the initial magnetic susceptibility ($\chi_{\text{initial}}$) and the magnetic susceptibility after the fourth step gives the pedogenic magnetic susceptibility ($\chi_{\text{pedogenic}}$), which is the susceptibility accounted for by magnetic minerals in the SP range formed during pedogenesis.

The CBD technique is based on the principle of reductive iron dissolution. Sodium dithionite, which acts as a reductant, adsorbs on to and reduces the iron atoms while it itself gets oxidised to sulphite. The ferrous iron goes into solution where it is chelated by sodium citrate. Sodium bicarbonate buffers the $\text{H}^+$ loss during the reaction
(Van Oorschot, 2001). Hence, after the treatment, all the pedogenic magnetic minerals of SP grain size will go into solution, leaving behind the coarse grained lithogenic fraction.

2.5 Particle Size Analysis

Particle size analysis was carried out on 46 samples from selected profiles. About 4 g of the soil sample was used for the analysis. The organic matter and carbonate contents were removed by treating the samples with 30 % H$_2$O$_2$ and 10 % glacial acetic acid respectively. Sand fraction was separated by wet sieving through an ASTM test sieve no. 230 (62 μm pore diameter). After removal of sand, the silt + clay fraction was transferred to a 1000 ml measuring cylinder. One g of sodium hexametaphosphate was added to prevent flocculation. After making it up to 1000 ml volume, the silt and clay fraction was determined using pipette analysis (Carver, 1971). The column containing silt and clay was stirred vigorously for about one minute using a long stirring rod. After 20 seconds of the stoppage of stirring, 20 ml of the liquid was pipetted out from 20 cm depth which contains the silt + clay fraction. After 3 hours 10 minutes, 20 ml of the liquid was pipetted out from 5 cm depth which contains only the clay fraction. The total weight of the clay fraction was calculated by multiplying the weight of clay by 50. A value of 1 g was subtracted from the value obtained to account for the weight of sodium hexametaphosphophate. Similarly, the total weight of the silt + clay fraction was also calculated. From these values, the total weight of the silt fraction was determined. The three fractions (sand, silt and clay) are expressed as percentages.

2.6 Organic Carbon Analysis

Organic carbon was determined for 41 samples from selected profiles. Soil samples were dried in a hot air oven at 40 °C. About 2 g of the soil sample was taken in a beaker and 1N diluteHCl added in small increments until effervescence stopped
(Schumacher, 2002). It was kept overnight at room temperature to facilitate complete removal of carbonates. The sample was washed with deionized water and the supernatant decanted thrice successively and then dried in the hot air oven at 100 °C. After the removal of inorganic carbon, the remaining carbon is organic. The organic carbon and nitrogen in the decarbonated samples was determined using a CHNS analyzer (Model: Elemental Vario EL III) at the Sophisticated Test and Instrumentation Centre, Cochin University of Science and Technology, Kochi.

2.7 Scanning Electron Microscope-Energy Dispersive Spectroscopic (SEM-EDS) Studies

Based on the magnetic data obtained, 5 samples from different soil profiles were selected for Scanning electron micrograph and Energy dispersive spectroscopic (EDS) studies. For this, the sample was dispersed and suspended in 500ml of deionized water using a magnetic stirrer. A small rare-earth magnet sealed in a zip-lock polythene cover was suspended in the beaker. The magnetic minerals that stuck to the polythene cover were collected in a small beaker and dried before carrying out SEM studies (Hounslow and Maher, 1999). The magnetic extract was sputter-coated with gold and examined at using an Analytical Scanning Electron Microscope JEOL-JSM-6380LA operated at 15–20 keV at National Institute of Technology Karnataka (NITK), Surathkal, India. An EDS helps to analyze the composition of a specific grain to determine the elemental concentrations. This analysis is presented as spectra, in which energies corresponding to different elements are shown.