Chapter 3

MATERIALS AND METHODOLOGY

This chapter contains the methods we have adopted in carrying out this research work.

Monitoring of soil and water quality parameters require accurate and sensitive analytical measurements. Because of their normally low concentration in natural systems, analytical methodology usually requires both high sensitivity and specificity.

3.1 Soil Chemical Analysis

The 18 essential nutrients for plants are classified into four groups (Brady and Weil, 1999). Measurements, which involve characterization of the soil solution and its constituents and of the composition of the inorganic and organic phases in soil, are broadly termed chemical. For any one element, numerous procedures or variations of procedures can be found in the literature (Walsh and Beaton, 1973; Page, 1982; Westerman, 1990). The procedures adopted by us are discussed separately in the following sections.

3.1.1 Soil Sample Collection

The value of a soil analysis result is no better than the quality of the samples analyzed. Soils are naturally variable horizontally as well as vertically, requiring careful consideration in terms of sampling technique. Topography and soil type are common factors for determining sampling boundaries for collecting a single soil composite. There are statistical concepts in soil sampling that will determine which method of sampling

best defines the area under test evaluation (Radojevivc and Baskin, 1999). Proper procedures must be followed to collect representative soil samples. Proper random sampling or grid sampling can provide an accurate picture of the average nutrient level in the field. We have adopted Simple Random Sampling techniques to collect the soil samples.

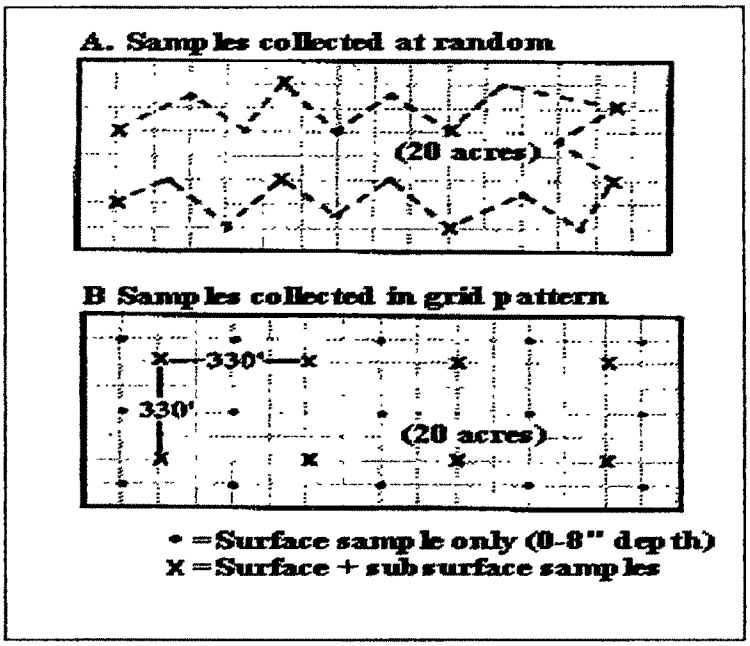

Figure3.1 Soil sample collection pattern 1

Since most of the tea gardens of the study are in hilly areas, soil may vary between top and bottom flat. We, have, therefore, taken separate samples from the top flat, the bottom flat, and the slope in the tea garden areas.

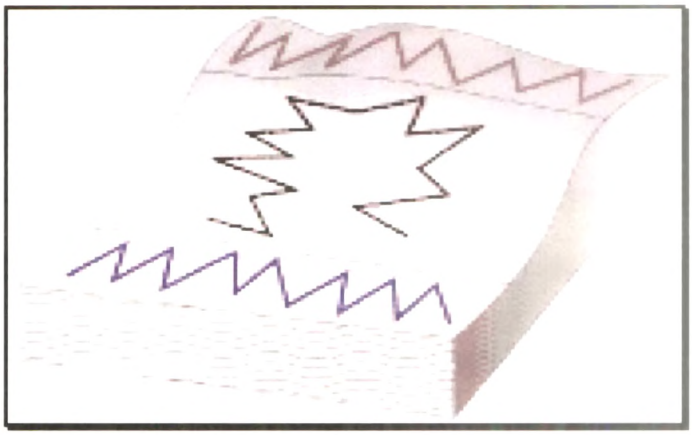

Figure 3.2 Soil sample collection pattern 2

The depth of sampling is determined by several factors. Surface (tillage layer) samples are used for determining soil pH, organic matter, phosphorus, potassium, sulphate-sulfur, and zinc. Usually the tillage layer is considered to be the 0-6 inch or the 0-8 inch depth. Sampling deeper than the tillage layer generally results in lower soil test values for organic matter, phosphorus, and zinc. Potassium and pH may increase, decrease, or remain the same with tests from deeper samples. For getting good measure of the average nutrient status in the field, a "V" shaped cut of 0 to 6-inch depth at random locations was made in each sampling sites and one inch of soil on either side of pit is scraped and collected. These samples were mixed well and about ½ kg of representative composite samples was prepared.

We have used screw type auger for soil collection. Post hole augers are used for collecting soil samples from the paddy fields near the tea gardens. The samples are packed in cloth bag of size 20 cm x 10 cm and kept in a cool environment for analysis.

3.1.2 Soil Sample Preparation:

3.1.2.1 Drying

The field collected soil samples after assigning identification number were air-dried in oven set at 100 F (38^0C) for 12 hours. The drying process were done promptly and rapidly to minimize microbial activity.

3.1.2.2 Crushing/Grinding

Following drying, the soil sample was crushed by hand using a wooden pestle in a heavy porcelain mortar and screened through a 2 mm sieve. Although crushing and sieving can be a mixed process, sample size reduction was necessary to ensure that the sample was thoroughly mixed. About 250 gm of the well prepared soil sample is kept in a cardboard container for analysis.

3.1.2.3 Sample Measurement

Various measures are used in soil testing laboratories (Soil and Plant Analysis Council, 2002). These measures consist of tall cylindrical spoons, calibrated to hold the required weight of the soil as given below:

Organic Carbon: We measure equivalent to 1 gm of soil.

Available phosphorous: We measure equivalent to 2.5-5.0 gm of soil.

Available potassium: We measure equivalent to 5 gm of soil.

Soil pH and Conductivity: We measure equivalent to 10.0 gm of soil etc.

3.1.3 Soil Texture

The objective of determining textural composition of soils is to know the percentage of soil materials contained in different grain size fractions viz., sand, silt and clay and to classify the soils to any particular textural group so that dominant grain sizes present in the soil can be identified easily. Textural compositions of soils are estimated by means of mechanical analysis (Black, 1965). This consists essentially of two distinct operations viz. dispersion of the soil to ultimate grain sizes and grading the dispersed grains according to their groups.

3.1.4 Bulk density of soil

Bulk density (ρ_b) is a measure of the mass of particles that are packed into a volume of soil. The measurement of ρ_b provides a relative value of the porosity and compaction of a soil. Bulk density was measured by cylindrical core method (Arshad, M. A *et al.*, 1996).

Bulk Density = Mass of oven dry soil core in gram/Volume of soil core in cm^3

3.1.5 Soluble Salts: Conductivity Method (1:5 soil: water ratio)

Soluble salts in soil are estimated by measuring the specific conductivity of a water extract of soil with conductivity meter (Bower *et al.*, 1965, Jackson, M. L., 1958). The value is corrected at $25^{O}C$ and reported as millisiemens/cm (mS/cm). The most easily interpretable conductivity values are those on a saturation extract, prepared from a saturated soil paste. It is also useful to obtain conductivity readings on soil: water mixtures at other ratios, usually 1:1 or 1:2 or 1:5, separating soil as much as possible by settling or centrifugation. The soil: water ratio used during our study is 1:5.

3.1.6 Soil pH (1:5 soil: water ratio)

The pH is defined as the negative log of the hydrogen ion activity. Since pH is logarithmic, the H-ion concentration in solution increases ten times when its pH is lowered by one unit. The pH range normally found in soils varies from 3 to 9. Significance of pH lies in its influence on availability of soil nutrients, solubility of toxic nutrient elements in soil, physical breakdown of root cells, cation exchange capacity in soils whose colloids (clay/humus) are pH dependent, and on biological activity. pH reflects whether a soil is acid, neutral, basic or alkaline. Soil pH (1:5 soil:water) is measured with Digital pH meter (Peech, M. 1965, Schofield *et al.*, 1955)

3.1.7 Organic Matter

Organic carbon present in organic matter is oxidized to CO_2 in the presence of $K_2Cr_2O_7$ and H_2SO_4. $K_2Cr_2O_7$ produces nascent oxygen which combines with organic carbon producing CO_2 (Walkley, A. 1947, Jackson, M. L. 1958). The excess potassium dichromate not reduced by the organic matter of soil is determined by titration with standard ferrous ammonium sulphate. Nitrates may interfere when present more than 1/20th of the carbon content.

a. Dichromate ion reacts with carbon as follows:

$$2Cr_2O_7^{2}\ 3C^{o} + 16H \rightarrow 4Cr^{3+} + 3CO_2 + 8H_2O$$

b. Ferrous ion reacts with dichromate as follows:

$$6Fe^{2+} + Cr_2O_7^{2} + 14H^{+} \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$

3.1.8 Total Kjeldahl Nitrogen

The Total Kjeldahl Nitrogen (TKN) method (Bremner *et al.*,1982.) is based on the wet oxidation of soil organic matter and botanical materials using sulphuric acid and digestion catalyst and conversion of organic nitrogen to the ammonium form. Ammonium is determined using the diffusion-conductivity technique. The procedure does not quantitatively digest nitrogen from heterocyclic compounds (bound in a carbon ring), oxidized forms such as nitrate and nitrite, or ammonium from within mineral lattice structures. The method has a detection limit of approximately 0.001% N and is generally reproducible within 8%.

3.1.9 Available Phosphorus

The available phosphorus in soil is determined by the method of Bray and Kurtz (1945). The combination of 0.025 N HCl and 0.03 N NH_4F extracts acid-soluble forms of P is largely calcium phosphate and a portion of Al and Fe phosphates. The fluoride ions complex trivalent Al and Fe in acid solution and thereby release P held by Al and Fe. The P extracted from soil is a measure of labile P. P in the soil extract is determined calorimetrically using a photoelectric colorimeter after developing molybdenum blue colour using 660 mμ red filter (Klett No. 66), the intensity of which varies with the P concentration .

3.1.10 Potassium

Ammonium acetate is used to extract exchangeable K in soil as ionic diameter of NH_4^+ is such that exchangeable K in soil is readily displaced by NH_4^+. K in the extract is atomized into blue flame of a flame photometer so that it gets excited on gaining energy and emits radiation of a certain wave length in proportion to the concentration of K. the light emitted strikes a photocell after passing through a filter. The photocell converts

light energy into electrical energy which is measured by a galvanometer. The galvanometer is first calibrated by atomizing solution varying in K concentration from 0 to 20 ppm (Toth and Prince, 1949). A direct reading Perkin Elmer flame photometer is used.

3.1.11 Calcium and magnesium

Calcium and magnesium in soil samples have been estimated by EDTA titrimetric method (Tucker *et al.,* 1961). The EDTA acts as a chelate. The end point is indicated by metal sensitive indicators which are also chelate. The Ca indicator is murexide and the Mg indicator is Eriochrome Black T (EBT). In the presence of free Ca, murexide gives a wine red colour which turns to purple when free Ca has been completely complexed by EDTA. When EBT is used as an indicator, EBT gives a wine red colour in the presence of free Mg, and when Mg is completely complexed by EDTA, EBT gives a clear blue or green colour. As Ca EDTA complex is more stable than Mg EDTA complex, it is possible to determine Ca + Mg by EDTA titration using Mg indicator. The colour developed at the end point is the result of reaction of Mg with EBT, but it is indicative that the reaction of Ca with EDTA has been completed. The EDTA forms chelate with Ca, Mg and other metals and interferences from other metals have been removed by either by chelation or precipitation. Some metal ions like Fe^{3+} are needed to be reduced prior to chelation. In Ca determination, Mg gets precipitated as $Mg(OH)_2$ on adjusting the pH of the solution to 12 or more by the addition of 4N NaOH. In Mg determination, the interference of Ca is removed by precipitation of Ca by adding alcohol on introducing 1mL of 6N H_2SO_4 which effect flocculation of $CaSO_4$ formed.

3.1.12 Sulphate

Sulphate-S in soil is determined by tubidimetric method (Bardsley and Lancaster,1960) . Sulphate in soil is extracted by 0.5N CH_3COONH_4 in acetic acid solution employing a 1:2.5 extractant (w/v)in the presence of activated charcoal free from sulphur. The soil extract is treated with acid seed solution (1:1 HCl containing20 ppm of SO_4-S) and crystals of $BaCl_2$ to develop $BaSO_4$ turbidity for the measurement of absorbance by UV spectrophotometer at 420 nm. The value of sulphate – S can be obtained by multiplying the value of sulphate (in ppm) by 0.333

3.1.13 Chloride

Chloride is the most recent addition to the list of essential elements. Most of the chlorides in the soil are soluble in water and determined directly in soil. The most common method is titrimetric, involving direct titration of the soil solution with $AgNO_3$ using K_2CrO_4 as an indicator. A 1:5 soil solution is prepared by adding 20 g of soil in 100ml distilled water and stirred mechanically for one hour at regular interval. Suspension is filtered through Whatman No.50 filter paper using Buchner funnel.

50 ml of the filtrate is titrated in an alkaline medium (pH 8.2) until the bright lemon-yellow color just stars to turn orange. A dark reddish-orange color will appear if excess silver nitrate is added. A consistent choice of endpoint is really what is required. When Cl^- in solution is exhausted through precipitation as AgCl, the red precipitate of Ag_2CrO_4 sharply signals the end point. Thiosulphate, cyanide, sulphite and sulphide are likely to interfere with this method. These can be eliminated by oxidation with a 30% hydrogen peroxide solution before titration with $AgNO_3$. The Chloride content is calculated as.

$$\%\text{Chloride} = (A \times N \times 35.5)/\text{ml of soil solution} \times 2$$

Where, A = Volume of silver nitrate in ml, required to titrate the sample

N= Normality of the $AgNO_3$

Multiplying the values in % by 1000, we get the values in mg/100g

3.1.14 Iron

Fe from soil is extracted by 1.0 N CH_3COONH_4 (pH 4.8) by following the procedure of Olson (1965). The Fe in soil extract is reduced to Fe^{2+} with $NH_2OH.NH_4Cl$ and then Fe^{2+} is reacted to form a red coloured complex [tri-(1,10)-phenanthroline ferrous ion, $Fe(C_{12}H_{10}N_2)_3^{++}$]. The absorbance of the red coloured complex is measured at 490 mμ wavelength using a photoelectric colorimeter. Since1.0 N CH_3COONH_4 (pH 4.8) extract in most soils are slightly coloured because of extraction of organic matter, a correction is made for the colour with each soil. This is done by running a check for each soil, to which all the reagents except orthophenantroline is added and its absorbance is deducted from the absorbance of soil extract with all the reagents including orthophenantrolone.

3.1. 15 Estimation of Pb, Mn, Cu , Zn , by AAS

Atomic Absorption (AA) occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analytes present in known standards can be used to determine unknown sample concentrations by measuring the amount of light they absorb. Atomic spectroscopy is the technique for determining the elemental composition of an analyte by its electromagnetic or mass spectrum. Performing atomic absorption spectroscopy requires a primary light source, an atom source, a monochromator to

isolate the specific wavelength of light to be measured, a detector to measure the light accurately, electronics to process the data signal and a data display or reporting system to show the results. The light source used is a hollow cathode lamp (HCL) or an electrode-less discharge lamp (EDL). Pb, Mn, Cu, Zn, Cd, B and Al were analysed by using an atomic absorption spectrometer (Perkin Elmer AAnalyst 200) with flow injection analyze mercury hydride generation system (Model FIAS-100). Standard procedure is followed for estimation of each metal (APHA, 1995).

Instrument	Metal	Wavelength (HCL) in nm	Width in mm	Flame composition
AAS (Perkin Elmer Model AAnalyst 200) with FIAS 100 facility	Mn	279.48	1.8/0.6	Air – C_2H_2 AAS
	Cu	324.75	2.7/0.8	Air – C_2H_2 AAS
	Zn	213.86	2.7/1.8	Air – C_2H_2 AAS
	Pb	283.30	2.7/1.05	Air – C_2H_2 AAS

3.2 Water Chemical Analysis

The present research work is largely confined to monitor drinking water quality in the tea garden areas of undivided Darrang district. Since such chemical parameters are very large and new parameters have found entry into the standard methodology in recent

times, monitoring whole parameters are merely an impossible task. So, considering various factors such as laboratory facilities, availability of reagents, the following chemical parameters have been selected for monitoring the water quality.

The first step for any test is getting a reliable, representative sample. The need for careful sampling techniques varies according to the constituent being tested. For the present study, twenty eight drinking water samples were collected at different sites in and around the five tea gardens of Darrang district to assess the qualitative changes in various parameters. Samples were collected by random selection during and combined together in clean and sterile one-litre polythene cans to obtain a composite sample and stored in an ice box (Laxen and Harrison, 1981). Samples were protected from direct sun light during transportation to the laboratory (Tata, 1987). All probable safety measures were taken at every stage, starting from sample collection, storage, transportation and final analysis of the samples to avoid or minimize contamination. Temperature, pH and conductivity were determined quickly after sampling. Samples were protected from direct sun light during transportation (Tata, 1987). The physical parameters studied are Temperature, Colour, Odour, Taste, Conductivity, Total Solid (TS), Total Dissolved Solid (TDS) and Total Suspended Solid (TSS). The chemical parameters studied are pH, Alkalinity, Chloride (Cl^-), Sulphate (SO_4^{--}), Nitrate (NO_3^-), Total Hardness, Calcium (Ca), Magnesium (Mg) and Iron (Fe), Dissolved Oxygen (DO), Phosphate (PO_4^{-3}), Fluoride (F^-), Arsenic (As), Lead (Pb), Nickel (Ni), Cadmium (Cd), Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn). Analytical techniques as described in "Standard Methods for the Examination of Water and Wastewater" (Clesceri *et al.*, 1992) are adopted for physico-chemical analysis of water samples.

3.2.1 Colour and temperature

Colour test is basically carried out by comparison with a known standard. The colour of water samples of the study are observed through naked eye by taking 50 ml of the sample in a beaker. The temperature of water was done immediately at the time of sample collection with a mercury thermometer.

3.2.2 Odour :

Pure water is odourless. In the present work, only a qualitative estimation of odour is attempted.

3.2.3 Turbidity :

Turbidity was measured immediately after collection of the sample by turbidimeter (Elico, CL-52D). The values were expressed in NTU (Nephelometric Turbidity Unit) and calibration was done with standard turbidity suspension. The instrument works on the principle that when light is passed through a sample having suspended turbidity, some of the light is scattered by the particles. The scattering of the light is generally proportional to the turbidity (Trivedy, R. K., 1990).

3.2.4 Electrical Conductivity:

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. Conductance is not a pollution indicator, in fact, it reflects the degree of mineralization of water. Conductivity is measured by using a digital conductivity meter (Wilcox, 1950, Clesceri *et al.,* 1992). Conductivity cell is rinsed with atleast three portions of 0.01 N KCl solutions. Temperature of a fourth portion is adjusted to 25.0 ± 0.1°C. Then conductivity is measured which should be 1413 μS/ cm.

If not so the conductivity meter is adjusted to read this value using the cell constant knob. After noting the cell constant, the cell is rinsed with one or more portions of sample. Temperature of a final portion is adjusted to 25.0 ± 0.1°C and the conductivity value is measured. Digital conductivity meter (Elico, CM-180) was used to measure conductivity of the water sample

3.2.5 pH

The measurement of *p*H is performed electrometrically using a *p*H meter having a glass and reference calomel electrode (Jackson, 1958). The principal of the probe requires the glass electrode to adsorb a layer of the sample onto its surface; the resultant potential difference being a function of the hydrogen ion (H+) concentration in the sample and the electrolyte contained within the electrode (Wetzel and Likens 1991). Prior to measurement the *p*H meter is calibrated using a freshly made buffer solution (pH 7) and the slope of the electrode adjusted against a pH 4 buffer. Temperature compensation is adjusted manually according to the ambient sample temperature. The electrode is thoroughly rinsed with distilled water, well flushed with the sample, and allowed to stand for several minutes without agitation before the *p*H value is determined. This method is most accurate and almost free from interferences. It is imperative to follow strictly the manufacturer's direction.

pH of water samples was determined immediately after collection. The digital pH meter (Elico, LI-127) was used to measure the pH.

3.2.6 Total Alkalinity:

Alkalinity of water is characterized by the presence of all hydroxyl ions capable of combining with the hydrogen ion. Free hydroxyl ions and hydrolysis of salts of weak acids and strong bases are responsible for alkalinity in natural water. The number of

milliequivalents of acid used in the titration to combine all the hydroxyl ions, is called as total alkalinity (Trivedy R.K,1990).

This method is applicable to drinking, ground, surface, and saline waters that can be characterized as carbonate systems. It is suitable for all concentration ranges of alkalinity. This method (A.P.H.A, 1989) describes the standard and sample analysis of waters for total alkalinity by titration. Total alkalinity in drinking, surface, saline, and ground water samples is a function of the carbonate, bicarbonate, and hydroxyl ion concentrations. Titration of representative sample aliquot with standardized sulfuric acid to pH 4.5 or sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity. For low alkalinity samples, the titration is continued to reach a second end-point 0.3 pH-units below the initial end-point. Based upon the volume of acid used, the alkalinity in the sample is calculated and reported in units of mg/L $CaCO_3$. For total alkalinity measurements, the sample must not be filtered, diluted, concentrated, or altered prior to titration. If the sample is altered, then the results must be qualified accordingly. For example, if the sample is filtered, the results might be considered as "dissolved" alkalinity. The measurement process is not affected by sample pre-treatment.

3.2.7 Solids

Solids refer to matter suspended or dissolved in water. Solid analyses are important in the control of biological and physical assessing compliance with regulating agency.

- Total solids (TS) are determined by drying a known amount of a sample at a temperature of 103 to 105 °C till a constant weight was obtained. Results can be expressed in mg/L or percent by weight.

$$TS\ (mg/L) = (W_2 - W_1) \times 1000/V$$

Where, W_2= Final weight of the beaker and residue in gram

W_1 = Initial weight of the beaker in gram

V = volume of sample in ml.

- If the original sample is filtered through a tared glass-fiber filter, which is then dried, the weight of the material captured on the filter is used to figure the total suspended solids (TSS).
- The total dissolved solids (TDS) can be estimated from the difference between the total solids and the total suspended solids, but the official method calls for drying the filtrate (the liquid which passes through the filter) in a dish at 180 ^{0}C.

3.2.8 Dissolved oxygen

DO was measured by WINKLER'S IODOMETRIC Method in this method DO is allowed to react with iodide to form I_2 which is titrated against standard $Na_2S_2O_3$ solution and $MnSO_4$ is added in alkaline medium. NaN_3 is added to avoid interferences of oxidizing agents like NO_2^- and SO_3^{-2}

DO = (ml x N) of sodium thiosulphate x 8 x1000/V_2 x (V_1-V)/ V_1

Where, V_1 = volume of sample bottle

V_2 =volume of contents titrated

V = Volume of $MnSO_4$ and KI added

3.2.9 Chloride

Chloride is measured by Argentometric Method (A.O.A.C., 1950). Silver nitrate plus chromate indicator plus chlorides yield a white precipitate of silver chloride. Once all Cl^- ions have been precipitated, the silver nitrate then reacts with the chromate indicator to form an orange precipitate of silver chromate.

a) $AgNO_3 + K_2CrO_4 + Cl^- \text{----------} > AgCl$

(white precipitate)

b) $AgNO_3 + K_2CrO_4 \text{----------} > Ag_2CrO_4$

(orange precipitate)

The sample is titrated in an alkaline medium (pH 8.2) until the bright lemon-yellow color just stars to turn orange. A dark reddish-orange color will appear if excess silver nitrate is added. A consistent choice of endpoint is really what is required. When Cl^- in solution is exhausted through precipitation as AgCl, the red precipitate of Ag_2CrO_4 sharply signals the end point. Thiosulphate, cyanide, sulphite and sulphide are likely to interfere with this method. These can be eliminated by oxidation with a 30% hydrogen peroxide solution before titration with $AgNO_3$. The Chloride content is calculated as.

(Chloride (mg/L) = A x N x 35.5/ml of sample

Where, A = Volume of silver nitrate in ml, required to titrate the sample

3.2.10 Nitrate

Nitrate – nitrogen in water is determined by phenol disulphonic acid method using UV spectrophotometric technique (Elico, SL-159) and absorbance is measured at 410nm. At first, water sample were filtered and then 50 ml was evaporated until it became completely dry. The residue was dissolved in phenol disulphonic acid and then diluted to 50 ml and liquor ammonia was added to develop a yellow colour. The nitrate

content was read directly by operating the instrument in photometry mode calibrating against a blank and a standard potassium nitrate solution.

3.2.11 Phosphate

Phosphate – phosphorous in water is determined by using UV spectrophotometric technique (Elico, SL-159).The phosphate in water react with ammonium molybdate and form complex heteropoly acid (molybdophosphoric acid), which gets reduced to a complex of blue colour and the absorbance of light by this blue colour is measured at 690nm and the concentration of phosphate is determined by comparing it with standard phosphate solution of potassium hydrogen phosphate (K_2HPO_4) (Trivedi R. K., 19xx)

3.2.12 Sulphate

Sulphate is measured by Turbidimetric method. Sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in HCl medium. The concentration of the sulphate can be determined from the absorbance of the light by $BaSO_4$ and then comparing it with standard Na_2SO_4 curve on a spectrophotometer at 420 nm.

3.2.13 Total Hardness

This method (complexometric titration with EDTA) is applicable to drinking, surface, and saline waters and domestic and industrial wastes. The method (Clesceri *et al.*, 1992) is suitable for all concentration ranges of hardness; however, in order to avoid large titration volumes, the procedures use a sample aliquot containing not more than 25 mg $CaCO_3$. The quantitation limit (QL) is 5 mg/L $CaCO_3$ hardness. Calcium and magnesium ions are the primary sources of hardness in aqueous samples. Samples are

titrated with a disodium ethylenediamine tetraacetate solution (EDTA solution). In alkaline condition EDTA reacts with Ca and Mg to form a soluble chelated complex. Ca and Mg ions develop wine red colour with Eriochrome black 'T' under alkaline condition. When EDTA is added as a titrant the Ca and Mg divalent ions get complexed resulting in sharp change from wine read to blue which indicates end point of the filtration. The pH for this titration has to be maintained at 10.0 ± 0.1. At a higher pH, i.e., about 12.0 Mg ion precipitates and only Ca ion remains in solution. At this pH Murex indicator form a pink colour with Ca ++ When EDTA is added Ca ++ gets complexed resulting in a change from pink to purple which indicates end point of the reaction. Based upon the volume of EDTA solution used, the total hardness in the sample is calculated and reported in units of mg/L $CaCO_3$.

Total Hardness (mg/L, CaCO3) = ml of EDTA used x 1000/ ml of sample

3.2.14 Calcium and Magnesium

Calcium (Ca) and Magnesium (Mg) are determined by EDTA titration (Cheng & Bray, 1951; Diehl *et al.* 1950). The EDTA titration is convenient as well as quite sensitive. Calcium and magnesium ions are sequestered by the addition of EDTA as EDTA forms complexes with Ca, Mg and divalent heavy metals. The interference from heavy metals is prevented by introducing heavy metal complexing reagents *e.g.* disodium diethyl dithiocarbamate, KCN, K.Na-ferrocyanide, hydroxylamine hydrochloride and triethanolamine. EDTA titration in the presence of Ca-indicator, Murexide (ammonium purpurate) which gives a color change from pink to purple at the end point determines Ca and EDTA titration in the presence of Mg-indicator (Eriochrome Black T) determines Ca^{+2} Mg as Ca-EDTA complex has a higher stability than Mg-EDTA complex. Mg is conveniently determined by subtraction of Ca from

Ca^{+2} Mg. The distilled water used in EDTA titration should be free from calcium and the buffer solution as well as Ca and Mg indicators should be freshly prepared.

The calcium content was calculated as

Ca (mg/L) = X. 400.8/ml of sample

Calcium and magnesium form a complex of wine red colour with Eriochrome black T at pH 10. The EDTA has got a stronger affinity for Ca^{+2} and Mg^{+2}, the former complex is broken down and a new complex of blue colour is formed. The value of Mg^{+2} is obtained by subtracting the value of calcium from the total of Ca^{+2} and Mg^{+2}. Magnesium was estimated as

Mg (mg/L) = (y – X) x 400.8/ ml of sample x 1.645

Where,

y = EDTA used in hardness determination

X= EDTA used in calcium determination for the same volume of sample.

3.2.15 Iron

Iron in water sample is measured by Phenanthroline Method (Clesceri *et al.*, 1992). The detection limit is 50µg Fe. The ferric form of iron is reduced to ferrous form by boiling with HCl and hydroxylamine hydrochloride. Later 1,10 phenanthroline is added at pH between 3.2 and 3.3 to form soluble chelated complex of orange red colour with iron. The colour obeys Beer's Law and the intensity of colour is independent of pH between 3 and 9. Zinc and polyphosphate interferences are eliminated by the acid digestion. If a hot plate is to be used for the digestion, extreme caution must be taken to prevent any bumping of the sample. If sample loss occurs due to bumping that sample or standard cannot be used for the analysis. A fume hood must be used for this type of digestion to exhaust caustic fumes and to contain samples in case of an accident. The

resulting intensity of the orange red solution is measured calorimetrically using a UV-visible spectrophometer (Elico, SL -159) at 510 nm.

3.2.16 Fluoride

Fluoride content in the water samples were determined by using SPADNS METHOD. Fluoride reacts with the coloured complex of zirconyl acid and SPADNS [Sodium -2(para-sulphopherylaze) 1,8 dihydroxy -3,6 napthalene disulphonate] to form colourless $[ZrF_6]^{-2}$ and release the dye. Fluoride can be estimated on the basis of this reaction by colorimetric measurement of the dye. Before employing the SPADNS method fluorides were separated from the water samples distilling them in presence of conc. H_2SO_4 and soft glass beads to obtain fluorosilicic acid . The absorbance measurements were carried out at 570 nm using UV spectrophotometer (Elico, SL-159) and fluoride concentrations were read directly operating the instrument in photometry mode calibrating against a blank and standard sodium fluoride solution.

3.2.17 Sodium and potassium

The metals sodium and potassium were determined with a flame photometer (Elico, CL-22D) , using standard calibration procedure.

3.2.18 Copper, Lead, Manganese, Arsenic, Zinc & Cadmium:

All these metals are determined by Atomic Absorption Spectrophotometer (AAS) technique with the help of an instrument Perkin Elmer AA 200. For digestion and pre-concentration of the water samples, standard methods (APHA, 1995) were followed. Chemicals used for the purpose is analytical grade (Merck, Mumbai). For As and Pb light source is Electrodeless discharge lamp (EDL)and for other metals like Cu, Cd, Mn,

Zn and Ni is Hollow Cathode Lamp (HCL).Wave length and flame composition for the detection of different heavy metals has been listed below:

Instrument	Metal	Wave length in nm	Flame composition
Perkin Elmer A Analyst 200	Arsenic	193.7	Acetylene-argon
	Copper	324.8	Air-C_2H_2 AAS
	Lead	217.0	Air- C_2H_2 ASS
	Nickel	232.0	Air- C_2H_2 AAS
	Manganese	279.5	Air- C_2H_2
	Cadmium	228	Air- C_2H_2
	Zinc	213.0	Air- C_2H_2 ASS

3.3 Data Analysis

Data analysis and presentation, together with interpretation of the results and report writing, form the last step in the soil and water quality assessment process. It is this phase that shows how successful the monitoring activities have been in attaining the objectives of the assessment. It is also the step that provides the information needed for decision making, such as choosing the most appropriate solution to a water or soil quality problem, assessing the state of the environment or refining the water and soil quality assessment process itself.

In this study, the tools for data analysis are mainly experimental, aimed at defining possible relationships, trends, or interactions among the measured variables of interest. The observed parameters are related graphically. Sample data are subjected to statistical treatment using normal distribution statistic. Details of these may be found in standard books on statistics and software packages (Meloun, M *et al.*, 1992). Given

below are some of the important features of these techniques that were used in the present study.

Normal distribution analysis (NDA): A univariate data set may be represented as, f (x_1; x_2; . . . ; x_i; . . . ; x_n) x_i in the present context means a parameter x for the i^{th} water or soil sample. x_i is the parameter under study in the scope of the present study. A wide variety of commonly used statistical procedures, including the mean, standard deviation, and Analysis of Variance (ANOVA), require the data to be normally distributed for the statistics to be fully valid. The normal distribution is important because:

- it fits many naturally occurring data sets,
- many non-normal data sets readily transform to it
- aspects of the environment which tend to produce normal data sets are understood,
- its properties have been extensively studied, and
- average values, even if estimated from extremely non-normal data, can usually form a normal data set.

Some of the properties of a normal distribution are:

- the values are symmetrical about the mean (the mean, median and mode are all equal),
- the normal distribution curve is bell-shaped, with only a small proportion of extreme values, and
- the dispersion of the values around the mean is measured by the standard deviation.

Assuming that sampling is performed randomly, the conditions that tend to produce normally distributed data are:

- many "factors" affecting the values: in this context a factor is anything that causes a biological, chemical or physical effect in the aquatic or soil environment, the factors occur independently: in other words the presence, absence or magnitude of one factor does not influence the other factors,
- the effects are independent and additive, and
- the contribution of each factor to the variance is approximately equal.

Some more statistical estimates derived from the normal distribution mentioned above were also made in the present study for analysing soil and water quality data and have been shown below.

Median, range and percentiles: The median *M*, range *R* and percentiles *P*, are non-parametric statistics which may also be used to summarise non-normally distributed water quality data. The median, range and percentiles have similar functions to the mean and the standard deviation for normally distributed data sets.

The median is a measure of central tendency and is defined as that value which has an equal number of values of the data set on either side of it. It is also referred to as the 50^{th} percentile. The main advantage of the median is that it is not sensitive to extreme values and, therefore, is more representative of central tendency than the mean.

The range is the difference between the maximum and minimum values and is thus a crude measure of the spread of the data, but is the best statistic available if the data set is very limited.

A percentile P is a value below which lies a given percentage of the observations in the data set. For example, the 75th percentile *P*75 is the value for which 75 per cent of the observations are less than it and 25 per cent greater than it. Estimation is based on supposing that when arranged in increasing magnitudes, the n data values will, on average, break the parent distribution into $n + 1$ segments of equal probability. The

median and percentiles are often used in water quality assessments to compare the results from measurement stations.

Mean, standard deviation, variance and coefficient of variation: The mean, and the corresponding standard deviation *s* are the most commonly used descriptive statistics. The arithmetic mean is a measure of central tendency and lies near the centre of the data set. It is expressed in the same units as the measurement data. Occasionally, the Mode, or the Modal value is quoted. This is the value which occurs most frequently within the data set. It is a useful description for data which may have more than one peak class, i.e. when the data are multi-modal, but is otherwise little used.

The variance is the average squared deviation of the data values from the mean. The standard deviation *s* is the square root of the variance, and is a measure of the spread of the data around the mean, expressed in the same units as the mean. The coefficient of variation *cv* is the ratio of the standard deviation to the mean and is a measure of the relative variability of the data set, regardless of its absolute magnitudes.

Kurtosis: Kurtosis is an indicator of the relative sharpness or flatness of the peak compared to normal distribution. Positive kurtosis indicates a sharp distribution while negative kurtosis indicates a flat one.

Skewness: Skewness typifies level of asymmetry of a distribution around its mean. Positive skew indicates an asymmetric tail extending towards higher values while a negative skew is a pointer towards an asymmetric tail extending towards lower values.

Confidence limits: Since the error statistics give some indication of the reliability of the data estimates, they can be used to define a range about the statistic of interest, with some confidence that this range covers the "true" mean μ. The bounds of this confidence interval are the confidence limits of the statistic.

Student's t-test: This test assumes that the variances of the two data sets being compared are approximately the same, and that the variables are independently distributed. This application of the *t*-test, called the 2-sample *t*-test, can be used in water quality management to test for compliance with water quality objectives (or standards or

guidelines) or for assessing the effectiveness of water pollution control measures. Student's t-test is performed by taking the null hypothesis that the mean soil and water quality parameters in the study area are within the ICAR and WHO guideline values. The test may also be used for a wide variety of purposes as it relies on the universal property that a deviation divided by a standard deviation is distributed as t with n - 1 degrees of freedom; and that the variance of a difference is the sum of the individual variances:

Correlation analysis: Pearson's correlation coefficient is a measure of linear association among different variables. Correlation coefficient ranges between -1 (a perfect negative relationship) and +1 (a perfect positive relationship). A value of 0 indicates no linear relationship. If the correlation coefficient is nearer to +1 or -1, it shows the probability of linear relationship between the variables. Since the directions of association of the measured variables are unknown in advance, two-tailed test of significance was carried out.