CHAPTER 3

MATERIAL AND METHODS

3.0 MATERIALS AND METHODS

3.1. Study area

Location and climate

The study area i.e Kheralu Taluka is located in the north-eastern part of the Mehsana District at the foothills of Aravalli range. It is located between 23.82N to 23.98N latitude and 72.48E to 72.79E longitude (Study map) covering an area of 334.24km. The climatic condition of the study area is semi-arid with temperature varying from 42°C to 20°C Rainfall is scanty about 350mm.

Physiography

The region is a plain (almost flat) with a gentle slope, towards the south. It has a low hilly region towards the north. The soils of the region contain various proportions of clay, silt, sand, gravel and pebbles with intercalations of kankar ($CaCO_3$ concretion) and the total thickness ranges up to 12 m below ground level (b.g.l.). The black cotton soils are dominant. The area has a sub-dendritic drainage of ephemeral nature. Surface runoff occurs only during the rainy season. There is both surface and subsurface water irrigation, resulting in the recirculation of groundwater.

Geology

The geological setup for the Kheralu taluka shows thick alluvium almost throughout the study area. The north-eastern part of the taluka is foothills of Aravalli showing the traces of the Ajabgarh metasedimentary rocks occurring as a small area in the north-eastern portion. The latter consist of calc-gneiss and para-gneiss, which have been intruded by basic rocks. The granites occur in the northeastern part and are highly weathered, giving rise to clay formation. Over the granites lie the Himmatnagar formation, comprising sandstone, conglomerate and shale. These formations are also highly weathered. The tertiary rocks are not exposed anywhere in the entire Mehsana District as they are overlain by thick soil and alluvium (Fig. 2). The geological cross-sections reveal that the average thickness of sandy clay may be 35–40 m at a depth of 125 m below the ground surface. The main mineral resources in the district are china clay and fire clay. Occurrence of bentonite beds in the alluvial deposits may be the result of deposition of bentonite, washed from the granitic terrain, towards the east and northeast by winds

(Phadtare, 1981). The five main types of soil in the district are saline-alkali soil, calcareous sandy loams, calcareous sandy soil, non-calcic brown soils and non-calcic red-brown soils (UNDP/CGWB, 1976), as shown in Fig. 3. The geological formations in the investigated area, namely granite gneisses (associated with a limited occurrence of schists) and charnockites, are Precambrian. The granite gneisses cover almost a half of the area towards the north-west and the charnockites occupy the remaining part. Dikes, and pegmatite, quartz and granite veins, which occur to a limited extent, are present in the rocks.

Hydrogeology

The area has a small river Rupen entering from the western part in the Taluka. In addition Sabarmati river passes besides the village Dedasana in the Northern area of the taluka. In its flow, groundwater mainly follows the topographic gradient. Rainfall is the main source of groundwater recharge in the investigated area (Rao, 2002).

PLATE 2

A: Map of India

B: Map of Gujarat

C: Map of Mehsana

D: Map of **Kheralu taluka**

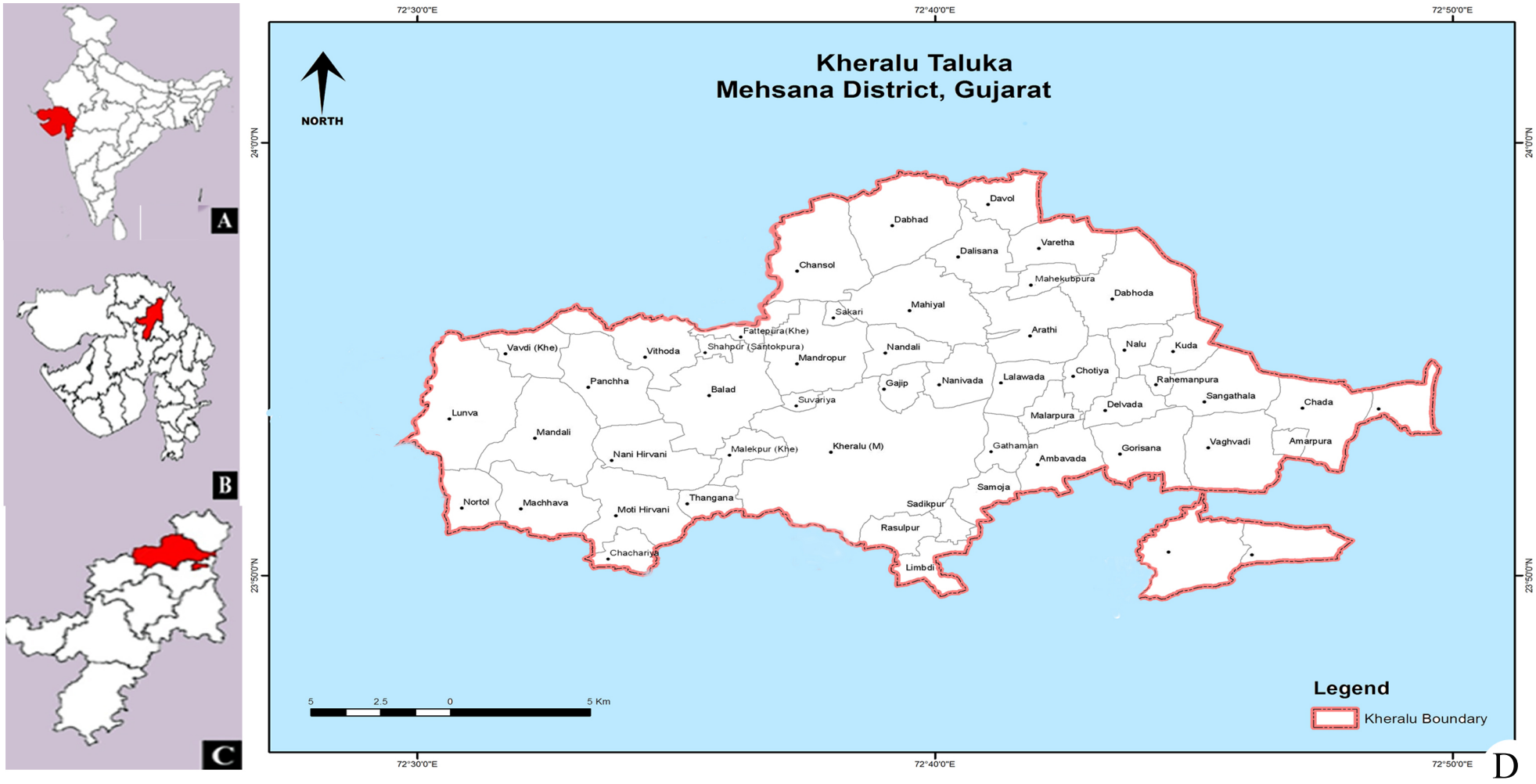
A
B
C
D
NORTH
Kheralu Taluka
Mehsana District, Gujarat
72°30'0"E
72°40'0"E
72°50'0"E
24°0'0"N
23°50'0"N
Davol
Dabhad
Varetha
Dalisana
Chansol
Mahekubpura
Dabhoda
Mahiyal
Sakari
Arathi
Fattepura(Khe)
Vavdi (Khe)
Vithoda
Shahpur (Santokpura)
Nandali
Nalu
Kuda
Mandropur
Panchha
Balad
Gajip
Nanivada
Lalawada
Chotiya
Rahemanpura
Suvariya
Sangathala
Chada
Lunva
Malarpura
Delvada
Mandali
Malekpur (Khe)
Kheralu (M)
Gathaman
Gorisana
Vaghvadi
Amarpura
Nani Hirvani
Ambavada
Samoja
Nortol
Machhava
Thangana
Moti Hirvani
Sadikpur
Rasulpur
Chachariya
Limbdi
5
2.5
0
5 Km
Legend
Kheralu Boundary

PLATE 2

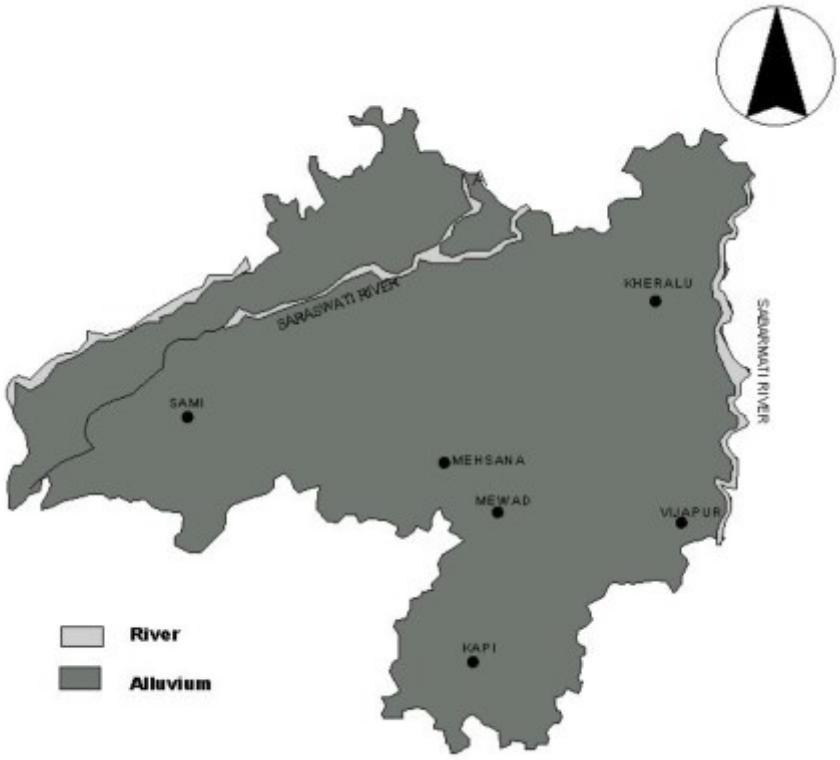

Figure 1: Geology of Mehsana district

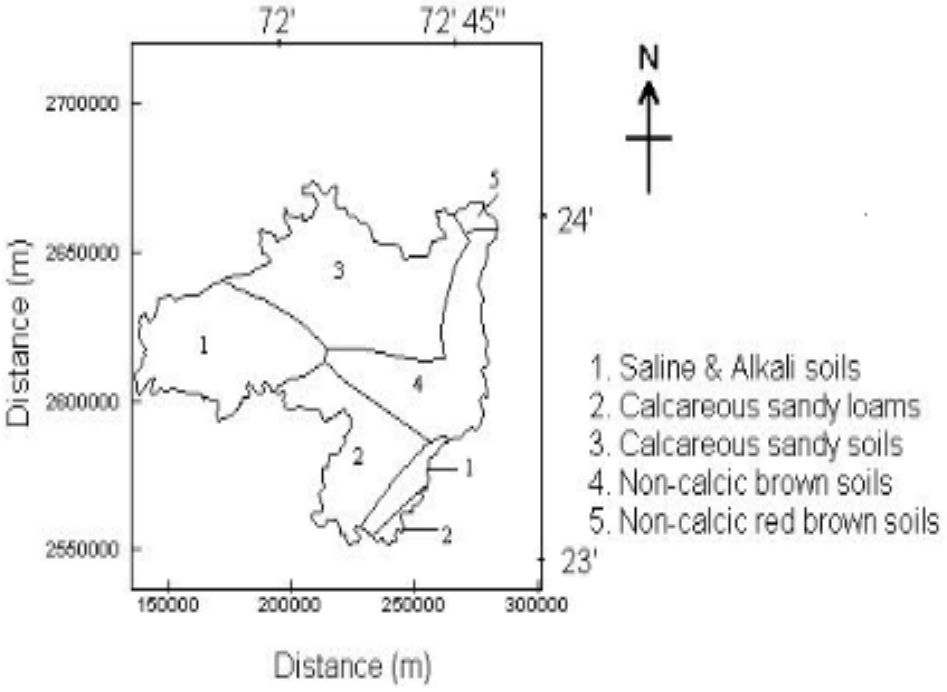

Figure 2: Generalized soil map of Mehsana district

Part I: Study area survey and Community health assessment

3.2. Survey method

To develop the knowledge about the impact of fluoride in the study area, survey methods was adopted. Both primary and secondary data were collected from the study area.

Primary data were collected from the locals in the study area. Group interview with semi-structured questionnaire were the tools adopted for the study. The aim of the survey was to collect data regarding the fluoride affected population.

Secondary data were collected from the local governmental and non-governmental organizations. It involved information about various bore wells in Kheralu Taluka, their depth and probable fluoride concentration. In addition to this information regarding fluorosis cases were collected from PHC's and CHC's.

Based on the collected data, sampling locations were selected for groundwater sampling. The water sampling from 3 bore-wells per village was collected, where selection criteria were the depth of bore-wells and utility aspect of a community. They were assessed for fluoride content.

Further, the water samples were tested for physiochemical parameters. Thereafter fluoride concentration in bore-well irrigated field soil and crops/grains were also assessed.

3.3. Water Analysis

A Randon stratified sampling was considered for groundwater sampling. From the entire Taluka, three random sampling points from each village were opted for the water analysis during pre-monsoon and post monsoon season.

3.3.1. Groundwater sample collection

The sampling procedure was employed as per the standard methods prescribed by APHA (1995-1998). The groundwater samples were collected for various physico-chemical examinations. The groundwater was collected directly from the outlet of the borewell. The water was pumped out of borewell through submersible motors. After pumping for about 1 min the water was collected in a pre-cleaned, sterilized polyethylene bottles of 1

L capacity. The sample bottles were preserved at a temperature below 10°C in a bigger container. The samples immediately dispatched to the laboratory for various physicochemical analyses.

3.3.2. Fluoride analysis and map genearearation for the study

Analysis (using Ion selective electrode, A-star 410)**:**

Reagents and standards

- Stock fluoride solution: dissolved 221mg anhydrous NaF and diluted to 1000mL. 1mL = 100µgF^-
- Standard fluoride solution: Diluted stock solution 10 times with distilled water to obtain 1mL = 10µg F^-
- Total Ionic Strength Adjustment Buffer (TISAB): Placed approximately 500mL distilled water in a 1L beaker, added 57mL glacial acetic acid, 58g NaCl and 4g 1, 2-cyclohexylenediamine tetraacetic acid. Stirred to dissolve. Place beaker in a cool water bath and added slowly 6N NaOH (about 125mL) with stirring, until pH is between 5 and 5.5. Transferred to a 1L volumetric flask and made up the volume to the mark.

Calibration

- Take 50mL of each 1ppm and 10ppm fluoride standard. Add 50mL TISAB (or 5mL if conc. TISAB is used) and calibrate the instrument. Check the electrode slope with the ion meter (59.16mV for monovalent ions and 29.58mV for divalent ions at 25°C).

Procedure

1. Calibrate the instrument as explained above.
2. Transfer 50 mL of sample to a 150 mL plastic beaker. (Check pH of solution if above or below 7.0 neutralize it using acid or base)

PLATE 3

PLATE 3

A: Groundwater sample from Delwada

B: Groundwater sample from Santokpura

PLATE 4

PLATE 4

A: Groundwater sample from Fatepura

B: Groundwater sample from Malapura

3. Add 5ml of TISAB solution to the sample and stir thoroughly
4. Rinse electrode, blot dry and place electrode in the sample.
5. Note down the steady reading on the meter.
6. After use place electrode in 1 ppm solution

Mapping (By Inverse Distance Weighting-IDW):

This interpolation method estimates a point using the nearest sample points, which are weighted by a power proportional to the inverse of their distance from the estimated point. The higher the power the stronger the influence of the closer sample points.

3.3.3. Physico-chemical analysis of Water

pH

Reagents

- 0.05 M Potassium hydrogen phthalate ($KHC_3H_4O_4$, Mol. Wt. 204.22): Dissolve 10.21 gm AR grade potassium hydrogen phthalate in warm water and making volume to 1 L. This gives a pH of 4.00 at 25°C and can be used as a standard buffer.

- Buffer solution pH 6.86: Potassium dihydrogen phosphate + Disodium hydrogen phosphate, each 0.025 M – Dissolve 3.40 gm of potassium dihydrogen orthophosphate and 4.45 gm disodium hydrogen orthophosphate dihydrate (Sorenson's salt – $Na_2HPO_4.{}_2H_2O$) to 1 L in distilled water.

- Buffer solution pH 9.2: Dissolve 3.81 gm sodium tetraborate (A.R.) in water and dilute to 1000 ml.

Procedure

1. Turn the pH meter ON and allow it to warm for 15 minutes.

2. Standardize the glass electrode using a standard buffer of pH 7.0 and calibrate with the buffer pH = 4 or pH = 9.2.

3. Take 50 ml of filtered water sample in 10 ml beaker and immerse the glass and calomel electrodes or combined electrode of the pH meter. Never allow the lower portion of glass electrodes to touch the bottom of the beaker.

4. While recording pH, switch the pH meter to pH reading, wait for 30 seconds and record the pH value to the nearest 0.1 unit. Put the pH meter in standby mode immediately after recording.

5. Remove the electrodes after each determination and carefully blot them dry with filter paper before the next determination. Standardize the glass electrodes after every ten determinations.

6. Keep the electrodes in distilled water, when not in use and ensure that the reference electrode always contains saturated potassium chloride solution in contact with solid potassium chloride crystals.

Total dissolved solids

Procedure

1. Weigh dried evaporating dish.

2. Filter the well-mixed sample under vacuum through a membrane filter or Gooch Crucible.

3. Transfer 100mL or more, depending upon the concentration of dissolved solids, in a weighed evaporating dish.

4. Evaporate to dryness on a steam bath. Dry the evaporated sample for at least 1 hour in an oven at 180±2°C. Cool in a desiccator and weigh.

5. Repeat the drying until constant weigh is obtained or weight loss is less than 0.5mg.

Calculation

$$\text{Total dissolved solids (mg/l)} = \frac{A - B * 1000}{C} \qquad \text{…[Equation 1]}$$

Where, A = weight of dried residue + dish
B = weight of dish
C = mL of filtrate used

Total Alkalinity (By Titrimetric method)

Chemicals and reagents6

- Standard sulphuric acid solution (0.02N): 30 ml of concentrated H_2SO_4 was mixed into 970ml of distilled water. This would give the stock solution of H_2SO_4 (1N). 20ml of the stock solution was added into 980 ml of water. This would give the working solution of 0.02N H_2SO_4.

- Phenolphthalein indicator (0.5%): 0.5g of Phenolphthalein powder was dissolved in 100ml of 50% alcohol.

- Methyl orange indicator (0.05%): 50mg of methyl orange powder was added in distilled water and made it up to 100ml.

Procedure

1. 100 ml of water sample was taken in a conical flask and 7-10 drops of phenolphthalein indicator were mixed with it.

2. (a)In some samples, carbonates were absent as there was no colour change appeared after addition of phenolphthalein indicator.

   (b) In the remaining samples, colour changed to pink depending on the presence of carbonates.

3. (a) No titration is required

   (b) In burette (0.02N) H_2SO_4 was taken and was titrated with the pink colour solution. Note the end point value on decolouration of this sample.

4. After the decolouration of the above water sample, 10 drops of methyl orange indicator was added. Then, the solution appeared yellow and was neutralized with (0.02N) H_2SO_4. Then the colour of the solution changed to brick red which was the end point of bicarbonate.

Calculation

Total alkalinity is the sum of phenolphthalein alkalinity and Methyl orange alkalinity.

$$\text{Phenolphthalein alkalinity } (mg/l) = \frac{A * 1000}{V} \qquad \ldots[\text{Equation 2}]$$

$$\text{Total alkalinity } (mg/l) = \frac{T * 1000}{V} \qquad \ldots[\text{Equation 3}]$$

Where, A = volume of titrant used against phenolphthalein indicator

V = volume of sample

T = total volume of titrant used

Once, the phenolphthalein and total alkalinities are determined, three types of alkalinities, i.e. hydroxide, carbonate and bicarbonate are easily calculated from the table given as under:

Values of P and T	Type of Alkalinity		
	OH^-	CO_3^{2-}	HCO_3^-
P = O	0	0	T
P<1/2T	0	2*P	T – 2P
P = 1/2T	0	2P	0
P>1/2T	2P - T	2(P – T)	0
P = T	T	0	0
Where, P = phenolphthalein alkalinity and T = Total alkalinity			

Once carbonate and bicarbonate alkalinities are known, then their conversions to milligrams CO_3^{2-} or HCO_3^-/L are possible.

mg CO_3^{2-}/L = Carbonate alkalinity mg $CaCO_3$/Lx0.6 …[Equation 4]

mg HCO_3^-/L= Bicarbonate alkalinity mg $CaCO_3$/Lx1.22 …[Equation 5]

Total Hardness (Calcium and Magnesium)

Chemical and Reagents:

- Ammonia Buffer Solution: 13.5 g of NH_4Cl was dissolved in 114ml of NH4OH. Then total volume was made 200 ml by adding distilled water.
- Eriochrome lack-T (indicator): The indicator was prepared by dissolving 0.5g of EB-Tin 100ml of 80% ethyl alcohol.
- EDTA solution (ethylene-diamine-tetra-acetic acid) (0.01): This solution was prepared by dissolving 3.723g EDTA salts in 1 litre of distilled water.
- Murexide indicator (ammonium purpurate): 0.2g of ammonium purpurate and 100g of sodium chloride were mixed and grinded thoroughly to form a fine powder.

Procedure:

Procedure for Total Hardness:

1. Take 100 ml of sample in conical flask
2. Add 1 ml Ammonia Buffer Solution
3. Add a pinch of EBT indicator and titrate with 0.01 N EDTA to a pure turquoise blue without any traces of red. This titre value may be considered as "T".

Procedure for Calcium Hardness:

4. Take 100 ml of sample in conical flask
5. 1ml of NaOH was added to the above solution to raise PH to 12.0
6. Add a pinch of Murexide indicator and titrate with 0.01N EDTA to a pure turquoise blue without any traces of red. This titre value may be considered as "A".
    1. Calculation:

$$\text{Total hardness (mg/L)} = \frac{T * 1000}{V} \quad \text{…[Equation 6]}$$

$$\text{Calcium hardness (mg/l as } CaCO_3\text{)} = \frac{A * 1000 * 1.05}{V} \quad \text{…[Equation 7]}$$

$$\text{Calcium (mg/l as CaCO)} = \frac{A * 400 * 1.05}{V} \quad \text{…[Equation 8]}$$

$$\text{Magnesium harndess (mg/l as } CaCO_3 = TH - Ca(H) \quad \text{…[Equation 9]}$$

$$\text{Magnesium (mg/l as } CaCO_3) = \text{Mg hardness} * 0.2431 \quad \text{…[Equation 10]}$$

Sodium

Reagents and standards:

- Deionised distilled water: Use deionised distilled water to prepare all reagents and calibration standards and as dilution water.
- Stock sodium solution: Dissolve 2.542 g NaCl dried at 140°C and dilute to 1000mL with water, 1 mL = 1 mg Na.
- Intermediate sodium solution: Dilute 10 mL stock sodium solution with water to 100mL; 1 mL = 100µg Na. Use this intermediate solution to prepare calibration curve in sodium range of 1 to 10 mg/L.

- Standard sodium solution: Dilute 10 mL intermediate sodium solutions with water to 100 mL; 1.00 mL = 10µg Na. Use this solution to prepare calibration curve in sodium range of 0.1 to 1 mg/L.

Procedure

1. Pre-treatment of polluted water and wastewater samples: Filter the sample passing through a 0.45 µm membrane filter.
2. Instrument operation: Because of differences between makes and models of instruments, it is impossible to formulate detailed operating instructions. Follow manufacturer's recommendation for selecting proper photocell and wavelength, adjusting slit width and sensitivity, appropriate fuel and air or oxygen pressures and the steps for warm-up, correcting for interferences and flame background, rinsing of a burner, igniting sample and measuring emission intensity.
3. Direct-intensity measurement: Prepare a blank and sodium calibration standards in stepped amounts in any of the following applicable ranges: 0 to 1.0, 0 to 10, or 0 to 100 mg/L. Starting with the highest calibration standard and working toward the most dilute, measure emission at 589 nm. Repeat the operation with both calibration standards and samples enough times to secure a reliable average reading for each solution. Construct a calibration curve from the sodium standards.

Calculation

Na (mg/L) = (Na (mg/L) in portion) x dilution factor …[Equation 11]

Potassium

Reagents and Standards

- Reagent water deionised distilled water: Use this water for preparing all reagents and calibration standards and as dilution water.

- Stock potassium solution: Dissolve 1.907g KCl dried at 110ºC and dilute to 1000mL with water; 1mL = 1mg K.

- Intermediate potassium solution: Dilute 10mL stock potassium solution with water to 100mL; 1 mL = 0.1 mg K. Use this solution to prepare calibration curve in potassium of 1 to 10 mg/L.

- Standard potassium solution: Dilute 10mL intermediate potassium solution with water to 100mL; 1mL = 0.01 mg K. Use this solution to prepare calibration curve in potassium range of 0.1 to 1 mg/L.

Procedure

1. Pre-treatment of polluted water and wastewater samples: Filter the sample passing through a 0.45 μm membrane filter.
2. Instrument operation: Because of differences between makes and models of instruments, it is impossible to formulate detailed operating instructions. Follow manufacturer's recommendation for selecting proper photocell and wavelength, adjusting slit width and sensitivity, appropriate fuel and oxidant gas pressures and the steps for warm-up, correcting for interference and flame background, rinsing of a burner, igniting a flame and measuring emission intensity.
3. Direct-intensity measurement: Prepare a blank and potassium calibration standards in a stepped amount in any of the following applicable ranges: 0 to 1.0, 0 to 10, and 0 to 100 mg /L. Determine emission intensity at 766.5 nm. Aspirate calibration standards and a samples enough time to secure a reliable average reading for each. Construct a calibration curve from the potassium standards. Determine potassium concentration of the sample from the calibration curve. Where a large number of samples must be run routinely, the calibration curve provides sufficient accuracy.

Calculation

K (mg/L) = (K (mg/L) in portion) x Dilution factor …[Equation 12]

Chloride

Chemicals and Reagents

- Silver Nitrate Solution (0.02N): 3.40g of $AgNO_3$ was dissolved in distilled water to make 1 litre solution and it was stored in dark glass bottle.

- Potassium Chromate Indicator: 10g of $K_2Cr_2O_4$ was dissolved in 20ml of distilled water and few drops of 0.02N $AgNO_3$ was added to produce a red precipitate. The solution was filtered and then diluted with distilled water to make a 1 L solution.

Procedure

1. Transfer 100 ml of water sample to a 150 ml of conical flask.
2. If alkaline adds 0.01N H_2SO_4 with methyl orange to neutralize the amount of carbonate and bicarbonate and provide 1 ml in excess.
3. Add few drops of potassium chromate indicator making it dark yellow.
4. Titrate the contents against 0.02N $AgNO_3$ solution with continuous stirring till the first brick red tinge appears. Note the volume of the $AgNO_3$ required (ml.)
5. Run a blank of 100 ml of distilled water and subtract from the titre value to avoid error due to any impurity of chemicals.

Calculation

$$\text{Chloride } (\text{mg/l}) = \frac{(\text{V} - \text{B}) * \text{N} * 35.45 * 1000}{\text{V}} \quad \text{…[Equation 13]}$$

Where,

Where, V = volume of titrate (ml) $AgNO_3$

B = volume of blank titrate (ml) $AgNO_3$

N = Normality of titrant (0.02)

V = volume of sample (ml)

Sulphates

Chemicals and Reagent

- NaCl-HCl solution: 240 g of NaCl in a double distilled water was dissolved and 20ml of HCl was added to it and diluted with more of distilled water to make volume 1 litre.
- Glycerol-ethanol solution: 50ml of Glycerol was added to 100ml of ethyl alcohol and was shaken well.
- Barium chloride(dry crystal)

- Standard sulphate solutions: 0.147 g of anhydrous sodium sulphate was dissolved in distilled water to make the volume 1 litre. The solution contains 100mg sulphate per liter. Standard of various strengths was prepared by diluting this stock solution.

Procedure

1. The sample was filtered through filter paper (Whatman No: 1) and 50ml of the filtrate was taken in the flask.
2. 10ml of NaCl–HCl solution, 10 ml of Glycerol-ethanol solution and 0.15g of $BaCl_2$ was added to 50ml filtrate solution and was stirred with the help of a magnetic stirrer for about an hour.
3. The absorbance against a distilled water blank at 420 nm was measured using a spectrophotometer.
4. In a similar way, the standard sulphate solution of different strengths was processed and absorbance for each was recorded.
5. The sulphate content of the sample in mg/L was obtained by comparing the absorbance of samples with the standard curve

3.3.4. Water Quality Index

WQI indicates the quality of water in terms of index number which represents overall quality of water for any intended use. It is defined as a rating reflecting the composite influence of different water quality parameters were taken into consideration for the calculation of water Quality index (WQI). The indices are among 68 the most effective ways to communicate the information on water quality trends to the general public or to the policy makers and in water quality management. In formulation of water quality index the relative importance of various parameters depends on intended use of water. Mostly it is done from the point of view of its suitability for human consumption.

The calculation of WQI was made using weighed Arithmetic index method (Brown et al, 1972) in the following steps:

Let there be "n water quality parameters and quality rating (q_n) corresponding to nth parameter is a number reflecting relative value of this parameter in the polluted water with respect to its standard permissible value. q_n values are given by the relationship.

$$Q_i = 100 \frac{(V_n - V_i)}{(V_s - V_i}$$ …[Equation 14]

Where, V_s = Standard value,
V_n = observed value
V_i = ideal value

In most cases V_i = 0 except in certain parameters like pH, dissolved oxygen etc.

Calculation of unit weight: The Unit weight (Wn) to various water Quality parameters are inversely proportional to the recommended standards for the corresponding parameters.

$$W_i = K/S_n$$ ...[Equation 15]

Where W = unit weight for nth parameter
S_n = standard permissible value for nth parameter
k = proportionality constant.

WQI is calculated by the following equation.

$$WQI = \sum_{n=1}^{n} Q_i W_i / \sum_{n=1}^{n} W_i$$ …[Equation 16]

The suitability of WQI values for human consumption according to Mishra & Patel, 2001 are rated as follows.

WQI Value	**Water classification**
0-25	Excellent water
26-50	Good water
51-75	Bad water
76-100	Very Bad water
100 and above	Unfit for drinking purpose

3.4. Fluoride in Soil and Crop plants

From the calculated value for approximate sampling numbers in consideration to the errors 40% around samples were selected from the entire Taluka for soil analysis and its 25% were selected for crop analysis.

3.4.1. Soil sampling

The field was divided into quadrats and random spots were marked on them (No of samples for each field depends on size, generally 10 to 20 spots were taken for one composite sample depending on the size of the field). From the spotted area, surface litter was scraped followed by cutting a soil into a V-shaped pit of upto 10 to 15 cm slice of soil. A uniform thick slice of soil was collected from the surface to the plough depth from each place and was kept in a clean bucket.

The soil from the bucket was then transferred onto a piece of clean paper or cloth and thoroughly mixed. Thereafter soil samples were evenly spread and divided into 4 quarters. Two quadrates were left out and rest were again mixed. Process was repeated till only about half kg of the soil was left, a collection was done and was put in a clean cloth bag. Each bag was properly marked to identify the sample. Details of the sample information were written on a sheet attached to the bag.

3.4.2. Plant sampling

By composite sampling technique, ear of *Triticum aestivum* L. and *Pennisetum typhoides* L. were collected from the same bore-well irrigated fields from which soils were taken. Grains from the ear were separated, collected in a big container, thoroughly mixed, packed in dry clean polyethylene bags and proceeded for fluoride analysis. Details about each sample were noted in the information sheet attached to the bag.

PLATE 5

PLATE 5

A: Soil collection from Malarpura

A: Soil collection from Fatepura

PLATE 6

PLATE 6

A: Crop collection from Malarpura

A: Crop collection from Fatepura

PLATE 7

PLATE 7

A: Crop collection from Delwada

A: Crop collection from Gathaman

3.4.3. Fluoride analysis in various matrices

Sample Digestion

By McQuaker and Gurney (1977)

It involves following steps:

1. 0.5 gram dried, grounded sample was taken in a 130 ml crucible
2. Moistened slightly with distilled water.
3. To this 8 ml, 16N NaOH was then added
4. The crucible was then placed in hot air oven at 200˚C for 1 hour.
5. After NaOH was solidified, the crucible was placed in a muffle furnace at 200˚C for 2.5 hours. Temperature is then raised to 600˚C and kept for 30 minutes.
6. The crucible was then cooled, 10 ml distilled water was added and heated slightly to dissolve solid NaOH cake.
7. 8 ml conc. HCl was added to adjust the pH between 8 and 9.
8. The content was then transferred to a 100 ml flask, diluted to the volume using distilled water and filtered through Whatman's filter paper no. 40.

Analysis (using Ion selective electrode, A-star 410)**:**

Reagents and standards

- Stock fluoride solution: dissolved 221mg anhydrous NaF and diluted to 1000mL. 1mL = 100µgF^-
- Standard fluoride solution: Diluted stock solution 10 times with distilled water to obtain 1mL = 10µg F^-
- Total Ionic Strength Adjustment Buffer (TISAB): Placed approximately 500mL distilled water in a 1L beaker, added 57mL glacial acetic acid, 58g NaCl and 4g 1, 2-cyclohexylenediamine tetraacetic acid. Stirred to dissolve. Place beaker in a cool water bath and added slowly 6N NaOH (about 125mL) with stirring, until

pH is between 5 and 5.5. Transferred to a 1L volumetric flask and made up the volume to the mark.

Calibration

Take 50mL of each 1ppm and 10ppm fluoride standard. Add 50mL TISAB (or 5mL if conc. TISAB is used) and calibrate the instrument. Check the electrode slope with the ion meter (59.16mV for mono valent ions and 29.58mV for divalent ions at 25°C).

Procedure

7. Calibrate the instrument as explained above.
8. Transfer 50 mL of sample to a 150 mL plastic beaker. (Check pH of solution if above or below 7.0 neutralize it using acid or base)
9. Add 5ml of TISAB solution to the sample and stir thoroughly
10. Rinse electrode, blot dry and place electrode in the sample.
11. Note down the steady reading on the meter.
12. After use place electrode in 1 ppm solution

3.4.4. Physico-chemical analysis of Soil

Soil pH (pH meter (1:2) dilution)

Reagents

- 0.05 M Potassium hydrogen phthalate ($KHC_3H_4O_4$, Mol. Wt. 204.22): Dissolve 10.21 gm AR grade potassium hydrogen phthalate in warm water and making volume to 1 L. This gives a pH of 4.00 at 25oC and can be used as a standard buffer.
- Buffer solution pH 6.86: Potassium dihydrogen phosphate + Disodium hydrogen phosphate, each 0.025 M – Dissolve 3.40 gm of potassium dihydrogen orthophosphate and 4.45 gm disodium hydrogen orthophosphate dihydrate (Sorenson's salt – $Na_2HPO_4.2H_2O$) to 1 L in distilled water.

- Buffer solution pH 9.2: Dissolve 3.81 gm sodium tetraborate (A.R.) in water and dilute to 1000 ml.

Procedure

1. Weigh 20 gm of 2.0 mm air dry soil into a beaker. Add 50 ml of distilled water and stir with a glass rod thoroughly for about 5 minutes and keep for half an hour.
2. In the meantime turn the pH meter ON, allow it to warm up for 15 minutes. Standardize the glass electrode using standard buffer of pH = 7 and calibrate with the buffer pH = 4 or pH = 9.2.
3. Dip the electrodes in the beakers containing the soil water suspension with constant stirring.
4. While recording pH, switch the pH meter to pH reading, wait 30 seconds and record the pH value to the nearest 0.1 unit. Put the pH meter in standby mode immediately after recording.
5. Remove the electrodes from soil suspension and clean the electrodes with distilled water.
6. Rinse the electrodes after each determination and carefully blot them dry with filter paper before the next determination. Standardize the glass electrodes after every 10 determinations.
7. Dip the electrodes in distilled water, when not in use and ensure that the reference electrode always contains saturated potassium chloride solution in contact with solid potassium chloride crystals.
8. Three to four drops of toluene are added in standard buffer solutions to prevent growth of mould.

Electrical Conductivity (EC) (EC meter (1:5) dilution)

Reagent

- 0.01N Potassium chloride solution: Dry a small quantity of A.R. grade Potassium chloride at 60°C for 2 hours. Weigh 0.7456 gm of it and dissolve in freshly prepared distilled water and make to one litre. This solution gives and electrical conductivity of 1411.8 x 10-3 i.e. 1.41 dS/m at 25°C.

Procedure

1. Calibrate the conductivity cell with the help of standard KCL solution and determine the cell constant.
2. The soil water suspension of 20 gm: 50 ml ratio prepared for the determination of pH can also be used for conductivity measurements. After recording the pH, allow the soil water suspension in the beaker to settle for additional half an hour
3. After the calibration dip the conductivity cell in the supernatant liquid of the soil water suspension. Read the conductivity of test solution in proper conductance range
4. Remove the cell from soil suspension, clean with distilled water and dip into a beaker of distilled water.

Calculations:

$EC_e25 = ECT \times K \times ft$ …[Equation 17]

Where EC_e25 is the conductivity of the extract at 25ºC.
K = Known Conductivity of 0.01N KCL/ Conductivity of 0.01N KCL measured

Soil organic carbon (modified Walkely-*Black method)*

Reagents

- 1 N potassium dichromate: Dissolve 49.04 AR grade $K_2Cr_2O_7$ (dry) in distilled water and make up the volume to one litre.
- Concentrated sulphuric acid (Sp. Gravity 1.84, 96%): If the soil contains chloride, fAthen 1.25% silver sulphate may be added in H_2SO_4.
- Orthophosphoric acid (Sp. Gravity 1.75, 85%)
- Sodium Fluoride (chemically pure)
- 0.5 N Ferrous ammonium sulphate – Dissolve 196.0 gm of AR grade ferrous ammonium sulphate in distilled water, add 20 ml of concentrated H_2SO_4 and make volume to one litre. The ferrous ammonium sulphate should be from a fresh lot and light green in colour.
- Ferroin indicator

Procedure

1. Weigh 1 gm. of 0.5 mm sieved soil into dry 500 ml conical flask. Add 10 ml of $K_2Cr_2O_7$ into the flask with pipette and swirl.
2. Add rapidly with a burette 20 ml conc. H_2SO_4 and swirl gently until soil and reagents are mixed then more vigorously for one minute.
3. Allow the reaction to proceed for 30 min on asbestos sheet to avoid burning of table due to release of intense heat due to reaction of sulphuric acid.
4. Add slowly 200 ml of distilled water, 10 ml of concentrated orthophosphoric acid and add about 0.2 gm NaF (one small teaspoon) and allow the sample to stand for 1.5 hrs. The titration end point is clear in a cooled solution.
5. Just before titration add 1 ml ferroin indicator into the conical flask. Titrate the excess $K_2Cr_2O_7$ with 0.5 N ferrous ammonium sulphate till the colour flashes from yellowish green to greenish and finally brownish red at the end point.
6. Simultaneously blank test is run without soil.

Calculations

% Organic carbon = (B – S) x N x 0.003 …[Equation 18]

Where, B = ml of std. 0.5 N ferrous ammonium sulphate required for blank.

S = ml of std. 0.5 N ferrous ammonium sulphate required for soil sample.

N = Normality of std. ferrous ammonium sulphate (0.5N)

Phosphorous

Reagents

- 0.5M $NaHCO_3$ – Dissolve 42.0 gm of P-free sodium bicarbonate in about 500 ml of hot distilled water and dilute to 1 litre. Adjust the pH to 8.5 using dilute NaOH or dilute HCL. Prepare fresh solution before use.
- Activated Charcoal – wash pure activated charcoal or commercially available Darco G-60 with acid to make P-free, even if having traces of P.
- Ammonium Paramolybdate $[(NH_4)_6MO_7O_{24}.4H_2O]$ – Dissolve 12.0 gm of ammonium paramolybdate in 250 ml of distilled water to get solution ‘A’. Prepare solution ‘B’ by dissolving 0.2908 gm of potassium antimony tartarate $(KSbO.C_4H_4O_6)$ in 100 ml of distilled water. Prepare one litre of 5N H_2SO_4 (14

ml of concentrated H_2SO_4 diluted to 1 lit.) and add solutions "A" and "B" to it. Mix thoroughly and make the volume to 2 lit with distilled water. Store in amber coloured bottle in dark and cool compartment. (Reagent C).

- Ascorbic Acid Solution – Dissolve 1.056 gm of ascorbic acid in 200 ml of molybdate tartarate solution (reagent C) and mix well. This ascorbic acid (reagent D) should be prepared as required because it does not keep more than 24 hrs.
- P - nitrophenol indicator – Dissolve 0.5 gm of p-nitrophenol in 100 ml of distilled water to get approximately 5N H_2SO_4.
- Standard P Solution (Stock Solution) – Analytical grade (AR) KH_2PO_4 is dried in an oven at 60°C for one hour and after cooling in desiccator, weigh 0.4393 gm and dissolve in about 500 ml distilled water (shake the content until the salt dissolves.) Add 25 ml of approximately 7N H_2SO_4 and make the volume to 1 lit. Add 5 drops of toluene to diminish microbial activity. This gives 100 ppm stock solution of P (100 mg/ml).
- P solution (5 ppm) – Pipette out 5 ml of stock solution of P and make up the volume to 1 lit with distilled water. This solution contains 5 mg P/ml (i.e. 5 ppm solution).
- Hydrochloric Acid (0.02 N) – Dilute 1.8 ml of concentrated HCl to 1 lit.
- Standardization of sodium hydroxide (NaOH) – Pipette out 10 ml of 0.02 potassium hydrogen pthalate in a 250 ml conical flask. Add 3 drops of phenolphthalein indicator. The end point is appearance of pale permanent pink colour.

Procedure

1. Weight 2.5 gm of soil sample in 150 ml plastic conical flask, add pinch (0.3 gm) of phosphate free activated charcoal AR grade. Add 50 ml of Olsen reagent and shake for 20 minutes exactly on platform type shaker at 180 rpm.
2. Filter the contents immediately through filter paper. Transfer 5 ml of aliquot into 25 ml volumetric flask.
3. Pipette out 5 ml of filtrate into 25 ml volumetric flask. Add 4 ml of the freshly prepared ascorbic acid and ammonium molybdate solution. Shake well and keep it for 30 minutes then make the volume.

4. Prepare the standard curve using 0, 1, 2, 3, 4 and 5 ml of 5 ppm standard P solution into 25 ml volumetric flask and develop the colour using the same procedure as above. The corresponding P concentration will be 0, 0.2, 0.4, 0.6, 0.8 and 1 ppm.
5. Measure the absorbance and colour intensity at 882 nm after half an hour.
6. Run a blank method sample with the extracting solution.

Potassium (Olsen method)

Reagents

- Neutral normal ammonium acetate solution – Take 58 ml of glacial acetic acid in 500 ml volumetric flask. Also, take 71 ml of concentrated ammonium hydroxide solution in another 500 ml volumetric flask. Dilute both the solutions with distilled water upto the 2/3 volume and mix both in 1 lit. flask then adjust pH to 7.0 and finally make up the volume to 1 lit. For bringing pH of solution to 7, add dilute acetic acid or ammonium hydroxide, or dissolve 77 gm/lit. NH4OAC and adjust pH to 7 by acetic acid or ammonium hydroxide.
- Standard potassium stock solution (100mg K/ml) – Dissolve 1.908 gm chemically pure KCl in distilled water, make up the volume to 1 L. This solution contains 1000mg/ml of K. It serves as standard stock solution. Also prepare secondary stock solution of 100mg K/ml from this primary stock solution by taking 10 ml and making 100 ml volume.
- Working solution – Pipette 0, 0.5, 1, 2, 4, 6, 8 and 10 ml of 100 mg K/ml solution in 100 ml volume flask separately and make up the volume with NH_4OAC solution. This gives 0, 0.5, 1, 2, 4, 6, 8 and 10 mg/ml respectively.

Procedure

1. Add 25 ml of NH_4OAC extracting solution to a conical flask containing 5 gm air dry soil sample.
2. Shake on a reciprocating shaker at 200 to 220 oscillations per minute for 5 min and filter.
3. Determine potassium as indicated in preparation of standard curve, dilute if necessary.

Sodium

Reagents

- 1N Ammonium Acetate - Dissolve 77.08 gm of ammonium acetate in distilled water and make the volume to 1 lit. Adjust the pH to 7.0 with glacial acetic acid or ammonia solution.
- Standard Sodium Solution – Dissolve 2.542 gm of dried NaCl (AR at 110°C for 1 hr) in distilled water and make the volume to 1 lit. i.e. 1000 ppm Na solution. 10 ml of 1000 ppm solution was diluted to 100 ml. The concentration of the sodium is 100 ppm.
- Take 2, 4, 6, 8 and 10 ml of 100 ppm Na solution in separate 100 ml volumetric flask and make up volume with distilled water. Thus 2, 4, 6, 8 and 10 ppm Na solutions are maintained and readings are taken on the flame photometer.

Procedure

1. Weigh 5 g of 2 mm sieved soil sample in 250 ml plastic conical flask.
2. Add 25 ml of the neutral 1N ammonium acetate solution and shake for 30 minutes on mechanical shaker at 110 rpm. Filter through whatman No. 1 filter paper.
3. Take the readings on flame photometer.
4. Feed the working standard solution and prepare a standard curve.
5. If the sample reading is not found within the standard reading range, in that case, the appropriate dilution of the filtrate may be made to bring the reading within standard range.

Available Sulphur

Reagents

- Mono-calcium phosphate extracting solution (500 mg P/litre): Dissolve 2.035 g of $Ca(H_2PO_4)_2.H_2O$ in 1 litre of water.
- Gum acacia-acetic acid solution: Dissolve 5g of chemically pure gum acacia powder in 500 ml of hot water and filter in hot condition through Whatman No.42 filter paper. Cool and dilute to one litre with dilute acetic acid.

- Barium chloride: Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use. • Standard stock solution (2000 mg S/litre): Dissolve 10.89g of oven-dried AR grade potassium sulphate in 1 litre water.
- Standard working solution (10 mg S/litre): Measure exactly 2.5 ml of the stock solution and dilute to 500 ml.
- Barium sulphate seed suspension: Dissolve 18 g of AR grade $BaCl_2$ in 44 ml of hot water and add 0.5 ml of the standard stock solution. Heat the content to boiling and then cool quickly. Add 4 ml of gum acacia-acetic acid solution to it. Prepare a fresh seed suspension for estimation everyday.
- Dilute nitric acid (approx 25%): Dilute 250 ml of AR grade conc. HNO_3 to one litre.
- Acetic-phosphoric acid: Mix 900 ml of AR grade glacial acetic acid with 300 ml of H_3PO_4 (AR grade)

Procedure

1. Weigh 20 g of soil sample in a 250 ml conical flask. Add 100 ml of the monocalcium phosphate extracting solution (500 mg P/litre) and shake for one hour. Filter through Whatman No.42 filter paper.
2. Take 10 ml of the clear filtrate into a 25 ml volumetric flask.
3. Add 2.5 ml of 25% HNO_3 and 2 ml of acetic-phosphoric acid. Dilute to about 22 ml, stopper the flask and shake well, if required.
4. Shake the $BaSO_4$ seed suspension and then add 0.5 ml of it, and 0.2 g of $BaCl_2$ crystals. Stopper the flask and invert three times and keep.
5. After 10 minutes, invert 10 times. Again after 5 minutes invert for 5 times.
6. Allow to stand for 15 minutes and then add 1 ml of gum acacia-acetic acid solution.
7. Make up the volume to 25 ml, invert 3 times and keep aside for 90 minutes.
8. Invert 10 times and measure the turbidity intensity at 440 nm (blue filter).
9. Run a blank side by side.
10. Preparation of standard curve:
11. Put 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ml of the working standard solution (10 mg S/litre) into a series of 25 ml volumetric flasks to obtain 25, 50, 75, 100, 125 and 150 µg S.

12. Proceed to develop turbidity as described above for sample aliquots.
13. Read the turbidity intensity and prepare the curve by plotting readings against sulphur concentrations (in µg in the final volume of 25 ml).

Calculation

$$Available\ sulphur\ in\ soil\ (\text{mg/kg}) = \frac{W * 100}{10 * 20} = \frac{W}{2} \qquad \text{…[Equation 19]}$$

Where, W stands for the quantity of sulphur in mg as obtained on X-axis against an absorbance reading (Y-axis) on standard curve

DTPA Extraction for Fe, Zn, Cu, Mn

Reagents

- Deionized water
- Diethylenetriaminepentaacetic acid (DTPA)
- Triethanolamine (TEA)
- Calcium chloride ($CaCl_2$)
- HCl (12M)
- ICP or ICP/MS grade 1000 ppm Zn, Fe, Cu, Mn and B standards dissolved in HCl or nitric acid
- DTPA extractant composition
- 0.005M DTPA
- 0.01M calcium chloride ($CaCl_2$)
- 0.1M TCA (triethanolamine, $(HOCH_2CH_2)_3N$))

To prepare 20L of extract:

- Carboy calibration: On a digital balance, weigh 15,000 and 20,000 g (0.0 g) deionized water (diH_2O) into a 20 L carboy. Draw a mark on the carboy at the water level and label 15L and 20 L, respectively.
- Place a 1.5” stir bar into the calibrated carboy. Add diH_2O into the carboy to the 15L mark.
- Using a digital balance, weigh 39.89 g (0.00 g) diethylenetriaminepentaacetic acid (DTPA) and add to the carboy. Weigh 29.44 g (0.00 g) calcium chloride

($CaCl_2$) and add to the carboy. Measure 265 mL triethanolamine (TEA) with a 500 mL graduated cylinder and add it to the carboy. Measure 85 mL HCl (12M) with a 100 mL graduated cylinder and add it to the carboy.

- Add diH_2O to bring the volume to the 20 L mark.
- Place carboy on a magnetic stirrer and stir overnight (minimum of 8 hr) at a speed of ~600 rpm.
- Measure pH of the extractant. Adjust the pH to 7.3 (repeat pH measurement 30 minutes of mixing). Add 9 mL HCl (12M) per each pH unit over 7.3. Add 9 mL TEA per each pH unit under 7.3

Standard Preparation

1. All standards should be made from NIST traceable ICP/ICP-MS grade primary standards. The following four or five multi-element standards and associated blank-matrix solution will provide a wide adequate elemental concentration ranges for most soils including heavily manured and garden soils.
2. Fill a 1000 mL Class A volumetric flask with about 250 mL of the DTPA or DTPA-Sorbital Extractant
3. Using Class A pipettes, transfer the appropriate volume of each primary standard to the volumetric flask.
4. Bring to volume with the extraction Solution. Cap flask and invert and shake 20 times. Transfer standard to labelled polypropylene or similar sealable containers.

Procedure

Extraction

1. Either gravimetric or volumetric sampling maybe used as long as a 1:2 ratio of soil to extractant is maintained.
2. Ensure clean and dry extracting cups or flasks are available.
3. Label extraction containers with appropriate laboratory number or other sample information.
4. Weigh or transfer 20 g of soil to appropriate extraction cup or flask. Laboratories that primarily process light textured soils may elect to use 10 gram

sample, however, these laboratories must ensure adequate filtrate for ICP determination.

5. Dispense 40 mL of the DTPA or DTPA-Sorbital extracting solution into each sample cup using the appropriate calibrated dispenser.
6. Shake the samples for 2 hr using the 200 rpm shaker table

Filtration

1. Following the two hour shaking period, decant samples into appropriate filters/filter assemblies using medium-fine (Whatman No. 2 equiv.) porosity filter paper.
2. Transfer filtrate to number/referenced test tubes for ICP analyses.

Analysis

Place standards and samples in appropriate order on to ICP sample racks. Calibrate and standardize ICP and method as appropriate per laboratory QA/QC protocols and manufacturer's recommendations.

Calculations

The dilution factor for this method is 2. Normal reporting for micronutrient concentrations is in parts per million (ppm or mg/kg) on the soil basis. Therefore, the final ICP analysis result of the extract is multiplied by 2 to provide mg/kg of soil.

3.4.5. Fluoride concentration in crop plants

Bio-concentration factor (BCF)

It is a common parameter for estimating the F concentration in vegetables and subsequently human exposure through consumption of vegetables which is defined as the ratio between the concentration of F in the edible part of the vegetable and F concentration in soil (Jha, Nayak, and Sharma 2011).

$$\mathbf{BCF} = \frac{\mathbf{Fluoride\ concentration\ in\ vegetation}}{\mathbf{Fluoride\ concentration\ in\ soil}} \quad \text{…[Equation 20]}$$

Estimated Daily Intake analysis

The exposure doses of fluoride in terms of estimated daily intake (EDI) in population due to the consumption of edible part (fruit) of *T. aestivum and P. typhoides* were calculated on average fluoride concentration found in crops during this study by using generic equation (USEPA 1992).

$$EDI = \frac{C \times IR \times EF \times ED \times AF \times CF}{BW \times AT} \quad \text{…[Equation 21]}$$

Where,

C = Concentration of F in vegetables (mg/kg)
IR Ingestion or Intake Rate (mg/day)
EF Exposure frequency (days/year)
ED Exposure duration (year)
AF Absorption Factor (unitless)
CF Conversion Factor (10^{-6}kg/mg)
BW Body weight (kg)
AT Averaging time (days)

Part II Fluoride removal by adsorbent

3.5. Defluoridation Study

3.5.1. Adsorbent study

Preparation of Adsorbent

The ripened Tamarindus indica fruit were collected from the tree situated in Malekhpur villages of Kheralu taluka, Mehsana District, Gujarat. The seeds were separated from the pulp manually. Thereafter these seeds were thoroughly rinsed with distilled water to remove remaining pulp and other impurities, and were dried at room temperature. Finally the dried seed were grounded to a fine powder in a local flour mill. The resulting material was used as an adsorbent for adsorption studies.

Characterization of adsorbent

Zero Potential Charge

The point of zero charge of the adsorbent was determined by the solid addition method (Freundlich, 1906). A 50ml of 0.1M KNO_3 solution transferred into a series of 100 ml conical flask. The initial pH (p_0^H) values of the solution was adjusted from 1.0 to 12 by adding either 0.05N HNO_3 or 0.1N KOH. Then 0.5 g of TISP was added to each flask which was securely capped immediately. The flasks were then placed into a constant temperature water bath shaker and shaken for 24 h. The pH values of the supernatant liquid were noted after 24 h. The change of pH were noted down. The point where no pH change is observed is considered as Zero potential charge for material.

Fluoride analysis

As explained in 3.4.3

Material study

The crude biochemical of seeds viz: Carbohydrates (by cold water extraction of polysaccharide; Khuller's method), proteins (by extracting soluble protein in alkaline NaOH; Alkaline method) and lipids (by soxhlet extraction with ether; AOAC method) of

seed was extracted. These extracts along with the residual material were utilized for defluoridation study.

Instrument study

FT-IR spectroscopy

The FTIR spectrum of TISP before and after the adsorption of fluoride metal was recorded at the Department of Applied Chemistry, The M.S. University Baroda. The spectra of samples (in the forms of KBr pellets) were obtained with a frequency range of 4000–400 cm^{-1}.

XRD

X-Ray Diffraction Studies of TISP before and after the adsorption of fluoride metal was recorded at Department of Metallurgical and Materials Engineering, The M. S. University of Baroda. X-ray powder diffraction data studies were carried out with X-rays of 40kV/30mA in the continuous scanning 2Theta/Theta mode.

3.5.2. Batch model studies

Batch adsorption experiments were carried out by shaking a known quantity of the adsorbents (TISP), along with the fluoride ion solution taken in conical flasks, optimizing each parameter taken into consideration to maximum possible removal of fluoride ion.

The solutions after adsorption were filtered using the cloth prior to analysis, to remove the adsorbent from the solution mixture. The concentration of metal ions in the filtrate was analysed using ion selective electrode. Each determination was repeated thrice, and the result obtained is the average of the values. The data obtained in these batch studies were used to calculate the percentage removal of the fluoride ions, by using the following mass balance relationship:

$$\%\, removal = \left(\frac{C_0 - C_e}{C_0}\right) * 100 \qquad \text{…[Equation 22]}$$

Where C_0 and C_e are the initial and equilibrium concentrations (mg/L) of the fluoride ion solution respectively.

Effect of Shaker speed

To find out the effect of shaker speed required for the removal of fluoride ion experiments were carried out. 100mL of solutions 10 mg/l fluoride ion solution containing 0.5 g *T. indica* seed powder were rotated at speed ranging from 75 to 300 rpm. After defined period of agitation, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case.

Effect of pH

To find out the optimum pH for maximum removal fluoride ion experiments were carried out by varying the pH of the aqueous fluoride solutions over the range 1.0 to 12.0 ± 0.2. After the optimum equilibration time of agitation, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

Effect of Particle size of TISP powder

To find out the effect of particle size required for the removal of fluoride ion, experiments were carried out using 100mL of fluoride ion solutions of concentration 10 mg/L containing 0.5 g *T. indica* seed powder at pH 7.0 ± 0.2 for ranging particle size from 0-50, 50-75, 75-150, 150-250, 250-500 micron sizes. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed and adsorption capacity in each case was calculated.

Effect of adsorbent dose

To find out the effect of dosage required for the removal of fluoride ion experiments were carried out using 100mL of fluoride ion solutions of concentration 10 mg/L containing *T. indica* seed powder ranging from 0.025g to 0.2g per 100ml at pH 7.0 ± 0.2. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

Effect of agitation time

To find out the effect of equilibrium time required for the removal of fluoride ion experiments were carried out using 100mL of fluoride ion solutions of concentration 10

mg/L containing 0.5 g *T. indica* seed powder at pH 7.0 ± 0.2 for ranging periods from 30 to 360 minutes. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

Effect of initial fluoride concentration

To find out the effect of equilibrium time required for the removal of fluoride ion experiments were carried out using 100mL of fluoride ion solutions of concentration 10 mg/L containing 0.5 g *T. indica* seed powder at pHt 7.0 ± 0.2 for ranging periods from 30 to 360 minutes. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

Effect of temperature

To find out the effect of temperature required for the removal of fluoride ion experiments were carried out using 100mL of fluoride ion solutions of concentration 10 mg/L containing 0.5 g *T. indica* seed powder at P^H 7.0 ± 0.2 for temperature of 25°C, 35°C and 45°C . After defined period of agitaton time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

Effect of co-ions

To find out the effect of co-ions interference in removal of fluoride ion experiments were carried out using 100ml of fluoride solution of along with various co-ions (like sulphate, phosphate, nitrate, chloride, bicarbonates) of concentration ranging from 20 mg/l to 100mg/l of containing 0.5 g *T. indica* seed powder at P^H 7.0 ± 0.2. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage of fluoride ion removed in each case was calculated.

3.5.3. Adsorption isotherm

The capacity of adsorption isotherm provides a panorama of the course taken by the system under study in a concise form, indicating how efficiently an adsorbent will adsorb and allows an estimate of the economic viability of the adsorbents commercial

applications for the specified solute. Sorption isotherms usually describe the equilibrium relation between sorbent and sorbate. They give the equilibrium relationship between the quantity of metal sorbed, and that remaining in aqueous solution at a fixed temperature. By plotting solid phase concentration against liquid phase concentration, it is possible to predict the equilibrium isotherm. The isotherm thus yields certain constants whose values express the surface properties and affinity of the sorbent.

Langmuir isotherm

The Langmuir isotherm (Langmuir, 1918) was derived originally to study gas adsorption on activated carbon but lately has been successfully applied to study many metal adsorptions. The basic assumption of Langmuir theory suggests homogenous uptake of metal ion onto the monolayer of adsorbent with no further interaction between adsorbed ions. In addition, the model assumes uniform energies of adsorption onto the surface and no transmigration of the adsorbate. The non-linear form of equation for Langmuir adsorption isotherm is of the form:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad \text{…[Equation 23]}$$

where q_e is the equilibrium metal concentration adsorbed per unit weight of adsorbent; C_e is the residual metal concentration in the solution; q_m is the maximum specific uptake corresponding to sites saturation and K_L is Langmuir constant. The Equation can be linearized as follow:

$$\frac{1}{q_e} = \left(\frac{1}{q_m K_L}\right)\left(\frac{1}{C_e}\right) + \frac{1}{q_m} \quad \text{…[Equation 24]}$$

The constants q_m and K_L in the Langmuir isotherms can be determined by plotting $\frac{1}{q_e}$ vs $\frac{1}{q_m}$ from above equation. In addition to this the essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor R_L, which is given by the following equation is given by the following equation

$$R_L = \frac{1}{1 + K_L C_o} \quad \text{…[Equation 25]}$$

where C_o (mg/L) is the initial concentrations of fluoride in aqueous solution, and K_L (L/mg) is the Langmuir constant.

Freundlich isotherm

The Freundlich isotherm model is the well-known earliest relationship describing the adsorption process. This model applies to adsorption on heterogeneous surfaces with the interaction between the adsorbed molecules, and the application of the Freundlich equation also suggests that sorption energy exponentially decreases on completion of the sorptional centers of an adsorbent. This isotherm is an empirical equation, and can be employed to describe heterogeneous systems and is expressed as follows:

$$Q_e = K_F C_e^{1/n} \quad \text{…[Equation 26]}$$

The equilibrium can be linearized by taking logarithms to find the parameters KF and n,

$$\ln Q_e = \mathrm{Ln}K_F + \frac{1}{n}\ln C_e \quad \text{…[Equation 27]}$$

The values of KF and n are calculated from the slope and intercept of the plot. Where K_F is the Freundlich constant ((mg/g) (l/mg) (1/n)) related to the bonding energy. 1/n is the heterogeneity factor and n (g/L) is a measure of the deviation of adsorption from linearity. This value indicates the degree of nonlinearity between the solution concentration and adsorption as follows: when n = 1, adsorption is linear; n < 1, adsorption is a chemical process; n > 1, adsorption is a physical process.

Dubinin–Radushkevich isotherm model

Dubinin and Radushkevich equation (Hall, Eagleton, Acrivos and Vermeulen, 1996) is represented in a linear form by equation.

$$q_e = q_m e^{-\beta\varepsilon^2} \quad \text{…[Equation 28]}$$

Where q_m is the Dubinin-Radushkevich monolayer capacity (mg/g), β is a constant related to the adsorption energy, and ε is the Polanyi potential which is related to the equilibrium concentration as follows:

$$\varepsilon = RTln\left(1 + \frac{1}{C_e}\right) \quad \text{…[Equation 29]}$$

Where, R is the gas constant (8.314 J/mol.K) and T is the absolute temperature (K). The constant β gives the mean free energy, E, of the adsorption per molecule of the adsorbate,

when it is transferred to the surface of the solid from infinity in the solution, and can be computed using the relationship:

$$E = \frac{1}{\sqrt{2\beta}} \quad \text{…[Equation 30]}$$

The final form of the Dubinin-Radushkevich isotherm is expressed as follows:

$$q_e = q_m e^{-\beta\left(RTln\left(1+\frac{1}{C_e}\right)^2\right)} \quad \text{…[Equation 31]}$$

The Dubinin-Radushkevich isotherm constants, the monolayer capacity q_m and adsorption energy (β) were calculated from the plot of qe versus Ce.

When one mole of an ion is transferred to the adsorbent surface, and the E value is less than 8 kJ/mol, it indicates physical adsorption (Rieman and Walton 1970). When E is between 8 and 16 kJ/mol, it indicates ion-exchange (Helfferich 1962), and when E is between 20 and 40 kJ/mol, it indicates chemisorption (Rieman and Walton 1970).

3.5.4. Kinetics study

Predicting the rate at which adsorption takes place for a given system is probably the most important factor in adsorption system design, with adsorbate residence time and the reactor dimensions controlled by the system's kinetics. A number of adsorption processes for pollutants have been studied in an attempt to find a suitable explanation for the mechanisms and kinetics for sorting out environment solutions. In order to investigate the mechanisms of adsorption, various kinetic models have been suggested. In recent years, adsorption mechanisms involving kinetics-based models have been reported. In order to investigate the adsorption processes of onto AHC four kinetic models were used.

The pseudo-first-order model

The pseudo-first order model assumes that the rate of change of solute uptake with time is directly proportional to difference in saturation concentration and the amount of solid uptake with time. It was the earliest equation describing the adsorption rate based on the adsorption capacity. The differential form of the equation is given by Lagergren (1898),

$$\frac{dq}{dt} = K_i'(q_e - q_t) \quad \text{…[Equation 32]}$$

where qe and qt are the amounts of dye adsorbed (mg/g) on adsorbent at equilibrium time and at various time t (min) respectively and kf is the rate constant of pseudo-first order kinetics (1/min). Integrating above equation for the boundary conditions.

$$\log(q_e - q_t) = log q_e - \frac{K_1 \mathrm{t}}{2.303} \quad \text{…[Equation 33]}$$

This is the linear form of the pseudo-first order model.

The pseudo second-order model

The sorption kinetics following pseudo-second order model was given by Ho and McKay (1997). The various assumptions of Ho pseudo-second order model are

- The energy of adsorption for each ion is the same and independent of surface coverage
- The sorption occurs only on localized sites and involves no interactions between adsorbed ions
- The rate of adsorption is almost negligible in comparison with the initial rate of adsorption

The differential equation has the following form:

$$q_t = \frac{q_e^2 K_2' t}{1 + q_e K t} \quad \text{…[Equation 34]}$$

where k_s is the rate constant of the pseudo-second order kinetics (g/mg/min). The linear form of the pseudo-second order model is given by:

$$\frac{t}{q_t} = \frac{1}{(K_2 {q_e}^2)} + \frac{t}{q_e} \quad \text{…[Equation 35]}$$

The equilibrium adsorption capacity (q_e) and the pseudo second-order constant, k_2 for the metals under study for the adsorption by AHC can be determined experimentally from the slope and the intercept of plot of t/q_t versus time.

Intraparticle diffusion model

Weber and Morris (1963) introduced a simpler expression to obtain the diffusion rate coefficient, k_i

$$q_t = k_i * t^{0.5} \quad \text{…[Equation 36]}$$

The significant feature of this expression is that the linear plots of q_t vs. $t^{0.5}$ should pass through the origin (zero intercept). Thus the intra-particle diffusion model can be easily tested through the above plots provided they have zero intercept, which indicates a controlling influence for the diffusion process on the kinetics. The rate coefficient, k_i (mg/g/min$^{0.5}$) could be obtained from the slope of the plots.

Liquid film diffusion model

When the flow of the reactant through the liquid film surrounding the adsorbent particles is the slowest process determining kinetics of the rate processes, the liquid film diffusion model Boyd *et al.* (1947) given by the simple relation,

$$\ln(1 - F) = -K_{fd}t \quad \text{…[Equation 37]}$$

Where F is the fractional attainment of equilibrium (=q_t/q_e) and K_{fd} (min^{-1}) is the film diffusion rate coefficient.

A linear plot of −ln (1−F) vs. t with zero intercept suggests that the kinetics of the adsorption process is controlled by diffusion through the liquid film.

3.6. Defluoridation study

Batch Defluoridation studies were carried out with best optimized model in groundwater samples. The pre-treatment and post-treatment fluoride was analysed. In addition to fluoride various parameters like pH, TA and TH was measured in order to understand the effect of bio-adsorption on to groundwater.