CHAPTER 2

MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 Survey and selection of groundwater samples from North Gujarat and Saurashtra

2.1.1 Selection of the Districts from the study area and mapping of Fluoride in groundwater

In India, high Fluoride concentrations in groundwater (>1.5 mg/l) occur in 14 Indian states, namely Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamilnadu, Uttar Pradesh and West Bengal affecting a total of 69 districts, according to some estimates. In Gujarat, high Fluoride contamination is observed in some districts like, Amreli, Rajkot, Jamnagar, Sabarkantha, Banaskantha, Mehsana, and Patan. Base data on ground water samples was collected from Gujarat Water Supply and Sewerage Board (2009-12) for Mehsana, Patan, Jamnagar, Amreli, and Rajkot districts.

Based on the collected data from GWSSB, three districts were selected namely Mehsana, Amreli and Rajkot. From each district, one taluka with maximum Fluoride concentration was selected for further study. Fluoride content in 30 ground water samples of each taluka was estimated using Fluoride ion selective electrode (Orion ion meter-Model 920 –A).

2.1.1.1 Groundwater sample collection

The Sampling procedure was employed as per the standard methods prescribed by APHA (1995-1998). The groundwater samples were collected for analysis of Fluoride. Groundwater samples were collected from different bore well, tube well and well. Random stratified sampling technique was selected for selection of bore well, tube well and well.

The water was pumped out of bore well and tube well using submersible motors. After pumping for about 1 min. the water was collected in pre-cleaned, sterilized polyethylene bottles of one liter capacity. The samples were preserved at temperature

below 10°C in bigger container. The samples immediately dispatched to the laboratory for Fluoride analysis.

2.1.1.2 Fluoride analysis

Analysis : (using ion selective electrode, Orion ion meter-Model 920 –A):

Principle

When the Fluoride electrode is dipped in sample which concentration is to be measured, a potential is established by the presence of Fluoride ions by any modern pH meter having an expanded milivolt scale.

The Fluoride ion selective electrode can be used to measure the activity or concentration of Fluoride in aqueous sample by use of an appropriate calibration curve. However, Fluoride activity depends on the total ionic strength of the sample. The electrode does not respond to bound or complexed Fluoride. Addition of a buffer solution of high total ionic strength containing a chelate of complex aluminium preferentiality overcomes these difficulties.

Apparatus and equipment

a. Ion meter (field/laboratory mode) or pH/mV meter for precision laboratory measurements

b. Reference electrode (calomel electrode)

c. Fluoride-sensitive electrodes

d. Magnetic stirrer

Reagents and standards

a. Stock Fluoride solution: dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100µgF^-

b. Standard Fluoride solution: Dilute stock solution 10 times with distilled water to obtain 1mL = 10µg F^-

c. Total Ionic Strength Adjustment Buffer (TISAB): place approximately 500mL distilled water in a 1L beaker, add 57mL glacial acetic acid, 58g, NaCl and 4g 1, 2-cyclohexylenediamine tetraacetic acid. Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125mL) with stirring, until pH is between 5 to 5.5. Transfer to a 1L volumetric flask and make 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 (59.16 mV for monovalent ions and 29.58 mV for divalent ions at 25°C)

Procedure

1. Calibrate the instrument as explained above.
2. Transfer 50 to 100mL of sample to a 150mL plastic beaker. (Check pH of solution if above or below 7.0 neutralize it using acid or base)
3. Add 5 ml of TISAB solution to the sample and stir thoroughly.
4. Rinse electrode, blot dry and place in the sample. Stir thoroughly and note down the steady reading on the meter.
5. After use place electrode in 1 ppm solution.

Calculation

The concentration in mg/l is obtained directly from the specific ion meter.

2.2 Bioremoval of Fluoride

2.2.1 Screening of Plant materials, checking Fluoride removal capacity and selection of suitable bioadsorbent

In-vitro experiments were carried out to check Fluoride removal efficiency of different plant materials. These were *Moringa oleifera* L., *Cocos nucifera* L. and *Oryza sativa* L. were tried. For *Moringa oleifera* - Bark, *Moringa oleifera* – Seed, *Cocos nucifera* - Shell, *Cocos nucifera* - Mature Fruit Fiber and *Oryza sativa* – Husk were used. Initial Fluoride concentration was kept constant; i.e. 1.5 mg/l and final concentration was determined after incorporating the plant material. Volume of initial Fluoride concentration for each experiment was kept constant; i.e.150 ml. Standard dose of each plant part was kept constant i.e. 0.75 g and all experiments were carried out for 4 hours time duration. In these experiments, air dried, sun dried and oven dried plant part were used. In air dried method, plant part was kept as such for 24 hours for air drying process. For sun dried treatment, plant parts were kept in sun light for 6-7 days for drying process. Plant parts were kept for 24 hours at 80 °C in oven for oven dried treatment. Out of the tested biomaterials *Moringa oleifera* L. (bark) and *Moringa oleifera* L. (seed) were found to give satisfactory results. Therefore, to check bioremoval capacity of these two plant materials towards Fluoride removal in Groundwater samples as such, they were selected for further study.

2.2.1.1 Preparation of Adsorbent

The ripened *Moringa oleifera* L. fruits were collected and seeds were separated. Seeds were dried at sunlight for one day. Bark of *Moringa oleifera* L. was collected and sundried for 6-7 days. Both sundried bark and seed were grounded to a powder by motor and pastel. The resulting material was used as an adsorbent for adsorption studies.

2.2.2 In-vitro Optimization Study

Batch optimization study using *Moringa oleifera* bark powder (MBP) and seed powder (MSP)

Batch adsorption experiments were carried out by taking a known quantity of the adsorbents (MBP and MSP), along with the Fluoride ion solution, optimizing parameter (contact time, adsorbent dose, initial Fluoride ion concentration) taken into consideration for maximum possible removal of Fluoride ion.

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

$$\% \text{ removal} = \left(\frac{C_0 - C_e}{C_e}\right) \text{X } 100$$

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

Effect of adsorbent dose

To find out the effect of dosage required for the removal of Fluoride ion, experiments were carried out using 100 ml Fluoride ion solutions of concentration ranging from 1-5 mg/l containing *Moringa oleifera* bark and seed powder ranging from 0.12 g to 1.0 g for agitation time ranging from 120 to 600 minutes. At the end of the agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage Fluoride ion removal.

Effect of agitation time

To find out the effect of equilibrium time required for the removal of Fluoride ion experiments were carried out using 100 ml of Fluoride ion solutions of concentration ranging from 1-5 mg/l containing 0.12 g to 1.0 g *Moringa oleifera* bark and seed powder for agitation time period from 120 to 600 minutes. At the end of agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage Fluoride ion removal.

Effect of initial Fluoride ion concentration

To find out the effect of initial Fluoride ion concentration for the removal of Fluoride ion experiments were carried out using 100 ml of Fluoride ion solutions of concentration ranging from 1-5 mg/l containing *Moringa oleifera* bark and seed powder ranging from 0.12 g to 1.0 g for agitation time period from 120 to 600 minutes. At the end of agitation time, the solutions were allowed to settle and supernatant was collected for analysis of percentage Fluoride ion removal.

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 sorbet. They give the equilibrium relationship between the quantity of metal adsorbed 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 it 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 :

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

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:

$$\frac{1}{q_e} = \left(\frac{1}{q_m K_L}\right)\left(\frac{1}{C_e}\right) + \frac{1}{q_m}$$

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.

$$R_L = \frac{1}{1 + K_L C_o}$$

Where C_o (mg/l) is the initial concentration 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:

$$Q_e = K_F C_e^{1/n}$$

The equilibrium can be linearized by taking logarithms to find the parameters K_F and n.

$$\ln Q_e = \mathrm{Ln}K_F + \frac{1}{n} \ln C_e$$

The values of K_F 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 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.

2.2.3 Physicochemical characterization of groundwater samples with special reference to Fluoride

For in vivo bioremoval study, 30 groundwater samples (well/ tube well/ bore well) from 30 villages from each taluka i.e. Satlasana, Lilia and Wankaner were collected seasonally (pre and post monsoon) and were analyzed for potable parameters as well as Fluoride content which followed the methods described in APHA-AWWA-WPCB, 1998 .Out of these 30 groundwater samples, five samples were selected randomly from each taluka for correlation analysis and piper diagram.

The villages selected from Satlasana taluka were Dharoi, Mota Kothasana, Dharavania, Dholu and Vajapur. From Lilia taluka, Punjapadar, Putaliya, Sanaliya, Eklera and Timbri were selected and Satapar, Jodhapar, Garida, Shekharadi and Amarsar were selected from Wankaner Taluka.

pH

Principle:

The pH stands for "potential of hydrogen". It is the measure of intensity of acidity and alkalinity and the concentration of hydrogen ions in water. The pH value is expressed as the negative logarithm of the hydrogen ion concentration.

The electrometric determination of pH by a pH meter is based on measuring the e.m.f. (milivolts) of a pH cell both a reference buffer and then with a test solution. The change in the potential difference at 25°C for 1 pH unit is 59.1 mV. pH of water is measured on a scale of 0 to14. This scale is actually indicates that the water is pure (neutral) or acidic or alkaline.

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 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 – $Na_2HPO_{4.2}H_2O$) 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.

- Apparatus:

i. pH meter with glass electrodes

ii. Thermometer

iii. Glass beaker (100 ml)

iv. Glass rod

Procedure :

1. Turn the pH meter on and allow it to warm for 15 minutes.
2. Standardize the glass electrode using 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 Alkalinity (By Titrimetric method)

Principle:

Alkalinity is the measure of the buffering capacity of water or the capacity of bases to neutralize the strong acids and characterized by the presence of hydroxyl ion capable of combining hydrogen ion. It does not refer to pH but instead refers to the ability of water to resist change in pH. The buffering materials are primary the Bicarbonates and Carbonates.

Total alkalinity was estimated as carbonate and bicarbonate alkalinity (also called as phenolphthalein and methyl orange alkalinity respectively) and addition of both provide total alkalinity.

Total alkalinity (mg/l) = phenolphthalein alkalinity + Methyl orange alkalinity

Carbonate and Bicarbonate ions in the sample can be determined by titrating it with against standard sulphuric acid (H_2SO_4) using phenolphthalein and methyl orange as indicators. Addition of phenolphthalein gives pink red colour in the presence of Carbonates and titration with H_2SO_4 converts these CO_3^- into HCO_3^- and decolourises the red colour as shown below.

$$2\ Na_2CO_3 + H_2SO_4 \rightarrow 2\ NaHCO_3 + Na_2SO_4$$

Thus the Carbonates neutralization is only half way. These Carbonates along with the already present ones are then determined by continuing the titration using methyl orange indicator which gives yellow colour in presence of Bicarbonates. On complete neutralization of Bicarbonates the yellow colour will change to red.

$$2\ NaHCO_3 + H_2SO_4 \rightarrow Na_2CO_3 + 2H_2O + 2CO_2$$

Obviously the Bicarbonate titer value will be less if Carbonates were not present (absence of pink colour). In such a situation, either the same aliquot is used for Bicarbonate titration or a fresh sample is analyzed for this. If Carbonates are present and neutralized, the volume of H_2SO_4 used in the first phase (carbonate titration) is to be doubled to get the actual volume needed for complete neutralization of the Carbonates.

Chemicals and reagents

i. 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 stock solution was added into 980 ml of water. This would give the working solution of 0.02N H_2SO_4.

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

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

Procedure

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

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

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

iii. (a) No titration is required

(b) In burette (0.02N) H_2SO_4 was taken and was titrated with the pink colour solution.

iv. 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.

$$\text{Phenolphthalein alkalinity } (mg/l) = \frac{A * 1000}{V}$$

$$\text{Total alkalinity } (mg/l) = \frac{T * 1000}{V}$$

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 Total alkalinity			

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

$$\text{mg } CO_3^{-2}\text{ /L} = \text{Carbonate alkalinity mg } CaCO_3\text{/L x } 0.6$$

$$\text{mg } HCO_3^-\text{/L} = \text{Bicarbonate alkalinity mg } CaCO_3\text{/L x } 1.22$$

Total dissolved solids

Principle:

The filterable residue is the material that passes through a standard glass filter disk and remains after evaporation and drying at 180°C.

Apparatus and equipment

a. Evaporatory dish (porcelain) – 100/200mL

b. Drying oven – equipped with thermostatic control capable of maintaining the temperature within 2°C range.

c. Desiccator – provided with desiccants

d. Analytical balance – 200mg capacity of weighing to 0.1mg

e. Filter holder – Gooch crucible adapter or membrane filters

f. Suction flask – 500mL capacity

Procedure

1. Weigh dried evaporating dish.

2. Filter the well-mixed sample under vacuum through 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 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

$$\text{Total dissolved solids } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{A} - \text{B} * 1000}{\text{C}}$$

Where:

A = weight of dried residue + dish

B = weight of dish

C = mL of filtrate used

Total Hardness (Calcium and Magnesium)

Principle

In general practice the hardness is measured as the concentration of only Calcium and Magnesium (as $CaCO_3$) which are far high in concentration over other cations. The most common method of Calcium and Magnesium determination in irrigation water is by complexometric titration using sodium salt of ethylene-diamine tetra acetic acid. (EDTA).

Ethylene diamine tetra acetic acid (EDTA) form soluble complexes with calcium and magnesium ions at an optimum pH of 10.0 and thus removing them from solution without precipitation. The reaction is stoichiometric and essentially instantaneous at temperature near 60°C and the complexes formed are very stable. At the same pH the dye erichrome blue-black B has a turquoise blue colour in the absence of Calcium and Magnesium ions but forms red compounds with them which are less stable than the EDTA-Ca and EDTA-Mg complexes. The formation of Ca and Mg complexes at pH 10.0 is achieved by using ammonium hydroxide-ammonium chloride buffer.

A number of polyvalent ions are preferably complexed by EDTA as these are less dissociated than those of Ca and Mg and thus included in the titration. Fortunately, the concentration of such interfering metals e.g. Fe, Cu, Pb, Cd, Zn, Co and Mn is quite low and negligible in most waters and can be ignored. However, the interference, if high, can be prevented by using 2% solution of NaCl. If the sample is made strongly alkaline (pH about 12.0), Magnesium is selectively precipitated as magnesium hydroxide. At the same pH Patton and Reeder's indicator / ammonium purpurate (murexide) forms a red compound with Calcium ions but is not affected by Magnesium present as magnesium hydroxide. If EDTA is then closely added, the Calcium ions are gradually transferred from the dye complex to the more stable EDTA complex until when all have been transferred, the liquid acquired a pure

turquoise blue colour. The reaction is virtually instantaneous at normal room temperature.

Chemical and Reagents:

1. Ammonia Buffer Solution: 13.5 g of NH_4Cl was dissolved in 114ml of NH_4OH. Then total volume was made 200 ml by adding distilled water.
2. Eriochrome Black-T (indicator): The indicator was prepared by dissolving 0.5g of EB-Tin 100ml of 80% ethyl alcohol.
3. 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.
4. Murexide indicator (ammonium purpurate) : 0.2g of ammonium purpurate and 100g of sodium chloride were mixed and grinded thoroughly to form 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:

1. Take 100 ml of sample in conical flask
2. 1ml of NaOH was added to the above solution to raise pH to 12.0

3. 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".

Calculation:

$$\text{Total hardness } (mg/L) = \frac{\text{T} * 1000}{V}$$

Where,

T and A = volume of titrant

V = volume of sample

$$\text{Calcium hardness } (\text{mg/l as } CaCO_3) = \frac{\text{A}*1000*1.05}{\text{V}}$$

$$\text{Calcium } (\text{mg/l as } CaCO\ ) = \frac{\text{A}*400*1.05}{\text{V}}$$

$$\text{Magnesium harndess } (\text{mg/l as } CaCO_3 = \text{TH} - \text{Ca(H)}$$

$$\text{Magnesium } (\text{mg/l as } CaCO_3) = \text{Mg hardness} * 0.2431$$

Sulphate

Principle

The turbidimetric methods are used successfully for drinking the ground and surface water. Sulphate ion is precipitated in an acetic acid medium by adding barium chloride. This is the most routine and lesser time consuming method of sulphate estimation.

Chemicals and Reagent

i. NaCl-HCl solution: 240 g of NaCl in a little distilled water was dissolved and 20ml of HCl was added to it and diluted with more of distilled water to make volume 1 litre.

ii. Glycerol-ethanol solution: 50ml of Glycerol was added to 100ml of ethyl alcohol and was shaken well.

iii. Barium chloride(dry crystal)

iv. Standard sulphate solutions: 0.147 g of anhydrous sodium sulphate was dissolved in distilled water to make the volume 1 litre. The solutions contain 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 filtrate was taken in 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 spectrophotometer.
4. In 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.

Sodium

Principle:

Trace amounts of Sodium can be determined by flame emission photometry at the wavelength of 589 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at 589 nm is approximately proportional to the concentration of the element. If alignment of the wavelength dial with the prism is not precise in the available photometer, the exact wavelength setting, which may be slightly more or less than 589 nm, can be determined from the maximum needle deflection and then used for the emission measurements. The calibration curve may be linear but has a tendency to levels off at higher concentrations.

Apparatus:

i. Flame photometer

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 0.45µm 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 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. Determine sodium concentration of sample from the calibration curve. Where a large number of samples must be run routinely, the calibration curve provides sufficient accuracy.

Calculation

Na (mg/l) = (Na (mg/l) in portion) x dilution factor

Fluoride

As mention above in 2.1.1.2

2.2.4 In-vivo experiment for Bioremoval study for defluoridation capacity of the MBP and MSP

Batch defluoridation studies were carried out with best optimized model in groundwater samples. The pre-treatment and post-treatment Fluoride was analyzed.

2.3 Estimation of biochemical parameters in the test plants

Estimation of various biochemical parameters like Chlorophyll, Carbohydrate, Protein and Proline was done in the plants of of *Triticum aestivum* L. and *Pennisetum glaucum* R.Br grown in in-vitro condition. All parameters were estimated during their vegetative phase. Chlorophyll was estimated in leaves while Carbohydrate, Protein and Proline was estimated in root, stem and leaf of the test plants.

Chlorophyll : (Arnon,1949)

Carbohydrate : Anthrone method (Sadasivam and Manikam, 1991)

Protein : (Thimmaiah 1951)

Proline : (Thimmaiah 1999)

2.4 Bioaccumulation of Fluoride in crop plants

Seeds of *Triticum aestivum* L. var. GW 496 and *Pennisetum glaucum* R.Br. var. Proagro 9444 were collected from the field and grown in medium size pots.They were treated with different concentration; 1,2,3,4 and 5 mg/l Fluoride in the form of NaF solution. Each treatment involved 15 pots with three replicates. Control set up of the experiment was also done for *Triticum aestivum* L. var. GW 496 and *Pennisetum glaucum* R.Br. var. Proagro 9444 in which the plants were supplemented with water having no Fluoride content. After maturity, grains were collected and preserved for sample digestion (McQuaker and Gurney 1977) for Fluoride analysis.

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, Orion ion meter-Model 920 –A)**:**

Reagents and standards

d. Stock Fluoride solution: dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100µgF^-

e. Standard Fluoride solution: Dilute stock solution 10 times with distilled water to obtain 1mL = 10µg F^-

f. Total Ionic Strength Adjustment Buffer (TISAB): place approximately 500mL distilled water in a 1L beaker, add 57mL glacial acetic acid, 58g, NaCl and 4g 1, 2-cyclohexylenediamine tetraacetic acid. Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125mL) with stirring, until pH is between 5 to 5.5. Transfer to a 1L volumetric flask and make 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 (59.16 mV for monovalent ions and 29.58 mV for divalent ions at 25°C)

Procedure

1. Calibrate the instrument as explained above.
2. Transfer 50 to 100mL of sample to a 150mL plastic beaker. (Check pH of solution if above or below 7.0 neutralize it using acid or base)
3. Add 5 ml of TISAB solution to the sample and stir thoroughly.
4. Rinse electrode, blot dry and place in the sample. Stir thoroughly and note down the steady reading on the meter.
5. After use place electrode in 1 ppm solution.

2.5 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

For the surface morphological determination, seeds of control and Fluoride treated (5mg/l) of *Triticum aestivum* L. and *Pennisetum glaucum* R.Br. were taken and SEM and EDX analysis were performed. These were analysed by SEM (JEOL Japan - 6490) and EDS (EDS-133) instruments for SEM and EDAX respectively.