Materials and Methods
3.1 STUDY PERIOD

Samples have been collected on three major seasons such as summer, rainy, and winter during the period of investigation (2013-2015). The analysis of samples for all the parameter except for heavy metals was done in the laboratory of Science S.S. Jain Subodh P.G. College Jaipur (Raj.). The analysis of heavy metals was outsourced from the Analyst Labs Pvt Ltd. Jaipur.

3.2 SAMPLING

The ground water sampling was collected from the existing dug well and bore well. While, the waste water samples were collected from the existing industries effluent of respective industrial area. The sampling wells were completed at depths of 5 m to 6.5 m below ground. The samples collected were small in volume, enough to accurately represent the whole water. The water samples tend to alter itself to the new environment. The main objectives of water quality assessment are to:

- Characterize the status and trends in water quality.
- Investigate the causes for the observed conditions and trends.
- Identify the area specific problems of water quality and provide assessments in the form of management to evaluate alternatives that help in decision-making

3.2.1 Types of sampling

Normally three types of sampling are taken up for collecting water samples.

- **Grab or Catch sampling:** the sample is collected at a particular time and place that represents the composition of the source at that particular point and time.
- **Composite sampling:** a mixture of grab samples is collected at the same sampling point at different time intervals.
- **Integrated sampling:** a mixture of grab samples collected at different points simultaneously.
3.2.2 Sampling frequency

Monthly sampling was the highest frequency which would permit sample analysis within the recommended holding times for each constituent and allow preparation for the next run on a routine basis. It was required to carefully judge the timing of each step of the procedure to maintain the sampling frequency within a day of the biweekly timing.

The quality of water changes with time in a water due to different natural and human induced factors. The monitoring has to be done in a way that records all the changes in the quality. The sampling frequencies generally considered while monitoring are:

✓ Monthly sampling for one year.
✓ Consecutive day sampling during study period.
✓ Hourly sampling for 24 hours.
✓ Monthly sampling at predefined time

I have done grab sampling during three seasons in a year pre monsoon, post monsoon and winters.

3.2.3 Sampling container

I have considered sampling container made of non reactive material of sufficient capacity to store the sample and be free from other onsite contamination.

3.2.4 Sampling protocol

The actual sampling of the ground water would be done at flow rates of approximately 100 mL/min. Wells at both sites showed very good yields, with hydraulic conductivities in excess. From rough computations (3), it was clear that greater than 95% aquifer water could be obtained from pumps set at the top of the well screen in ten minutes or less by pumping at 1-2 L/min. Thus, all of the wells were pumped at a relatively low rate, ensuring hydrologic representativeness. This was verified for each well at the time of sampling by monitoring the well purging parameters: pH, temperature, conductivity, redox potential and dissolved oxygen.
3.2.5 Sample preparation

Sample Preparation Polyethylene bottles to be used for field collection were initially washed in a 5% Contrad 70 (R) detergent solution.

- The bottles were then rinsed with deionized water, followed by a rinsing with 50% HCl. The final step in bottle preparation was a complete rinsing with double-deionized water (resistivity > 17.8 megohms).
- Glass bottles for organic analyses were washed as above and then muffled at 450°C for 4 hours. All Teflon-lined bottle caps and septa were rinsed in methanol following the detergent washing procedure.
- Double deionized water was used for all cleaning, rinsing, blank and standard preparation for the inorganic parameters.
- Double distilled-in-glass water was used for the solutions and rinsing procedures for organic parameters. Standard solutions for calibration of inorganic analyses were prepared by serial dilution with class-A volumetric glassware.
- Stock solutions were obtained from commercial vendors if available, or prepared by dilution of pre-measured ampules or a high-purity analytical salt of the desired constituent.
- All stock and calibration standards were verified by comparison to NBS or USEPA reference standards, as well as by comparison to previous lots of standard.
- Analytical standard solutions were prepared for sample spiking and the control standards prepared for submission as “field standards”.


### Table 3-1: Sample handling, preservation and analysis

<table>
<thead>
<tr>
<th>Chemical parameter</th>
<th>Container type</th>
<th>Means of preservation</th>
<th>Maximum holding</th>
<th>Approximate time to analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity</td>
<td>HP</td>
<td>4°C</td>
<td>24 hours</td>
<td>Immediate</td>
</tr>
<tr>
<td>Winkler DO</td>
<td>B</td>
<td>Fix on site, store dark</td>
<td>8 hours</td>
<td>Immediate</td>
</tr>
<tr>
<td>NH₃</td>
<td>HP</td>
<td>4°C, 0.2% v/v H₂SO₄</td>
<td>28 days</td>
<td>&lt;3 days</td>
</tr>
<tr>
<td>NO₃NO₂-N</td>
<td>HP</td>
<td>4°C</td>
<td>8 hours</td>
<td>&lt;3 days</td>
</tr>
<tr>
<td>S²⁻</td>
<td>V</td>
<td>4°C, Zinc Acetate</td>
<td>7 days</td>
<td>&lt;3 days</td>
</tr>
<tr>
<td>SiO₂</td>
<td>HP</td>
<td>4°C</td>
<td>28 days</td>
<td>&lt;3 days</td>
</tr>
<tr>
<td>o-PO₄</td>
<td>HP</td>
<td>4°C</td>
<td>48 hours</td>
<td>&lt;4 days</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>HP</td>
<td>Frozen</td>
<td>48 hours</td>
<td>&lt;4 days</td>
</tr>
<tr>
<td>TOC</td>
<td>V</td>
<td>4°C, Gas Tight</td>
<td>24 hours</td>
<td>&lt;4 days</td>
</tr>
<tr>
<td>VOC</td>
<td>V</td>
<td>4°C, Gas Tight</td>
<td>24 hours</td>
<td>&lt;4 days</td>
</tr>
<tr>
<td>s-PO₄</td>
<td>HP</td>
<td>4°C, 0.2% v/v H₂SO₄</td>
<td>28 days</td>
<td>&lt;5 days</td>
</tr>
<tr>
<td>CH₄</td>
<td>S</td>
<td>4°C</td>
<td>24 hours</td>
<td>&lt;1 week</td>
</tr>
<tr>
<td>CI⁻</td>
<td>HP</td>
<td>4°C</td>
<td>28 days</td>
<td>&lt;1 week</td>
</tr>
<tr>
<td>Ca</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Fe</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>K</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Mg</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Mn</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Na</td>
<td>HP</td>
<td>4°C, 0.3% v/v HNO₃</td>
<td>6 months</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>HP</td>
<td>4°C</td>
<td>23 days</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>TOX</td>
<td>G</td>
<td>4°C, Gas Tight</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Footnotes:**

1. High density polyethylene (HP), glass septum vial (V), amber glass with PTFE-lined cap (S), polyethylene syringe (B), 800 bottle (B).
2. Sample preservation performed immediately upon collection.
3. Samples analyzed as soon after collection as possible. The times listed are the maximum times that samples may be held before analysis and still considered valid. Some samples may not be stable for the maximum time period given (68, 69).
4. Approximate time to analysis includes two days of field storage.

Source: Sampling Frequency for Ground-Water Quality Monitoring- EPA
### 3.2.6 Preservation of the sample

Till the samples are collected and analysed in the laboratory, physical, chemical and biochemical reactions may take place in the sample container leading to changes in the basic quality of the sample, thus it becomes mandatory to preserve the samples with chemicals such as alcohol and mercuric chloride. Highly unstable parameters such as pH, temperature, transparency, free carbon-di-oxide, dissolved oxygen, etc. are measured at the sampling site.

To preserve the sample, the samples were kept in dark, adding chemical preservative, lowering the temperature to slow down reactions, or combinations of these.

The preservation methodologies used are as follows:

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>PRESERVATIVE</th>
<th>Max. holding time</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>Cool, 4°C</td>
<td>4 hours</td>
</tr>
<tr>
<td>Calcium</td>
<td>Cool, 4°C</td>
<td>7 days</td>
</tr>
<tr>
<td>Chloride</td>
<td>Cool, 4°C</td>
<td>7 days</td>
</tr>
<tr>
<td>COD</td>
<td>Cool, 4°C</td>
<td>24 hours</td>
</tr>
<tr>
<td>Dissolved Oxygen*</td>
<td>Fix on site</td>
<td>6 hours</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Cool, 4°C</td>
<td>7 days</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Cool, 4°C</td>
<td>7 days</td>
</tr>
<tr>
<td>Nitrate + Nitrite</td>
<td>Cool, 4°C</td>
<td>24 hours</td>
</tr>
<tr>
<td>PH</td>
<td>None</td>
<td>6 hours</td>
</tr>
<tr>
<td>Phosphorus*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved</td>
<td>Filter on site using 0.45µm filter</td>
<td>24 hours</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Cool, 4°C</td>
<td>24 hours</td>
</tr>
<tr>
<td>Ortho</td>
<td>Cool, 4°C</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
### EXPERIMENT | PRESERVATIVE | Max. holding time
--- | --- | ---
Total | Cool, 4°C | 1 month
Potassium | Cool, 4°C | 7 days
Specific conductance | Cool, 4°C | 24 hours
Sodium | Cool, 4°C | 7 days

**Heavy metals**

| EXPERIMENT | PRESERVATIVE | Max. holding time |
--- | --- | ---
Cadmium | 2 ml conc. Nitric acid/L sample | 6 months |
Chromium | 2 ml conc. Nitric acid/L sample | 6 months |
Copper | 2 ml conc. Nitric acid/L sample | 6 months |
Iron | 2 ml conc. Nitric acid/L sample | 6 months |
Lead | 2 ml conc. Nitric acid/L sample | 6 months |
Nickel | 2 ml conc. Nitric acid/L sample | 6 months |
Zinc | 2 ml conc. Nitric acid/L sample | 6 months |

### Table 3.3: Analytical method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type of method</th>
<th>Std. Methods (7)</th>
<th>USEPA (68)</th>
<th>USGS (72)</th>
<th>Other^{***}</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>E</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductance</td>
<td>E</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOC/VOC</td>
<td>P</td>
<td>505b</td>
<td>415.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOX</td>
<td>APT</td>
<td>506</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Alkalinity</td>
<td>PT</td>
<td>407C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NO₃⁻ + NO₂⁻)-N</td>
<td>AS</td>
<td>353.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>AS, PT</td>
<td>426D</td>
<td>(see text)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiO₂</td>
<td>AS</td>
<td>00995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>AAS</td>
<td>365.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>AAS</td>
<td>365.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>AAS</td>
<td>365.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>AAS</td>
<td>365.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>AAS</td>
<td>365.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>E</td>
<td>365.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>RT, E</td>
<td>4218, 421F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe(II)</td>
<td>S</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe(total)</td>
<td>AAS</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>AAS</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄</td>
<td>S</td>
<td>7&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>GC-FID</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt;sup&gt;-&lt;/sup&gt;</td>
<td>S</td>
<td>427</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻-N</td>
<td>S</td>
<td>419</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^{* Types of methods: AAS = atomic absorption spectrophotometric; APT = adsorption/pyrolysis, titrimetric; AS = automated spectrophotometric; E = electrometric; GC-FID = gas chromatographic method of flame ionization detection; P = persulfate oxidation; PT = potentiometric titration; RT = redox titration; S = spectrophotometric.

^{**} Method number corresponding to, or most closely to, that used in this project.

Source: Sampling Frequency for Ground-Water Quality Monitoring- EPA

#### 3.3 METHODOLOGY:

3.3.1 The measurement of temperature

a) Temperature of samples was analyzed with the help of thermometer. The thermometer was submerged directly in the water sample for adequate time, so that the constant reading can be achieved.

b) The readings were noted in the separate registered, the marking was done properly to prevent any error.

3.3.2 The measurement of pH value (electrometric methods)

pH value is the logarithm of reciprocal of hydrogen ion activity in moles per liter. In a solution, deviations in pH value from 7 are majorly due to hydrolysis of salts of strong bases and weak acids or vice versa. The pH value range in water is generally between 6 and 8. The calorimetric method is simple and less expensive, and is accurate for work.
3.3.2.1 Principle

The pH value is determined by measurement of the electromotive force of a cell consisting of an indicator electrode (an electrode responsive to hydrogen ions such as a glass electrode) immersed in the test solution and a reference electrode (usually mercury/calomel electrode). Contact between the test solution and the reference electrode is usually achieved by means of a liquid junction, which forms part of the reference electrode. The electromotive force is measured with a pH meter, that is, a high impedance voltmeter calibrated in terms of pH. 2.1.1 Several types of electrodes have been suggested for electrometric determination of pH value. Although the hydrogen gas electrode is recognized as primary standard the glass: electrode in combination with calomel electrode is generally used with reference potential provided by saturated calomel electrode. The glass electrode system is based on the fact that a change, of 1 pH unit produces an electrical change of 59.1 mV at 25°C. The active element of a glass electrode is a membrane of a special glass. The membrane forms a partition between two liquids of differing hydrogen ion concentration and a potential is produced between the two sides of the membrane which is proportional to the difference in pH between the liquids.

3.3.2.2. Apparatus

1. pH meter – Madeup of glass and reference electrode, with temperature compensation.

Figure 3.2: pH meter
3.3.2.3 Sample handling and preservation
1. Samples were analyzed onsite.

3.3.2.4 Procedure

After calibrating the instrument with standard solution, Rinse and gently wipe the electrodes with solution, then immerse them into the sample beaker and stir at a steady rate to provide homogeneity. Rate of stirring should minimize the air transfer rate at the air-water interface of the sample. Note and record sample pH and temperature. However, if there is a continuous drift, take a second reading with the fresh aliquot of sample without stirring and report it as the pH value.

3.3.3 Methodology for measurement of turbidity

3.3.3.1 Principle

The measurement is based on comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions.

3.3.3.2 Apparatus

Sample Tubes - The sample tubes were clear and colorless glass.

Turbidimeter - The turbidimeter shall consist of a nephelometer with a light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter should be so designed that little stray light reaches the detector in the absence of turbidity and should be free from significant drift after a short warm-up period.
3.3.3.3 Procedure

Turbidimeter Calibration is the first step to measure the turbidity. The steps were as per the instrument instruction.

Shake the sample to disperse the solids. Watch until air bubbles disappear. Transfer sample into turbidimeter tube and read turbidity directly from the instrument scale or from calibration curve.

3.3.4 Methodology for measurement of total solids

Put the glass fiber filter on the membrane filter or place into bottom of a suitable Gooch crucible with wrinkled surface up. As vacuum is applied, wash the dish with three consecutive 20 ml volumes of distilled water. Eliminate traces of water by continuing apply vacuum after water has passed through. Eliminate filter from membrane filter and dry in an oven at 103-105°C for 1 hour. Transfer to a desiccators and weigh after half an hour. Repeat the drying cycle until a constant mass is obtained (mass loss is less than 0.5 mg in successive weighing). Weigh immediately before use. After weighing, handle the filter or crucible filter with forceps or tongs only.

Figure 3.4: Total solids apparatus
3.3.5 **Methodology for measurement of total suspended solids**

a) Heat the evaporating dish to 180°C for 1 hour. Cool, weigh and store in desiccators.

b) Select volume of the sample which has residue between 25 and 250 mg, preferably between 190 and 200 mg. This volume may be estimated from values of specific conductance.

c) Pipette the volume to a weighed evaporating dish placed on a steambath.

d) After evaporation of water from the residue, moved the dish to an oven 181°C and dried. Drying for a long duration (usually 1 to 2 hours) is done to eliminate necessity of checking for constant mass.

e) Weighing the dish as soon as it was cooled avoiding residue to stay for long time as some residues.

![Figure 3.5: Total suspended solids apparatus](image)

**Figure 3.5: Total suspended solids apparatus**

![Figure 3.6: Pictorial presentation of total suspended solid test procedure](image)

**Figure 3.6: Pictorial presentation of total suspended solid test procedure**
3.3.6 Methodology for measurement of biological oxygen demand

3.3.6.1 Theory

The Biochemical Oxygen Demand (BOD) is the oxygen required by the organism to degrade the complex organic matter to simple organic matter.

3.3.6.2 Methods

I. Dilution Method
II. Manometric Method

**Dilution method**

**APPARATUS**

i. Incubation Bottles (250-300 ml)
ii. Air Incubator (20°C)
iii. DO meter
iv. Burette

![Figure 3.7: BOD incubator](image-url)
Chapter-3 Materials and Methods

REAGENTS
a) Phosphate buffer
b) Mohr’s salt
c) Magnesium sulfate solution
d) Calcium chloride solution
e) Ferric Chloride solution
f) Acid and alkali solution
g) Sodium sulfate solution
h) Nitrification inhibitor

1. Freshly prepared dilution water is to be used.
2. The contaminant growth is stimulated by the phosphate buffer thus, it was added on the first day of the water was used for testing.
3. Distilled water was allowed to equilibrate in the incubator or with outside air for at least 24 hours at 20°C before use.
4. BOD bottle was poured slowly by adding sufficient dilution water. There should not be any air bubble in the bottle.
5. When volumes of sample used exceed 150 ml, additional nutrients was added to sample bottle.
6. An additional 0.1 mL of nutrients was added for each 50 mL of sample used in excess of 150 mL.
7. The 2 bottles was completely poured with dilution water as blank

DISSOLVED OXYGEN (DO) CALCULATION
1. DO meter was calibrated.
2. The DO all the sample water and blacks was determined and recorded.
3. The samples and the dilution water blanks were placed in a 20°C incubator for 5 days.
4. After 5 days the DO was calculated of the 2 blanks and the sample bottle
3.3.7 Chemical oxygen demand (COD)

3.3.7.1 Theory

The COD is used to determine the oxygen equivalent of the organic equivalent of the organic matter content of a sample. Potassium dichromate is the most excellent oxidant due to its strong oxidizing capability.

3.3.7.2 Methods

I. Open Reflux Method

II. Closed Reflux, Titrimetric Method
Chapter-3 Materials and Methods

III. Closed Reflux, Colorimetric Method

The COD was determined as per the SOP under standard methods for the examination of water and wastewater 20th edition with the help of above mentioned apparatus. The formulae used for COD calculation is given below:

Where:
COD, mg/L = (A-B) x M x 8000 / Volume of Sample in ml
A: mL of titrant used for sample
B: mL of titrant used for blank
M: normality of ferrous ammonium sulfate

3.3.8 Methodology for measurement of heavy metals and Cn

Nickel, Chromium, Calcium, Cadmium, Iron, Zinc, Copper, lead, Cyanide and were determined using Atomic Absorption Spectrometer (model AA6800-SHIMADZU) according to APHA, 1998.

Figure 3.9: Atomic Absorption Spectrometer