CHAPTER-3

MATERIALS AND METHODS

The present study “Investigation on drinking water quality parameters from different sources in Bhiwani District Haryana” was carried out in the Department of Energy and Environmental Sciences, Chaudhary Devi Lal University, Sirsa. The detail methodologies adopted to achieve the objectives are as follows:

The sampling was done manually and conducted during summer (May), monsoon (August) and winter (January) seasons of 2013 and 2014 from different blocks of Bhiwani district namely Bhiwani, Tosham, Bawani Khera, Charkhi Dadri and Loharu.

The various physico-chemical parameters such as color, odour, taste, pH, turbidity, dissolved oxygen (DO), biochemical oxygen demand (BOD), total dissolved solids (TDS), total hardness (TH), electrical conductivity (EC), total alkalinity, calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), carbonate (CO$_3^{2-}$), bicarbonate (HCO$_3^{-}$), chloride (Cl$^-$), nitrate (NO$_3^-$), sulphate (SO$_4^{2-}$), phosphate (PO$_4^{3-}$) and fluoride (F$^-$) concentration of the water samples were determined. The microbiological parameters such as Heterotrophic Plate Count (HPC), MPN index/100 were determined by following the methodology of APHA, 2005 and Cappuccino and Sherman, 2008. To check the suitability of water for irrigation purpose, an In vitro plant response study was conducted by using two radish (Raphanus sativus L.) varities RD-68 and radish hill queen by following the methodology of Aery, 2010.
Figure 3.1: Map of Bhiwani district showing sampling locations covering all the studied blocks

3.1 Sampling locations

Table 3.1: Details of sampling sites of Bhiwani block

<table>
<thead>
<tr>
<th>Code given</th>
<th>Sampling Site</th>
<th>Source</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Rudra Colony</td>
<td>H.P.</td>
<td>28° 80'9&quot; N</td>
<td>76° 11'1&quot; E</td>
</tr>
<tr>
<td>B2</td>
<td>Sector-13</td>
<td>H.P.</td>
<td>28° 82'0&quot; N</td>
<td>76° 13'3&quot; E</td>
</tr>
<tr>
<td>B3</td>
<td>Sarogian Dhani</td>
<td>H.P.</td>
<td>28° 79'8&quot; N</td>
<td>76° 13'4&quot; E</td>
</tr>
<tr>
<td>B4</td>
<td>Tosham by pass</td>
<td>B.W.</td>
<td>28° 80'8&quot; N</td>
<td>76° 11'6&quot; E</td>
</tr>
<tr>
<td>B5</td>
<td>Water works</td>
<td>B.W.</td>
<td>28° 80'9&quot; N</td>
<td>76° 14'8&quot; E</td>
</tr>
<tr>
<td>B6</td>
<td>Mitathal road</td>
<td>B.W.</td>
<td>28° 81'4&quot; N</td>
<td>76° 15'9&quot; E</td>
</tr>
<tr>
<td>B7</td>
<td>Sector-13</td>
<td>Tap</td>
<td>28° 81'2&quot; N</td>
<td>76° 14'0&quot; E</td>
</tr>
<tr>
<td>B8</td>
<td>Naya bazar</td>
<td>Tap</td>
<td>28° 79'7&quot; N</td>
<td>76° 13'6&quot; E</td>
</tr>
<tr>
<td>B9</td>
<td>Vidhya Nagar</td>
<td>Tap</td>
<td>28° 80'3&quot; N</td>
<td>76° 14'8&quot; E</td>
</tr>
<tr>
<td>B10</td>
<td>Jui Feeder Canal</td>
<td>Canal</td>
<td>28° 81'0&quot; N</td>
<td>76° 11'5&quot; E</td>
</tr>
<tr>
<td>B11</td>
<td>Gujrani Miner</td>
<td>Canal</td>
<td>28° 81'7&quot; N</td>
<td>76° 12'5&quot; E</td>
</tr>
<tr>
<td>B12</td>
<td>Bamla</td>
<td>B.W.</td>
<td>28° 80'6&quot; N</td>
<td>76° 24'5&quot; E</td>
</tr>
<tr>
<td>B13</td>
<td>Dhangar</td>
<td>H.P.</td>
<td>28° 66'4&quot; N</td>
<td>75° 92'3&quot; E</td>
</tr>
<tr>
<td>Code given</td>
<td>Sampling Site</td>
<td>Source</td>
<td>Latitude</td>
<td>Longitude</td>
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<td>------------</td>
<td>-----------------------</td>
<td>--------</td>
<td>---------------</td>
<td>-----------------</td>
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<tr>
<td>T1</td>
<td>Bapora</td>
<td>H.P.</td>
<td>28° 81'5&quot; N</td>
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<tr>
<td>T2</td>
<td>Dang Khurd</td>
<td>H.P.</td>
<td>28° 86'0&quot; N</td>
<td>76° 00'2&quot; E</td>
</tr>
<tr>
<td>T3</td>
<td>Tehsil</td>
<td>H.P.</td>
<td>28° 86'6&quot; N</td>
<td>75° 91'6&quot; E</td>
</tr>
<tr>
<td>T4</td>
<td>Water works</td>
<td>B.W.</td>
<td>28° 87'6&quot; N</td>
<td>75° 92'0&quot; E</td>
</tr>
<tr>
<td>T5</td>
<td>New Anaj Mandi</td>
<td>B.W.</td>
<td>28° 86'6&quot; N</td>
<td>75° 91'5&quot; E</td>
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<tr>
<td>T6</td>
<td>Siwani Road</td>
<td>B.W.</td>
<td>28° 86'6&quot; N</td>
<td>75° 91'6&quot; E</td>
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<tr>
<td>T7</td>
<td>Bhiwani Chowk</td>
<td>Tap</td>
<td>28° 87'0&quot; N</td>
<td>75° 91'8&quot; E</td>
</tr>
<tr>
<td>T8</td>
<td>Water works</td>
<td>Tap</td>
<td>28° 87'4&quot; N</td>
<td>75° 91'6&quot; E</td>
</tr>
<tr>
<td>T9</td>
<td>Market near Bus Stand</td>
<td>Tap</td>
<td>28° 87'2&quot; N</td>
<td>75° 91'6&quot; E</td>
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<tr>
<td>T10</td>
<td>Dang Miner</td>
<td>Canal</td>
<td>28° 85'0&quot; N</td>
<td>76° 01'1&quot; E</td>
</tr>
<tr>
<td>T11</td>
<td>Nigana Feeder</td>
<td>Canal</td>
<td>28° 85'7&quot; N</td>
<td>76° 12'1&quot; E</td>
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<tr>
<td>T12</td>
<td>Miran</td>
<td>H.P.</td>
<td>28° 84'8&quot; N</td>
<td>75° 73'7&quot; E</td>
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<tr>
<td>T13</td>
<td>Kairu</td>
<td>H.P.</td>
<td>28° 69'9&quot; N</td>
<td>75° 87'0&quot; E</td>
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<tr>
<td>T14</td>
<td>Rodha</td>
<td>H.P.</td>
<td>28° 74'3&quot; N</td>
<td>75° 73'3&quot; E</td>
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<table>
<thead>
<tr>
<th>Code given</th>
<th>Sampling Site</th>
<th>Source</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK1</td>
<td>Tosham Road</td>
<td>H.P.</td>
<td>28° 93'5&quot; N</td>
<td>76° 03'3&quot; E</td>
</tr>
<tr>
<td>BK2</td>
<td>Haryana Gramin Bank</td>
<td>H.P.</td>
<td>28° 94'5&quot; N</td>
<td>76° 04'3&quot; E</td>
</tr>
<tr>
<td>BK3</td>
<td>Shaikhi Pur Pana</td>
<td>H.P.</td>
<td>28° 95'2&quot; N</td>
<td>76° 04'2&quot; E</td>
</tr>
<tr>
<td>BK4</td>
<td>Hansi Road</td>
<td>B.W.</td>
<td>28° 97'6&quot; N</td>
<td>76° 01'9&quot; E</td>
</tr>
<tr>
<td>BK5</td>
<td>Near B.K. College of Education</td>
<td>B.W.</td>
<td>28° 93'5&quot; N</td>
<td>76° 04'2&quot; E</td>
</tr>
<tr>
<td>BK6</td>
<td>Tosham Road</td>
<td>B.W.</td>
<td>28° 93'4&quot; N</td>
<td>76° 03'4&quot; E</td>
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<tr>
<td>BK7</td>
<td>Near Railway Station</td>
<td>Tap</td>
<td>28° 93'8&quot; N</td>
<td>76° 03'3&quot; E</td>
</tr>
<tr>
<td>BK8</td>
<td>Ward- 2</td>
<td>Tap</td>
<td>28° 95'7&quot; N</td>
<td>76° 03'1&quot; E</td>
</tr>
<tr>
<td>BK9</td>
<td>Hansi Chungi</td>
<td>Tap</td>
<td>28° 96'0&quot; N</td>
<td>76° 02'9&quot; E</td>
</tr>
<tr>
<td>BK10</td>
<td>Sunder Branch upstream</td>
<td>Canal</td>
<td>28° 98'9&quot; N</td>
<td>76° 02'7&quot; E</td>
</tr>
<tr>
<td>BK11</td>
<td>Sunder Branch downstream</td>
<td>Canal</td>
<td>28° 98'6&quot; N</td>
<td>76° 01'5&quot; E</td>
</tr>
<tr>
<td>BK12</td>
<td>Jamalpur</td>
<td>H.P.</td>
<td>28° 96'3&quot; N</td>
<td>75° 95'6&quot; E</td>
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<tr>
<td>BK13</td>
<td>Aurangnagar</td>
<td>B.W.</td>
<td>28° 99'4&quot; N</td>
<td>75° 98'6&quot; E</td>
</tr>
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</table>
Table 3.4: Details of sampling sites of Charkhi Dadri block

<table>
<thead>
<tr>
<th>Code given</th>
<th>Sampling Site</th>
<th>Source</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>Arya Hindi Sanskrit Mahavidhalya</td>
<td>H.P.</td>
<td>28° 60’7” N</td>
<td>76° 27’9” E</td>
</tr>
<tr>
<td>CD2</td>
<td>Jhadu Chowk</td>
<td>H.P.</td>
<td>28° 59’4” N</td>
<td>76° 27’2” E</td>
</tr>
<tr>
<td>CD3</td>
<td>Dhani Phatak</td>
<td>H.P.</td>
<td>28° 59’3” N</td>
<td>76° 28’3” E</td>
</tr>
<tr>
<td>CD4</td>
<td>Rawaldhi</td>
<td>B.W.</td>
<td>28° 63’3” N</td>
<td>76° 28’6” E</td>
</tr>
<tr>
<td>CD5</td>
<td>Champapuri</td>
<td>B.W.</td>
<td>28° 60’3” N</td>
<td>76° 27’8” E</td>
</tr>
<tr>
<td>CD6</td>
<td>Dhikara Road</td>
<td>B.W.</td>
<td>28° 60’4” N</td>
<td>76° 26’7” E</td>
</tr>
<tr>
<td>CD7</td>
<td>Parshu Ram Chowk</td>
<td>Tap</td>
<td>28° 59’6” N</td>
<td>76° 27’3” E</td>
</tr>
<tr>
<td>CD8</td>
<td>Vivek Nagar</td>
<td>Tap</td>
<td>28° 60’8” N</td>
<td>76° 28’0” E</td>
</tr>
<tr>
<td>CD9</td>
<td>Railway Phatak</td>
<td>Tap</td>
<td>28° 60’2” N</td>
<td>76° 27’7” E</td>
</tr>
<tr>
<td>CD10</td>
<td>Indira Canal upstream</td>
<td>Canal</td>
<td>28° 60’9” N</td>
<td>76° 23’6” E</td>
</tr>
<tr>
<td>CD11</td>
<td>Indira Canal downstream</td>
<td>Canal</td>
<td>28° 60’8” N</td>
<td>76° 23’5” E</td>
</tr>
</tbody>
</table>

Table 3.5: Details of sampling sites of Loharu Block

<table>
<thead>
<tr>
<th>Code given</th>
<th>Sampling Site</th>
<th>Source</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Boosting Station 1</td>
<td>B.W.</td>
<td>28° 43’0” N</td>
<td>75° 80’8” E</td>
</tr>
<tr>
<td>L2</td>
<td>Boosting Station 2</td>
<td>B.W.</td>
<td>28° 43’7” N</td>
<td>75° 81’1” E</td>
</tr>
<tr>
<td>L3</td>
<td>Boosting Station near Shamshan Ghat</td>
<td>B.W.</td>
<td>28° 43’5” N</td>
<td>75° 81’5” E</td>
</tr>
<tr>
<td>L4</td>
<td>Parvezpur</td>
<td>B.W.</td>
<td>28° 50’7” N</td>
<td>75° 73’1” E</td>
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<tr>
<td>L5</td>
<td>Gokalpura</td>
<td>H.P.</td>
<td>28° 65’4” N</td>
<td>75° 66’4” E</td>
</tr>
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<td>L6</td>
<td>Kharkhari</td>
<td>H.P.</td>
<td>28° 55’3” N</td>
<td>75° 78’9” E</td>
</tr>
</tbody>
</table>

3.2 Physico-Chemical Analysis

3.2.1 Water Sampling

Water samples from different sources i.e (hand pump, bore well, Municipal supply tap water and canal) were collected in 2 litre plastic containers and prior to collection as part of our quality control measures all the bottles were washed with non-ionic detergent and rinsed three times with de-ionized water. After the water sampling was performed, each sampled bottle was labeled according to sampling location and all the samples were preserved at 4°C till further investigation (APHA, 2005). It was ensured every time that bottle satisfies following requirement i.e
- Free from contamination.
- Resistant to any internal pressure.
- Don't affect water characteristics.

### 3.2.2 Analytical methods:

The chemical analysis of water samples were carried out in accordance with standard analytical methods (APHA, 2005). All the reagents used in the present study were of Analytical grade (AR) and double distilled water was used throughout the study. The samples were brought to laboratory within 5 hours of sampling and kept in dark and cool place to avoid any change in the physicochemical properties. Summary of methods is given in Table.

#### Table 3.6: Methods of determination of various parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Methods</th>
<th>Instrument used</th>
<th>Reference</th>
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<tbody>
<tr>
<td>pH</td>
<td></td>
<td>Electrometric method</td>
<td>pH meter (ELICO LI614 pH analyzer)</td>
<td>APHA, 2005, Shalu et al. 2015</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>Turbidity meter</td>
<td>Turbidity meter (Model 341E)</td>
<td>APHA, 2005, Trivedi et al. 2010</td>
</tr>
<tr>
<td>DO</td>
<td>mg/l</td>
<td>Azide Modification</td>
<td></td>
<td>APHA, 2005, Gupta et al. 2017</td>
</tr>
<tr>
<td>Total Hardness</td>
<td>mg/l</td>
<td>EDTA titration method</td>
<td></td>
<td>APHA, 2005, Kaur et al. 2017</td>
</tr>
<tr>
<td>Calcium (Ca&lt;sup&gt;2+&lt;/sup&gt;)</td>
<td>mg/l</td>
<td>EDTA titration method</td>
<td></td>
<td>APHA, 2005, Kaur et al. 2017</td>
</tr>
<tr>
<td>Magnesium (Mg&lt;sup&gt;2+&lt;/sup&gt;)</td>
<td>mg/l</td>
<td>EDTA titration method</td>
<td></td>
<td>APHA, 2005, Kaur et al. 2017</td>
</tr>
<tr>
<td>EC</td>
<td>mS/cm</td>
<td>Conductivity meter</td>
<td>ELICO CM 183</td>
<td>APHA, 2005, Shalu et al. 2015</td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>mg/l</td>
<td>Titration method</td>
<td></td>
<td>APHA, 2005, Nagaraju et al. 2014</td>
</tr>
<tr>
<td>Carbonate (CO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;)</td>
<td>mg/l</td>
<td>Titration method</td>
<td></td>
<td>APHA, 2005, Nagaraju et al. 2014</td>
</tr>
<tr>
<td>Bicarbonate (HCO&lt;sub&gt;3&lt;/sub&gt; -)</td>
<td>mg/l</td>
<td>Titration method</td>
<td></td>
<td>APHA, 2005, Nagaraju et al. 2014</td>
</tr>
<tr>
<td>Sodium (Na&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>mg/l</td>
<td>Flame photometer</td>
<td>Model no. 128 systronics</td>
<td>APHA, 2005, Garg et al. 2009</td>
</tr>
<tr>
<td>Potassium (K&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>mg/l</td>
<td>Flame photometer</td>
<td>Model no. 128 systronics</td>
<td>APHA, 2005, Garg et al. 2009</td>
</tr>
<tr>
<td>Chloride</td>
<td>mg/l</td>
<td>Silver nitrate method</td>
<td>APHA, 2005, Garg et al. 2009</td>
<td></td>
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<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Nitrate (NO₃⁻)</td>
<td>mg/l</td>
<td>UV spectrophotometric method</td>
<td>UV-1800, Shimadzu APHA, 2005, Shalu et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Sulphate (SO₄²⁻)</td>
<td>mg/l</td>
<td>Spectrophotometric (Turbidimetric method)</td>
<td>UV-1800, Shimadzu APHA, 2005, Janardhana et al. 2013</td>
<td></td>
</tr>
<tr>
<td>Phosphate (PO₄³⁻)</td>
<td>mg/l</td>
<td>Spectrophotometric (Stannous chloride method)</td>
<td>UV-1800, Shimadzu APHA, 2005, Shalu et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Fluoride</td>
<td>mg/l</td>
<td>Spectrophotometric (SPADNS dye method)</td>
<td>UV-1800, Shimadzu APHA, 2005, Bishnoi &amp; Arora, 2007</td>
<td></td>
</tr>
</tbody>
</table>

3.3 The details of methods are given below:

3.3.1 pH (APHA, 2005, 4500-H⁺ B)

Reagents

**Buffer solution of 4.0 pH (Thallate buffer):** 10.2 gram of potassium hydrogen thallate was dissolved in one liter double distilled water.

**Buffer solution of 7.0 pH (Phosphate buffer):** 3.4 gram of potassium dihydrogen phosphate and 4.5 gram of disodium hydrogen phosphate was dissolved in one liter double distilled water.

**Buffer solution of 9.2 pH (Borax Buffer):** 3.81 gram of borax was dissolved in one liter double distilled water.

Readymade buffer tablets of pH 4.0, 7.0 and 9.0 used for making 100 ml buffer solution.

**Procedure**

The pH meter was calibrated with buffer solution of pH 94, 7, 9.2) followed by determination of pH in water samples.

3.3.2 Turbidity (APHA, 2005, 2130 B)

Reagents

**Hydrazine sulphate:** Dissolve 1.00 gm hydrazine sulfate in double distilled water and dilute to 100 ml in a volumetric flask.
Hexamethylenetetramine: Dissolve 10.00 gm hexamethylenetetramine in double distilled water and dilute to 100 m in a volumetric flask.

In a 100 ml volumetric flask, mix 5.0 ml hydrazine sulphate and 5.0 ml hexamethylenetetramine and mix (kept for 24 hrs at 25 ± 3°C). The turbidity of this suspension is 400 NTU.

Procedure

The turbidity was first calibrated with suspension of 40 NTU and then turbidity of the samples was determined.

3.3.3 Electrical conductivity (EC) (APHA, 2005, 2510 B)

The EC (in mS/cm) of water samples was estimated by using ELICO CM 183 EC-TDS analyzer. The EC meter was calibrated with standard KCl solution (0.1N). The standard KCl solution of 0.1N was prepared by dissolving 0.747 of KCl (AR grade) in 100 ml double distilled water. The EC of standard solution was set at 12.48 mho cm⁻¹. After calibration of instrument, EC of samples was recorded.

3.3.4 Total Dissolved Solids (TDS)

TDS were calculated indirectly making use of EC. To calculate TDS formula given by United States Salinity Lab Staff (1954) was used.

TDS (ppm) = 640 × EC (mS/cm)

3.3.5 Dissolved oxygen and Biochemical oxygen demand (APHA, 2005, 4500-O C)

It is a measure of the amount of oxygen required by microbes while stabilizing decomposable organic matter.

Procedure

As soon as the water samples were brought to the laboratory, different dilutions of the unfiltered water samples were prepared in BOD bottles (300 ml capacity).

In two set for each water sample in BOD bottles (300 ml capacity), 1 ml MnSO₄, 1 ml alkali iodide azide reagent were added and mixed it by inverting bottle for few times. To this solution, added 1 ml of conc. H₂SO₄ and mixed it again to dissolve the precipitate and determined the initial dissolved oxygen in first set of BOD bottle by using sodium thiosulphate and starch indicator and kept the second BOD bottle for
incubation in BOD incubator at 20 °C for five days. After five days, remove the bottle from the incubator and determine the DO from the incubated set of bottles and the observation was recorded accordingly.

**Calculation**

\[
DO \text{ in mg/l} = \frac{N_1 \times V_1 \times 8000}{V}
\]

*Where*

\[V = \text{volume of water sample taken (ml)}\]

\[V_1 = \text{volume of titrant (Sodium thiosulphate) used (ml)}\]

\[N_1 = \text{Normality of the titrant (0.025)}\]

\[\text{BOD}_5 \text{ in mg/l} = D_1 - D_5\]

\[D_1 = \text{initial DO in the sample (mg/l)}\]

\[D_5 = \text{DO after five days of incubation (mg/l)}\]

**3.3.6 Total Hardness (TH) (as CaCO}_3\) (APHA, 2005, 2340 C)**

Total hardness of water samples was determined by using EDTA titration method.

**Reagents**

*Standard EDTA solution (0.01 M)*: 3.723 g of disodium salt of EDTA was dissolved in double distilled water to make the final volume one litre.

*Ammonium buffer solution:* 16.9 g of NH\(_4\)CI was dissolved in 143 ml of NH\(_4\)OH and finally distilled it to 250 ml with double distilled water.

*Erichrome Black-T (EBT) indicator:* 0.5 g of EBT was dissolved in 100 ml of 80% ethyl alcohol.

**Procedure**

50 ml of sample was taken in titration flask. To this, added 2 ml of ammonia buffer solution and a pinch of EBT indicator and titrated it against EDTA solution till wine red colour changed to blue. Repeated the experiment to get three concordant readings.
Standardization of EDTA solution

It was done prior to titration of EDTA with water samples; EDTA was standardized by using standard hard water (CaCO₃ solution) to find the actual molarity of EDTA solution. Procedure of standardization was same as given for titration of water samples (above).

**Calculations**

\[
\text{Total hardness (as CaCO}_3\text{)} = \frac{M_2 \times V_2 \times 1000 \times M.W.}{V_1}
\]

Where,

\(M_2 = \text{Molarity of standardized EDTA solution}\)

\(V_2 = \text{Volume of EDTA solution used}\)

\(V_1 = \text{Volume of sample taken}\)

\(M.W. = \text{Molecular weight of CaCO}_3(100)\)

**3.3.7 Calcium (Ca}^{2+}\text{)**

Calcium content of water samples was estimated following the complexometric titration method (EDTA)

**Reagents**

*EDTA solution (0.01 M)*: 3.723 g disodium salt of EDTA was dissolved in double distilled water to make the volume 1 litre. EDTA solution was standardized as given in total hardness estimation.

*Standard NaOH solution (IN)*: 4 g of NaOH was dissolved in double distilled water to make 100 ml solution.

*Murexide indicator (solid)*

**Procedure**

25 ml of water sample was taken in titration flask. To this, added 1 ml of I N NaOH solution to raise the pH and a pinch of solid murexide. Titrated it against standard EDTA solution till the colour changed from pink to purple. Repeated the experiment to get three concordant readings.
3.3.8 Magnesium (Mg$^{2+}$)

Magnesium content of the water samples was calculated indirectly using Ca$^{2+}$ and TH content of water samples by following formula

$$Mg^{2+} \text{ (ppm)} = [\text{Total hardness} - (Ca^{2+} \times 2.5)] \times 0.243$$

3.3.9 Total Alkalinity (as CaCO$_3$) (APHA, 2005, 2320 B)

Reagents

*Standard sulphuric acid solution (N/50):* 1.1 ml of conc. H$_2$SO$_4$ was mixed with double distilled water and made the volume 2 litres.

H$_2$SO$_4$ solution was standardized using standard NaOH solution.

*Phenolphthalein indicator*

*Methyl orange indicator*

Procedure

25 ml of water sample was taken in a 100 ml conical flask and 2 drops of phenolphthalein indicator was added, if pink colour appeared titrated it against standardized H$_2$SO$_4$ solution until sample become colourless. The volume of acid used as 'A' ml was recorded. To the same solution, further 2-3 drops of methyl orange indicator was added and titrated it further with standard H$_2$SO$_4$ untill colour changed from light yellow to red. Again the volume of acid consumed as 'B' ml was recorded. The whole experiment was repeated thrice to have three concordant readings.

Calculations

$$\text{Total alkalinity (as CaCO}_3\text{)} = \frac{N_2 \times V_2 \times \text{Eq.wt of CaCO}_3 \times 1000}{V_1}$$

(in mg/l)

Where

\[N_2 = \text{Normality of standardized } H_2SO_4\]

\[V_2 = \text{Total volume (A+B) of acid used}\]

\[V_1 = \text{Volume of sample taken, Equivalent weight of CaCO}_3 = 50\]
3.3.10 Carbonate (CO$_3^{2-}$)

CO$_3^{2-}$ content of the samples was estimated indirectly by making use of total alkalinity.

\[
CO_3^{2-} (mg/l) = \frac{N_2 \times V_2 \times \text{eq. wt. of } CO_3^{2-} \times 1000}{V_1}
\]

Where

\[N_2 = \text{Normality of standardized } H_2SO_4\]
\[V_1 = \text{Volume of sample taken (100 ml)}\]
\[V_2 = \text{Volume (2 A) of acid used.}\]

'A' is the volume of acid used for phenolphthalein indicator.

Equivalent weight of CO$_3^{2-} = 50$.

3.3.11 Bicarbonate (HCO$_3^{-}$)

HCO$_3^{-}$ content of water sample was also estimated indirectly making use of recorded total alkalinity estimation readings.

\[
HCO_3^{-} (mg/l) = \frac{N_2 \times V_2 \times \text{eq. wt of } HCO_3^{-} \times 1000}{V_1}
\]

Where

\[N_2 = \text{Normality of standardized } H_2SO_4\]
\[V_1 = \text{Volume of sample taken}\]
\[V_2 = (B-A) \text{ ml.}\]

\[V_1 = \text{Volume of sample taken}\]

Equivalent weight of HCO$_3^{-} = 61$

Note: These formulae for CO$_3^{2-}$ and HCO$_3^{-}$ are applicable only when B>A. This situation prevailed in the present study.

3.3.12 Chloride (Cl$^{-}$) (4500-Cl B)

Chloride in the water samples was determined by argentometric titration method.

Reagents

*Standard AgNO$_3$ solution (0.0141 N)*: 2.397 of AgNO$_3$ was dissolved in 1 litre double distilled water.
5% potassium chromate (K₂CrO₄) indicator solution: 5g of K₂CrO₄ was dissolved in 100 ml double distilled water.

Procedure

25 ml sample was taken in a titration flask and 3-4 drops of K₂CrO₄ indicator were added. Titrated it against AgNO₃ solution till yellow colour changed to light brick red. The whole experiment was repeated thrice to have three concordant readings.

Calculations

\[ CT \text{ (mg/l)} = \frac{N_2 \times V_2 \times 35.5 \times 1000}{V_1} \]

Where,

- \( N_2 \) = Normality of standard AgNO₃ solution (0.0141N)
- \( V_2 \) = Volume of AgNO₃ solution used (in ml).
- \( V_1 \) = Volume of sample taken (25 ml).

3.3.13 Nitrate (NO₃⁻) (APHA, 2005, 4500-NO₃⁻ B)

Nitrate content of water samples was determined by UV spectrophotometric method.

Reagents

Nitrate Stock Solution (100ppm): 721 mg potassium nitrate KNO₃ was dissolved in double distilled water. Made upto 1000 ml in volumetric flask (1.0 ml = 0.1 mg nitrate nitrogen) i.e. 100 ppm NO₃-N.

Nitrate Standard Solution (10ppm): Pipetted 100 ml Nitrate Stock solution into a 1000 ml volumetric flask and made final volume with double distilled water.

Hydrochloric acid solution (1N)

Procedure

100 ppm potassium nitrate solution was prepared by dissolving 722 mg of KNO₃ in one litre double distilled water. It was further diluted to make 10 ppm potassium nitrate solution. The standard solutions of NO₃⁻ concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (1-10 ppm) were prepared using the standard stock solution. To obtain 50 ml clear sample, filtration was done, 1 ml HCl solution was added to all standards and water samples
and mix thoroughly. Absorbance was obtained by setting the spectrophotometer at wavelength of 220 nm and standard curve was plotted.

The nitrate concentration of water samples was calculated by comparing the sample absorbance with standard curve.

3.3.14 Sulphate (SO$_4^{2-}$) (APHA, 2005, 4500-SO$_4^{2-}$ E)

Sulphate was estimated by precipitating it in acid medium in the form of Barium sulphate (BaSO$_4$) by the addition of barium chloride (BaCl$_2$). The sulphate estimation was carried out by taking 100 ml of water sample in a 500 ml conical flask and 20 ml of buffer solution was added and then stirred on magnetic stirrer. After few minutes 1 spatula full of BaCl$_2$ was added to the conical flask and stirred again in a magnetic stirrer for 1 minute. The absorbance of the solution was recorded in a spectrophotometer at a wavelength of 420 nm.

3.3.15 Phosphate (PO$_4^{3-}$) (APHA, 2005, 4500-P D)

Phosphate was determined by following the stannous chloride method using UV Vis. Spectrophotometer.

Reagents

*Ammonium Molybdate Solution:* (a) 2.5 g of Ammonium Molybdate was dissolved in 15 ml of double distilled water. (b) 28 ml of conc. H$_2$SO$_4$ was diluted with double distilled water to make it 40 ml.

Solutions 'a' and 'b' were mixed and the final volume was made 100 ml with double distilled water.

*Stannous Chloride Solution:* 2.5 g of SnCl$_2$ was dissolved in 10 ml of HCl to make up the volume 100 ml with double distilled water.

*Standard Phosphate Solution:* 0.4388 g of KH$_2$PO$_4$ was dissolved in 100 ml double distilled water to make a solution of 1000 ppm of Phosphate (PO$_4^{3-}$).

Procedure

Standard solutions of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ppm were prepared by the standard stock solution. To each 50 ml of standard solution and samples 2 ml of ammonium molybdate and 5 drops of SnCl$_2$ solution were added.
A blue colour complex appeared in presence of phosphate. The spectrophotometer was set at 690 nm and the readings were obtained. A standard curve was prepared between \( \text{PO}_4^{3-} \) concentration and absorbance. The phosphate concentration of the sample was estimated by comparing the absorbance values of sample from the standard curve.

### 3.3.16 Sodium (Na\(^+\)) and Potassium (K\(^+\)) (APHA, 2005, 3500-Na B, 3500-K B)

These both cations were detected by digital flame photometer Model no. 128 systronics company by using 10 and 100 ppm of stock solution of sodium and potassium.

**Reagents**

**Standard stock solution of Na\(^+\) (1000 ppm):** Dissolved 0.2543 gm of NaCl (AR grade) in 100 ml double distilled water.

**Standard stock solution of K\(^+\) (1000 ppm):** Dissolved 0.191 gm of KCl (AR grade) in 100 ml double distilled water.

### 3.3.17 Fluoride (F\(^-\)) (APHA, 2005, 4500-F\(^-\) D)

Fluoride was determined by following SPADNS method using UV Vis spectrophotometer.

**Reagent:-**

- **Fluoride stock solution:** Dissolve 221.0 mg anhydrous sodium fluoride (NaF) in double distilled water and made upto final volume 1000 ml in volumetric flask (1.00 ml= 0.1mg).

- **Fluoride standard solution:** Diluted 10 ml stock fluoride solution to 100 ml in a volumetric flask with double distilled water (100 ml=0.01mg).

**Acid Zirconyl – SPADNS reagent**

- **Zirconyl acid reagent:** 133 mg zirconyl chloride octahydrate (ZnOCl\(_2\) 8H\(_2\)O) was dissolved in 25 ml double distilled water. To this solution, 350 ml of concentrated HCl was added and diluted it to 500 ml with double distilled water.

- **SPADNS solution:** 455 mg SPADNS (Sodium-2(Parasulphophenylazo)- 1, 8-dihydroxy – 3, 6 napthalene disulphanate) was dissolved in double distilled
water and diluted to 500 ml. SPADNS solution and Zirconyl acid reagent were mixed in 1:1 ratio. This is known as zirconyl SPADNS reagent.

**Reference solution**

100 ml of double distilled water was taken in a beaker. To this, 10 ml of SPADNS solution was added. 7 ml of conc. HCl was diluted to 10 ml double distilled water and added to above solution. Mixed well and used this solution as reference to set spectrophotometer.

**Procedure**

Diluted 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml fluoride standard solution to 50 ml with double distilled water to make 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ppm fluoride standards. 10 ml acid zirconyl SPADNS reagent was added to all standards and water samples and mixed well. The spectrophotometer was set at 570 nm and the readings were obtained. A standard curve was prepared between fluoride concentration and absorbance. Fluoride concentration of the sample was estimated by comparing the absorbance values of sample from the standard curve.

**3.4 Water quality index**

The drinking water quality index (WQI) is intended to provide an easy to understand “ranking of water quality” (eg good, excellent, moderate, poor). The water quality index was calculated for assessing the suitability of water for drinking purpose. For calculation of WQI three steps are followed.

**Step 1-** In the first step, each of the essential parameters has been assigned a weight ranging from 1 to 5 depending on the relative importance of parameter with respect to drinking purpose.

We compute relative weight of each parameter from the following equation.

\[
W_i = \frac{W_i}{\sum_{i=1}^{n} W_i} \quad ..........(1)
\]

\(W_i\) is calculated according to its relative importance in the overall quality of drinking water

**Step 2-** In the Second Step, a quality rating scale (qi) for all the parameter is calculated by dividing its concentration in each water sample (Ci) by its respective standard (Si)
according to the drinking water guideline recommended Indian Standard and WHO drinking water standards. The results are then multiplied by 100 to get appropriate WQI

\[ q_i = \left( \frac{C_i}{S_i} \right) \times 100 \]  

\[ \text{Where } C_i = \text{Concentration of each parameter } S_i = \text{Respective Indian standard value } q_i = \text{Quality rating} \]

**Step 3-** The Sub Index (SIi) of water sample is determine for each parameter, by which WQI determined as per following equation

\[ S_i = W_i \times q_i \]  

\[ WQI = \sum S_i \]  

\[ \text{Overall } WQI = \frac{\sum q_i W_i}{\sum W_i} \]

\[ \text{Where } q_i = \text{Quality rating } W_i = \text{Relative weight} \]

### 3.5 Microbial methodology

#### 3.5.1 Water sampling

Water samples from different sources i.e (hand pump, bore well, tap and canal) were collected in 500 ml pre sterilized glass bottles (Figure 4.37) during summer (May), monsoon (August) and winter (January) seasons of 2013 and 2014. Sampling for bacteriological analysis was done aseptically with care, ensuring the omission of any external contamination of samples. After the water sampling was performed, each sampled bottle was labeled according to sampling location and all the samples were preserved at 4°C till further investigation.

Microbiological analysis is divided into four parts:

a. Estimation of heterotrophic plate count of all the studied water samples on seasonal basis for the year (2013-14 and 2014-15).

b. MPN based microbiological analysis for Total coliforms, Faecal coliforms and *E. coli* of all the studied water samples on seasonal basis for the year (2013-14 and 2014-15).

c. Morphological and biochemical characterization of the bacterial isolates.
d. Identification of the bacterial isolates from different water samples

3.5.2 Materials

Water samples (collected and stored in pre sterilized glass bottles (Schott Duran), Lauryl Tryptose Broth (LTB) (Himedia), Brilliant Green Bile Broth (BGBB) (Himedia), Eosine Methylene Blue (EMB) agar (Himedia), Nutrient agar (NA) (Himedia), sterilized test tubes (Borosil), pre sterilized disposable petri dish (Tarsons 90 mm), pre sterilized 1 ml micro-tips (Tarsons), micro pipettes (accupipet 100-1000µl), Glass spreader, Durham’s tubes, Laminar flow (Horizontal), BOD incubator (NSW), Adsorbent cotton, Conical flask (Borosil), Triclogel, Ethanol, spirit lamp, denatured spirit and Digital colony counter.

3.5.3 Serial dilution method (Cappuccino and Sherman, 2008)

The serial dilution technique was employed according to cappuccino and Sherman, 2008 to estimate the bacterial population from different water samples representing different blocks. Bacterial isolates were isolated at $10^{-3}$ dilution on pre-sterilized petri dish dispensed with sterilized Nutrient agar medium. Petri dishes were incubated in BOD incubator at 37 °C till 48 hrs. The developed bacterial colonies were counted using digital colony counter.

3.5.4 Pure culture of bacteria

Streak- plate method

Developed colonies on NA were isolated and further purified by streak plate method and incubated at 37 °C temperature till the bacterial cultures are ready for further studies. Slants having NA were prepared for storage and further identification of selected bacterial isolates by following the method described by Cappuccino and Sherman, 2008.

3.5.5 Total coliform bacteria

The total coliform bacteria in the samples were estimated using multiple tube fermentation technique (Cappuccino and Sherman, 2008). MPN index values were calculated by following the MPN- index value as given in Cappuccino and Sherman, 2008.
3.5.6 Presumptive test

The most probable number (MPN) of the number of bacteria present in water samples were enumerated by following the MPN (Index) table. Double strength and single strength Lauryl Tryptose Broth (LTB) were prepared as per the standard procedure using double distilled water. The media was poured in five test tubes of three dilutions each i.e. 10 ml, 1 ml and 0.1 ml. In one set 5 ml each of double strength media was dispensed and in the second and third set 5 ml of the single strength media was dispensed in each tube containing inverted Durham’s tube. The tubes were then sterilized in an autoclave for 15 minutes at 15 lbs and allowed to cool. 10 ml of the water sample was added with a sterilized micro pipette and sterilized tips to the first set of tubes containing double strength media and 1 ml and 0.1 ml of water sample to the second set of tubes containing single strength media. The tubes were then incubated for 48 hours at 37°C. After 48 hours each tube were gently agitate and examined for bacterial growth, gas formation and acidic reaction (shades of yellow colour). Growth with acidity and gas formation signifies a positive presumptive reaction. MPN/100 ml was calculated by following the formula given by (APHA, 2005):

\[
\text{MPN/100 ml} = \frac{\text{MPN value (from table)}}{10} \times 10 \\
\text{Largest volume tested in dilution series used for MPN determination}
\]

3.5.7 Confirmatory test

The confirmatory test was performed by preparing Brilliant Green Bile Broth (BGBB) tubes (APHA, 2005 and Cappucino and Sherman, 2008). Medium was prepared as per the standard procedure, sterilized in an autoclave at 15 lbs pressure for 15 minutes and dispensed into pre sterilized tubes. Transfer the culture from positive presumptive tube into each BGBB tube containing Durham’s tube by using sterile tips and micro pipette and incubated for 48 hrs at 37 °C.

3.5.8 Completed test

Completed test is the final analysis of the water sample confirming the presence of *E. coli*. It was used to examine the coliform appeared positive on the BGBB media by spreading on Eosine Methylene Blue (EMB) agar plates and incubated for 48 hrs at 37 °C.
3.5.9 Morphological and biochemical characterization of the bacterial isolate

Morphological characterization of the bacterial isolates was performed by following the method described by Cappuccino and Sherman, 2008.

3.5.10 Biochemical characterization

In the present study biochemical characterization tests for the isolated strains were carried out by following the method described by Cappuccino and Sherman (2008).

**Indole Test**

Tryptophan broth (Himedia) was used for this test after sterilization in an autoclave at 15 lbs for 15 minutes. Approximately 4 ml of broth was used per test tubes. After inoculation with inoculum, tubes were incubated at 37 °C for 24 to 48 hrs. After incubation, 1 ml Kovac’s reagent was added to each test tube. Presence and absence of cherry red colour on the top layer of tubes after addition of reagent confirms the positive or negative test for indole.

**Methyl Red Test**

MRVP Broth (Himedia) was used for methyl red test. 5 ml of broth was poured in each tube. After sterilization in an autoclave at 15 lbs for 15 minutes, tubes were inoculated with inoculum and incubated at 37 °C for 24 to 48 hrs. After incubation, 5 drops of methyl red indicator was added to each tube and colour of medium was noted. If the colour of the medium remains red after addition of indicator it confirms a positive test while decolorization of medium from red to yellow colour confirms negative test.

**Voges Proskauer Test**

MRVP Broth was used for this test. 5 ml of broth was poured in each tube. After sterilization in an autoclave at 15 lbs for 15 minutes, tubes were inoculated with inoculum and incubated at 37 °C for 24 to 48 hrs. After incubation, Baritts reagent was added to each tube and colour of medium was noted. Development of pink colour confirms positive test while no change in colouration confirms negative test.

**Carbohydrate Fermentation Test**

Phenol red broth (Himedia) was used for carbohydrate fermentation test. 4 ml of the medium was poured into each tube. The tubes were sterilized in an autoclave at 15 lbs for 15 minutes. After sterilization, the tubes were inoculated with the inoculum and
incubated at 37 °C for 24 to 48 hrs. After incubation, change in colour of the medium was noted. Development of yellow colour confirms positive test while no change in the colour confirms a negative test.

**Citrate Utilization Test**

Simmon citrate agar (Himedia) was used for this test. Approximate 4 ml of medium was poured into each test tube and sterilized in an autoclave at 15lbs for 15 minutes and prepared the agar slants. The slants were inoculated with the inoculum and incubated at 37 °C for 24 to 48 hrs and change of colour of medium was noted. Development of blue colour confirms positive test while no change in colouration confirms negative test.

**Catalase Test**

For this test, inoculum was placed on a clean glass slide by using sterilized inoculating needle. A drop of hydrogen peroxide (H₂O₂) was placed on the inoculum with the help of dropper and observed the appearance of oxygen bubbles within one minute. Appearance of bubbles indicates the positive test while no bubbles indicates the negative test.

**Gelatine Liquefaction Test**

Nutrient Gelatin (Himedia) was used for this test for the identification of micro-organisms. 4 ml of medium was taken into screw cap tubes and were sterilized at 15 lbs for 15 minutes and then medium was allowed to solidify. The inoculum was stabbed into the medium with the help of a sterilized inoculating loop and incubated at 37 °C for 48 hrs. After incubation, tubes were placed in a refrigerator at 4 °C for half an hour. Inoculated tubes that remain liquefied even after refrigeration shows positive test as it indicates hydrolysis of gelatin. Tubes that remain solidified even after refrigeration shows a negative test. Proteus vulgaris will show positive test and *E. Coli* will show negative test.

**Hydrogen Sulfide (H₂S) Test**

Dispensed SIM agar (Himedia) into test tubes & sterilized in an autoclave at 15 lbs for 15 minutes. The Inoculum was inoculated by stabbing. Plates were then incubated at 37 °C for 24 to 48 hrs and observed any change. Presence of black colouration along the line of stabbing indicates a positive test while no colour change indicates a negative test.
**Starch Hydrolysis**

Sterilized starch agar was poured into sterile petri dishes. After solidification, a single streak of inoculum was made in the centre of the plates and incubated at 37 °C for 48 hours. After incubation, flood the surface of the plates with the iodine solution and observed the change. A clear zone surrounding the colonies shows a positive test and dark blue colouration of the medium indicates a negative test.

**Urease Test**

Sterilized urea agar base (Himedia) was used for this test and dispense the medium into sterile test tubes. The tubes were inoculated with the inoculum and incubated in an incubator for 24 hrs. at 37 °C and observed any colour change. Development of deep pink colour indicates positive test while negative tubes do not show any color change.

**Oxidase Test**

Sterilized trypticase soy agar was used for oxidase test and poured into sterile petri dishes. A single line streak inoculation of the inoculums was made on the agar surface by using sterile technique and plates were incubated at 37 °C for 24 to 48 hrs. After incubation, oxidase reagent was added and noted the colour. Change of colour to purple indicates positive test while no colour change indicates negative test.

**Phenylalanine deaminase test**

Phenylalanine agar (Himedia) was used for phenylalanine deaminase test to differentiate proteus species from other members of enterobacteriaceae as phenyl pyruvic acid was formed from phenylalanine. Tubes containing medium were then sterilized at 15 lbs for 15 minutes. After sterilization, tubes were inoculated and incubated at 37 °C for 24 to 48 hrs. After incubation, ferric chloride (FeCl₃) was added to each tube and observed the colour change. Production of green colour indicates a positive test while no colour change indicates negative test.

Test kit KB013 (Himedia) for *Bacillus* species and KB002 (Himedia) for Gram negative rods were used to characterize the bacterial isolates (Figure 4.38).

**3.5.11 Gram staining**

Gram staining was performed by following the methodology discussed in Cappuccino and Sherman, 2008 to characterize the bacterial isolates on basis of gram stain and shape.
3.5.12 Identification of the bacterial isolates

The purified microbial cultures were phenotypically identified from IMTECH Chandigarh.

3.6 Methodology for plant study

3.6.1 Selection of seeds

The commercially available radish (Raphanus sativus L.) varieties RD-68 and Radish Hill Queen were procured and selected for the study. Seeds with uniform size, colour and weight were chosen for the experimental work.

3.6.2 Pot culture

For In vitro plant study, pot culture experiment was conducted in the laboratory of Environmental Toxicology and Microbiology research lab, EES department, CDLU, Sirsa by following the methodology of Aery, 2010. Plastic pots (15 cm height and 25 cm width) were used. The pots are prepared with sieved and dried soil. Surface sterilization was conducted for seeds of both test varieties of radish (Raphanus sativus L.) with 0.1% mercuric chloride (HgCl₂) for 30 seconds and vigorously rinsed with double distilled water for three times. Ten seeds were sowed in each pot in duplicate of each variety irrigated with water of different sources to study the response of both the test varieties.

3.6.3 Growth parameters

Growth parameters i.e. Germination percentage, Seedling vigour, Plant length, Plant fresh weight and dry weight were analysed by following the methodology of Aery, 2010.

3.6.4 Biochemical analysis

Chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid were estimated in plant samples by following the methodology of Arnon, (1949), Aery, 2010 and Qados, (2011).

3.6.5 Total Phenolic Content

Estimation of Total Phenolic Content was performed by following the Folin-ciocalteau method as described by Singleton et al. (1999), Aery, 2010, Rastgoo and Alemzadeh (2011).
3.7 **Hydro-geochemical characterization**

Aquachem scientific software version 2012.1 was employed to prepare the Piper, Durov, Schoeller and Wilcox plots for water analysis of all the studied water samples.

3.8 **Statistical analysis of the data**

Statistical analysis of the data obtained during physico-chemical and microbiological analysis was carried out by IBM SPSS software version 20.