2. MATERIALS AND METHODS
2.1 Sample stations
In the present study, three transects were chosen. They are 1) Helipad, 2) Light House and 3) Lagoon area. Four stations viz. nearshore, 2.5, 5 & 12.5 km in open sea were fixed on a line perpendicular to the coast at every transect of Helipad and Light House while three stations were fixed in Lagoon area (Fig. 1). In Lagoon stations were fixed at nearshore, centre and reef.

2.1.1 Sample collection: Surface samples were collected from all the stations during December 1997 (post monsoon), April 1999 (pre monsoon), August 2000 (monsoon) and May 2001 (monsoon).

2.1.2 Samples for microbiological analysis: Water samples for bacteriological analysis were collected in sterilised glass bottles with an overlapping rim of 300ml capacity. These samples were preserved in an icebox and analysed within three hours of collection.

2.1.3 Samples for physico-chemical analysis: Samples were collected using a clean plastic bucket. Samples for Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD) were collected in narrow mouthed glass-stoppered bottles of 125ml and 300ml capacities respectively without turbulence and agitation and fixed by adding 0.5 to 1 ml of Winkler A (WA) and Winkler B (WB) and the bottles were kept in dark until analysis. Samples for the analysis of nutrients were collected in clean plastic bottles and acidified with 2 N HCl. All the wares were cleaned by soaking in 6 N HCl and subsequently washed in double distilled water. Samples for petroleum hydrocarbon analysis were collected in 2.5litre amber coloured bottle (Strickland and Parsons, 1972; Grasshoff, 1983 & APHA, 1995).

2.1.4 Samples for productivity and plankton: Samples for primary productivity measurement were collected in three BOD bottles of 300ml capacity of which two are light bottles and third one is dark bottle. One of the light bottles is treated as control and the other as light bottle. All the bottles were filled through a polythene tube fitted with a coarse nylon net piece of 180μ pore size for avoiding the entry of zooplankton. Phytoplankton and zooplankton samples were collected in plastic bottles using standard plankton nets of mesh size 55μ and 180μ respectively. 10litre water was filtered through the phytoplankton net and a 250 ml aliquot was collected. One-litre samples were collected in dark coloured plastic bottles for the estimation of chlorophyll pigment in order to analyse the phytoplankton standing crop. Zooplankton samples were collected in 250ml plastic bottles by the subsurface hauling.
Fig. 2: Map showing well stations at Kavaratti Island
2.2 Collection of dug well water samples

Water samples were collected from 30 dug wells and 2 tube wells covering the entire Island (fig.2). Samples for bacteriological analysis were collected using sterilised BOD bottles tied in a clean rope where as samples for physico-chemical analysis were collected using clean plastic bucket. The samples for bacteriological analysis were immediately brought to the Lakshadweep Science & Technology/ PWD laboratory for analysis. Depth, diameter, presence/absence and proximity of leach pits/septic tanks to the dug wells were also noted. Possible pollution sources in the surroundings like the presence of poultry farm, coconut husk retting centre etc., were also noted down. The mode of withdrawal of water such as using conventional or mechanised mode was also noted.

2.2.1 Details of well stations

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Address of the wells</th>
<th>Location [10°-N; 072°-E]</th>
<th>Total Depth (m)</th>
<th>Diameter (m)</th>
<th>Potability status</th>
<th>Mode of withdrawal</th>
<th>Distance of septic tanks/leach pit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well near J.B School</td>
<td>34°52.86' 38°47.7&quot;</td>
<td>3.45</td>
<td>1.35</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>Well near Ottavathil Palli</td>
<td>34°8.16' 38°57.12&quot;</td>
<td>3.77</td>
<td>1.50</td>
<td>Yes</td>
<td>Conventional</td>
<td>8 m</td>
</tr>
<tr>
<td>3</td>
<td>Near Chekkilam House</td>
<td>34°18.78' 38°35.04&quot;</td>
<td>2.90</td>
<td>1.36</td>
<td>Yes</td>
<td>Pumping</td>
<td>10 m</td>
</tr>
<tr>
<td>4</td>
<td>c/o Kadat Palli</td>
<td>34°27.48' 38°29.82&quot;</td>
<td>2.60</td>
<td>1.13</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>Well near Bader Palli</td>
<td>34°17.16' 38°21.12&quot;</td>
<td>5.48</td>
<td>1.67</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>Well c/o Puthiya Palli</td>
<td>34°1.44' 38°10.86&quot;</td>
<td>4.50</td>
<td>1.34</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
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<tr>
<td>7</td>
<td>Well c/o Purathi Palli</td>
<td>33°47.82' 38°17.94&quot;</td>
<td>2.90</td>
<td>1.22</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>Well c/o Andam Palli</td>
<td>33°39.72' 38°30.78&quot;</td>
<td>3.45</td>
<td>1.45</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
</tr>
<tr>
<td>9</td>
<td>Well c/o Circuit House</td>
<td>33°28.08' 38°16.68&quot;</td>
<td>4.0</td>
<td>1.90</td>
<td>Yes</td>
<td>Pumping</td>
<td>15 m</td>
</tr>
<tr>
<td>10</td>
<td>Well c/o Govt. Press</td>
<td>33°38.58' 38°3.78&quot;</td>
<td>2.52</td>
<td>1.80</td>
<td>No</td>
<td>Conventional</td>
<td>10 m</td>
</tr>
<tr>
<td>11</td>
<td>Well c/o old Police barracks</td>
<td>33°20.46' 37°44.64&quot;</td>
<td>4.40</td>
<td>1.40</td>
<td>No</td>
<td>Pumping</td>
<td>10 m</td>
</tr>
<tr>
<td>12</td>
<td>Well c/o MPSAF Quarter</td>
<td>33°16.44' 37°54.36&quot;</td>
<td>4.05</td>
<td>1.46</td>
<td>Yes</td>
<td>Conventional</td>
<td>20 m</td>
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<tr>
<td>13</td>
<td>Well near Helipad</td>
<td>32°34.92' 37°6.24&quot;</td>
<td>1.70</td>
<td>76</td>
<td>Yes</td>
<td>Conventional</td>
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28
<table>
<thead>
<tr>
<th>No.</th>
<th>Well Name</th>
<th>Lat</th>
<th>Long</th>
<th>Depth</th>
<th>Diameter</th>
<th>Type</th>
<th>Flow</th>
<th>Pumping</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>14</td>
<td>Public well opposite S.B. School</td>
<td>34°6.54'</td>
<td>38°14.82'</td>
<td>5.12</td>
<td>3.75</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ujarana Palli</td>
<td>34°8.16'</td>
<td>38°44.94'</td>
<td>1.0</td>
<td>1</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>TI Madrasa near north J.B School</td>
<td>33°52.86'</td>
<td>38°47.7'</td>
<td>3.45</td>
<td>1.35</td>
<td>No</td>
<td>Conventional</td>
<td>Nil</td>
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</tr>
<tr>
<td>17</td>
<td>Near Kamiyana Palli</td>
<td>34°3.12'</td>
<td>38°34.08'</td>
<td>3.12</td>
<td>1.05</td>
<td>Yes</td>
<td>Conventional</td>
<td>20 m</td>
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<tr>
<td>18</td>
<td>Marakara Palli</td>
<td>34°6.12'</td>
<td>38°25.14'</td>
<td>3.14</td>
<td>1.66</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>SB School premises</td>
<td>34°5.52'</td>
<td>38°18.24'</td>
<td>2.65</td>
<td>1.42</td>
<td>Yes</td>
<td>Conventional</td>
<td>Nil</td>
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<tr>
<td>20</td>
<td>Front of PWD central stores</td>
<td>33°41.7'</td>
<td>37°59.22'</td>
<td>5.35</td>
<td>1</td>
<td>Yes</td>
<td>Pumping</td>
<td>20 m</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Handicraft premises</td>
<td>33°43.86'</td>
<td>38°6.48'</td>
<td>3.05</td>
<td>1.78</td>
<td>Yes</td>
<td>Pumping</td>
<td>10 m</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Behind Post office quarters</td>
<td>33°35.16'</td>
<td>38°24'</td>
<td>4.05</td>
<td>1.04</td>
<td>Yes</td>
<td>Pumping</td>
<td>15 m</td>
<td></td>
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<tr>
<td>23</td>
<td>Vetenery hospital premises</td>
<td>33°23.94'</td>
<td>38°5.16'</td>
<td>2.83</td>
<td>1.25</td>
<td>No</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
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<tr>
<td>24</td>
<td>PWD lab premises</td>
<td>33°9.9'</td>
<td>37°42.6'</td>
<td>3.05</td>
<td>1.5</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Puthi Illam, humairath house</td>
<td>34°16.26'</td>
<td>38°55.02'</td>
<td>3.37</td>
<td>2 x 2</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Mukkari Illam Beefuthumma house</td>
<td>34°15.42'</td>
<td>38°48.54'</td>
<td>4.42</td>
<td>1.70</td>
<td>Yes</td>
<td>Pumping</td>
<td>8 m</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Kavarom Kakkada</td>
<td>34°24.96'</td>
<td>38°44.7'</td>
<td>3.08</td>
<td>1.02</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Bebiyoda</td>
<td>34°33'</td>
<td>38°43.5'</td>
<td>2.0</td>
<td>1.33</td>
<td>Yes</td>
<td>Pumping (occasionally)</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Chamayatha pura</td>
<td>34°36.24'</td>
<td>38°30.84'</td>
<td>2.70</td>
<td>1.40</td>
<td>Yes</td>
<td>Pumping</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Thithottam</td>
<td>34°28.44'</td>
<td>38°22.56'</td>
<td>4.52</td>
<td>1.20</td>
<td>Yes</td>
<td>Pumping</td>
<td>13 m</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Shallow tube well in PWD lab compound</td>
<td>33°9.9'</td>
<td>37°42.6'</td>
<td>9.35</td>
<td>No</td>
<td></td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Deep tube well in PWD lab compound</td>
<td>33°9.9'</td>
<td>37°42.6'</td>
<td>15.4</td>
<td>No</td>
<td></td>
<td>Nil</td>
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</tr>
</tbody>
</table>
2.3 Analytical techniques

2.3.1 Microbiological analysis

Both the sea water and dug well samples were analysed for Total Viable Count (TVC), Total coliforms (TC), Faecal coliforms (FC), Faecal streptococci (FS), *Salmonella* like organisms (SLO), *Shigella* like organisms (SHLO), *Proteus*, *Klebsiella* like organisms (PKLO), *Vibrio cholera* like organisms (VCLO), *Vibrio parahaemolyticus* like organisms (VPLO) and *Pseudomonas aeruginosa* like organisms (PALO). Analysis of seawater samples was carried out using spread plate technique with 0.1 to 0.5 ml sample. Membrane filter method with 1.0 to 100ml sample was used for analysis of well water.

i) Total Viable Count (TVC)

The estimation of total viable count was carried out on Nutrient Agar medium at 37°C for 24 - 96 hrs. All the colonies in the range of 30 to 300 numbers were counted as TVC.

**Nutrient Agar media**

**Ingredients (Grams/litre)**: Beef extract, 3.0; Peptic digest of animal tissue, 5.0; Agar, 15.0; Final pH 6.8 ± 0.2

23.0 grams of the dehydrated medium (M/s Hi-Media Lab. Ltd., Bombay, India) was suspended in 1000 ml of distilled water, boiled to dissolve completely, sterilised by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to < 45°C and 12-15 ml of the cooled medium was poured over sterile petri plates.

ii) Total coliforms (TC)

The enumeration of total coliforms was carried out using Mac Conkey’s Agar at 37°C for 24-48 hrs. Colonies having shades of pink to red colours were counted as TC.

**Mac Conkey Agar**

**Ingredients (Grams/litre)**: Peptic digest of animal tissue, 17.0; Protease peptone, 3.0; Lactose, 10.0; Bile salts, 1.5; Sodium chloride, 5.0; Neutral red, 0.03; Agar, 15.0; Final pH 7.1 ± 0.2.

55.1 grams of the dehydrated medium (M/s Hi-Media Lab. Ltd., Bombay, India) was suspended in 1000 ml of distilled water, boiled to dissolve completely, sterilised by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to < 45°C and 12-15 ml of the cooled medium was poured over sterile petri plates.
iii) Faecal coliforms (FC)
The faecal coliforms were estimated using M-FC Agar at 44.5°C for 24 hrs. Seawater and
dug well samples were analysed for faecal coliforms using Membrane filter technique
(APHA, 1995).

M-FC Agar
Ingredients (Grams/litre)
Tryptose, 10.0; Protease peptone, 5.0; Yeast extract, 3.0; Sodium chloride, 5.0; Lactose, 0.2.

2 grams of the dehydrated medium (M/s Hi-Media Lab. Ltd., Bombay, India) was
suspended in 990 ml of distilled water, heated to dissolve completely, 10 ml of rosolic acid
was added and heated for one more minute. Cooled to < 45°C and 12-15 ml of the cooled
medium was poured over sterile petri plates.

iv) Salmonella like organisms (SLO)

Salmonella like organisms were screened using XLD (Xylose Lysine Deoxycholate) Agar
medium for 24 - 48 hrs at 37°C and identified as red colonies with black centre.

v) Shigella like organisms (SHLO)

Shigella like organisms were screened using XLD (Xylose Lysine Deoxycholate) Agar for
24 - 48 hrs at 37°C and the red colonies were identified as SHLO.

vi) Proteus & Klebsiella like organisms (PKLO)

Proteus & Klebsiella like organisms were screened using XLD (Xylose Lysine
Deoxycholate) Agar medium for 24-48 hrs at 37°C and yellow colonies are identified as
PKLO.

Xylose Lysine Deoxycholate Agar (XLD)
Ingredients (Grams/litre)
Yeast extract, 3.0; L-Lysine, 5.0; Lactose, 7.5; Sucrose, 7.5; Xylose, 3.5; Sodium chloride,
5.0; Sodium Deoxycholate, 2.5; Sodium thiosulphate, 6.8; Ferric ammonium citrate, 0.8;
Phenol red, 0.08; Agar, 15; Final pH, 7.4 ± 0.2

56.68 grams of the dehydrated medium was suspended in 1000 ml of distilled water and
boiled to dissolve completely. Cooled to < 45°C and 12-15 ml of the cooled medium was
poured over sterile petri plates.

vii) *Vibrio cholera* like organisms (VCLO)

*Vibrio cholera* like organisms was screened using TCBS (Thiosulphate Citrate Bile Salt Sucrose Agar) medium at 37°C for 24 hrs. VCLO looks yellowish and were counted.

viii) *Vibrio parahaemolyticus* like organisms (VPLO)

*Vibrio parahaemolyticus* like organisms were screened using TCBS (Thiosulphate Citrate Bile Salt Sucrose Agar) medium at 37°C for 24 hrs. *V parahaemolyticus* like organisms form greenish colonies.

TCBS Agar

**Ingredients (Grams/litre)**

- Peptone, special, 10.0; Yeast extract, 5.0; Sodium thiosulphate, 10.0; Sodium citrate, 10.0; Sodium cholate, 3.0; Oxgall, 5.0; Sucrose, 20.0; Sodium chloride, 10.0; Ferric citrate, 1.0; Bromothymol blue, 0.04; Agar, 15; Final pH, 8.8 ± 0.2

89.0 grams of the dehydrated medium was suspended in 1000 ml of distilled water and boiled to dissolve completely. Cooled to < 45°C and 12-15 ml of the cooled medium was poured over sterile petri plates.

ix) *Pseudomonas aeruginosa* like organisms (PALO)

PALO were isolated using Cetrimide Agar at 37°C for 24 - 48 hrs. Colonies of PALO are colourless.

Cetrimide Agar

**Ingredients (Grams/litre)**

- Beef extract, 10.0; Peptone, 10.0; Sodium chloride, 5.0; Cetrimide, 0.3; Agar, 12; Final pH, 7.3 ± 0.1.

37.5 grams of the dehydrated medium was suspended in 1000 ml of distilled water and sterilised by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled to < 45°C and 12-15 ml of the cooled medium was poured over sterile petri plates.

x) Faecal streptococci (FS)

Faecal streptococci were enumerated on M-Enterococcus Agar medium at an incubation temperature of 37°C for 24 - 48 hrs. The FS colonies were identified as maroon coloured.
M-Enterococcus Agar
Ingredients (Grams/litre)
Casein enzymic hydrolysate, 15.0; Papaic digest of soyameal, 5.0; Yeast extract, 5.0;
Dextrose, 2.0; Dipotassium phosphate, 4.0; Sodium azide, 0.4; 2, 3, 5 triphenyl tetrazolium
chloride, 0.1; Agar, 15; Final pH, 7.2 ± 0.2

41.5 grams of the dehydrated medium (M/s Hi-Media Lab. Ltd., Bombay, India) was
suspended in 1000 ml of distilled water and boiled to dissolve completely. Cooled to <
45°C, 0.5 ml of Tween 80 and 2.0 ml of sodium carbonate was added, and 12-15 ml of the
cooled medium was poured over sterile petri plates.

2.3.2 Physico-chemical parameters
i) Temperature
Temperature measurements were made with calibrated mercury filled Celsius thermometer
with an accuracy of 0.1°C and results were reported to the nearest 0.1 or 1.0°C depending
on need.

ii) pH
Principle
The basic principle of electrometric pH measurement is determination of the activity of the
hydrogen ions by potentiometric method. pH was measured using a standard pH meter.

iii) Total suspended solids
Principle
A well mixed sample is filtered through a pre weighed 0.45 micron cellulose nitrate filter
and the residue retained on the filter is dried to a constant weight at 103 105°C. The
increase in weight of the filter represents the total suspended solids.

Procedure
a) Preparation of glass-fiber filter disk.
Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with
3 successive 20 ml portions of distilled water. Continue suction to remove all traces of
water, and discard washing. Remove filter from filtration apparatus and transfer to
Aluminum or stainless steel planchet as a support. Dry in an oven at 103 –105°C for 1 hr. Cool in a dessicator to balance the temperature and weighed.

**Calculation**

\[
\text{Milligrams of total suspended solids/litre} = \frac{(A - B) \times 1000}{\text{Volume of sample ml.}}
\]

Where, 

- A = weight of filter + dried residue
- B = weight of filter

**iv) Dissolved Oxygen (DO)**

DO in water samples were determined by Winkler method. Dissolved oxygen in water reacts with manganese hydroxide in strongly alkaline medium forming manganese (trivalent) hydroxide. When acidified to a pH less than 2.5, the manganese hydroxide dissolves to liberate divalent manganese. Iodine, equivalent to the original dissolved oxygen content of the water is liberated and is titrated against a standard thiosulphate solution using starch as indicator.

**Reagents**

Winkler- A (WA): Dissolved 400g manganous chloride in 1000ml distilled water (DW) and stored in a polyethylene bottle.

Winkler-B (WB): Separately dissolved and mixed together, 360g potassium Iodide and 100g sodium hydroxide in 1000ml DW Stored in a polyethylene bottle.

Hydrochloric acid (50%): Carefully added 50ml conc. HCl to 50 ml DW. Stored in a ground stoppered glass bottle.

Sodium thiosulphate solution (Approximately 0.02 N): Dissolved 5g sodium thiosulphate in DW and made up to 1000ml in a volumetric flask. Stored in a ground stoppered glass bottle.

Starch indicator solution: Dissolved 1g starch in 100ml hot distilled water, quickly heated the suspension to boil in order to complete the dissolution of starch, and cooled.

Standard iodate solution (0.01N): Accurately weighed 0.3567g of potassium iodate (AR) and dissolved in 1000ml DW in a volumetric flask.
Analysis
Fixed DO by adding 0.5ml of WA followed by 0.5ml of WB in a 125ml DO bottle containing the sample. Shaken well and allowed the precipitate to settle. 1ml of 1:1H₂SO₄ was added and shaken vigorously till the precipitate dissolves. 100ml of the clear solution was transferred to conical flask and titratred against thiosulphate solution till a pale yellow color appears. Added 1 ml starch solution and continued the titration until the blue color disappeared. The burette reading was noted.

Calculation. The amount of DO in 1L of sample was given by

\[
\text{DO (mg/l) = } 8 \times N \times BR \times V/V-1 \times 1000/a \quad \text{Where } N = \text{Normality of thiosulphate solution,} \\
\text{BR = Burette reading, } V = \text{Volume of the sampling bottle, } a = \text{Volume of the sample titrated} \\
\]

Note: The factor V/V-1 was included to correct the volume of reagents added to the sample bottle.

v) Inorganic phosphate
Phosphate in sea water is allowed to react with acid – ammonium molybdate, forming a phosphomolybdate complex, which is reduced by ascorbic acid in the presence of antimonyl ions (to accelerate the reaction) to a blue coloured complex containing 1:1 atomic ratio of phosphate and antimonyl ions. The extinction of the blue colour is measured at 882 nm using 5cm cell. To avoid interference by silicate, the pH is kept below 1.

Reagents
1. Sulphuric acid (9.0 N)  Added carefully 250 ml concentrated sulphuric acid to a 1 litre volumetric flask containing 750 ml distilled water and dilute to 1000 ml.
2. Ammonium molybdate solution: Dissolved 1.25 g ammonium molybdate tetra hydrate (AR) in 125 ml distilled water. Stored in a plastic or glass bottle.
3. Potassium antimonyl tartrate solution: Dissolved 0.5 g potassium antimonyl tartrate (AR) in 20 ml distilled water. Stored in glass bottle.
5. Ascorbic acid solution: Dissolved 10 g ascorbic acid in 50 ml distilled water and add 50 ml 9.0 NH₂SO₄. The reagent was stored in an amber coloured bottle, in refrigeration, and could be used for a week.

6. Phosphate standard solution: Weighed 0.1361 g of potassium dihydrogen phosphate (AR) already dried at 110 °C in an oven and cooled in a dessicator and dissolved in 100 ml DW containing 1 ml of 9.0 N H₂SO₄. This solution contains 10,000 µmol PO₄³⁻/L.

7. Oxidation solution:
   Procedure: Transferred 5 ml of phosphate standard solution and a 500 ml volumetric flask and the volume made with distilled water. This solution contained 100 µmol PO₄³⁻/L and then diluted 10 times to get 10 µmol PO₄³⁻/L. 25 ml DW was taken in three tubes for blank determination. Prepared working standard solutions of 2.5µmol PO₄³⁻/L and 5 µmol PO₄³⁻/L and measured out 25 ml each in 3 test tubes and added to each tube, 0.5 ml of ascorbic acid reagent and mixed well. 0.5 ml of mixed reagent was added, mixed and kept for 10 minutes to develop blue complex. Absorbance of blank A (b) and standard A (st) was measured at 882 nm in a spectrophotometer using distilled water as the reference. 25 ml of the sample was taken in glass tube and the reagents added as in the case of standard solutions. Measured the absorbance A(s) in 5 cm cuvette at 882 nm. When the samples were turbid, the absorbance A(t) was measured separately on addition of 0.5 ml ascorbic acid.

Calculations

\[ F = 1.0 \]

\[ A(st)-A(b) ; \text{ Where } A \text{ (st) = Means absorbance of standards,} \]

\[ A(b) = \text{Means absorbance of blanks.} \]

\[ \text{PO}_4^{3-} \text{ mmol/L} = F \times A \text{ (s)} - A \text{ (t)} - A \text{ (b)} ; \text{ where A(s) = Mean absorbance of sample.} \]

\[ A(t) = \text{Mean absorbance of turbidity.} \]

vi) Determination of reactive silicate

Principle

The seawater sample is allowed to react with molybdate under conditions, which result in the formation of silicomolybate, phosphomolybate and arsenomolybate complexes. A
reducing solution, containing metol and oxalic acid is then added which reduces the silicomolybdate complex to give a blue reduction compound and simultaneously decomposes any phosphomolybdate so that interference from phosphate and arsenate is eliminated.

**Reagents**

1. Molybdate reagent: Dissolved 4.0g of analytical reagent quality ammonium paramolybdate, (NH)\(_4\)\(\text{Mo}_7\)\(\text{O}_{24}\).\(\text{H}_2\)\(_2\)O in about 300ml of distilled water. Added 12 ml of concentrated hydrochloric acid, mixed and made the volume to 500ml with distilled water. Store the solution in polythene bottle.

2. Metol—sulphate solution: Dissolved 6.0g of anhydrous sodium sulphite, Na\(_2\) SO\(_3\), in 500ml of distilled water and then added 10ml of metol (p-aminophenol sulphate).

3. Oxalic acid solution: Prepared a saturated oxalic acid solution by shaking 50g of analytical reagent quality oxalic acid dihydrate (COOH)_2, 2H\(_2\)O with 500ml distilled water; decant the solution from the crystals for use.

4. Sulphuric acid solution (50%v/v): Poured slowly 250ml of concentrated H\(_2\)SO\(_4\) into 250ml of distilled water, cooled and made up to 500ml with distilled water.

5. Reducing agent: Mixed 100ml of metol-sulphate solution with 60ml of oxalic acid solution. Added slowly with mixing 60ml of the 50 % sulphuric acid solution and made the mixture to a volume of 300ml with distilled water.

**Procedure**

1. Added 10ml of molybdate solution into a dry 50ml conical flask and poured 25ml of water sample into the conical flask, mixed solutions and allowed to stand for 10min.

2. Added 15ml of reducing agent and mixed immediately.

3. After 2hrs measured the extinction (O.D) of the solution in a 1cm cell against distilled water at a wavelength of 810nm.

**Standard**

a) Dissolved 0.96g of silicofluoride, Na\(_2\)SiF\(_6\) in 100ml distilled water and diluted to exactly 1000ml and stored this stock solution in a polyethylene container.

1ml= 5 \(\mu\)g at Si

b) Diluted 10ml of the stock solution to 500ml with synthetic water (25g NaCl + 8 g MgSO\(_4\). 7H\(_2\)O/litre of distilled water).
25ml of the diluted standard solution is taken and carried out the silicon determination as given in the procedure.

d) Measure the extinction (O.D) in a 1cm cell at 810nm. Approximate factor value:1.O.D.

vii) Total phosphorus and Total nitrogen

Principle
Total phosphorus represents all forms of dissolved inorganic and organic species of phosphorus. Organically bound phosphorus is completely decomposed to phosphate by a strong oxidising agent (alkaline persulphate). Inorganic forms of lower oxidation state are also oxidised to inorganic phosphate. The pH of the reaction is maintained at 9.7 so that after oxidation the pH remains between 4 and 5.

Reagents
1. Ammonium free water (double distilled water)
2. Sodium hydroxide (0.375 M): Dissolved 15.0 g sodium hydroxide and diluted to 1000 ml with double distilled water, stored in a tightly stoppered polythene bottle.
3. Oxidising reagent: Dissolved 5.0 g purified potassium persulphate (K2S2O8) and 3.0 g phosphoric acid (H3PO3) (AR) in 0.375 N NaOH, mixed well and stored in a tightly stoppered polythene bottle and covered with aluminium foil.
4. Standard stock organic nitrogen solution: Dissolved EDTA disodium salt (0.1862 g) in ammonia free distilled water (100 ml) and stored in a glass bottle and refrigerated. The standard contained 10μmol organic nitrogen/ml.

Calibration and determination of blank
Separately diluted each of the stock solutions of phosphate and organic nitrogen with 100 ml of ammonium free distilled water each. Pipetted out 10 ml of each solution into 250 ml volumetric flasks and volume made with ammonia free distilled water. This diluted standard solution contained 40μmol organic nitrogen/litre.

50 ml each of ammonia free distilled water and combined standard solutions were measured out in triplicate in oxidation flasks. Added 5.0 ml oxidising reagent to all the six flasks and autoclaved for 30 minutes. Swirled the flasks to dissolve any precipitate and allowed to cool. The contents of the flasks were transferred to 50 ml volumetric flask
ands diluted to the mark with distilled water. This solution contained 4 μmol NO₃=N (total)/litre.

Calculate the calibration factor (F total NO₃ μmol) = \( \frac{4.0}{A(\text{st}) - A(\text{b})} \);

Where \( A(\text{st}) \) = mean absorbance of standard and \( A(\text{b}) \) = mean absorbance of blanks

For total phosphorus calibration, 25 ml digested solution was measured in glass tubes and proceed for calibration. The calibration factor (F total P) was found out from

\[ F_{\text{total P}} = \frac{4.0}{A(\text{st}) - A(\text{b})} \]

50 ml seawater sample was taken and added 5.0 ml oxidising agent and digested as described above. For total nitrogen analysis 5 ml sample was diluted to 10 times and digested. Transfer 25 ml of digested sample into clean graduated tubes and the absorbance \( A(\text{s}) \) was found out at 882 nm.

Calculated total nitrogen and total phosphorus from

Total N (μmol)/l = \( F_{\text{total NO₃}} \times A(\text{s}) - A(\text{b}) \)

Total P (μmol)/l = \( F_{\text{total P}} \times A(\text{s}) - A(\text{b}) \); Where \( A(\text{s}) \) = mean absorbance of the sample and \( A(\text{b}) \) = mean absorbance of the blank. The results were reported in two decimal.

viii) Ammonia-nitrogen

Ammonia ( NH₃⁺,NH₄⁺ ) in sea water is allowed to react with Hypochlorite in moderately alkaline solution to form Monochloramine in presence of phenol, catalytic amounts of Nitroprusside ion and excess of Hypochlorite, gives an Indophenol Blue complex between pH 8 and 11.5. To prevent precipitation of Calcium and Magnesium Hydroxide and Carbonate present in sea water Citrate buffer is added. The Absorbance is measured spectrophotometrically at 630nm.

Reagents

1. NaOH (1N)
2. Phenol Reagent (Phenol + Sodiumnitroprusside)
3. Tri-Sodium citrate buffer
4. Hypochlorite Reagent
Standard Stock Solution was prepared by using dry Analar grade Ammonium Chloride and a drop of Chloroform was added for preservation.

ix) Determination of nitrite and nitrate
The method of nitrite determination depends on reaction with an aromatic amine, sulfanilamide to form a diazonium compound, which is then coupled with N-(1-naphthyl)ethylenediamine, to form an azo dye. The absorbance of the dye is measured spectrophotometrically at 540 nm. The nitrate is first reduced to nitrite before its determination. The reduction is carried out in reductor of cadmium granules. The cadmium is either coated with Hg or Cu. The conditions are so adjusted that the nitrate is quantitatively reduced to NO$_2^-$ but not further to NO.

Nitrite nitrogen
The nitrite in seawater was allowed to react with sulfanilamide in an acid solution. The resulting diazocompound was reacted with N-(1-naphthyl) – ethylene diamine to form a highly coloured azo dye, the absorbance of which was measured at 540 nm.

Reagents
Double distilled water (check for absence of nitrite)
Sulfanilamide solution (1%): dissolved 2.5 g sulfanilamide in 25 ml conc. hydrochloric acid and made upto 250 ml with distilled water. Stored in an amber coloured glass bottle.
n-(1-naphthyl) – ethylene diamine dihydrochloride (1%) Dissolved 0.25 g in 250 ml water and stored in an amber coloured glass bottle. Nitrite standard solution: Dissolved 0.690 g anhydrous sodium nitrite (dried at 100°C for 1 hr) in 100 ml water and diluted to 1000 ml in a volumetric flask. The solution contains 10 µmol NO$_2$-N/ml; the solution was stored in an amber glass bottle. Nitrite working standard solution: Pipetted out 10 ml of stock solution into a 500 ml volumetric flask and diluted to 500 ml mark. Transferred 5.0 ml of this solution to another 500 ml flask and the volume made upto the mark. This standard has 2 µmol NO$_2$-N/l.

Procedure
25 ml each of distilled water and the working standard solution was taken in three glass tubes each. To each tube added 0.5 ml of sulfanilamide, mixed and kept for 4 minutes. 0.5 ml of n-(1-naphthyl) – ethylene diamine dihydrochloride solution was added and allowed
the reaction to proceed for 10 minutes after mixing the contents. The absorbencies of blanks and standards were measured in a 5.0 cm cell against water as reference at 543 nm.

Calculations
Calculated the factor from the relation,
\[
F = \frac{\text{Conc. of standard solution}}{A(\text{st}) - A(\text{b})} \quad \text{where } A(\text{st}) = \text{Mean absorbance of standards}
\]

\[
A(\text{b}) = \text{Mean absorbance of blanks}
\]

Calculated the concentration from the relation
\[
\text{Nitrite-N mmol/l} = F [A(s) - A(b)] \quad \text{where } A(s) = \text{Mean absorbance of the sample}
\]

The results were reported to one place of decimal.

Nitrate-Nitrogen
The nitrate in seawater is reduced, almost quantitatively to nitrite, by passing through a column containing copperised cadmium filings. The nitrite thus produced is determined by diazotising with sulfanilamide and coupling with N- (1-naphthyl)-ethylene diamine as described for NO₂-N. Nitrite in the sample will pass through the reduction column without change. Hence the total nitrate plus nitrite will be determined by the method. Nitrate can be found by difference.

Reagents
Double distilled water (checked for absence of nitrate and nitrite)
Ammonium chloride buffer: Dissolved 10.0 g of ammonium chloride (AR) in 1000 ml water. Stored in a polyethylene bottle and the pH adjusted to 8.5 with ammonia.
Sulfanilamide solution and N-(1-naphthyl)-ethylene diamine dihydrochloride solution: Refer the case of NO₂-N. Nitrite standard solution: Dissolved 1.011 g dry potassium nitrite (previously dried at 105°C to constant weight) in 100 ml water and made upto 1000 ml in distilled water. The stock solution contained 10 μmol/ml NO₃-N.
Nitrite working standard: The stock solution is diluted in a volumetric flask to prepare a standard having a concentration of 5 μmol NO₃-N/l. For this 5 ml of the stock solution was diluted to 1000 ml and out of this 10 ml was again diluted to 100 ml with distilled water.
Cadmium metal filings: 40-60 mesh size (E Merck No. 120882)
Copper sulphate solution (1%): 10 g CuSO₄.5H₂O dissolved in 1000 ml water.
Preparation of the reductor

Commercially available granulated cadmium (E Merck) was sieved and the fraction between 40 and 60 mesh was used. Removed the traces of iron particles, if any, from the cadmium filings by a magnet. Washed with acetone to remove grease and oil. Washed with 2N HCl with stirring to make the metal surface free from oxides, washed with copious amounts of water to remove all the chloride ions. Placed the cadmium in a 125 ml stoppered glass bottle and filled with copper sulphate solution. Stoppered the bottle taking care that without the air bubbles trapped inside. The bottle was rotated slowly for 2 minutes and the copper sulphate solution drained out. Washed thoroughly with distilled water until the water drained out was clear. The copperised cadmium granules were kept under water.

Packaging the reductor column

Placed a plug of glass wool at the bottom end of the column and the reservoir was filled with distilled water. The copperised cadmium filings were transferred into the column reservoir. Allowed the filings to fall freely into the column taking care that no air cavities was formed. After transferring the filings in the column, placed another piece of glass wool on the top of the filings. The cadmium filings were kept under water always to avoid getting dried.

Activation of column

Passed 50 ml of activator solution plus 50 ml of buffer through the column at a rate of 50 ml in 8 minutes and discarded these elute. Another 50 ml water plus 50 ml buffer passed through the column. Stopped the elution, leaving the water just above the filings in the column.

Procedure

Blank: Passed 50 ml water plus 50 ml buffer through the column. Discarded the first 25 ml and collected next two portions of 25 ml in the stoppered glass tubes and preserved.

Standard: Passed 50 ml of standard 5 μmol NO₃⁻N/l plus 50 ml buffer through the column at the rate mentioned above. Discarded the first 25 ml and collected the next two portions of 25 ml and preserved. The elution was continued till the water level was just above the filings.

Blank: Passed 50 ml water plus 50 ml buffer through the column at the same rate. The first 25 ml was discarded and the next two 15 ml portions preserved. Elution continued till the water level was just above the filings.
Sample analysis

Passed 50 ml sample plus 50 ml buffer through the column, rejected the first 25 ml and the next two portions preserved.

Blank: Collected two 25 ml portions after discarding 25 ml on passing 50 ml water plus 50 ml buffer through the column. The samples were preserved and the column left with the buffer solution.

There are six blanks, two standards and two sample solutions. 1 ml of sulfanilamide was added to each tube and kept for four minutes. Added 1 ml N-(1-naphthyl) ethylene diamine dihydrochloride, mixed and waited fore 10 minutes. Measured the absorbance at 540 nm in a 1 cm cell against water.

Calculations

Factor F for nitrate was calculated from the formula

\[ F = \frac{\text{Conc. of standard solution}}{A(st) - A(b)} \]

where \( A(st) \) = Mean absorbance of standards;

\( A(b) \) = Mean absorbance of blanks

The concentration of nitrate + nitrite was calculated from the relation

\[ C(NO_2+NO_3) \ \mu\text{mol/l} = F \times [A(s) - A(b)] \]

where \( A(s) \) = Mean absorbance of samples

The values for nitrate was corrected using the relation

\[ C(NO_3) \ \mu\text{mol/l} = C(NO_2+NO_3) - C(NO_2) \]

where the \( C(NO_2) \) was the concentration of nitrite in \( \mu\text{mol/l} \) determined earlier.

x) Biochemical Oxygen Demand (BOD)

BOD is the amount of oxygen required by microorganisms to stabilise the degraded substances in water. Here the dissolved oxygen is analysed by Winkler method both at the start and after incubation at 20° C in a BOD incubator for five days. The amounts of DO reduced after 5 days is calculated in mg/l and termed as BOD.

Reagents: Same as in DO.

Procedure: Three BOD bottles were filled with sample making sure that no air bubble was trapped inside. DO in one bottle was found out as initial DO and the remaining two bottles incubated in a BOD incubator for 5 days to find out the final DO. The difference in the ‘Initial DO’ and the ‘Final DO’ was calculated as the measure of BOD, expressed in mg/l.
xi) Petroleum hydrocarbon
Petroleum Hydrocarbon in surface seawater is defined as the compounds of crude oil and oil products containing straight, branched, saturated and aromatic rings of hydrocarbon in combination with S, N, O, Ni and Olefinic Hydrocarbons. Petroleum hydrocarbon in seawater was extracted with n-Hexane. The extract was evaporated to dryness in a Rotary evaporator at 30°C under reduced pressure and the concentrate was taken up in n-Hexane and Fluorescence was measured at 360nm with excitation at 310nm (Fluorescence spectrophotometer).

xii) Dissolved trace metals
For the determination of dissolved trace metals, a larger volume of water was required. For this purpose, a prefiltration by GF/C and a subsequent Millipore filtration was undertaken. Prior to filtration the GF/C filter papers were cleaned with 0.01 N HCl and rinsed with distilled water. 800ml of water samples was used for the pre-concentration of metals as described by Sen Gupta et. al., (1978). The organic extract was back extracted with HNO3 and the aqueous layer (A) collected. The organic layer, after back extraction was treated with perchloric acid, evaporated to dryness and re-dissolved in 0.1N HCl (B). Both the extracts (A+B) were taken in a beaker, evaporated to dryness and made to a volume of 8ml using 0.1 N HCl and analysed for metals using AAS (Perkin Elmer, AA Analyst 200). Metals free seawater was prepared by extracting twice using this technique. Spiked metal standards were added to the metal free seawater and extractions were done as described for water samples. For each set of spiked standards, three replicate analyses were conducted and calibration curve drawn. Five replicate analyses were done for one sample from each set collected during different seasons. These gave coefficients of variation of 4.20% for Cu, 6.4-8.2% for Zn, 5.2 -6.8% for Cd, 2.3 -4.4% for Pb, 6.4-9.3% for Ni and 3.1-5.26% for Fe.

xiii) Conductivity
Conductivity is measured using a standard conductivity meter.

xiv) Chloride
Chloride is measured by argentometric method.
Argentometric method

In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of AgNO₃ titration of chloride. AgCl is precipitated quantitatively before red Ag₂CrO₄ is formed.

Sulphide, thiosulphate and sulphate ions interfere but can be removed by treatment with H₂O₂. Orthophosphate in excess of 25 mg/l interferes by precipitating as silver phosphate. Iron in excess of 10 mg/l interferes by marking the end point.

Reagents

K₂CrO₄ solution: Dissolved 50 g K₂CrO₄ in distilled water and diluted to 1 litre with distilled water.

Standard AgNO₃ solution (0.0141N): Dissolved 2.395 g AgNO₃ in distilled water and diluted to 1 litre. Standardised against 0.0141N NaCl and stored in a brown coloured bottle. 1 ml of this solution = 500 mg of Cl⁻

Standard NaCl solution (0.0141N): dissolved 824 mg of dried NaCl in distilled water and diluted to 1000 ml. 1 ml solution = 500 mg of Cl⁻

Procedure

Use 100 ml sample or a suitable portion sample diluted to 100 ml. Directly titrated the samples in the pH range 7 to 10. Adjust sample pH 7 to 10 with H₂SO₄ or NaOH if it is not in this range. Added 1 ml of K₂CrO₄ indicator. Titrated with standard AgNO₃ titrant to pinkish yellow end point.

Standardize AgNO₃ titrant and establish reagent blank value by above mentioned titration method.

Calculation

Milligrams of Cl/litre = \frac{(A-B) \times N \times 35450}{Volume \ of \ sample \ in \ ml} \ \ where, \ A = \text{Volume of AgNO₃ for sample} \ \ B = \text{Volume of AgNO₃ for blank} \ \ N = \text{Normality of AgNO₃}
xv) Total solids
Total solids refer to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include total suspended solids, the portion of total solids retained by a filter, and total dissolved solids, the portion that passes through the filter.

Principle
A well-mixed sample was evaporated in a pre weighed dish and dried to constant weight in an oven at 103 to 105 °C. The increase in weight over that of the empty dish represents the total solids.

Preparation of evaporating dish
If volatile solids are to be measured ignite clean evaporating dish at 550 ± 50°C for 1 hr in a muffle furnace. If only total solids are to be measured, heat clean dish to 103 – 105 °C, for 1 hr. Store dish in dessicator until needed. Then weighed.

Sample analysis
Choose a sample volume that will yield a residue between 2.5 mg – 200 mg. Transfer a measured volume of well-mixed sample to pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven. Dry the evaporated sample for at least 1 hr in an oven at 103 - 105 °C, cool the dish in dessicator to balance temperature and weighed.

Calculation
Milligrams of total solids/litre = \( \frac{(A - B) \times 1000}{\text{Volume of sample in ml}} \)

Where,

\( A = (\text{weight of dried residue + dish}) \)
\( B = (\text{weight of dish}) \).

xvi) Total alkalinity
The total content of substances in water that causes an increased concentration of hydroxide ions either upon dissociation or as a result of hydrolysis is called alkalinity of water. Total alkalinity of water is determined by the amount of acid neutralised together with a methyl orange indicator for water titration. Total alkalinity of water is not only due to the presence of bicarbonate, carbonate, hydroxide ions, but also due to other ions that react
with acid, including humates. The presence of salts of some weak organic acids (humates) also leads alkalinity to natural waters.

**Reagents**
1. 0.02N H₂SO₄ ie 1 ml 0.02N H₂SO₄ = 1 mg CaCO₃
2. Phenolphthalein indicator
3. Methyl orange indicator

**Procedure**

100ml of filtered sample was taken in a conical flask and few drops of alcoholic phenolphthalein indicator were added to it. Titrated the solution with 0.02N H₂SO₄ until become colourless. If the sample was colourless after the addition of phenolphthalein indicator, added few drops of aqueous methyl orange indicator and titrated against the acid till an orange pink colour was obtained at the end point. The nature of alkalinity was then predicated from the titration as follows:-

1. If the titration to the phenolphthalein end point was zero, alkalinity was regarded as due to bicarbonate alone.
2. When there was no further titration to the methyl orange end point after the phenolphthalein end point, the alkalinity was only due to hydroxides.
3. When the phenolphthalein end point titration was half the total titration, only carbonate alkalinity was expected to be present.
4. When the phenolphthalein end point titration was greater than half the total titration, the alkalinity is due to both carbonate and hydroxides.
5. When the phenolphthalein end point was less than half the total titration, the alkalinity was due to carbonate and bicarbonates.

**Calculation**

\[
\text{Milligrams of CaCO}_3/\text{l} = \frac{\text{Volume of acid (titrant)} \times 1000}{\text{Volume of sample taken}}
\]

**xvii) Total hardness**

The hardness of water is due to the ions of calcium, magnesium and strontium dissolved in water and is determined by complexometric titration. Because calcium and magnesium are usually present in significant concentrations in natural water, hardness is defined as
the characteristic of water that represents the total concentration of just the Ca and Mg ions expressed as CaCO₃. Hardness of water is of two types temporary or carbonate hardness and permanent or non-carbonate hardness.

**Principle**
Ethylene diamine tetra acetic acid (EDTA) and its sodium salts form a chelated soluble complex when added to solution of certain metal cations. If a small amount of dye such as Erichrome Black T is added to an aqueous solution containing Ca and Mg ions at a pH of 10 ± 0.1, then the solution becomes wine red. If EDTA is added as a titrant, the Ca and Mg ions will be complexed. When all of the Mg and Ca and has been complexed, the solution turns from wine red to blue. This is the end point of the titration. Mg ions must be present to yield a satisfactory end point in the titration.

**Reagents**
- **Buffer solution**: dissolved 16.9 g of NH₄Cl in 143 ml of NH₄OH, diluting this to 250 ml with deionised water.
- **Erichrome Black T indicator**: 0.2 g dye was dissolved in 15 ml of triethanol amine with the addition of 5 ml of absolute ethanol to reduce the viscosity. Added two drops of indicator per 50 ml of the solution to be titrated.
- **Standard EDTA solution (0.01 M)**: Weighed out 3.723 g of A R sodium ethylene diamine tetra acetate dihydrate (EDTA) dissolved in water and diluted to 1000 ml. 1 ml 0.01 M EDTA solution = 1 mg of CaCO₃
- **Standard calcium solution**: Weighed out 1 g anhydrous CaCO₃ powder into a 500 ml Erlenmeyer flask. 1:1HCL was added, little at time, until all CaCO₃ has dissolved. Added 200 ml distilled water and boil for few minutes to expel CO₂. Cooled and added a few drops of methyl red indicator, and adjusted to the intermediate orange colour by adding (3N NH₄OH + 1; 1HCl) as required. This was diluted to 1000 ml, with distilled water. 1 ml Ca solution = 1 mg of CaCO₃.
- **Sodium hydroxide (0.1 M)**: 0.4 g NaOH was dissolved in 1000 ml distilled water.

**Procedure**
Selected a suitable volume of the sample. Added 1 to 2 ml of NH₄Cl-NH₄OH buffer solution to maintain the pH above 10. The absence of sharp end point colour change in the titration means that the indicator has deteriorated. One to two drops of the Erichrome
Black T indicator was added. EDTA solution is then added slowly with continuous stirring. At the end point, the colour changes from wine red to blue.

**Calculation**

Hardness as milligrams of CaCO$_3$/l = \( \frac{A \times B \times 1000}{\text{Vol. of sample in ml}} \)

where, \( A = \text{Vol. of EDTA in ml required for the sample} \)

\( B = \text{Milligrams of CaCO}_3 \text{ equivalent to 1 ml of EDTA titrant.} \)

**xviii) Calcium hardness**

**Principle**

When EDTA is added to water containing both Calcium and Magnesium, it first combines with Ca. Ca can be determined directly with EDTA, when the pH is made sufficiently high that the Mg is largely precipitated as the hydroxide and an indicator is used that combines with Ca only.

**Reagents**

1. Sodium hydroxide (1 N)
   
   4g NaOH is dissolved in 100 ml.

2. Murexide (ammonium purpurate)
   
   0.2g murexide was mixed with 100g NaCl and ground well to get a uniform coloured mixture. Titrate immediately after adding indicator because it is unstable under alkaline conditions. This indicator changes from pink to purple at the end point.

3. Standard EDTA solution (0.01M)
   
   Weigh out 3.723g of AR Sodium ethylene diamine tetra acetate dihydrate dissolved in water and diluted to 1000ml.

   1ml 0.01m EDTA solution = 1mg of CaCO$_3$.

**Procedure**

50ml sample or a smaller portion diluted to 50ml was used for the test so that the Ca content is about 5 to 10mg. Added 2ml NaOH solution to increase the pH to 12 to 13. Stirred the solution and added 0.1 to 0.2g of indicator. EDTA solution was slowly added with continuous stirring to the proper end point. 1 to 2 excess drops of the titrant were added to ascertain that no further colour change was occurred.
Calculation

Milligrams of Calcium/l = \( \frac{A \times B \times 0.4008}{\text{Vol. of sample in ml}} \)

Calcium hardness as milligrams of CaCO₃/l = \( \frac{A \times B \times 1000}{\text{Vol. of sample in ml}} \);

Where, \( A \) = Volume of EDTA in ml; \( B \) = Milligrams of CaCO₃ equivalent to 1 ml of EDTA titrant at the Ca indicator end point.

xix) Magnesium

Magnesium is estimated as the difference between total hardness and Calcium as CaCO₃, if interfering metals are present in non-interfering concentrations in the Calcium titration and suitable inhibitors are used in the Hardness titration.

Milligrams of Magnesium/l = \( \left[ \text{Total hardness (as milligrams of CaCO₃/l)} - \text{Calcium hardness (as milligrams of CaCO₃/l)} \right] \times 0.243 \)

xx) Fluoride

Fluorine, also of the halogen group, is quite from other elements in the group in its geochemistry. Unlike chloride, bromine and iodine, many of the compounds of fluorine have a low solubility. Natural concentrations of fluoride commonly range from about 0.01 to 10.0 ppm. A few water samples have been analysed which have more than 10.0 ppm, the highest reported is 67 ppm from the Union of South Africa.

It is estimated by spectrophotometrically using Spadens reagent

xxi) Sulphate

Sulphate is determined following turbidimetric method using BaCl₂ salt to precipitate sulphate present in the sample as Barium sulphate. The measurement is carried out spectrophotometrically at 420nm using sulphuric acid as standard.

Calculation

\( \text{mg of SO}_4^{2-} = \text{Mg SO}_4^{2-} \times 1000/\text{vol. of sample} \)
2.3.3 Biological parameters

i) Primary Productivity
Productivity was measured by light and dark oxygen bottle experiment after six hours \textit{in-situ} incubation. The dissolved oxygen of the control bottle is measured initially and the difference in dissolved oxygen content in the light and dark bottles is calculated per day using Winkler method.

\textbf{Calculation}

\begin{equation}
\text{Gross primary productivity} = \frac{(\text{Light DO} - \text{Dark DO}) \times (12/32)}{\text{PQ}} \times \text{Time of incubation} \\
\end{equation}

Where PQ = photosynthetic coefficient which is 1.25

12/32 is a factor used to convert oxygen to carbon ie, 1 mole of O$_2$ (32 g) is released for each mole of carbon (12 g) fixed.

ii) Chlorophyll $a$
Chlorophyll concentration is one of the indicators of phytoplankton standing crop.

\textbf{Reagents}

1. MgCO$_3$ suspension 1%
2. 90 \% aqueous acetone

The water sample collected for chlorophyll was filtered through Whatman GF/C filter paper of 0.47\textmu m wetted with 1ml of MgCO$_3$ suspension to prevent the pigment degradation. Filtration was carried out with the help of vacuum pump. The paper was taken out with a clean forceps and ground in a mortar and pestle with 2-3ml acetone and added to a test tube with 90\% acetone to make up the final volume to 10 ml. The tube was covered with aluminum foil and kept in refrigerator at 4\textdegree C for 24 hrs to complete the extraction of the pigment. The extract was then centrifuged at 5000rpm for 20 minutes. The supernatant was then transferred to a photometric cell and the absorbance measured with 664 nm and 750 nm. Acidified the extract in the cell with 0.1 ml, 0.1 N HCl, gently agitated and read at 665 nm and 750 nm after 90 seconds.

\textbf{Calculation}

\begin{equation}
\text{Chlorophyll a (mg/m}^3\text{)} = 26.7 (664b-665a) \frac{V_1}{V_2} x 1 \\
\end{equation}

where $V_1$ = volume of extract in litres
V<sub>2</sub> = volume of sample in m<sup>3</sup>

l = light path

664b and 665a = Optical Density of 90% acetone before and after acidification.

Phaeophytin (mg/m<sup>3</sup>) = 26.7 (1.7 x 665a-664b) (V<sub>1</sub> / V<sub>2</sub>) x l

Where, 26.7 is the absorbance correction and is equal to A x K

And A = absorbance coefficient for chlorophyll a at 664 nm = 11

K = Ratio expressing correction for acidification

iii) Phytoplankton

Phytoplankton samples were concentrated by membrane filtration technique using Whatman No. 42 filter and the concentrate was used for identification under biological microscope (Olympus CH 20)). Triplicate analyses were carried out for each sample. The density was expressed in cell nos/litre and the abundance of genera was noted following the method of Santhanam et al., 1987.

iv) Zooplankton

Zooplankton identification was carried out with the help of binocular stereo zoom microscope (Olympus SZ 60) and biomass was found out by displacement volume measurement. The zooplankton samples were filtered through a piece of net of same mesh used for the collection. The filtered sample was then transferred to a measuring cylinder having a known volume of 4% formalin. From the difference between the initial and final reading, the volume of sample was calculated and expressed value in ml/m<sup>3</sup>