Chapter – 2:

MATERIALS AND METHODS

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1. Study area

In Vadodara city there are a large number of water bodies. All of these are fresh water bodies. Some of them are natural urban ponds and some of them are artificial tanks. Temple is located near most of them and is surrounded by the slum area and human habitation; such ponds are used by inhabiting people around it for various anthropogenic activities like bathing, washing clothes, fishing, and dumping sewage etc. (Parikh, 2011).

The selection of the criteria of the study sites were depended on availability of fish and water throughout the study period.

For the present study following ponds were selected as the study sites:

1. Dhobi talav
2. Majamtalav
3. Danteshwar pond
4. Sama pond

In this research work two such ponds i.e. Dhobi talav and Majamtalav were majorly investigated for the period of two year. For some period work also has been carried out for Danteshwar pond and Sama pond (Fig. 1.1).

Table 1.1: Details and geo-location of the study sites

Sr. No.	Study sites	Type and Location	Surroundings	Latitude	Longitude
1.	Dhobi talav	Perennial and natural urban pond, located near Swami Taioon Ram temple, Warasiya, Vadodara	No slum area, permanent residences, less affected by anthropogenic activity and other wastes.	22°18'45.31"N	73°13'9.43"E
2.	Majamtalav	Perennial and natural urban pond, located near Tulsiwadi, Vadodara	3/4 parts of pond covered by slums and puccha houses, influenced by inhabiting people through various activity	22°18'45.69"N	73°12'28.13"E
3.	Danteshwar talav	Perennial and natural urban pond, located near Pratapnagar railway station, Vadodara	1/4 parts of pond covered by slums and influenced by anthropogenic activity	22^{0}27'60.15" N	73^{0}21'04.21"E
4.	Sama talav	Perennial and natural urban pond, located near Urmi school, Vadodara	Majorly covered by slum area and puccha houses, much affected by anthropogenic activity	22^{0} 20' 55.4" N	73^{0}12' 17.7" E

Fig 1.1 Representation of the study sites

Site-1 Dhobi Talav

Site- 2 Majam Talav (Hathi Talav) Tulsiwadi

Site-3: Danteshwar Talav

Site-4 Sama Talav

Study Site-1 Dhobi Talav

Study Site-2 Majam Talav

Study Site-3 Danteshwar Talav

Study Site-4 Sama Talav

Fig 1.2 A full view of Tilapia (*Oreochromis mossambicus*)

2. Parametric analysis

Such analyses are essential for assessment of hydro-biological status, environmental condition, quality of water and soil and the structure of an ecosystem. A healthy aquatic ecosystem is dependent on the maintenance of physico-chemical properties of water and its biological diversity. Water quality plays an important role in aquaculture because its imbalances can cause stress, poor growth and mortality of culture species (Boyd, and Tucker, 1998). To assess the environmental condition and hydro-biological status of study sites, physico-chemical analysis of water and soil samples was carried out throughout the study period for research work.

2.1 Water analysis

For assessment of physico-chemical status of water, the study sites were studied monthly. Water samples were collected from various sites at random from the ponds in the morning time between 7 to 8 am in plastic bottles. Water temperature was recorded by standard centigrade thermometer on site. For the estimation of dissolved oxygen, water samples were collected separately in 300 ml BOD bottles and oxygen was fixed by using Winkler's reagent at the time of sampling on field. pH was recorded by standard pH meter. All other remaining parameters were analyzed immediately on return to the laboratory by titrometric methods. The physico-chemical parameters for water quality were assessed using standard methods (APHA, 1998), details for the same in presented in (Table 2.1).

Table – 2.1: Methods for various water quality parameters

Sr. No.	Parameter	Method	Instrument
1	pH	Electrometric method	pH meter
2	Temperature	Laboratory or Field method	Mercury Thermometer
3	Acidity	Titrimatric Method	Titration assembly
4	Alkalinity	Titrimatric	Titration assembly
5	Chloride	Argentometric Method (Titrimetric method)	Titration assembly
6	Total Hardness (TH)	Titrimetric Method	Titration assembly
7	Total Solids (TS)	Filtration method	Oven, Beaker
8	Dissolved Oxygen (DO)	Winkler's method - Azide modification method	BOD bottle, Titration assembly
9	Total Phosphorus	Ammonium Molybdate method.	Spectrophotometer
10	Nitrate	Cadmium reduction method.	Spectrophotometer

pH:

pH indicates the acidic or basic nature of water. It represents the negative logarithm of H^+ ions and one of the most important parameters studied to evaluate the chemistry of water (Ramchandra *et al.*, 2002). The estimation of pH was carried out by dipping the digital pH meter probe (pH scan, Eutech Instruments) about 10 cm below the surface of water.

Temperature (Temp):

The surface water temperature was recorded through standard centigrade thermometer by dipping up to desired depth at the study sites.

Acidity:

Acidity of water is its quantitative capacity to react with strong base at a designated pH (APHA, 2004). The estimation was performed by titrimatric method. In 10ml of sample, 2 to 4 drops of phenolphthalein as indicator was added and titrated against 0.02 N NaOH solutions. A colour change from colourless to pink was taken as the end point.

Alkalinity:

Alkalinity of water is its ability to neutralize a strong acid. The chief component contributing to the alkalinity of most natural freshwaters are carbonates (CO_3^{-2}) and bicarbonate (HCO_3^{-1}). This was estimated by titrimatric method. Total alkalinity is the sum of Hydroxyl alkalinity and Bicarbonate alkalinity. Hydroxyl ions present in the sample as a result of dissociation or hydrolysis of solutes reacts with additions of standard acid. Thus alkalinity depends on the end point of pH.

Chloride (Cl^-):

The presence of chloride in natural waters can be attributed to the dissolution of salt deposits, irrigation drainage and sewage discharges. Chloride was determined by Argentometric method wherein the samples were titrated against standard silver nitrate solution using $K_2Cr_2O_4$ as an indicator. Silver Chloride was quantitatively precipitated before red silver chromate was formed, which is the end point.

Total Hardness (TH):

The Total Hardness is the sum of concentration of the alkaline earth metal cations present in it. Calcium and magnesium are the principal cations imparting hardness. TH was estimated by EDTA titrimatric method. In an alkaline condition, EDTA reacts with Ca^{++} and Mg^{++} to form a soluble chelated complex. They develop wine red colour with Eriochrome Black-T. When EDTA is added as a titrant, Ca++and Mg divalent ions get complexes resulting in a sharp change from wine red to blue which indicates end point of the titration. At higher pH, about 12.0, Mg ions precipitate and only Ca++ions remain in the solution. At this pH, Murexide indicator forms a pink colour with Ca++ions. When

EDTA is added Ca++ions form complex resulting in the change from pink to purple, which indicates end point of the reaction.

Total Solids (TS):

Total Solids gives the measure of ions dissolved in the water. TS is the term applied to the material residues left in the vessel after evaporation of water sample and its subsequent drying in the oven. Total solids include Total Dissolved Solids (TDS) and Total Suspended Solids (TSS). About 10ml well-mixed sample was taken into a pre-weighed evaporating dish and evaporated to dryness at 103-105°C. The evaporating disc was cooled accurately weighted to constant weight. The difference in two weights is considered as TS.

Dissolved Oxygen (DO):

Dissolve Oxygen acts as limiting factor in natural water bodies, because most of aquatic organisms die rapidly when oxygen in water becomes low or falls to zero. DO is inversely related to temperature and salinity, helps breakdown of organic detritus.DO is a very important parameter of water quality and is an index of physical and biological process of water. It is estimated by Winkler's method.

Nitrate (N):

Nitrate generally occurs in trace quantities in surface water but also attains high levels in some ground water. It is found in small amounts in fresh water and domestic waste water. Nitrates and Nitrites serve as nutrients, increasing amount of such nutrients creates eutrophication . Nitrate (NO_3-N) is estimated by Cadmium - reduction method.

Total Phosphorus (TP):

Phosphate occurs in natural water or in waste water. Small amount of phosphate arise from a variety of sources but primarily by biological processes from organic phosphorus. phosphorous are often identified a limiting nutrients in pond water for plankton production (Hecky & Kilham 1988).When present in large quantity in surface waters it is associated with the excessive growth of algae (Lcan, 1973; Ambasht and Ambasht, 1992). It is estimated by Ammonium molbydate method.

2.2 Soil quality analysis:

Monthly collection of soil samples up to the depth of 15 cm was done for the study the soil quality parameters. The soil samples were collected using scoop and were kept in thick quality polythene bags. The samples were dried in laboratory oven. The dried soil was grinded using mortar and pestle and then sieved through 2 mm mesh sized sieve and used for further analysis. The soil quality parameters such as pH; Phosphate-P and Nitrate-N were analyzed during the investigation of the study sites by using Standard methods.

pH:

Soil pH was analyzed through Richards (1954) method. According to this soil pH was determined by using analytical pH meter. This is composed of two electrodes.

- Glass electrode or indicator electrode.
- Calomel electrode or reference electrode.

When the both electrodes are dipped in aqueous solution under test, the potential difference between both the glass electrode and the calomel electrode is measured.

Procedure: Take 10 gm soils in 50 ml beakers add 20 ml of DW and stir intermittently with glass rod for 30 minutes. Determine pH of the soil suspension with the pH meter as per directions with the instruments. Stir the suspension in each beaker with the glass rod again just before taking the pH reading wash the electrodes with DW after each determination. Express pH to the nearest tenth of pH unit.

Nitrate-N (NO_3-N):

Nitrate is the highest oxidized form of nitrogen and its most important source is biological oxidation of nitrogenous organic matter. Sources of nitrogenous organic matter are domestic sewage, agriculture run off, metabolic wastes of aquatic community and dead organisms. Organic matter oxidized through nitrifying bacteria such as Nitrosomonas, Nitrobactor. Certain nitrogen-fixing bacteria (Azobactor) and algae (Anabaena, Nostoc) have capacity to fix molecular nitrogen in nitrates. Such nitrate can be estimated. Nitrate-N (NO_3-N) estimated by using phenol disulphonic method through

Calculation $NO_3\text{-N (mg/g)} = F \times V / 1000 \times W$

Phosphate-P (Po4-p):

Phosphorus occurs in natural ponds due to leaching of rocks and soil. Runoff domestic and agricultural wastes, industrial effluents and anthropogenic activities by human being are the major sources of phosphorus in water. The high concentration of phosphorus indicates pollution. Phosphate-p (Po_4-p)estimated by Ammonium molbydate method by using

Calculation Po_4-p (mg/g) = PS x V / 1000 W

3. Fish population analysis

Fish population majorly estimated through the morphology, morphometric and condition factor of fishes. Morphology and morphometric of fishes exists size, structure and growth while the condition factor indicates the condition (good or poor) or well being of fishes in population.

3.1 Morphological and morphometric analysis:

Morphologic and morphometric analysis has been done by taking length and weight relationship of fishes. For such analysis the fishes {Tilapia (*Oreochromis mossaimbicus*)} were collected from the study sites.The following measurements and observations were recorded for each sample. i. Total length in centimeters. ii. Standard length in centimeters. Length was measured with the help of thread and scale (in cm). iii. Total weight in grams. Weight was measured with help of an electronic weighing balance to the nearest 0.01 (in gm). After taking measurements the fishes were released in natural water bodies.

The relationship between the length (L) and weight (W) of fish was expressed by equation **W=a Lb, (Le cren, 1951)**

Where W= weight of fish in gram, L=total length (TL) of fish in cm. a=Constant (intercept), b = The length exponent (slope)

When expressed in Logarithm: Log W=Log a + b LogL i.e. y = A+Bx**,** where Y = Log W, B = n (regression coefficient) and X =Log L. The "a" and "b" values were obtained from a linear regression of the length and weight of fish.

The correlation (r^2) that is the degree of association between the length and weight was computed from the linear regression analysis: $\mathbf{R = r^2}$

3.2 Condition Factor analysis:

The condition factor (K) of the experimental fish was estimated by using Fulton's condition factor: K= W/L3 x 100 (**Fulton, 1904**).

Where K= condition factor, W= weight of fish (g), L= length of fish (cm).

All the observations were tabulated to various relevant statistical analyses to draw inference.