

# **Chapter – 2: MATERIALS AND METHODS**

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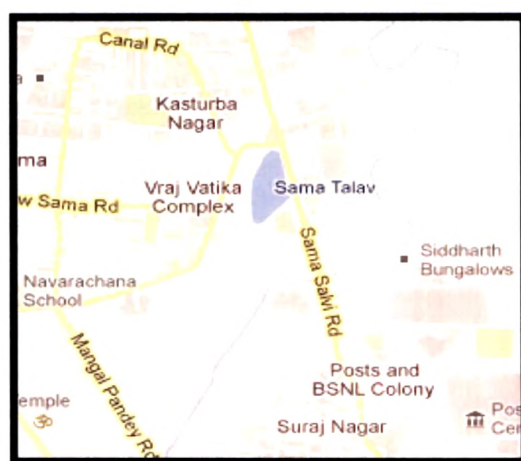
# 1 STUDY AREA

There are several ponds in and around Vadodara city within Vadodara Urban Development Authority (VUDA) area. Various urban ponds of Vadodara city were surveyed during the study period, but for this study only two ponds were selected i.e. Sama pond and Harni pond. Sama pond was subjected to detailed analysis, as site wise anthropogenic activities and its possible impact was recorded for only this pond. It is an urban pond which is utilized by surrounding inhabitants for various activities such as bathing, washing clothes, cleaning vegetables as well as vehicle washing and dumping sewage etc. Compared to other ponds of Vadodara city Sama and Harni ponds are more under the influence of urbanization, in future the situation may worsen and thus these two ponds were selected for study. Sama pond is geographically located besides a highway; hence immense pressure of transportation is recorded along the site. This easy access made the pond subjected to more activities compared to Harni pond. The Harni pond is away from highway and has comparatively lesser slum inhabitation. As this pond receives more amounts of general waste and untreated sewage from different sources, it was found having high organic status, visible through more vegetation and greenness of water.

**SAMA POND**, an urban pond is located in Vadodara district of Gujarat State. It is a perennial urban pond; the geo-location is 22° 20' 554"N latitude and 73° 12' 177"E longitude (Fig-2.1). Average depth of the pond is 2 meters with water storage capacity of approx. 1.2 lakh cubic meters at Full Reservoir Level (FRL).

Total free catchment area of the pond is 700 ha. Large part of the catchment area (80%) is agricultural land, however presently the area is under rapid urbanization as around 15% has residential constructions, 2.5% has plantations and 1% is small water bodies. In the south an outlet drain carries excess water downstream into Vishwamitri River. In recent years the flow of the pond downstream is blocked due to the construction of Urmi School. Also, the inlet of the pond has been blocked by buildings and the flow of the natural drain into the pond is obstructed leading to water stagnation. There are around 300 huts on one side of the pond.

Three different sites within Sama pond were selected for present study, on the basis of their anthropogenic interactions. Site-1 located near Urmi School, is less influenced by the anthropogenic activities. The Site-2 is along the highway, the activities like sewage dumping and washing vegetables is done regularly on this side. Site-3 is near the human residential area where washing clothes; utensils and bathing etc. are common features.



**Fig-2.1: Map showing location of Sama pond**



**PLATE 2.1: Map showing location of Sama pond**



SITE - 1



SITE - 2



SITE - 3

**HARNI POND** is located at 22° 20' 266" N latitude, 73° 13' 115" E longitude (Fig: 2.2). Total area of the pond is 11.6 ha. Average depth of the pond is 03 meters. Water storage capacity of the pond at FRL is about 3.5 lakh cubic meters. Bed of the pond is made up of sandy alluvium with good permeability. The pond has free catchment area of about 315 ha, large part of the catchments include wasteland (36%) followed by agricultural area (33.5%) and 16.9% area has concrete constructions, while another 12.8% area has plantations. The pond lies on the peripheral boundary of airport and has shops on one side. There are around 100 hut settlements on the North-Eastern side of the pond. The household sewage and domestic waste is diverted directly into the pond. Thereby increasing the organic load of the water and promoting the growth of algae responsible for deterioration of water quality, besides, propagation of microbes causing diseases. Like Sama pond, here on the bank local inhabitants wash their clothes, utensils etc.



**Fig-2.2: Map showing location of Harni pond**





**PLATE 2.2: Overview of Harni pond**



**Anthropogenic activities around Harni Pond**

## **2 PARAMETRIC ANALYSIS**

All organisms and the communities are directly or indirectly affected by the physical characteristics of their environment (Gillis et al., 2008). Thus the study of interaction between biotic and abiotic factors becomes essential to understand the community structure of an ecosystem (Donson and Travis, 1991). These characteristics with natural or manmade changes, determine the quality of water (Anonymous, 2003). The physico-chemical analysis of water and soil samples was carried out to study the ecological status of the ponds.

### **2.1 Water quality analysis:**

The water of these fresh water bodies is used for various human activities and so it becomes necessary to check the physico-chemical characteristics of the same. Monitoring the quality of water is important because clean water is necessary for human health and for the integrity of aquatic ecosystems. The study of physico-chemical properties of an aquatic ecosystem is important as fluctuations in water quality have an influence on the biotic communities (Aher et al., 2007).

For ecological assessment as planned for this study, regular visits to both the ponds were made; the sampling was done for water quality. The quality analysis for various parameters of the water samples was performed as per standard methods (APHA, 1998), details of the same presented in (Table-2.1).

**Table – 2.1: Methods and Instruments 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	Titrimetric Method	Titration assembly
4	Alkalinity	Titrimetric Method	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

Water samples were collected from the sites and stored in 1litre plastic bottles. These samples were taken to the laboratory for routine physico-chemical analysis on the same day. The estimation of dissolved oxygen (DO) was carried out by fixing the same in the sample on site by adding Winkler's reagent.



**pH:**

It indicates the acidic or basic nature of water. It is 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 water temperature was recorded by dipping the Mercury Thermometer up to the desired depth.

**Acidity:**

Acidity of water is its quantitative capacity to react with strong base at a designated pH (APHA, 2004). The estimation was performed by titrimetric 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 ( $\text{CO}_3^{2-}$ ) and bicarbonates ( $\text{HCO}_3^{-1}$ ). This was also estimated by titrimetric 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<sup>-</sup>):**

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 titrimetric 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 complex 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). 10ml well-mixed sample was taken into a pre-weighed evaporating dish and evaporated to dryness at 103-105°C. The evaporating dish was cooled and accurately weighted to constant weight. The difference in two weights is considered as TS.

#### **Dissolved Oxygen (DO):**

Oxygen dissolved in water, often referred as 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 (Nit):**

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. Nitrate ( $\text{NO}_3^-$ ) 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. Phosphorus has been identified as the limiting nutrient for phytoplankton development (Khan and Ansari, 2005) however; 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.

### **Statistical Analysis:**

Appropriate statistical analysis was carried out to analyze the results. Correlation coefficient is commonly used to establish the relation between independent and dependent variables (Nair et al. 2005). Correlation coefficient (r) was calculated to know the relationship between and among the parameters.

### **Bacteriological analysis:**

Bacteriological examination of water samples was done by using standard procedure (APHA, 2004). The bacteriological analysis of pond water has been conducted from the point of view of public health. The most important members of coliforms are *E.coli*. The Coliform test was done by using Nutrient agar, Mackonkey agar and EMB agar as substrates. Water samples were collected in a clean sterile screw cap bottle and were processed within one hour. The samples were taken upto 5 ml in test tube. The plate count technique was used in which the sample is diluted quantitatively and measured amounts of the dilutions are cultured in Petri dishes. The laboratory procedure involves making serial dilutions of the samples (1/100, 1/1000, 1/10000 etc.) on agar in a dish that is sealed and incubated. Typical media include Plate count agar for a general count or Mackonkey agar to count gram negative bacteria such as *E. coli*. The set of plates were incubated at 37° C for 24 hrs. At the end of incubation the colonies are counted visually. The unit of measurement is Colony Forming Units per millimeter (CFU/ml).

## **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 parameters analyzed for soil samples were pH, water retention capacity, soil organic matter and Total Phosphorus according to standard methods.

### **pH:**

pH of the soil is the measure of the hydrogen ion activity and depends largely on relative amount of the adsorbed hydrogen and metallic ions. It is measured using colour comparative method.

### **Water retention capacity:**

Water retention capacity is defined as the amount of water taken by unit weight of dry soil when immersed in water under standardized conditions. Specific amount of measured dry sediment sample was taken in the funnel lined with Whatmann filter paper no. 4. The filter paper was saturated with water so that it does not soak additional water. Specific amount of water was added gradually and allowed to drain slowly in the flask. The sample was kept for 3-4 hours so as to allow drainage of water. The amount of water drained in the flask was measured and final calculation was made for percentage water retention by the sample.



**Organic matter:**

Soil samples were air dried and finely powdered. A required amount of sample was taken from the bulk after mixing. The organic matter analysis was done by acid digestion of samples following titrimetric method commonly known as Walkly Black method (1934).

**Total Phosphorus:**

The availability of phosphorus is important to aquatic productivity owing to the fact that  $\text{PO}_4^-$  ions in soil form insoluble compounds with iron and aluminum under acidic conditions and with calcium under alkaline conditions, rendering the phosphorus ion unavailable to water body. However, phytoplankton helps in uptake of available phosphorus, which is stored for use in their cells, and as a result it helps in production of their population, which may directly or indirectly affect pond productivity.

### **3 BIODIVERSITY ANALYSIS**

The biodiversity account for the ponds was reported as taxonomy of various plankton, molluscans, fishes etc. Phytoplankton is one of the biological elements known to respond to the anthropogenic impact, expressed itself mainly as nutrient enrichment (e.g. Hutchinson, 1967). Plankton are important components of aquatic ecosystem and have diverse habitats.

### **3.1 Plankton:**

The planktonic forms were collected from the surface of the pond water with plankton net of 20 $\mu$  mesh size nylon cloth and were preserved for laboratory analysis. The phytoplankton was preserved in Lugol's iodine solution while zooplankton samples were preserved in 5.0% formalin. The preserved samples were used for qualitative and quantitative studies of plankton. The quantitative analysis of plankton was done by Lacky's drop count method (Lacky, 1938). The identification of plankton was done using descriptive and illustrative keys (Needham and Needham, 1962; Edmondson, 1963; Melanby, 1971; Tonapi, 1982; Adoni, 1985).

### **3.2 Molluscs:**

The molluscs were collected from the periphery of the ponds. Some of the specimen were living, so were narcotized and preserved in 5% formalin and later transferred to 70% alcohol, while some shells were found empty. The empty shells were cleaned and preserved for future reference. The taxonomic identification was carried out based on the standard literature (Subbha Rao *et al.*, 1989).

### 3.3 Population study of fish

The fish specimens were collected from the fishermen nearby the pond. The Total Length (TL) of the fish was measured from the tip of the snout to the caudal fin using meter rule calibrated in centimeters. Length was measured to the nearest centimeter. Fish weight was measured after blot drying with a piece of clean hand towel. Weighing was done with a digital weighing balance.

The relationship between the length (L) and weight (W) of fish was expressed by equation (Pauly, 1983):

$$W=aL^b$$

Where, W=Weight of fish in grams L=Total Length (TL) of fish in cm a=Constant (intercept) b=The Length exponent (slope)

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:

$$R = r^2$$

The condition factor (k) of the fish was estimated from the relationship:

$$K=100 W /L^3$$

Where,

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

## **4. HEALTH DATA**

An urban pond is a water sheet which is utilized by inhabiting population of near vicinity for various domestic purposes. Due to all these activities the status of the pond is altered to ecologically depleted state. Anthropogenic activities around the pond have degraded the water quality of the pond and which will lead to the contamination of the water as well as supportive medium for the parasites and pathogens. These will lead to water borne diseases. Contamination of the water reservoirs by the pathogens may affect the health of surrounding inhabitants. Health camps were attended regularly and the health data was collected monthly for water borne disease in the surrounding settlements through reliable sources.