5 METHODOLOGY

The research methodology and design of experiments has an important role to play for the success of the research work. There are various steps in the research work from where the error can occur (Eberhardt and Thomas, 1991). The error leads to deviation of the results from the actual scenario. These errors can occur right from the faulty experiment design to improper data management and analysis techniques, it can be affected by the inappropriate interpretation of results. To avoid error at each step of the research work, the pre-established and validated methodologies were adopted with study specific and site-specific modifications wherever needed (Collins *et al.,* 2004). For current study the adopted methodologies are divided into following parts:

5.1 Sampling Design and Procedure:

The water and sediment samples were collected from the study sites by standard procedures. The frequency of water sample collection from each sampling site once a month and the sediment samples were collected during the pre-monsoon (March – May) and post monsoon (October – December) period**.** One of the three reservoirs namely, Timbi, was already under investigation by the present author, as a part of pilot project since January, 2016 before the submission of current research work for acceptance. For the other two reservoirs viz., Dhanora and Vadadala, was incepted from August, 2016 to March 2018 (encompasses 20 months of study period with overlap of two seasons). For laboratory analysis, one composite sample was prepared per reservoir for water and sediment analysis. Reagent preparation and procedure for parameter estimation was carried out using the standard protocol (APHA, 1995). Parameter wise detailed methodology is described as follows:

5.1.1 *In situ* estimation and collection of water samples

For the analysis of water quality, the reservoirs were visited on monthly basis. As the reservoirs under investigation are shallow irrigation tanks, it can be considered that there was complete vertical mixing of water leading to more or less homogenized water quality (Jeppesen *et al.,* 1990). However, it is also noteworthy that bodies of still water viz., ponds, lakes, reservoirs have relatively low probability of horizontal equality in the water quality parameters, which mandates collection of multiple samples than to collect

a single sample which would result in the representativeness of the quality for the water sample collected (Myers, 2006). The parameters viz., Temperature, pH, Electrical Conductivity were measured in situ at the sites of sample collection (n=6). For analysis of Dissolved Oxygen (DO), the 300 ml BOD bottle (Durasil CG 118) was immersed in water and sample was collected. Care was taken while collection of the sample that the bubbling doesn't take place. The samples for DO analysis were then immediately fixed using 1 ml of $MnSO_4$ followed by 1 ml of Alkali Iodide Azide solution in each BOD bottle. Care was taken to keep the tip of the pipette immersed in the sample while discharging the solutions in the BOD bottle. Once done, the glass stopper was immediately placed over the mouth of the BOD bottle to ensure no gaseous exchange. For subsequent laboratory analysis the water samples were collected in pre cleaned 1 litre PET (Polyethylene Terephthalate) bottles using grab sampling technique. The sampling procedure was randomized to have probability sampling while ensuring fair representation of the samples (Kothari, 2004). Composite samples was prepared for each of the study site, appropriately labelled and were brought to the laboratory in an insulated box. This was warranted to avoid change in the concentration of water quality parameters due to temperature variation, if any (American Public Health Association, 1995).

5.1.2 Temperature

Temperatures were recorded with the help of mercury glass thermometer (Make: J.R. Mumbai). During each sampling event, the temperature of all the 6 samples were measured. This was performed before making a composite sample out of the 6 grab samples. The thermometer was kept immersed in the sample till the thermometer showed a constant height of Mercury which represented the temperature of the water. The average value of the same was calculated and presented as water temperature in the unit of °C (Degree Celsius) of that particular month.

5.1.3 pH

pH was recorded with the help of a digital pH meter (Make:J.R. Mumbai). Similar to the measurement of temperature, the pH of all the 6 samples were measured. Before preparation of a composite sample, smaller quantity (approximately 50 ml) of each sample was used to rinse a pre – cleaned 500 ml glass beaker (Borosil) and with the same, the beaker was given approximately 400 ml of sample was transferred in a 500 ml pre-cleaned beaker (Borosil). This was performed before making a composite sample out of the 6 grab samples. The average monthly value of the same was calculated and presented as pH (unit less).

5.1.4 Electrical Conductivity

Electrical conductivity (EC) was recorded with the help of a conductivity meter (Make: J.R. Mumbai). During each sampling event, the electrical conductivity of all the 6 samples were measured. This was performed before making a composite sample out of the 6 grab samples. The average value of the same was calculated and presented as EC of that particular month.

5.1.5 Dissolved Oxygen (Winkler's method)

For estimation of Dissolved oxygen, the sample was collected in a 300 ml BOD bottle with utmost care to avoid bubbling. The sample was fixed on site using 1 ml of $MnSO_4$ and 1 ml of alkali – iodide – azide reagent. The content was mixed well by shaking the bottle upside down in clockwise and anti-clock wise manner. At this stage, if white precipitates were formed, it indicated absence of DO. In presence of DO, brown precipitates were formed which are of Manganese Oxyhydrate. These precipitates are then dissolved in 1 ml of Concentrated Sulphuric acid. 50 ml of this content was transferred to a 250 ml conical flask. Few drops of 0.025 N Sodium Thiosulphate was added till the point when the content turned pale yellow. After that 2 ml of freshly prepared starch solution was added which turned the content to a blue colour solution. The content was titrated against 0.025 N Sodium Thiosulphate solution till the end point which is indicated by colour change in the solution from blue to colourless. The volume of Sodium Thiosuphate consumed during the titration was recorded and the DO was calculated using following formula. The titration was repeated for three (03) times taking 50 ml of sample.

$$DO\left(\frac{mg}{L}\right) = \frac{B.R. \times N \times 1000}{Volume\ of\ sample\ (ml)}$$

Where,

B. R. = Volume of Sodium Thiosuphate consumed during titration

N = Normality of Sodium Thiosuphate (0.025 N)

5.1.6 Chlorophyll – a

Varied sample volumes were collected for estimation of Chlorophyll – a. During the post monsoon season, the water was least turbid and the phytoplankton density appeared much lower visually. In such cases (depending upon the visual appearance of the water) relatively larger volumes (upto 2 litre) of water were collected. In the pre – monsoon

season, when the water appeared to be greenish, smaller sample volumes were collected (upto 200 ml). The above procedure was followed considering the density of phytoplankton cells in the water. The sampling bottles were then wrapped with paper and kept in ice box for further laboratory analysis. The samples were filtered with Glass fibre filter (Whatman GF/C) using vacuum filtration assembly. After filtration, the Filter paper was chopped into smaller pieces in a mortar and pestle. A pinch of $MgCO_3$ was added to it which assists in avoiding premature conversion of Chlorophyll to Phaeophytin. To this approximately 5 ml of 90 % aqueous acetone was added and the content was ground manually to the thick homogenized paste. 90 % aqueous acetone was added if the slurry appeared to be drying up. The content was then transferred to centrifuge tubes and were centrifuged at 5000 RPM for 5 minutes. The clear supernatant was transferred to a 50 ml volumetric flask. The centrifuge tubes were again filled with 90 % aqueous acetone and the procedure was repeated. The final volume of the extract was made upto 50 ml using 90 % aqueous acetone. The samples were then taken for spectroscopic analysis at Optical Density of 664 nm before acidification and at 665 after acidifying with 0.1 N HCl. The chlorophyll-a concentration was calculated using the following formula.

$$\text{Chlorophyll a } \left(\frac{\mu g}{l}\right) = \frac{26.7(664b - 665a) \times V1}{V2 \times L}$$

Where,

V1 = Volume of extract in ml

V2 = Volume of sample in L

L = light path lenth in cm (1 cm)

664b = optical density of extract before acidification

665a = optical density of extract after acidification

5.1.7 Nitrate – Nitrogen

An ultraviolet (UV) technique measures the absorbance of nitrate at 220 nm, which is suitable for screening uncontaminated water (low in organic matter). A second measurement made at 275 nm, may be used to correct the nitrate value (because 275 nm is not absorbed by nitrate, but absorbed by other matter). The nitrate calibration curve

follows the Beer's law up to 11 mg *$N0_3$-N/L.* Standard curve was made using standards in the range of 0 to 7 mg/L by diluting 0, 1,2,4,7, 10, 15, 20, 25, 30 and 35 mL to 50 mL with distilled water. 1 ml of HCl solution was added to 50 ml of filtered sample and standard solutions respectively. Absorbance of standard and sample was taken against distilled water at the wavelength of 220 nm and 275 nm. Reading at 220 nm gives $N0_3$-N reading while interference of dissolved organic matter can be determined at 275 nm. By subtracting twice of absorbance reading at 275 nm from the reading at 220 nm gives the value of NO_3-N only for the samples and standards.

5.1.8 Phosphate

Phosphate in the water sample was analysed by Stannous chloride colorimetric method. In acidic conditions, phosphorous occurring as orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid. It is reduced by stannous chloride to a blue colour complex. The intensity of the blue colour is measured, which is directly proportional to the concentration of phosphate present in the sample.

$$PO_4^{3-} + 12[(NH_4)_2MoO_4] + 24H^+ \xrightarrow{yields} \left[(NH_{4)})_3PO_4.12MoO_3\right] + 21NH_4^+ + 12\,H_2O$$

$$\left[(NH_{4)})_3PO_4.12MoO_3\right] + Sn^{2+} \xrightarrow{yields} (molybdenum\ blue) + Sn^{4+}$$

The preliminary sample treatment for acidification was done by taking 100 ml of the sample. Few drops of the phenolphthalein indicator was added to it, and the pink colour if developed was discharged by adding strong acid solution drop-wise. In the same solution, 4ml of molybdate reagent and 0.5ml of stannous chloride reagent was added with thorough mixing. After 10 minutes, the absorbance of the solution was taken at 690 nm against distilled water as blank. Same procedure was followed for making standard solution in the range of 0.3 to 2 mgP/L. Phosphate concentration was calculated as follows:

$$phosphate\ \left(\frac{mg}{L}\right) = \frac{(mg\ of\ P)}{ml\ of\ sample} \times 1000$$

5.1.9 Total Dissolved Solids

In natural water, large number of solids like carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium are present as cations and anions in the dissolved form contributing to the total dissolved

solids. TDS concentration affects the utility of water for drinking, irrigation and industrial purposes. Electric TDS meter was used to measure the concentration of total dissolved solids in water. Before making the composite sample, total dissolved solid was measured for each of the sample, and the average value of the same was calculated and presented as TDS of that particular month.

5.1.10 Total Suspended Solids

Total suspended solids is very important parameter to determine the health of natural water bodies. Suspended solid cut down light transmission and affect the photosynthesis of the aquatic flora, and in standing water bodies it may decrease the depth as some of the solids may sediment out. Total suspended solid was determined by filtering the sample using filtration apparatus through suction flask. After filtration, the filter paper was dried at 105°C in a hot air oven for about 1 hour. The final weight of the filter paper was taken to determine the concentration of total suspended solids in water.

$$TSS\ \left(\frac{mg}{L}\right) = \frac{(W_f - W_i) \times 1000}{Volume\ of\ sample\ (ml)}$$

5.1.11 Total Solids

Total solids is measure of all kind of solids i.e. suspended, dissolved and volatile solids. It is determined as the residue left after evaporation of the unfiltered sample. 100 ml of the sample is taken in pre-cleaned and dried beaker, and is heated up to dryness in hot air oven at 105°C for 2 hour. After the water is completely evaporated, the beaker is cooled to the room temperature in desiccator and final weight of the beaker is taken. Total solids are calculated using following formula.

$$Total\ Solids\ \left(\frac{mg}{L}\right) = \frac{(W_f - W_i) \times 1000}{Volume\ of\ sample\ (ml)}$$

In current study the Total solids were calculated by summing up the values of Total Suspended Solids and Total Dissolved Solids.

5.1.12 Chloride

Chloride in water sample was analysed by Mohr's method. The sample was titrated with silver nitrate solution using potassium chromate as indicator. On addition of silver nitrate, chloride get precipitated as white silver chloride and at the end point as the

concentration of chloride ion decreases, silver ions form reddish brown precipitate of silver chromate.

5.2 Sediment Sampling and analysis

Unlike water samples, there were no parameter to be assessed on site and analysis of parameters under investigation were analysed in the laboratory. The sediment samples from various locations (n=10) were collected using soil auger up to a depth of 15 cm during the pre-monsoon and post-monsoon period. While collecting samples from the exposed sediments, the overburden (mostly grasses and weeds) was removed by scrapping with a sickle before sample collection whereas in the case of submerged sediments the hydrophytes and their roots were removed from the sample and only the sediments were collected. Approximately 01 (one) kg of sediment samples were collected from each sampling point and the samples were transferred to zip lock air tight polythene bags. The samples were labelled on site for further identification in the laboratory. Similar to the water samples, the sediment samples were also transferred to the laboratory in an insulated box to prevent major temperature variation.

5.2.1 Temperature

Once the sediments were sampled, an electronic thermometer was inserted into the sample and temperature was recorded once the thermometer showed a constant value.

5.2.2 pH and Electrical Conductivity

pH of the soil is the measure of H^+ ion activity of the soil water system. It indicates whether the soil is acidic or neutral in nature. Since ions are the carriers of electrical conductance, the EC of the soil - water system rises according to the content of soluble salt. The measurement of the EC can be directly related to soluble salt concentration of soil at any particular temperature. 10 gram of the soil was taken in a beaker to which 50 ml of distilled water was added (in the ratio of 1:5). The soil suspension was stirred at the shaker for 30 min. pH of the solution was measured immediately with the pH meter probe and after 10 minutes, the electrical conductivity of the supernatant liquid was measured with the help of conductivity meter.

5.2.3 Organic Carbon

Organic carbon was determined by Walkley and Black (1934) method of rapid dichromate oxidation technique. Organic carbon is contained in the soil organic fraction, which consist of cells of microorganism, plants and animal residues at the various stage of decomposition, stable humus synthesized from residues, and highly carbonised compound such as charcoal, graphite, and coal. The organic matter in the soil gets oxidized by chromic acid (potassium dichromate plus conc. H_2SO_4) utilizing the heat of dilution of H_2SO_4. The unreacted dichromate is determined by back titration with ferrous ammonium sulphate (redox titration).

0.5 g of the oven dried soil sample (particle size<0.2 mm) was taken in 500ml conical flask. To the sample, 10 mL of 1N potassium dichromate and 20 mL of conc. H_2SO_4 was added with thorough mixing. The solution was allowed to stand for 30 minutes and then 200 ml of distilled water and 10ml of ortho-phosphoric acid was added. The solution was titrated with ferrous ammonium sulphate solution after the addition of 1 ml of diphenylamine indicator till the colour changes from blue-violet to green. Organic carbon in the soil sample was calculated as follows:

$$Organic\ Carbon\ (\%) = \frac{10(B-T)}{B} \times 0.003 \times \frac{100}{S}$$

Where,

B = volume of ferrous ammonium sulphate required for blank in mL
T = volume of ferrous ammonium sulphate required for sample in mL
S = weight of soil in g

5.2.4 Organic Matter

The organic matter in soil influences many properties of soil like capacity to supply nutrients, infiltration and retention of water, cation exchange capacity etc. For direct determination of organic matter, it should be separated from inorganic material which constitutes approximately 90% of the soil weight as mineral compound. Organic matter in given soil sample can be destroyed at the higher temperature and the loss in weight of the soil sample can be attributed to the loss of organic matter.

In the research carried out for estimation by organic matter showed that if the soil was exposed at the temperature of 430 ^{o}C for 24 hours, it did not destroy the inorganic carbon as $CaCO_3$. In the same experiments it was concluded that the temperature of 430 ^{o}C for ignition of organic matter was found to be optimal and it can be employed for efficient estimation of organic matter. The crucible containing 10 g of soil sample was placed in a muffle furnace and temperature was increased slowly to 430^{o}C for overnight. It was switched off and cooled to room temperature in the desiccator and was reweighted to measure the weight of the soil left in the crucible.

$$\%\ Loss\ on\ Ignition\ (430°C) = \frac{A - B}{A} \times 100$$

Where,

A= initial weight of crucible

B= final weight of crucible

Organic matter can also be calculated if the values of Organic Carbon are available. In that case, following formula can be used.

Organic matter (%) = % Organic carbon × 1.724

5.2.5 Phosphate

Phosphorous is key nutrient in soil and play an important role in plant productivity. Its availability in soil is controlled by several factors like soil organic matter level, soil pH, iron and aluminium content. Phosphorous is present in soil in two forms- organic and inorganic phosphorous. Inorganic phosphorous which occurs as orthophosphate are the primary forms of phosphorous taken up by plants. In the present study, Bray's method was used to estimate phosphate concentration in the soil sample. This method has been widely used as an index of available P in soil. The combination of HCL and NH_4 is design to remove easily acid-soluble P forms, calcium phosphate, and portion of aluminium and iron phosphates. The NH_4F dissolves aluminium and iron. Available phosphorous was extracted into solution by taking 5g of soil and 50 ml of the Bray's reagent in a 100 ml conical flask. After 5 minutes of thorough mixing, it was filtered through Whatman No. 42 filter paper. 5 ml of this extract was taken into a volumetric flask to which 5 ml of Dickman's and Bray's reagent was added. The content of the flask is diluted with distilled water, 1 ml of stannous chloride solution is added and then volume is made up to the

mark. The intensity of the blue colour developed was measured at 660 nm after 10 minutes and the concentration of phosphorous was determined from the standard curve.

The available P in kg/ha was calculated by using the following formula;

$$Bray'sP\left(\frac{kg}{ha}\right) = R \times \frac{50}{5} \times \frac{1}{5} \times 2.24$$

$$= \mu g \text{ of } P \times 4.48$$

Where, R = μ g of P in aliquot (obtained from standard curve)

5.2.6 Nitrate

In soil nitrogen is present as organic nitrogen, ammonical nitrogen, and nitrate and nitrite nitrogen. Nitrate is easily mineralising form of nitrogen present in soil, and signifies the amount of available nitrogen which can be easily absorbed by the plants. Major portion (90%) of soil nitrogen exists in combination with the organic matter. Only a negligible fraction of Nitrogen, which is inorganic in form is available to plant. Therefore organic-N mineralised to inorganic form is then available to plants. Estimation of organic carbon is usually used to measure available –N in soil.

Nitrogen mineralization test gives a measure of the amount of nitrogen that may become available to microbial decomposition of the total organic nitrogen present.

Organic matter $\xrightarrow{\text{mineralization process}}$ NH_4

The procedure involves distilling the soil sample with alkaline potassium permanganate solution and determination of the liberated ammonia which serves an index of the available / mineralising nitrogen status of soil. For this, 20g of the soil sample was taken in Kjeldahl flask to which 20 ml of distilled water, 100 ml of $KMnO_4$ and 100 ml of NaOH solution was added in sequence. The content was distilled in Kjeldahl assembly at steady rate and the liberated ammonia gas was collected in a conical flask containing boric acid cum mixed indicator solution. With the absorption of ammonia gas the pinkish colour of boric acid solution turns to green. About 100 ml of the distillate was collected in 30 minutes and the solution was titrated with 0.02N H_2SO_4. Available N was calculated from the following equation:

$$Available\ N\ (ppm) = \frac{(A - B) \times N \times 14\ \times 10^3}{W}$$

Where, A = volume of H_2SO_4 consumed for blank

B = volume of H_2SO_4 consumed for soil sample

N = normality of H_2SO_4

W = weight of soil in grams

5.3 Study of phytoplankton

Phytoplankton analysis, the surface (n=6) and subsurface (n=6; depth = 1 m) samples (volume= 1 l) were collected from the locations of water sampling sites twice a year before and after the monsoon. All the samples were mixed in a bucket and a representative sample was prepared. From this, a two litre sub sample was collected in pre-cleaned plastic bottle (Desai *et al.,* 2004) and kept in the ice box for transporting to the laboratory. Water samples were preserved using Lugol's solution (Britton and Greeson, 1978) leading to increased sedimentation rate which helps in concentrating the cells by gradual removal of clear supernatant. Identification and enumeration of phytoplankton were done with the aid of Sedgwick Rafter cell through light compound binocular microscope (Almicro), using standard monographs and manuals (Desai *et al.,* 2004; Van Vuuren *et al.,* 2006). The result was recorded as consolidated list of phytoplankton present during the study period.

5.4 Study of Avifaunal diversity

The avifauna were surveyed during the winter season at all the three reservoirs. To enlist the birds, the reservoirs were visited at least 10 times during winter season during March – 2016 to March – 2018. The survey periods were restricted to morning hours between 7:00 (time just after the dawn) to 11:00 (IST) as well as during the evening hours between 17:00 to 19:00 (dusk) where most of the birds are highly active and vocal (Sutherland, 2006). This enhances the encounter with the birds which are less active or dormant during the other times of the day. The periphery of the reservoirs were surveyed by walking and 20-30 minutes halts were made at almost equidistant and elevated random points along the boundary. The birds which were spotted on both the sides of the periphery were recorded which includes the wetland birds (such as Egrets, Ducks, Geese,

Herons etc.) as well as the birds which were present in near vicinity of the wetland (such as Bee-eaters, Black drongo, Hoopoe etc.).

Binoculars (Olympus, 8-16 X 40, Zoom DPS I) were used to spot the birds at distance which, otherwise, would be difficult to identify. Apart from the planned visits, birds were also recorded during the routine sampling schedule and were added to the consolidated list of bird species prepared during the winter months if they were not already listed. The calls of certain bird species were also taken into consideration while preparing the list even if the bird was not visible. For example, the birds with best camouflage in the bushes and difficult to spot such as Coppersmith barbet which can be easily identified by its robust heavy call "took-took-took-took-took". Multiple field guides were used for precise identification of birds (Ali *et al.*, 1995; Grimmett *et al.,* 2013). The result was recorded as consolidated list of birds cited during the study period.

5.5 Calculation of Beta Diversity

Beta diversity is a measure of change in the species diversity along an environmental gradient. Here, since the water bodies are similar in a number of conditions, it becomes important to identify if there are any factors which would still influence the variation in species diversity. Sorenson's Beta Diversity Index is a simple yet effective way of estimating the similarities between the communities at two or more locations which is possible even with the presence absence data. To check the similarity / dissimilarity among the reservoirs under investigation, Sorenson Index was calculated using the following formula.

$$Ss = \frac{2a}{2a + b + c}$$

Where,

Ss = Sorenson's similarity index

a = Total number of species common to both the sites

b = Total number of species present only at site one

c = Total number of species present only at site two

The Sorenson's Similarity Index is expressed as % value by multiplying the result fraction with 100.