CHAPTER 4
EXPERIMENTAL METHODOLOGY

From the literature survey conducted it was observed that limnological studies for trophic status assessment and lake management in Assam, India is very limited. Historical water quality data for the major surface water bodies are also not readily available. Keeping these factors in mind, this study aims at development of eutrophication models for the water bodies in Assam based on the studies conducted on artificially eutrophied prototype lakes. The experimental methodology adopted for the presented work is discussed in the next sections.

4.1 EXPERIMENTAL SET-UP

Three model tanks were constructed, two concrete tanks (Tank 1 and Tank 2) and one artificial pond (Tank 3) to simulate lake eutrophication scenario (Figure 4.1). Tank 1 and Tank 2 were rectangular in cross section with side and bed of the lake being constructed with concrete to prevent infiltration. Tank 3 was trapezoidal in cross section and was constructed in clayey soil to maintain steady water level like a natural pond. To prevent excessive ground water infiltration, a layer of bentonite clay was applied in the bottom and side surfaces of the model tank and was compacted properly. General description of the model tank constructed for experimental investigation is presented in Table 4.1. The tanks were initially filled with clear tap water and thereafter periodic application of waste water was done. Waste water was added at a constant interval of time to the artificial lakes and homogeneity in sampling and test run timings had been taken care of to minimize errors. Samples of wastewater, tank water prior to addition of waste water as well as tank water after addition of waste water were collected in standard procedure for conducting water quality investigations in the laboratory.

The experimental investigation was conducted on the artificial prototype lakes from an initial clear water state until the hypereutrophic state of the lakes was achieved. In due course of time complete deterioration of water quality has been observed with heavy algal growth, high turbidity, and anoxic conditions for the studied artificial lakes. During the study period proper care had been taken to maintain a controlled environment in the Tank 1 and Tank 2 for eutrophication to occur in an ideal condition. Heavy rainfall was also protected with shades of polyethylene sheets so that major dilution of water may not hinder the chemical process of eutrophication. So, the factors like sediment mixing and nutrient release, infiltration, dilution etc. was not supposed to influence the process of eutrophication. Only the effect of excessive input nutrient loading, leading to the process of eutrophication was replicated for the concrete bedded prototype lakes. But in case of Tank 3, such measures were not taken and eutrophication process was allowed to occur similar to a natural water body condition.

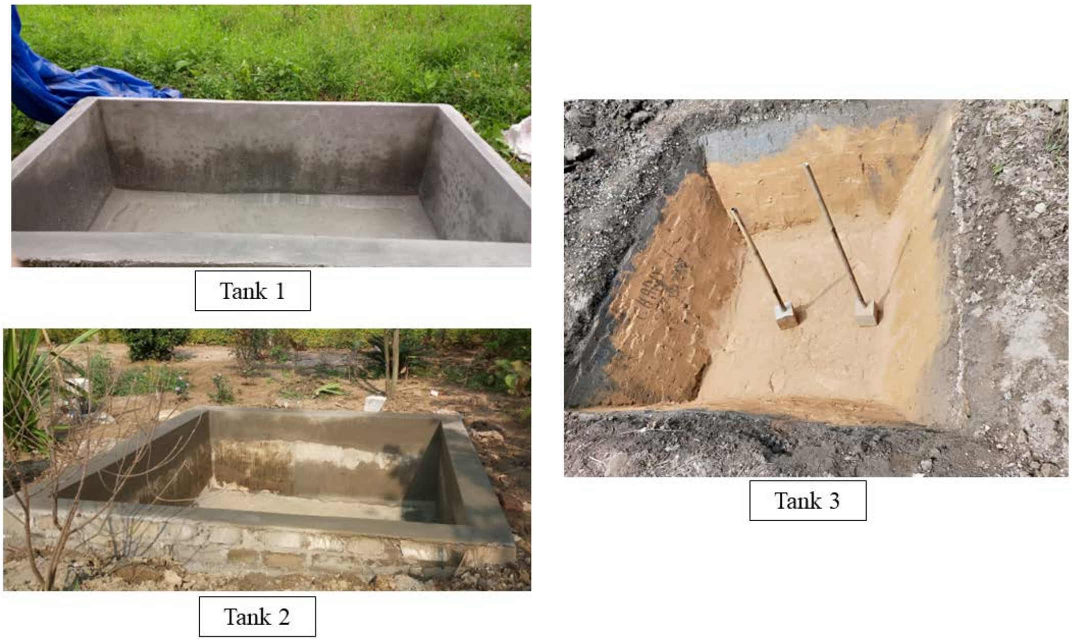

Figure 4.1: Prototype lakes constructed for experimental investigation

The process of experimental investigation on the prototype lakes was done in two trials. In the first trial Tank 1 and Tank 2 were used as prototype lakes and the eutrophication process was replicated successfully in due course of time. A second trial was conducted to validate the findings of the initial trial. In the second trial Tank 1 and Tank 3 were used to simulate the process of eutrophication. Here a concrete tank and artificial pond was used so as to understand the variations in the process of eutrophication occurring under controlled environment and natural situations. Details of experimental investigation, water quality parameters monitored, duration of the study etc. are presented in Table 4.2 below. During both the trials 5 litres of waste water was applied every fourth day interval to the prototype lakes Tank 1 and Tank 3 respectively. However, Tank 2 which had higher dimension and storage capacity, 8 litres of waste water application was done on a weekly basis.

Table 4.1: General description of test arrangement

	Tank 1	**Tank 2**	**Tank 3**
Location	Civil Engg. Department, Tezpur University, Tezpur, Assam, India	GIMT Tezpur Campus, Tezpur, Assam	Civil Engg. Department, Tezpur University, Tezpur, Assam, India
Tank Dimension	2.61m × 1.63m × 0.73m	2.5m × 2.5m × 0.85m	3.0m x 2.5m x 0.90m
Side Slope	Nil	Nil	1 (H) : 1.8 (V)
Initial Free Board	0.11 m	0.15 m	0.30 m
Initial Volume of clear water added	2.637 m^3	4.375 m^3	3.20 m^3

Table 4.2: General description of experimental trials investigated

Trial	**Model tanks used**	**Waste water application and sampling frequency**	**Water quality parameters investigated**	**Duration of investigation**
Trial 1	Tank 1	Every fourth day	pH, TDS, EC, BOD, DO, turbidity, TN, TP, SD, temp	March to November, 2018
	Tank 2	Every seventh day		March to December, 2018
Trial 2	Tank 1	Every fourth day	pH, TDS, EC, BOD, DO, turbidity, TN, TP, SD, temp, Chl-a	February to November, 2019
	Tank 3	Every fourth day		February 2019 to January, 2020

4. 2 WATER QUALITY PARAMETERS INVESTIGATED

To study the water quality parameters of the studied artificial lakes, water samples were collected in a standard procedure from a depth of approximately 10 cm below the water surface in plastic sample bottles. Thereafter sealed samplers were taken to laboratory for further analysis. Water quality parameters were investigated in the Environmental Engineering Laboratory of Civil Engineering Department, Tezpur University, Assam. Tests for water quality parameters pH, total dissolved solids (TDS), electrical conductivity (EC), biochemical oxygen demand (BOD), dissolved oxygen (DO), turbidity, total nitrogen as nitrite and nitrate (TN), total phosphorus (TP), Secchi depth (SD), water temperature, chlorophyll-a (Chl-a) etc. were regularly conducted after collection of samples. Apart from the artificial prototype lakes, water samples were collected from few natural water bodies in Assam and water quality parameters had been investigated which were utilized for model validation. The locations of the study area and the natural water bodies have been presented with Fig. 4.2.

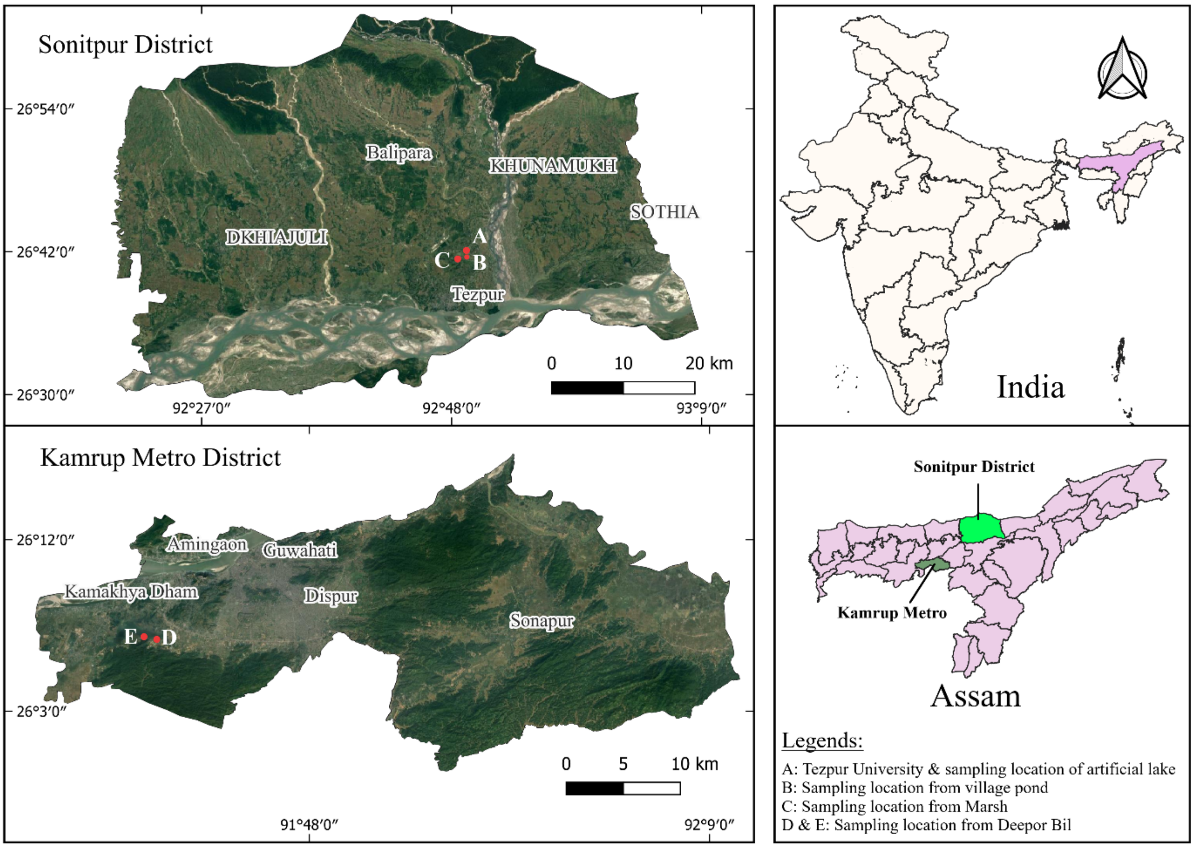

Figure 4.2: Location map showing study area and natural water bodies

4.2.1 Environmental Significance of investigated parameters

Environmental significance of the studied water quality parameters on surface water ecology can be described as follows:

pH: It refers to the acidity or alkalinity of a solution and is very important for assessing the quality of surface water. pH directly influences survival and health of aquatic life, chemical reactions in water, availability of nutrients and effectiveness of water treatment processes. Monitoring pH for surface water bodies is essential to ensure safety of water for both aquatic life and human consumption. It can serve as an indicator of pollution for factors such as acid rain, industrial effluents etc.

TDS: It is a very important water quality parameters that refers to the measurement of all organic and inorganic substances that are dissolved in water. TDS provides information about water salinity, nutrient availability, and helps in identification of pollution sources. High TDS levels can adversely affect the aquatic life with increase in osmotic stress, reduction in availability of oxygen etc.

EC: Electrical conductivity measures the ability of water to conduct electrical current and it is closely related to salinity and TDS. EC serve as an indicator of water quality changes over time and helps in detection of contamination levels caused by natural or anthropogenic factors. High EC values may negatively impact the aquatic ecosystem.

BOD: It represents the amount of oxygen required by microorganisms during decomposition of organic matter in water. High BOD levels have detrimental effect on aquatic life and it is an indicator of organic pollution in water. BOD is very essential parameter for designing waste water treatment processes and often it is used as regulatory parameter for protection of water resources.

DO: It is a measure of amount of free, non-compound oxygen dissolved in water and is a vital indicator of the health of the ecosystem. DO is a surface water quality parameter that has a direct impact on aquatic life. A too high or too low DO level can adversely affect the aquatic balance of the ecosystem. Monitoring DO levels aids in eutrophication risk assessment, aquatic life protection, pollutant detection, suitability of treatment process, and regulatory compliance of water bodies.

Turbidity: It refers to the clarity or haziness in water and is an important indicator of suspended particles in water, presence of pathogens and other contaminants that may adversely affect water quality. Highly turbid water affects the aesthetic beauty of the water body. Turbidity affects light penetration, sediment transport, and drinking water treatment process and as such monitoring of turbidity is essential for maintaining water quality and protecting ecosystems.

TN and TP: Total nitrogen and phosphorus are very important parameters in limnological studies due to their significant impact on ecosystems. Excessive levels of these two nutrients lead to heavy algal blooms leading to oxygen depletion and lake eutrophication. Monitoring of TN and TP is also essential for identification of pollution sources, evaluation of trophic status of water bodies and to adhere to the regulatory requirements.

SD: It is a popular method of water clarity or transparency measurement in lakes, invented by scientist Angelo Secchi from Italy [23]. SD provides information about light availability for photosynthesis, algal blooms, and nutrient enrichment. It is a simple and cost effective method to evaluate the overall health and condition of the water bodies. Monitoring SD provides critical information to understand the extent of eutrophication and subsequent guiding measures to restore ecosystem balance of the water bodies.

Temperature: Water temperature is an important factor for eutrophication assessment in lakes. It influences algae growth, stratification, oxygen depletion, nutrient cycling, and it varies seasonally. Other physio-chemical parameters like pH, TDS, DO, BOD, Chl-a etc. are greatly influenced by ambient water temperature. Water temperature monitoring helps in understanding the processes of eutrophication, assessing the risk for algal blooms and oxygen depletion. Water temperature, in conjunction with other data, provides a thorough understanding of the eutrophication process in lakes.

Chl-a: Chlorophyll-a is one of the most important indicators of eutrophication in surface water bodies. Chl-a is a pigment found in algae and aquatic plants that is essential for photosynthesis and it is used to estimate the algal biomass and productivity in the water bodies. Chl-a concentrations are closely related to nutrient enrichment particularly by TN and TP that leads to algal growth. Moreover, Chl-a data is extensively used to monitor the effects of different lake management and restoration strategies.

4.2.2 Experimental procedure

The tests for investigated water quality parameters were carried out according to protocol of Standard Methods [8]. All the tests were conducted in triplicate and average values were reported. The pH, TDS, EC, turbidity, and temperature values of samples were measured soon after collection of samples using standard digital electronic meters for each test. DO was determined by using Winkler Azide modification method. The 5-day BOD value was determined using the same method after determination of 0 day and 5 day DO values. This method is modification of the original Winkler method by introducing sodium azide to neutralize effect of nitrite in DO determination. The sample mixed with $MnSO_4$, and alkali azide reagent was titrated against standard sodium thiosulphate solution to get the DO concentration in the sample. For BOD determination another sample was stored for 5-day period in a BOD incubator and thereafter DO concentration was measured.

The TN concentration was measured as a sum of available nitrite and nitrate in the sample measured using a UV-spectrophotometer. The nitrate concentrations were estimated using ultraviolet spectrophotometric screening methods. This method is based on the fact that nitrate ions absorb ultraviolet light at specific wavelengths. So, nitrate estimation was done by measuring absorbance of sample at 220 nm. Nitrite concentration was determined using colorimetric method. In this method the sample containing nitrite is mixed with sulfanilamide and NED dihydrochloride which results in formation of reddish purple azo dye. The concentration of nitrite in the sample is proportional to the intensity of the color produced. The absorbance reading of the sample at 543 nm was taken to obtain nitrite concentration.

The TP concentrations as orthophosphate were determined as per vanadomolybdophosphoric acid colorimetric method using a UV-spectrophotometer. The principle of this method is that orthophosphate solution forms a yellow acid in presence of vanadium and the intensity of the color is proportional to concentration of phosphate. This color intensity was measured in the spectrophotometer within the range of wavelengths 400 to 490 nm.

For determination of Chl-a concentrations, Trichormatic or Spectrophotometric method was used. This method is based on the fact that light is absorbed at specific wavelengths by chlorophyll. Here optical densities of samples at 664, 647, and 630 nm were estimated and after correction for turbidity, Chl-a concentrations were calculated by using standard empirical equations prescribed in Standard Methods [8].

Secchi depth was measured in-situ following the methods suggested by Carruthers et. al. [23]. SD readings were taken with the help of a circular metallic disc of 15 cm diameter with four alternate black and white strips. The disk was attached to a rope and it was lowered in the lake water surface until it was no longer visible and the corresponding depth was recorded as SD value for the particular reading.

The details of the instrument and equipment used in this work are presented in Table 4.3.

4. 3 TROPIC STATUS INVESTIGATION

To justify occurrence of eutrophication in the studied artificial lakes and natural water bodies mathematically, the investigated parameters were used for trophic status evaluation following the protocol suggested by Carlson [20]. The trophic state index is a term used to quantify the trophic status or level of nutrient enrichment of a lake or other body of water based on other water quality parameters associated with eutrophication. It provides a numerical value indicating the lake's relative degree of eutrophication. Carlson's trophic status index (TSI) is calculated with Equations (4.1) to (4.3) below using SD, Chl-a, and TP values of the lakes. A lake is considered oligotrophic if the calculated TSI value is less than 40. For values in between 40 and 50, the lake is categorized as mesotrophic and eutrophic if the TSI value ranges from 50 to 70. For TSI values greater than 70, the considered lake is assumed to be in hypereutrophic stage [20, 154]. In the present study SD and TP were used for TSI calculation for the 1st trial and during 2nd trial SD, TP, and Chl-a were used for estimating the trophic status of investigated lakes.

$$TSI\ (SD) = 60 - 14.43.\,ln(SD) \tag{4.1}$$

$$TSI\ (Chl - a) = 30.56 + 9.81.\ln\,(Chl - a) \tag{4.2}$$

$$TSI\ (TP) = \ 4.14 + 14.43.\ln\,(TP) \tag{4.3}$$

Table 4.3: Instrument/Equipment used in the work

Instrument/Equipment	Manufacturer
De-ionized water purification system based on reverse osmosis	Model: RiOs 3, LOT No. BM3BA4017, Brand: Millipore
Digital pH meter	Model: µ pH System 361, M/S Systronics India Ltd., India
Digital EC/TDS/Temperature meter	Model: Conductivity TDS Meter 308, M/S Systronics India Ltd., India
Digital turbidity meter	Model: µC Turbidity Meter 135, M/S Systronics India Ltd., India
BOD incubator	M/S Labard Instruchem Private Limited, India
UV-Visible Spectrophotometer	Model: AU-2603 UV-VIS Double Beam Spectrophotometer, M/S Systronics India Ltd., India
Analytical balance	Model: MS304S/A01, M/S Mettler-Toledo India Private Ltd., India

4. 4 CALCULATION OF CORRELATION COEFFICIENT

The degree of linear relationship between two random variables are expressed by the correlation coefficient. It is a statistical indicator of the degree to which the data fits the regression line or the strength of the relationship between the two variables. It is a calculated value that shows the statistical relationship between two sets of random variables. Mathematically correlation coefficient (R) can be determined using the formula given in Equation 4.4.

$$R = \frac{\sum xy - \frac{\sum x \sum y}{N}}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{N}\right)\left(\sum y^2 - \frac{(\sum y)^2}{N}\right)}} \tag{4.4}$$

Where x and y represents the variables whose correlation coefficient needs to be determined. N is total number of data sets considered. Coefficient of correlation can range from values -1 to 1. A value of 1 implies that a linear equation accurately and favourably describes the connection, with all data points on the same line and y and x increases simultaneously. A score of -1 implies that all data points are connected by a single line, but as y value increases, the value of x decreases. A value of 0 shows that a linear model is unsuccessful since the variables do not have a linear relationship. In the present study, the investigated water quality parameters were utilized for determination of correlation coefficients with the target eutrophication indicators using Microsoft Excel spreadsheet.

4.5 SUMMARY OF EXPERIMENTAL METHODOLOGY

Three artificial prototype lakes were constructed and eutrophication scenarios were replicated in controlled environment by periodic addition of waste water. Continuous monitoring of water quality data had been done from an initial clear water stage to hypereutrophic state which were subsequently used for eutrophication model development. The process of artificial replication of eutrophication was done for two trails by considering two prototype lakes at a time. TSI was used to validate trophic status change in the lakes and correlation coefficient estimation was done on the investigated dataset to check linear dependency among variables.