## 05. MATERIALS AND METHODS

Chemical water properties not only modify the physical features of the medium but also have a major influence on the life forms, which in turn, change the chemical water quality in course of time. Thus, to understand the behaviour of an ecosystem from the holistic face of view, it is necessary that detail study on physical as well as chemical features of the system should be done. Hence, keeping this vital fact in mind, some important parameters are estimated in the research study.

### Collection Centres

For carrying out the investigation, ten (10) sites were randomly selected from lentic, transition and lotic sector (Table 2, Figure 4). The lentic zone of reservoir was located within the impervious dam area, which is relatively deeper and exhibited minimal flow and lotic sector is vital part where a riverine system joins to the reservoir. It has extensive flow of water during monsoon and minimum throughout post-monsoon. The intermediate or transition zone constituted between lotic and lentic sectors and this zone has maximum depth. For ease of calculation and tabulation, average values from three sites have been presented.

**Table no 2: Geolocation of sample collection sites.**

| Sl. No. | Classification of Doyang reservoir areas | Site points | GPS Coordinates |
|---|---|---|---|
| 1 | *Upper Stretch* (towards Doyang rivers) | Site -A | Lat $26^0$10'19.82"N Long $94^0$20'27.11"E |
| 2. | | Site -B | Lat $26^0$11'34.23"N Long $94^0$19'11.39"E |
| 3. | | Site -C | Lat $26^0$12'3.44"N Long $94^0$19'43.6"E |
| 4. | | Site -D | Lat $26^0$12'17.68"N Long $94^0$19'0.32"E |
| 5. | *Middle Stretch* (between the main reservoir and Doyang river) | Site -E | Lat $26^0$12'47.58"N Long $94^0$18'21.53"E |
| 6. | | Site -F | Lat $26^0$12'45.31"N Long $94^0$18'11.66"E |
| 7. | | Site -G | Lat $26^0$13'16.79"N Long $94^0$17'21.23"E |
| 8. | *Lower Stretch* (Main Doyang reservoir area where hydroelectric project is situated) | Site -H | Lat $26^0$14'0.27"N Long $94^0$17'0.83"E |
| 9. | | Site -I | Lat $26^0$13'49.24"N Long $94^0$15'55.83"E |
| 10. | | Site -J | Lat $26^0$14'23.1"N Long $94^0$15'59.87"E |

**Figure 5: Partial view of Doyang Reservoir, Nagaland during summer season.**

**Figure 6: Partial view of Doyang Reservoir during winter season.**


**Figure 7: A detail account of Doyang Reservoir by NEEPCO, GoI undertaking.**

**Figure 8: Installation for cage culture initiated by the Department of Fisheries and Aquatic Resources, Nagaland at Doyang Reservoir**

**Figure 9: A view of different sampling sites at Doyang Reservoir**

**Study Period**

For the key purpose of investigation, unsystematic sampling of water quality and biological study of the reservoir from selected points was executed for three years, starting from February, 2015 to January, 2018.

***Frequency of Sampling***

Sampling of core water parameters (2015 to 2018 in three phases): Month-wise Sampling of Fish species: Month-wise (data presented seasonally for fish sampling).

**Biotic Community**

**Collection of fish samples:**

During the study period, entire Doyang reservoir was studied in regular mode from the help of local fishermen and Govt. Officers. Total 10 different sites are highlighted for proper sampling but except those points random sampling was also done in upper, lower and middle stretch of doyang reservoirs. The small reservoir extensions, interlinked streams connecting to the reservoir are also surveyed out. The fishes caught were collected by me or from the local fishermen. Fishes are also opted from landing centres. Inhabitant freshwater ichthyofauna was collected using fishing gears viz., cast and drag net etc. Fishes are gathered secondarily from the fishermen and from fish market. The photos are occupied immediately after collection. Then the data sheets was prepared. The fishes are preserved in sterilized container with 4% formalin. After that they were examined and identified by following the methodologies after Fish Base (2017) and the available literature of Talwar & Jhingran, 1981. Photographs were clicked immediately after sampling of the fish specimens to obtain the actual body colour and contour patterns. Fishes were taxonomically identified from order to species apart from juveniles form.

***Taxonomic enumeration of fish biota***

Standard scientific protocols are followed for the identifications and inventorisation of the aquatic organisms. Taxonomic enumeration of fish species were done following standard keys like Dey (1994), Jayaram (1981), Talwar and Jhingran (1991) publications as well as Fishbase.

**Figure 10: Collection of fish sample through non-mechanized boat and interaction with the fisherman at Doyang reservoir.**

**Figure 11: Fish identification and water sample collection at Doyang reservoir.**

**Figure 12: Physico-chemical parameters analysis of water sample at Doyang reservoir.**

**Diversity Indices**

Species diversity composes with two components. These are species number and the species evenness. It can be known as species richness. The evenness denotes how the species abundances are scattered among the species. Over the years, diversity indices are used for characterizing species evenness and richness. Here PAST software has been used for data analysis.

***Margalef's richness index***

Margalef's richness index $R_1 = \frac{S - 1}{\ln (n)}$

Where, S stands for the number of species and *n* stands for the total number of individuals observed in the sample.

***The Shannon diversity index (H) (Kasturirangan, Aravamudan et al. 1996):***

Shannon's index calculates the diversity of species within community. It considers the evenness and abundance both. The pi is calculated at first, which means proportion of species within total species. This value then multiplied by natural logarithm of pi and total of the results is taken and finally multiplying with -1.

Shannon's diversity index $H = -\sum_{i=1}^{S} p_i \ln p_i$

Where H= Shannon's index of diversity

S= total taxa number within a community

pi= proportion of species

ln= natural logarithm

Shannon's equitability ($E_H$) is generally obtained after division of H by $H_{max}$ Equitability value ranges from 0 to 1 where 1 mean complete evenness.

Shannon's equitability index $E_H = \frac{H}{H\max} = \frac{H}{\ln S}$

Where $H_{max} = \ln S$

Disturbance in any ecological environment changes results to the diversity of any community.To preserve biodiversity in a particular area, one needs to understand the conservation and management strategies affecting the diversity. Biodiversity indices act as effective tools in getting the diversity status to the researchers and help the conservationist to protect the desired species.

***Simpson's Index (D) (Prasad, Ramachandra et al. 2002)****:*

Simpson's index determines probability among two individuals sampled randomly from a large community will be of same species.

Simpson's index $D = \frac{\sum n(n-1)}{N(N-1)}$

Where, n = species wise total individuals

N = total individuals.

D value ranges from 0 to 1.

In index, 0 = infinite diversity where 1 = no diversity. The higher value of D means lower diversity.

Simpson's Diversity Index 1-D:

***Margalef's richness index (Shannon 1948):***

Margalef's richness index= $\frac{(S-1)}{\ln(n)}$

Here S= number of taxa

***Chao-1(Simpson 1949):***

This index determines the total species richness.

$$Chao1 = S + \frac{F1(F1-1)}{2(F2+1)}$$

F1 = Singleton species number

F2=Doubleton species number

S= Taxa number

***Dominance Indices:***

Dominance indices are subjective toward the abundance of species, which are mostly common. Commonly applied dominance index is 1-D. It considers evenness and richness both.

***Species evenness:***

Species evenness means the available species numbers are close to each other in the environment. It quantifies the numerical equality of species for the specified community. Evenness of community is represented following the Pielou's evenness index (Margalef 1958):

$$J' = \frac{H'}{H'max}$$

Here H′ = Shannon diversity index

and H′max = maximum value of species diversity (in case every species are likely equal)

$$H'max = -\sum_{i=1}^{s} \frac{1}{S} \ln \frac{1}{S} = \ln S$$

S = total species number

J' value ranges 0 to 1. Lower the J' value means less evenness among the species in communities. Conversely it shows the dominancy of fewer species in that community.

***SHE analysis:***

SHE analysis deduces relationship among the S (the species richness), H (the Shannon index) and the E (the evenness) of the sampled data. Therefore, it contributes species number and their equitability to the changes in diversity. SHE analysis describes the changes in parameters with increasing of sampling. The output is a plot on spreadsheet of calculated S, H and E for all the selected samples. SHE analysis is useful for identifying ecotones (Chao 1984). It is most effective method in testing 'goodness-of-fit' to these models.

***Individual rarefaction:***

This model is used to compare diversity of different sampling sizes. Samples have to be similar taxonomically and obtained by standardized sampling from similar habitat.

This model estimates the expected species number from a very small sample size. The algorithm is taken from Pielou equation (Pielou 1966).

Rarefaction normally assesses species richness from sampling data. Rarefaction generates rarefaction curves which are obtained by plotting species number. These curves generally grow rapidly initially so long the most common species are found and it forms plateau after that getting rarest species sampled.

The formula used to this model is:

$$E(S_n) = \sum_{i=1}^{S} \left[ 1 - \frac{\left( \frac{N - N_i}{n} \right)}{\frac{N}{n}} \right]$$

Here = expected species number

N = total individuals of the rare taxa,

Ni =total individuals in each of the original taxa and n = sub-sample

The curve highlights on sampling efforts based on the number of species. The greater the sampling size the chance of getting the rare and less common species. It generates abundances on equivalency based on various sampling size. The total abundance imbalances are owing to sampling differences and not due to actual abundances (rarity) differences.

***Diversity profile:***

This profile compares diversities of several samples. The validity of the diversities via arbitrarily different diversity indices is not out of question. One sample may contain large taxa number while the other has higher Shannon index value. It can be minimized by defining a family of various indices based on single continuous parameter (Hayek and Buzas 2010).

PAST uses exponential of Renyi index, that depends on α value. α=0 means total species. α =1 is proportional to H, while α =2 is proportional to Simpson index.

$$\exp(H_\alpha) = \exp\left( \frac{1}{1-\alpha} \ln \sum_{i=1}^{s} p_i^\alpha \right)$$

This program plot several diversity profiles together. The obtained profile of the diversities cannot be compared if they cross each other.

### *Statistical Analysis*

Statistical analysis/tests were performed on data collected for abiotic and biotic factors by using Excel Programme, PAST software, Stat plus2009 and SPSS-16 and 17 Version. Correlation, regression and ANOVA were performed on the data generated.

### *Correlation model/regression model analysis:*

The Pearson Correlation matrix(r) between physicochemical parameter and fish/plankton availability also be work out using with the help of stat+ software and Microsoft Excel (2007) to correlate among them.

## Physico-Chemical Analysis of Water

### *Collection of water samples*

Sample water is collected from surface or 1ft. bellow from the surface at 10 sampling stations in each month by sinking the bottles at 9.30 A.M. – 10.30 A.M. The samples are collected at monthly intervals from all the mentioned sampling points during early morning and assessment for temperature, pH, transparency, conductivity, TDS, DO (dissolved oxygen), free CO2 and total alkalinity at site only. For the study of other hydrographical parameters, the samples water were collected and transported in sterilised plastic bottles, which are taken to the laboratory for additional analysis. Accordingly, analysis of a range of physico-chemical parameters like Dissolved oxygen (DO) by Winkler's method; Total alkalinity, Total Hardness (H), Free $CO_2$, TDS have been measured by standard methods (APHA, 2014). Air and water temperature will record by minimum and maximum thermometer and hydro-thermometer respectively and pH by digital pH meter (Cystronics Model – 335). Depth of water was recorded through the graduated meter scale near by the lock gate of the reservoir. Conductivity also measured by digital conductivity meter (Labtronics model – LT 16) and expressed by micro-mho/cm as per standard protocol. Transparency also was determined through the followed Secchi disc method. All the necessary data was brought together with help of Directorate Office, Nagaland and Kohima Science college, Government of Nagaland.

**Figure 13: In-situ data collection and physico-chemical parameter analysis during different time periods from different sites of Doyang reservoir.**

**Figure 14: Collection of fish samples from Doyang reservoir extensions and main reservoir.**

***Temperature***

Temperature plays a noteworthy role in aquatic ecosystem. It is usually determined by a centigrade thermometer marked with 0.01ºC graduation. Surface temperature was determined by collecting sample water in an plastic container and dipping the thermometer into the water, keeping it steady for about a minute and then noting the temperature. Temperature for water samples was determined straight away after collection. Final reading considered as average of three reading. As the solubility of $O_2$ is connected to the temperature condition, it should be noted for the dissolved oxygen samples before fixing. Simultaneously air temperature is recorded, using centigrade thermometer at study site.

***Transparency***

The water transparency of reservoir water was calculated by a model Secchi disc which is 20.0 cm in diameter (Adoni et al., 1985). The disc was merged in to the water till the visuality become disappear, followed by lifting up till it reappeared. The depths at which point the disk become disappearance and reappearance was noted down and a middling value of three readings was taken as standard value for that particular point as water transparency. A standard Secchi disc consists of a circular metal plate 20 cm in diameter, the top surface of which is divided into 4 equal quadrats, each of them being painted black and white alternately while the lower side of the plate is painted black to eliminate reflection of light from that side. The disc was lowered on the graduated line into water and the depth (d1) at which it fade away was noted. The disc was lifted up slowly and the depth (d2) at which it reappeared was noted. The reading (d1+d2)/2 in cm gave a measure of light penetration and is known as Secchi disc transparency.

***pH***

Portable electric digital pH meter (Systronic Model- 335) with glass electrodes, operated by batteries was used in the field. The electrodes were thoroughly cleaned and wiped with dry filter paper. The sample was occupied in a clean beaker and the electrodes were dipped into it. The instrument was operated with the instructions associated with it and the indicating pointer recorded the pH directly. Calibration of the scale might be necessary with a buffer solution supplied with the instrument.

***Dissolved oxygen***

The Winkler's titrimetric method determined the DO content of water which is described by APHA (2008).

**Reagents**

1. Alkaline iodide: Dissolve pure potassium hydroxide measuring 700g and 150g of reagent potassium iodide dissolved in 750 ml of double distilled water. Cool and fill up to 1 litre.
2. Manganous sulphate: Place 480g of $MnSO_4$. $4H_2O$ in a large beaker and add 250ml of double distilled water. The solution pours into a one-litre flask. Continue addition of water to the beaker until all the manganous sulphate is dissolved. Make the final volume up to one litre with distilled water.
3. Sulphuric acid which is concentrated (Sp. gr. 1.84).
4. 0.025 N Sodium thiosulphate: Dissolve 24.82g of crystalline $Na_2S_2O_3$. $5H_2O$ in 700ml distilled water, add 4g of borax ($Na_2B_4O_7.10H_2O$) as a stabilizer, when borax has dissolved fill the quantity up to one litre with distilled water. This gives 0.1 N $Na_2S_2O_3$. Keep it in a brown glass stopper bottle. This stock solution has to be standardized against 0.1 N $K_2Cr_2O_7$ solutions. Dry crystalline potassium dichromate in a hot oven at $125^0$C, cool and weigh accurately 4.904g and liquefy it in distilled water to compose one litre. In 250ml conical flask 25ml of solution have been placed, add 1ml of alkaline iodide reagent, acidify with 2ml concentrated $H_2SO_4$ and titrate the iodine liberated with 0.1N $Na_2S_2O_3$, adding starch as indicator near the closing point, effecting the titration when the blue coloured solution suddenly turns colourless. Adjust the strength of the thiosulphate to exactly 0.1N. Take 125ml of this 0.1 N stock solution and reduce it to 500ml to get 0.025 N $Na_2S_2O_3$.
5. Starch Solution: Add 30ml of 20% NaOH solution to a suspension of 2gm powdered starch in 350ml of distilled water. Stir the suspension until a thick syrup, almost clear solution is obtained. Neutralize the alkali with HCl using litmus as indicator. Acidify with 1ml glacial acetic acid. This starch solution is very stable.

**Procedure**

The stopper of 100ml sample bottle was removed carefully; 1ml manganous sulphate reagent and 1ml of alkaline iodide reagent were added by means of a 1ml pipette which was dipped to the base of the bottle and drawn out as the reagents were given. The stopper was changed

and then the bottle was inverted three to four times that the reagents mixed thoroughly. A flocculent precipitate which was formed, settled. Whitish colour of the precipitate indicates very poor oxygen; light brown colour indicates poor oxygen; brown to reddish brown indicate medium to high dissolved oxygen. For quantitative estimation, 1ml of conc. $H_2SO_4$ was added to precipitate. 50ml of this solution was transferred to a conical flask and placed on a white background. 0.025 N $Na_2S_2O_3$ was added drop wise till the colour turned pale yellow. Starch solution measuring 1ml was added to impart a blue colour and the titration was completed when the solution turned colourless.

**Calculation**

DO (mg/l) = Volume and normality (ml x N) of $Na_2S_2O_3$ used 8 ×1000/volume (ml) for titration.

***Free Carbon dioxide***

The analysis was done without delaying after collection of the sample to avoid the escape of $CO_2$.

Reagents

1. N/44 NaOH- 4 gm of AR grade NaOH was dissolved into prepared 0.1 N NaOH solution and standardized against 0.1 N $H_2SO_4$ by using phenolphthalein as indicator. Dilute the 100 cc of this 0.1 N NaOH to 440 ml with distilled water. This is the N/10 NaOH, which was stored in a polyethylene bottle.
2. Phenolphthalein indicator: phenolphthalein measuring 0.5gm was mixed in 100 ml containing 50% alcohol.

**Procedure**

50 ml of sample water was occupied in a Nessler's tube and then 2 drops of phenolphthalein reagent was added. If the sample turns pink, no free $CO_2$ is present, but if the $H_2O$ remains colourless, N/44 NaOH to be added drop after drop since a 10 ml measured off pipette and stirred gently with a rod till the colour turned pink.

**Calculation**

Free $CO_2$ (mg/l) = Volume (ml) of N/44 NaOH used x 10

***Specific conductivity***

Specific conductivity offers a standard method for the resolve of the dissolved salts in water collectively. The conductance of an electrolyte in solution being almost directly proportional of the ionic strength of that solution and the total conductivity is equal to the sum of the several conductivities resulting from the various ionisable salts present.

0.2N KCl solution was taken in a small beaker. The electrodes were submerged 1cm below the facade of the solution. The cell and fluid were established at a constant temperature of $25^0$C. The variable resistance (R) was adjusted so that it was equal to the resistance of the cell which was given as 0.002765 at $25^0$C. The cell constant (C) was determined from the relation 1/R X C = K or C = K/(1/R). The process was continual for water sample instead of standard KCl solution to get the cell resistance $R_1$. The specific conductivity of water sample = $1/R_1$XC (in reciprocal ohms or mho).

The specific conductivity was measured by WTW multilane $P_4$ method.

The conductivity probe of the kit was calibrated before use. The probe was directly immersed into water and reading was recorded.

***Total dissolved solids (TDS)***

The TDS level can be linked to the conductivity of the water, but the association is not a stable. TDS is the summation of the positively and negatively charged ions which are found in the water sample. So, the TDS analysis gives a qualitative data on dissolved ions (both +Ve and –Ve ions) but does not tell about the nature of the ions.

**Procedure**

Sample water is filtered by a rinsed and dried glass fibre filter. The filtrate (liquid) was collected and water rinsed in a flask. The minimum sample water volume should be 100 ml and a minimum of three rinses of 20 to 30 ml volumes should be used. Weight of container and volume of filtrate recorded (not to include the volume of the rinse water). The rinse water should be deionizer water. Container should not be touched by bare hands. The filtrate was transferred to a ceramic or glass container. The container should be weighed to the nearest 0.000g and place the container in the hot oven, which is set at $103^0$ C. The filtrate was added to the container and the sample allowed staying in the oven at $103^0$ C for 24 hours. The container was removed. After removing from the hot oven, the sample was placed

in desiccators to cool in a dry air environment for at least 3 to 4 hours. After the container cooled, the container was reweighed at least three times to the nearest 0.0000 g. The initial weight (in grams) of empty container was subtracted from the weight of container with the dried residue to obtain the increase in weight.

**Calculation**

A- Weight of clean dried container (0.0000 grams)

B- Weight of container and residue (0.0000 grams)

C- Volume of Sample (do not include rinse water) (100 ml)

Concentration (mg/l) = {(B - A)/ C} × (1000 mg/gm) × (1000 ml/l)

***Total alkalinity***

In water analysis three types of alkalinity are differentiated i.e. Hydroxide alkalinity, carbonate alkalinity and bicarbonate alkalinity. But total alkalinity is determined by titration method. These are determined by using separately two indicators, methyl orange and phenolphthalein for estimation of alkalinity. The alkalinities so determined are called 'P' and 'M' and then with the formulae containing 'P' and 'M', the three types of alkalinity are determined. For all practical purposes however methyl orange alkalinity known as M.O.A. gives a measure of the acid combining capacity of the water.

**Reagents**

1. 0.02 N $H_2SO_4$: 30ml of conc. $H_2SO_4$ (sp. gr. 1.84) was added to 1 litre of distilled water to get approximately 1 N stock solution. To make 0.2N solution, 20ml of this stock solution was taken and diluted to one litre. This standard was checked against 0.02N $Na_2CO_3$.
2. Phenolphthalein indicator: 0.5% solution in 50% alcohol.
3. Methyl orange indicator: 0.05 % aqueous solution.
4. Standard 0.02 N $Na_2CO_3$: 5.3 g of anhydrous $Na_2CO_3$ was dissolved in one litre of double distilled water to make 0.1N $Na_2CO_3$ stock solution. 50ml of this stock solution was diluted to 250ml to give 0.02 N $Na_2CO_3$.

**Procedure**

a) Phenolphthalein alkalinity

In a conical flask 50ml of the sample was taken over white surroundings. Then Two drops of phenolphthalein (indicator) was added to the sample. (If the sample remains colourless then P=0.) The sample turned pink and titrated against 0.02 N $H_2SO_4$, till a colourless end point.

$$P \text{ (as ppm } Ca_2\,CO_3) = \text{No. of ml of 0.02 N } H_2SO_4 \text{ x } 20$$

b) Methyl orange alkalinity

50ml of water sample was taken in a conical flask, 1-2 drops of methyl orange was added to it and titrated against 0.02 N $H_2SO_4$ until the end point was indicated by a change in colour from yellow to pink.

$$\text{Total alkalinity (ppm)} = \text{No. of ml of 0.02 N } H_2SO_4 \text{ x } 20$$

***Total hardness***

**Reagents**

1. Ammonia buffer: Take 16.9 gm ammonium chloride in 143ml liquor ammonia and then add 1.25g magnesium salt of EDTA and dilute it to 250ml. If Mg-EDTA is not available, dissolve 1.179 g Na-EDTA and 780 mg $MgSO_4$, 7 $H_2O$/644mg $MgCl_2$, $6H_2O$ in 50ml distilled water. Add this solution to 143ml of conc. ammonia soln. in which 16.9 g ammonium chloride has already been dissolved.
2. Standard EDTA- Dissolve 3.723g Disodium EDTA and volume make up to 1litre.
3. Indicator- Solid Eriochrome Black T may be used after mixing with inert salt like sodium carbonate in the ratio of 10:1.
4. Standard calcium solution- 1g calcium carbonate is taken in a beaker and adds 1:1 HCl slowly until Calcium carbonate dissolves. It is then diluted to 200ml with distilled water. To drive out the $CO_2$ it is boiled for 5-8 minutes, cooled and pH adjusted using 3N Ammonium Hydroxide to 7.0 and Methyl Orange indicator was used to made volume of 1 litre.

**Procedure**

Take 25ml water and add 1ml ammonia buffer.

Add a bit of Eriochrome Black T and titrate with 0.01 M EDTA till colour changes from magenta to green.

Calculation

Total hardness (ppm)= reading in ml x 40

***Chloride***

Potassium chromate reacts with silver nitrate for chloride titration. $AgCl_2$ can precipitate before red colour silver chromate is produced.

**Reagents**

1. $K_2CrO_4$ indicator: 5 g of $K_2CrO_4$ in double distilled water. Adding $AgNO_3$ solution till exact red precipitate formed. Allowing for 12 hours for filter, dilute to 100 ml.

2. 0.0141N $AgNO_3$: Dissolve 0.2395 g silver nitrate in 100 ml distilled water.

3. 0.0141 N NaCl: Dissolve 82.4 mg NaCl (at $140^oC$) in 100 ml distilled water.

**Procedure:**

Take 25 ml water and add 5 drops of 5% potassium chromate. Titrate with 0.0141 N silver nitrate till colour changes from yellow to red.

**Calculation:**

Chloride (ppm) = reading in ml x 19.99 x factor of $AgNO_3$

**Figure 15: Stocking of IMC fish seeds at Doyang Reservoir by the Department of Fisheries and Aquatic Resources, Nagaland Government.**

**Figure 16: Collection of fish from Doyang reservoir by local fisherman.**

**Figure 17: Stocking of fish from Doyang reservoir at landing sites.**