CHAPTER 2

MATERIALS AND METHODS

2.1 Collection and characterization of lime sludge waste and marketed lime:

Lime sludge waste of Jagiroad Paper Mill was collected in plastic bags from the recent disposal site of the Jagiroad Paper Mill, Morigaon, Assam in order to analyze various physico-chemical properties. Marketed lime (Pure lime) used by the fish farmers was also collected from the local market. The collected waste and marketed lime (pure lime) were first dried in the laboratory and crushed into fine powder for analysis of various physico-chemical properties. The various physico-chemical properties were analyzed according to the standard methods of APHA (1998) and Trivedy et al. (1987).

The analytical procedure followed for the various estimations were given below:

2.2 Physico-chemical properties:

2.2.1 pH:

For determination of pH of lime sludge waste and pure lime 5 gms of lime sludge waste and pure lime has been taken in two beakers and then 25 ml of distilled water has been added into each beaker. The mixtures are then stirred and kept for about an hour with occasional stirring. The pH of the sample solutions were measured by pH meter -ElicoLI-127, after standardization of the electrode with standard buffers at pH 4.0 and pH 9.2. For the determination of the soil sample also the same procedure have been applied. For the determination of the water sample 25 ml of the sample was taken in a beaker and the pH value was determined as mentioned earlier.
2.2.2 Specific Conductivity:

For determination of Specific Conductivity of lime sludge waste and pure lime also 5 gms of soil has been taken in two beakers and then 25 ml of distilled water has been added into each beaker. The mixtures are then stirred for about an hour with occasional stirring. The Specific Conductivity was measured by Conductivity Meter-Elico CM180 after standardization of the electrode with 0.1N KCl solution. For the determination of the soil sample also the same procedure have been applied. For the determination of the water sample 25 ml of the sample was taken in a beaker and the conductivity value was determined as mentioned earlier.

2.2.3 Water Holding Capacity:

For determination of water holding capacity of lime sludge waste and pure lime, filter paper was placed in two different circular brass box so as to cover the whole perforated bottom of the brass box. Then the weight of each brass box plus filter paper \((W_1)\) was recorded. Then 5 gms of lime sludge waste and pure lime was transferred into each box in small portion, tapping the box gently after each addition. The boxes were then placed in two different Petri plates and water was added up to a depth of about 1cm into each plate and was kept for over night. Next day after 12-16 hrs, the boxes were removed and wiped it out dry from outside and then the weight were taken \((W_2)\).Then the boxes were dried in oven for 24 hrs at 105°C and before taking weight \((W_3)\) the boxes were cooled in desiccator. The amount of the water absorbed by the filter paper was also recorded separately by weighing a filter paper before and after saturating with water. The difference is the amount of water absorbed by the filter paper, \(W_4\).
Water Holding Capacity (%) = \( \frac{W_2-W_3-W_4}{W_3-W_1} \times 100 \)

Where, 
- \( W_i \) = weight of the brass box plus filter paper
- \( W_2 \) = weight of the brass box plus filter paper plus saturated sample
- \( W_3 \) = weight of the brass box plus oven dry sample
- \( W_4 \) = amount of the water absorbed by the filter paper

For the determination of the soil sample also same procedure has been applied.

2.2.4 Organic carbon (Walkey and Black Rapid Titration):

For the determination of organic carbon 1.00 gm of powdered lime sludge waste and pure lime were taken in two dry 500 ml conical flasks. 10 ml of 1N \( \text{K}_2\text{Cr}_2\text{O}_7 \) was pipetted in into each flask. Then 20 ml of \( \text{H}_2\text{SO}_4 \) was run in and swirled again two or three times into each flask. The flasks were allowed to stand for about 30 minutes and thereafter 200 ml of distilled water was added into each flask. After incorporation of 10 ml of \( \text{H}_3\text{PO}_4 \) and 1 ml diphenylamine indicator into each flask, the contents were titrated with ferrous ammonium sulphate solution till the colour flashes from blue violet to green. Simultaneously a blank was run without sample with the same chemicals.

\[
\frac{10(B-T)}{B} \times 0.003 \times \frac{100}{\text{Wt. of the sample}}
\]

Where, 
- \( B \) = Volume of FAS solution required for blank titration
- \( T \) = Volume of FAS solution required for sample titration

For the determination of soil sample also the same procedure has been applied.

2.2.5 Available phosphorous (Stannous Chloride Method):

1 gm of each air dry sample (lime sludge waste and pure lime) was taken in two different 500 ml conical flask and 200 ml of 0.002 \( \text{N} \) \( \text{H}_2\text{SO}_4 \) was added into each
flask. The suspensions were shaked for about half an hour with magnetic stirrer and then filtered through whatman 50 filter paper to get a clear solution. 50 ml of these clear solutions were taken in two different 100 ml dry conical flask and 2 ml of ammonium molybdate solution was added into each flask and then 5 drops of stannous chloride reagent was added to determine the concentration of phosphorous. The absorbances were taken at 690 nm wavelength on a spectrophotometer using double distilled water as blank with the same amount of chemicals. Readings were taken after 5 to 12 minutes of application of stannous chloride reagent. The concentrations of phosphorous were found with the help of standard curve. For the determination of phosphorous of the water sample 50 ml of sample solution was taken in a 100 ml dry conical flask and then same procedure has been applied.

2.2.6 Chloride (Argenometric Method):

20 gms of each sample (lime sludge waste and pure lime) and 100 ml of distilled water were taken to prepare a sample suspension. The mixture were shaked well with magnetic stirrer for about one hour and then filtered through whatman 50 filter paper using Buchner funnel and pump to get a clear solution. 50 ml of each clear solution was taken in 100 ml dry conical flask and then 2 ml of K₂CrO₄ solution was added into each flask. The contents were titrated against 0.02 N AgNO₃ until a persistent red colour appeared.

\[
\text{Chloride (ppm)} = \frac{(A \times N \text{ of AgNO}_3) \times 1000 \times 35.5}{\text{ml of sample}}
\]

Where, \( A = \) volume of 0.02 N AgNO₃ required for titration
For the determination of chloride of the water sample 50 ml of sample solution was taken in a 100 ml dry conical flask and then same procedure has been applied.

2.2.7 Calcium carbonate (Rapid Titration Method):

5 gms of dried sample (lime sludge waste and pure lime) were taken in two different beakers of 150 ml capacity and then 100 ml of 1.5N HCl was added with pipette into each beaker. Then the sample were allowed to settle for 1 hr. 20 ml of supernatant was taken out in two different dry conical flask of 100 ml capacity and 6-8 drops of Brom thymol blue indicator was added into each flask and titrated against 1.5 N NaOH until a blue colour appeared. Simultaneously a blank was also run without sample with the same chemicals.

\[
\text{CaCO}_3(\%) = (B-T) \times 5
\]

Where, \(B\) = Volume of 1.5 N NaOH solution required for blank titration

\(T\) = Volume of 1.5 N NaOH solution required for sample titration

2.2.8 Toxic metals (Pb, Zn, Cu Mn, Hg etc) (Atomic Absorption Spectrophotometer):

The powdered lime sludge waste and pure lime were digested in a microwave digester using nitric acid and hydrogen fluoride. After digestion the concentration of Pb, Zn, Cu, Mn, Hg etc were determined by atomizing aqueous samples in air acetylene flame in Shimadzu AAS 680 Atomic Absorption Spectrophotometer. Hollow cathode lamps of individual metals were used to produce respective resonance lines.

2.2.9 Available Calcium (Atomic Absorption Spectrophotometer):

The powdered lime sludge waste and pure lime were digested in a microwave digester using nitric acid and hydrogen fluoride. After digestion the
concentration of available calcium was determined by atomizing aqueous samples in air acetylene flame in Shimadzu AAS 680 Atomic Absorption.

2.2.10 Available sodium and available potassium (Flame Photometer):

10 gms of sample (lime sludge waste and pure lime) were taken in two different beaker of 250 ml and then 100 ml of 0.01 N Ammonium Acetate solution was added into each beaker. The contents were then stirred well with magnetic stirrer for about 1 hr. then the samples were kept for overnight to settle. The contents were filtered through whatman 42 filter paper and the filtrates were run in Flame photometer for Na and K with standard solution 40 ppm and 100ppm.

\[
\text{ppm of Na/K} = \frac{\text{reading} \times V}{\text{weight of sample}}
\]

Where, \( V \) = Volume of Ammonium Acetate

2.2.11 Total alkalinity (Titration Method):

For determination of total alkalinity 100 ml of water sample was taken in a conical flask and 2-3 drops of methyl orange indicator was added to it. Then the sample was titrated against 0.1 N HCl until yellow colour changed to pink at the end point.

\[
\text{Total alkalinity (ppm)} = \frac{B \times N \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}
\]

Where, \( B \) = volume of 0.1 N HCl required for titration

2.2.12 Total hardness (Titration Method):

For determination of total hardness 50 ml of sample was taken in a conical flask and 1 ml of buffer is added to bring the pH of the titrate between 10 ± 0.1. Few drops of Eriochrom Black T indicator was added to the sample. A wine red colour
appeared and then the sample was titrated against 0.01 M EDTA solution until the colour changed to blue which was the end point.

\[
\text{Volume of EDTA} \times 1000
\]
\[
\text{Total Hardness (ppm)} = \frac{\text{Volume of EDTA} \times 1000}{\text{ml of sample}}
\]

2.2.13 Free CO\textsubscript{2} (Titration Method):

For the estimation of Free CO\textsubscript{2} 50 ml of sample was taken in a conical flask and few drops of Phenolphthalein indicator was added to it. In case of absence of carbon dioxide the colour turned pink. When the sample remained colourless it was then titrated against 0.05 N NaOH solution until a pink colour appeared at the end point.

\[
\text{A} \times \text{N of NaOH} \times 1000 \times 44
\]
\[
\text{Free CO}_2 \text{ (ppm)} = \frac{\text{A} \times \text{N of NaOH} \times 1000 \times 44}{\text{ml of sample}}
\]

Where, A = Volume of 0.05 N NaOH for titration

2.2.14 Dissolved oxygen (Winkler's Iodometric Method):

For determination of dissolved oxygen, water sample was collected in 300 ml BOD bottle. Oxygen was fixed with 2ml MnSO\textsubscript{4} (Manganese Sulphate) and 2 ml alkaline KI (Potassium Iodide) solution. The bottle was stoppered to avoid air entrainment and mixed the contents well by inverting the bottle repeatedly. Then the bottle was kept for sometime to settle down the precipitate. After the precipitate had settled, the mixing and settling was repeated. Finally 2 ml of conc. H\textsubscript{2}SO\textsubscript{4} was run down the neck of the bottle and the precipitate was dissolved by inverting the bottle repeatedly. After one hour, from the sample bottle 50 ml of treated sample was taken in a conical flask and few drops of freshly prepared starch solution was added as indicator. Then the
content was titrated against 0.025 N Na₂S₂O₃ (Sodium Thiosulphate) until the blue colour disappeared.

\[
\text{Dissolved Oxygen (ppm)} = \frac{A \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 8 \times 1000}{V_2(V_1-V)/V_1}
\]

Where, 
- \(A\) = Volume of 0.025 N Na₂S₂O₃ for titration
- \(V_1\) = Volume of sample bottle after placing the stopper
- \(V_2\) = Volume of the part of the contents titrated
- \(V\) = Volume of MnSO₄ and alkaline KI added

2.3 Bio-chemical properties:

2.3.1 Protein Extraction and Estimation:

Protein estimation was determined by the standard methods as recommended by Lowry et al. (1951) with some modifications.

To estimate the protein 5 gms of fish flesh was taken and 20 ml of ice cold distilled water was added and homogenized. Then 5 ml homogenized content was taken in a centrifuge tube and 2.5 ml of 30% TCA (Trichloro Acetic Acid) was added and centrifuged for about 3-4 mins. at 3000 rpm. Discarding the supernatant the precipitate was again centrifuged with 2.5 ml of 30% TCA (Trichloro Acetic Acid). Discarding the supernatant the precipitate was centrifuged with 10% TCA for about 3-4 mins. Discarding the supernatant the precipitate was again centrifuged with 10% TCA. Discarding the supernatant the precipitate was centrifuged with 3 ml alcohol (Absolute Alcohol). It was repeated again with 3 ml alcohol. After discarding the supernatant the precipitate was centrifuged with alcohol and chloroform in the ratio 3:1. It was repeated again. After discarding the supernatant the precipitate was centrifuged with 2.5 ml ether. After discarding the supernatant the precipitate was allowed to dry at room temperature.
and then 1 ml of 1 N NaOH (Sodium Hydroxide) was added and incubated overnight at 37°C. The next day 1 ml of conc. HCL was added to reprecipitate and again centrifuged to separate precipitate. After discarding the supernatant the precipitate was centrifuged twice with 5% TCA. After discarding the supernatant the precipitate was dissolved in 2.5 ml 1N NaOH and waited for about 10-15 mins. Then the content was diluted with distilled water in a volumetric flask of 50 ml. This is known as the tissue extract. From this tissue extract 1 ml was taken in 3 test tubes. Then 5 ml of 2% Na₂CO₃ and 1 ml of 0.5% CuSO₄ in 1% Na-K tartarate was added and waited for about 10 mins. After that 0.5 ml Folin-phenol was added to each test tube and waited for about 30 mins. The tissue became ready for protein estimation. For standard solution BSA was taken. 100 mg of BSA was dissolved in 100 ml of distilled water. Then, 100 μg of BSA concentration was prepared by adding 9 ml of distilled water in 1 ml of BSA making a total of 10 ml. Then a set of three test tubes were taken and 1 ml of 100 μg of BSA concentration was poured and 5 ml of 2% Na₂CO₃ in 0.1 N NaOH and 1 ml of 0.5% CuSO₄ in 1% Na-K tartarate were added and waited for about 10 mins. After that 0.5 ml Folin-phenol was added to each test tube and waited for about 30 mins. The test tubes were kept in dark place. Simultaneously a blank was also prepared by using distilled water instead of BSA solution with same chemicals. The optical density of the tissue extract and the standard were taken at 650 nm wavelength on a spectrophotometer. The blank was adjusted to zero, prior to reading.

\[
\text{Protein (μg/ml)} = \frac{\text{Conc. of standard x Optical density of tissue}}{\text{Optical density of standard}}
\]

It was then expressed in mg/gm.
2.3.2 Lipid Extraction and Estimation:

Lipid estimation was determined by the standard methods as recommended by Folch et al. (1957) with some modifications.

First the abdominal portion of the fish with viscera and liver was taken and weighted and massarated in a mortar grinder with sufficient amount of sodium sulphate so as to absorb water. The massarated tissue was washed with acetone so that the lipid dissolved in acetone. This process was repeated at least three times to ensure that there was no lipid left in the tissue. The solvent (acetone) was kept in an oven dried beaker with mouth closed with paper for overnight. The weight of the beaker was taken before transferring the solvent. Then the oils that settled at the bottom was incubated and was weighted. This gave the amount of lipid present in a known quantity of fish tissue. It is then expressed in terms of percentage.

2.4.1. Determination of the dosage of lime sludge waste required to maintain an alkalinity within desirable range:

Lime sludge waste of paper mill was added in beakers of one liter capacity in different concentrations (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, and 1%) to determine the required dose. Marketed lime was also added in different concentrations (0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45% and 0.5%). After application of lime sludge waste and marketed lime the pH of the experimental beakers were determined. The concentration of lime sludge waste and marketed lime in which the pH is same was determined from the results. This will be the required dose of lime sludge waste and marketed lime to maintain an alkalinity within the desirable range.
2.4.2. Assessment of the impact of the lime sludge waste and marketed lime on water quality and fish growth in laboratory condition:

After optimizing the required dose of lime sludge waste and marketed lime, four aquaria of same size (of 210 liters capacity) were taken to study the impact of lime sludge waste and pure lime on water quality and fish growth in laboratory condition. Each aquarium was first washed thoroughly with tap water, placed in a plain surface and filled with 165 liters of water (tap water). Before filling with tap water, the different physico-chemical properties viz. pH, specific conductivity, total alkalinity, total hardness, free CO₂, dissolved oxygen chloride, etc of the tap water were analyzed as mentioned earlier. After filling with the tap water the pH of the four aquaria were analyzed as stated above. After analyzing the pHs of the four aquatic media, 22.5 gm lime sludge waste was added in the first aquarium based on the results of a set of pilot study and in second aquarium 15 gm pure lime was added simultaneously. One aquarium was kept as control without applying lime sludge waste or pure lime and one aquarium was kept as stock. The pH of the four aquaria after applying lime sludge waste and pure lime was determined. Fresh and live fingerlings of Indian Major Carp (*Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*) ranging from 4-6 cm (in length) and 8-10gm of weight were collected in plastic bags filled with water from local fresh water resources. The collected fishes were introduced in one aquarium where lime sludge waste or pure lime was not added and acclimatized in the laboratory condition for a period of one month to recover from stress and fed with commercial fish food in well-aerated water. After acclimatization twenty fishes (five fingerlings of *Catla catla*, ten fingerlings of *Labeo rohita* and five fingerlings of *Cirrhinus mrigala*) were taken out and added into each
aquarium (first three aquaria) and keeping one aquarium as stock. The various physico-
chemical properties of the aquatic media were determined before addition of fishes as
mentioned earlier. The fishes were cultured in the laboratory condition for a period of six
months. During the study period the fishes were fed daily with commercial fish food at a
recommended dose (4% of their body weight as recommended by Degani et al., 1989 and
Khan et al., 1991). The mortality of the fish if any was also noted during the course of
culture practice. Monthly assessment of the various physico-chemical properties were
also done by the standard methods described as earlier. After six months the length (in
cm) and weight (in gm) of the reared fishes were determined and recorded. This
experiment has been repeated in the laboratory for about three times.

2.5 Assessment of the impact of the lime sludge waste on growth and development of
plankton:

2.5.1 Application of different concentration of Lime sludge waste and certain
amount of cow-dung:

Water containing plankton was first collected from a local pond of
Guwahati, Assam. Five beaker of one litre capacity were taken and each beaker was first
washed thoroughly and placed in a plain surface where sufficient light is available. All
the beakers were filled with collected water up to one litre mark. Before filling with
water, the different physico-chemical properties of the collected water were analyzed by
following the standard methods mentioned earlier. The different types of plankton present
in the collected water were also determined and identified up to the genus with the help
of Lime sludge waste was added in the first four beaker in different concentrations viz.
1%, 2%, 5% and 10% (based on the results of a set of pilot study). One beaker was kept as control without applying lime sludge waste. 10 gm of cow dung was also added in each beaker as organic manure (based on the results of a set of pilot study). The different physico-chemical parameters of the five beakers were determined after addition of lime sludge waste and cow dung by following the standard methods mentioned earlier. All the beakers were kept under lighting condition for a period of one month. The pH of the five aquatic media were observed at an interval of seven days. Growth, occurrence and type of plankton were also observed and identified at an interval of seven days as mentioned earlier. Both qualitative and quantitative determination of plankton were made. Counting of plankton was done with the help of Sedgewick Rafter Cell. The physico-chemical parameters of the water of the five beakers were again determined at the end of the experiment by following the methods mentioned earlier.

2.5.2 Application of different concentration of cow-dung and certain amount of lime sludge waste:

Based on the results of the above mentioned experiment another experiment was done to study the impact of lime sludge waste on plankton. Here, 5 gms of lime sludge waste was added in the five aquaria and cow dung was also added in the first four aquaria in different concentration 1%, 2%, 5%, 10% (based on the results of a set of pilot study). One aquarium was kept as control without application of cow dung. Before applying cow dung and lime sludge waste, the physico-chemical properties of the collected water were analyzed. The different types of plankton present in the collected water were also determined. The different physico-chemical parameters of the five beakers were determined after addition of lime sludge waste and cow dung by following
the standard methods mentioned earlier. All the beakers were kept under lighting condition for a period of one month. The pH and temperature of the aquatic media were observed at regular interval. Growth, occurrence and type of planktons were also observed and identified at regular interval as mentioned earlier. Both qualitative and quantitative determination of plankton was made. Counting of plankton was done with the help of Sedgewick Rafter Cell. The physico-chemical parameters of the five beakers were again determined at the end of the experiment by following the methods mentioned earlier.

2.6 Assessment of the toxicity of lime sludge waste on fish:

Six well-cleaned rectangular glass tanks of 10 liter capacity were taken and each tank was first washed thoroughly with tap water and placed in a plain surface. After washing, each tank was filled with tap water up to the mark. The different physico-chemical properties of the tap water were analyzed before filling the tanks by following the standard methods mentioned earlier. After analyzing the physico-chemical properties lime sludge waste was added into the first five tanks in different concentrations viz. 1%, 5%, 10%, 15% and 20% (based on the results of a set of pilot study). One tank was kept as control without applying lime sludge waste for comparison. Fingerlings of *Labeo rohita*, approximately of same size and weight were collected from the market and were allowed to acclimatize in laboratory condition in well aerated water for about 15 days before starting the experiment. Three fishes of same size (7-10cm) and weight (7.5-10.5gm) were introduced in each aquarium and were cultured for a period of one month under laboratory conditions. The fishes were fed daily with commercial fish food at a recommended dose as mentioned earlier. The mortality of the fish if any was also noted.
and the dead fish was removed immediately during the course of culture practice. The different physico-chemical parameters of the culture water was analyzed before addition of the fishes and also at the end of the experiment by standard methods as mentioned earlier. Bio-chemical parameters like protein estimation and lipid were also determined as mentioned earlier.

2.7 Assessment of the impact of lime sludge waste on water and soil quality and also on fish growth in experimental ponds:

2.7.1 Rearing of Fishes:

Two ponds of approximately same size (25m x 20m x 2.5m) were prepared. The collected lime sludge waste of paper mill was first crushed into fine powder and added in one pond at an optimized dose (based on the results obtained in the experiment done in the aquarium) before starting the rainy season keeping one pond as a control without applying lime sludge waste. After 30 days of addition of lime sludge waste live fingerlings of Indian Major Carps (Labeo rohita, Cirrhinus mrigala, Catla catla and Labeo bata) were collected and added at a recommended dose (Jhingran, 1991) into each pond. Fish food (Rice bran and mustard oil cake at 1:1 ratio) was given daily at a recommended dose (Jhingran, 1991). The mortality of the fish if any was also noted and the dead fish was removed immediately during the course of culture practice.

2.7.2 Physico-chemical properties of the water and soil:

The different physico-chemical properties of the pond water viz. pH, specific conductivity, total alkalinity, total hardness, free CO₂, dissolved oxygen etc and soil viz. pH, specific conductivity, water holding capacity, organic carbon etc were analyzed before and after addition of lime sludge waste by following the standard
methods as mentioned earlier. Monthly assessment of the different physico-chemical properties of the water and soil of the two ponds were also done by following the standard methods as mentioned earlier.

2.7.3 Harvesting of the cultured fishes:

After seventy five days, 56 specimens of *Cirrhinus mrigala*, 52 specimens of *Labeo rohita*, 12 specimens of *Catla catla* and 30 specimens of *Labeo bata* were collected from each pond with the help of cast and drag net in early morning. The total body weight (in gm) of each specimen was recorded in the laboratory with the help of electronic balance up to three decimal points. The total body length (in cm) of each specimen was also recorded up to two decimal points in order to determine the length-weight relationship.

**Length-Weight Relationship:**

Length-weight relationship study helps to understand the growth dynamics of the fish specially whether the growth in fish is normal or abnormal. For normal growth of fish, length and weight are interrelated. This means, at ripe time the fish must acquire proper weight with increasing length to make it more economically viable. This length-weight relationship was first advocated in fish by Harvard Spencer, 1841 in the form of “Cube Law”.

**Cube Law:**

Length and weight of fishes may be determined with accuracy. Weight of the fishes may be considered as a function of the length

\[ W = L^3 \]

Where, \( W \) = weight of the fish

\( L \) = length of the fish
Subsequently fishery biologists advocated two conditions for the fish to exhibit this law—(i) uniform size in length— in the sample where study is done, the fish should be of more or less uniform size and (ii) constant specific gravity — if specific gravity of fish is constant then cube law can be easily followed.

Unfortunately a fish or any animal is continually prone to change its bodily proportions during life time. But in practice it is found that fish size varies according to weight, age etc so that the simple cube law expression does not hold throughout the life history and growth of fish. So unfortunately the cube was modified for more satisfactory expression of length-weight relationship. This is as follows-

\[ W = cL^n, \]

Where \( W \) = Weight of the fish
\( c \) = constant
\( L \) = length of the fish
\( n \) = an exponent to be calculated empirically by experiment

\( n \) is also known as growth coefficient. It actually determines the growth dynamics of the fish. It is the value of \( n \) which tells us whether the fish is having normal/abnormal growth.

(i) when \( n = 3 \), growth is called normal / isometric growth
(ii) when \( n \neq 3 \), growth is called allometric / not normal
(iii) when \( n > 3 \), growth is called positive allometric
(iv) when \( n < 3 \), growth is called negative allometric

In fishery science, positive allometric can be allowed as it means fish is gaining more weight but negative allometric is never entertained as it means underweight.
Thus, when $n \neq 3$, it means it deviates cube law

when $n = 3$, it means fish exhibit cube law

For practical purpose, this relationship is usually expressed in its logarithmic form (Le Cren, 1951):

$$\log W = \log c + n \log L$$

where,

$W =$ weight of the fish

$L =$ length of the fish

$$\log c = \frac{\sum \log W \cdot \sum (\log L)^2 - \sum \log L \cdot (\sum \log L \cdot \log W)}{N \cdot \sum (\log L)^2 - (\sum \log L)^2}$$

$$n = \frac{\sum \log W \cdot N \cdot \log c}{\sum \log L}$$

where, $N =$ number of specimen

Plate 3: Collecting Lime Sludge Waste