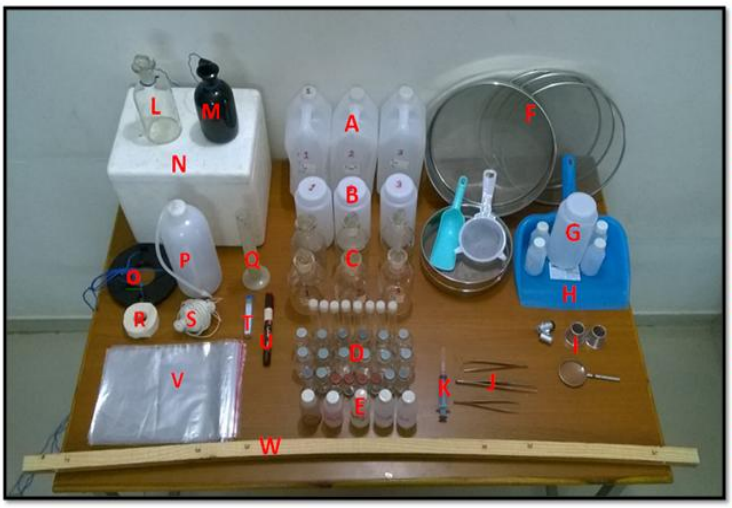
CHAPTER 4

MATERIALS AND METHODS

4.1 Preparatory Work

Before proceeding for the field work, necessary sampling equipments were procured, sample preservatives were prepared and a general sampling kit for sampling of water, sediment, Primary Production estimation and Benthic Macroinvertebrates was prepared **(Plate.4.1 and 4.2).** Also, field datasheets formats for collection of various samples like water samples, sediments samples and for Biomonitoring were prepared.



A. Carboys for Water Sample - General Parameters
B. C.O.D. Bottles
C. D.O. and B.O.D. Bottles
D. Glass Vials for Storing Benthic Macro Invertebrates
E. Preservatives: Alk. Azide, $MnSO_4$, H_2SO_4, Formaldehyde, Ethanol
F. Sieves for Benthic Macro Invertebrate collection
G. Bottles for Benthic Macro Invertebrate Collection
H. Plastic Scoop for Soil Sampling
I. Magnifying Devices for Field Use
J. Forceps for picking Benthic Macro Invertebrates
K. Syringe for adding Preservatives
L. Light Bottle
M. Dark Bottle
N. Insulated Box
O. Weight with rope
P. Wash Bottle
Q. Measuring Cylinder
R. Plaster Roll
S. Nylon Rope
T. pH Strip
U. Permanent Marker
V. Polythene Zip Bags for Soil Sample Collection
W. Wooden Plank with Hooks for Suspending Light Dark Bottles

Plate 4.1 Preparation of General Requriemnts for Sampling and Field Work

Plate 4.2 (A to B) Sampling Kit and Moving Towards Thol for Field Work on Bike

4.2 Physico-chemical Assessments and Trace Metal Analysis

Water quality was assessed by studying various physico-chemical parameters. Seasonal sampling covering three seasons viz. summer, winter and monsoon during the year 2015 – 2019 for all three locations was planned with sampling design and frequency as reflected in **Table no. 4.1.** Surface water grab samples were collected for physical and chemical analysis from all three different locations. Water samples for different Physico-chemical parameters were collected by dipping clean plastic bottle/bucket just under the water surface. pH and temperature were measured on site. Dissolved Oxygen and COD were preserved at the sites itself using Winkler's reagents (Manganese Sulphate i.e. $MnSO_4$ + Alkali Iodide Azide i.e. NaOH-NaI-NaN_3) and Sulphuric acid respectively. The collected samples were brought to the laboratory on the same day and stored in a refrigerator for further analysis. The water samples were analyzed for General Parameters (29), viz. Temperature -Ambient and Water (^oC), pH, Colour (Hazen), Turbidity (NTU), Conductivity (µs/cm), Total Solids (mg/L), Total Dissolved Solids (mg/L), Total Suspended Solids (mg/L), Ammonical Nitrogen (mg/L), Total Kjeldahl Nitrogen (mg/L), Nitrate (NO_3-N mg/L), Nitrite (NO_2-N mg/L), Phosphate (PO_4^{-2} mg/L), Alkalinity as $CaCO_3$ (mg/L), Total Hardness as $CaCO_3$ (mg/L), Sodium (mg/L), Potassium (mg/L), Calcium (mg/L), Magnesium (mg/L), % Sodium (%), Sodium Absorption Ratio (SAR milimole/L), Flouride (mg/L), Chloride (Cl^- mg/L), Sulphate (SO_4^{-2} mg/L), Dissolved Oxygen (mg/L), Chemical Oxygen Demand (mg/L), Biochemical Oxygen Demand 3 days 27^oC (mg/L) and Total Organic Carbon (mg/L) (Parikh and Mankodi, 2011; CPCB, 1978). The physico-chemical

parameters of water samples were assessed as per Standard Methods for Examination of Water and Waste Water (WEF, APHA AWWA, 2012). TOC was measured on TOC analyzer (Shimadzu make Model TOC_L). A small volume of water sample is inserted in a high temperature combustion chamber (680 ^{o}C) in presence of Pt catalyst. The sample gets oxidized to CO_2 and H_2O. The CO_2 from oxidation is measured by NDIR sensor. The water samples were also analyzed for Trace Metals (9) for the year 2016-2017 and 2017 -2018. The concentration of Trace Metal was analyzed using ICPMS (Agilent make model 7700X). The water samples were filtered through 0.2 µm milllipore filter paper and were then acidified / digested to a pH of <2 using supra pure Nitric Acid and Hydrochloric acid. These pre-treated water samples were passed through Plasma torch which generates temperature of 10000^{o}C that results into atomization followed by ionization and the separated ions are detected by Mass Spectrometer.

The obtained average values of water quality parameters were compared with BIS Drinking Water Specifications (IS 10500:2012), with analytical range prescribed in FAO guidelines for irrigation water (Raychaudhari et al., 2014) and also with CPCB effluent discharge standards for Inland Surface Waters.

Table 4.1 Sampling Plan for the Study Period

Year	2015 - 2016											2016 - 2017											2017 – 2018											2018 - 2019	
Season	SUMMER-I			MONSOON-I				WINTER-I				SUMMER-II				MONSOON-II				WINTER-II				SUMMER-III				MONSOON-III				WINTER-III				SUMMER-IV	
Month	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Parameters	W B P/R PP	-	W	-	W B P/R PP	-	W	-	W B P/R PP	-	W	-	W S B P/R PP	-	W S	-	W S B P/R PP	-	W S	-	W S B P/R PP	-	W S	-	W S B P/R PP	-	W S	-	W S B P/R PP	-	W S	-	W S B P/R PP	-	W S	-	W S B P/R PP
W = Water Sample, S = Sediment Sample, B = Biomonitoring, P/R = Photosynthesis Respiration Ratio, PP = Primary Production																																				

4.3 Water Quality Index

A water quality index is a number or a grade that expresses overall water quality at a particular location and time. It is based on several water quality parameters. The index turns complex water quality data into understandable, simple and usable information for the public and the policy makers (Alobaidy et al., 2010; Gor & Shah, 2014; Sharma & Reddy, 2013 and Singh et al., 2015). The Water Quality Index (WQI) was calculated by taking into consideration the irrigation water standards of BIS, FAO Guide lines for irrigation water, criteria for designated best use as lay down by CPCB and through expert opinion on relative significance of the parameter (Raychaudhari et al., 2014). The index presented here is specifically in context with its uses for irrigation and for sustenance of wild life. It thus gives an idea about the suitability of Thol Wetland water for a particular use or about the water related regional problems. The water parameters considered for calculating WQI are pH, Conductivity, TDS, Nitrate (NO_3-N), Phosphate , Alkalinity as $CaCO_3$, Total Hardness as $CaCO_3$, Sodium, Potassium, Calcium, Magnesium, % Sodium, SAR, Fluoride, Chloride and Sulphate (ICAR, 2014; BIS, 2002, **Table 4.2**).

Table 4.2 Irrigation Water Guidelines/Criteria Values and Weight Assigned

Parameter	Unit	FAO Guidelines (1985): irrigation water (Usual range)	BIS Standards (2002): Designated Use Class E (Max Values)	Weight (wi)	Relative Weight (Wi)
pH	-	6.0 – 8.5	6.5 -8.5	1	0.0323
Conductivity	µS/cm	0 – 3000	2250	4	0.1290
TDS	mg/L	0 – 2000	-	4	0.1290
Nitrate (NO_3-N)	mg/L	0 – 10	-	3	0.0968
Phosphate	mg/L	0 – 2	-	1	0.0323
Alkalinity as $CaCO_3$	mg/L	200	-	1	0.0323
Total Hardness as $CaCO_3$	mg/L	712*	-	1	0.0323
Sodium	mg/L	0 – 920	-	1	0.0323
Potassium	mg/L	0 – 2	-	5	0.1613
Calcium	mg/L	0 – 400	-	1	0.0323
Magnesium	mg/L	0 – 61	-	2	0.0645
% Sodium	-	-	60	2	0.0645
SAR	me/l	0 – 15	26	2	0.0645
Fluoride	mg/L	1.5*	-	1	0.0323
Chloride	mg/L	0 – 1065	600	1	0.0323
Sulphate	mg/L	0 – 960	1000	1	0.0323
			Total	31	

*Values as suggested in Research Bulletin No.71, Directorate of Water Management, ICAR, 2014.

The Water Quality Index (WQI) for Thol Wetland was calculated as below**.**

Assigning Weight: Weight (wi) was assigned to each water quality parameter based on its relative significance in the overall quality of water for irrigation and wildlife propagation.

Determination of Relative Weight (Wi): Relative Weight is determined from the equation $W_i = \frac{w_i}{\sum_{i=1}^{n} w_i}$

where Wi = Relative weight of parameter,

wi = weight of each parameter and

n = total number of parameters into consideration.

Determination of Quality Rating Scale: Quality Rating Scale (Qi) is developed by dividing concentration with its respective standard value laid down by BIS / CPCB and multiplying by 100. Thus, Qi = $\frac{C_i}{S_i}$ X 100

where Qi = Quality rating,
Ci = Concentration of each parameter and
Si = Standard value of parameter in mg/L as laid down by BIS / CPCB.

Determination of Sub-index: Sub-index (SI) for each water quality parameter is determined using the formulae SIi = Wi X Qi

The Water Quality Index (WQI) is computed using the formula WQI = $\sum SI_i$

where SIi = Sub-index of ith parameter
Qi = Quality rating based on concentration of ith parameter
n = the total number of parameters

The calculated WQI values are classified into four types (**Table 4.3)** as follows.

Table 4.3 Classification based on Water Quality Index for Irrigation

WQI value	Classification
< 50	Excellent
50 – 150	Good
150-300	Poor
>300	Very Poor

The irrigation water is classified (BIS IS: 11624, 1986) into four major groups **(Table 4.4)** based on hazardous effects of the Total Salt Concentration expressed as the Electrical Conductivity and Sodium Absorption Ratio (Alobaidy et al., 2010 and Sharma & Reddy, 2013). The US Salinity Laboratory has laid down the limits **(Table 4.4)** for conductivity with respect to irrigation (Zwart & Trivedi, 1994).

Table 4.4: EC & SAR Based Hazard Level Classification of Irrigation Water

US Salinity Laboratory*		US Federal Water Pollution Control Administration*		Irrigation water Classification Based on Hazardous Effects#		
Conductivity (µS/cm)	**Suitability for Irrigation**	**SAR**	**Hazard level**	**Conductivity (µS/cm)**	**SAR**	**Class**
< 250	Entirely Safe	< 8	Most probably Safe	<1500	<10	Low
250 -750 (moderately Saline)	Safe under practically all conditions	12- 5	Marginal Hazard	1500-3000	10-18	Medium
750 – 2250 (medium to highly Saline)	Safe only with permeable soils and moderate leaching	15-20	Serious Sodium Hazard	3000-6000	18-26	High
2250 – 4000 (highly Saline)	Unfit for irrigation	> 20	Detrimental because of Sodium Phytotoxicity	> 6000	> 26	Very High
4000 – 6000 (very highly Saline)	Unfit for irrigation					
>6000 (Excessively Saline)	Unfit for irrigation					

* Adapted from Zwart & Trivedi, 1994
# Based on Ramkrishnaiah et al., 2009, Abdul et al., 2010; and Anant, 2012

4.4 Biological Assessments

For an overall assessment of an ecosystem, physico-chemical studies have to be supplemented with biological assessments (Cairns & Pratt, 1993 and Zwart & Trivedi, 1994). The concept of Bio monitoring i.e. using the native organisms living in water bodies as sensitive indicators of prevailing water quality was used during the study. Central Pollution Control Board, Delhi has also established the role of biological parameters particularly Biomonitoring using Benthic Macroinvertebrates (CPCB, 1995, 2002 and 2016). They are indicators of water

quality as these groups exhibit varying sensitivity to different pollution levels. In this study, an attempt to integrate Bio monitoring and Physico-chemical monitoring for assessment of overall water quality of Thol Wetland is made. Hand net was used for collecting the benthic macro invertebrates. Ample care was taken to ensure that all indicator families of Benthic Macro-invertebrates present are actually encountered. This was accomplished by sampling all different (micro) habitats in a sizeable stretch of the water body (Bhadrecha et al., 2016; CPCB, 2016). At each location; the water sampling point (GPS location) was fixed as reference point and keeping 250 m stretch of the wetland bank on either side of it; a total of 500 m X 1 m width of wetland stretch was covered for Benthic Macro-invertebrate collection. This 500 m stretch was divided into 5 sub stretches of 100 m length and in each sub stretch; Benthic Macro-invertebrate collection was carried out starting from the water margin to open water side. Different sampling techniques were deployed for different micro habitats **(Plate 4.3)**. At each location, the sampling area was approached starting from downstream side to upstream side of the Wetland. All possible micro habitats such as aquatic vegetation on edges of the wetland banks, algae, pebbles, wetland bed, detritus, submerged and floating vegetations etc. were explored for collection of Benthic Macro-invertebrates by using hand net, sieve and hand picking with forceps (Bhadrecha et al., 2016; CPCB, 2002). Collection procedure **(Plate 4.3)** was repeated five times in each sub stretch. Five grab samples of silt was picked from the wetland bed by the plastic scoop/shovel and washed by wetland water in a sieve with mesh size 0.6 mm. The animals were then picked by forceps from the

sieve and transferred into the tray. In case of the sampling locations where the flow was observed, the sampling net was placed firmly on to the bed against the flow. The wetland bed was disturbed by standing before the net and disturbing the bottom with foot so that the animals are carried along with the flow into the net. The animals so collected into the net are then transferred into the tray. This procedure was repeated five times at each possible sampling site. Net was also moved all along the edges of grasses/emergent aquatic vegetation all along the wetland bank and the animals were collected and transferred into the tray with forceps. The water plants/floating plants present near the sampling area were uprooted and washed directly into the net or into white tray so as to detach the animals.

All animals thus collected were preserved in 4% formalin for further identification in laboratory. These samples were returned to the laboratory for further analysis. Benthic Macro invertebrates were sorted out using dissecting microscope/ compound microscope. Benthic Macro-invertebrates were identified up to family level using 'Appendix: 6 Taxonomic Key for Biological Water Quality Evaluation of the Manual on Integrated Water Quality Evaluation (Zwart and Trivedi, 1994) and other standard identification keys and literature. The abundance of each animal observed during sampling was also noted down. The biological parameters using Benthic Macro-invertebrates analyzed were BMWP (Bio Monitoring Working Party) Score or Saprobic Score and Sequential Comparison Index or Diversity Score (CPCB, 1995, 2002 & 2016).

Plate 4.3: Benthic Macroinvertebrates Sampling and Indicative Site Conditions

Diversity Score (Sequential Comparison): For calculation of Diversity Score, Benthic Macro invertebrate specimens were randomly dispersed on a plastic tray marked with a grid of 1 inch x 1 inch **(Plate 4.4).**

Plate 4.4: Biological Assessment of Benthic Macroinvertebrates in Laboratory

A sequential comparison of organisms in each square of the gridded tray was carried out from left to right direction and starting from the uppermost extreme left grid and ending at lowermost extreme right grid. While comparing, the organism is assigned a mark (run) of 1 if it is different from the just previous

organism and a mark of 0 if it is same. Since the method only involves a pair-wise comparison of sequentially encountered individuals and the differences of two specimen can easily be observed up to the family level, no special taxonomic skill is required. First observed animal is always different and scored as 1 run. When the next observed animal is different from the last, a new run starts. The encounter of an individual which cannot be discerned from the last does not increment the number of runs. Size differences only do NOT change the run. The number of runs will be the sum of all 1's.

$$\text{Diversity Score: } = \frac{\text{Number of Runs}}{\text{Total Number of Organisms}}$$

Biological Monitoring Working Party (BMWP) Score: A quantitative inventory up to 'family' level of taxonomic precision was carried out for Benthic Macroinvertebrates collected from different microhabitats of the study area **(Plate 4.3)** by using BMWP score card designed by Central Pollution Control Board. All possible families having saprobic indicator value are classified on a score scale of 1 to 10. The families which are most sensitive to pollution are on the top of the list and are getting a score of 10 while the most pollution tolerant families are getting a score of 1 and 2. The other intermediately sensitive families are placed in between the scoring scale of 10 to 1.The Saprobic scores of all the families were registered based on BMWP score. It was then averaged to produce Total BMWP Score. Abundance Scale of Families, A = Single (One Individual), B = Scarce (2-10 Individuals), C = Common (10-50 Individuals), D = Abundant (50-100 Individuals), E = Excessive (more than 100 individuals) is also noted based on field observation.

$$\text{Saprobic Score: } = \frac{\text{Grand Total Multiplied Score}}{\text{Grand Total Number of Families encountered}}$$

The Diversity Score and the Saprobic (BMWP) Score Card thus obtained for Thol Wetland during the Study Period is placed as **Annexure –VI.**

Biological Water Quality Criteria (BWQC)

To assess the actual health of water bodies, CPCB has derived a Biological Water Quality Criteria (BWQC) for water quality evaluation. This system is based on the range of Saprobic values and diversity of the benthic macro-invertebrate families with respect to water quality. To indicate changes in water quality to different grades of pollution level, the entire taxonomic groups, with their range of saprobic score from 1 to 10, in combination with the range of diversity score from 0 to 1 has been classified into five different classes of water quality **(Table 4.5).** The abnormal combination of Saprobic score and diversity score indicates sudden change in environmental conditions.

Table 4.5: Biological Water Quality Criteria (BWQC) Developed by CPCB

Range of Saprobic Score	Range of Diversity Score	Water Quality	Water Quality Class	Indicator Colour
7 and more	0.2 – 1.0	Clean	A	Blue
6 – 7	0.5 – 1.0	Slight Pollution	B	Light
3 – 6	0.3 – 0.9	Moderate	C	Green
2 – 5	0.4 – less	Heavy Pollution	D	Orange
0 – 2	0 – 0.2	Severe Pollution	E	Red

Criteria for Biological Water Quality Evaluation

The biological water quality evaluation was done by combining the observed saprobic score and diversity score and the biological water quality class was

determined through comparing the results with the ranges of Saprobic and Diversity score prescribed in Biological Water Quality Criteria (BWQC). It is to be mentioned that after taking into account the site conditions, Biomonitoring was carried out at Sampling Location 1 and location 2. It is also to be noted that Biomonitoring was not feasible at Location no. 3.

4.5 Primary Production and Production Respiration Ratio

A part from the Biomonitoring, Primary Production and Production Respiration Ratio are also important parameters for assessment of an aquatic ecosystem. In an aquatic ecosystem majority of primary production is accomplished by phytoplankton. The total amount of organic material produced by photosynthesis represents Gross Primary Production. As a portion of the organic material produced by photosynthesis is utilized in cellular Respiration, any excess production is referred to as Net Primary Production. It represents amount of organic material available to support consumers and decomposers (Odum, 1956). In other words it is an estimation of glucose ($C_6H_{12}O_6$) produced by photosynthetic autotrophs in a known volume of water over a specific time. The ratio of total primary production to total community respiration is used to classify communities quantitatively according to their predominantly heterotrophic or autotrophic characteristics (Cornell & Klarer, 2008; Zwart & Trivedi, 1995 and Pringault et al., 2007). An in-situ measurement of primary production and production respiration ratio of the Thol wetland using light dark bottle methodology (Ajayan & Parmeshwara, 2014; Ashok, 2015; Selvaraj, 2005; Kohler, 1988 and Sarma, 2016) was carried out at sampling location 1 and

Sampling location 3. The study was carried out for three seasons during the year 2015-2018. A set comprising three (one dark and two light bottles) 300 ml capacity bottles were taken. In each set, one of the bottles was double coated with black paint and labeled. Each of the bottles were carefully filled with wetland water sample by keeping the bottles at 45^{o} angle with the water surface avoiding shaking or splashing so that no oxygen is added to the water sample from outside. The bottles were suspended vertically from an indigenously made floating platform **(Plate 4.5)**. The whole setup was placed in the water column in the area having continuous exposure to sunlight. The dissolved oxygen of one of the light bottles representing the initial dissolved oxygen value was fixed with Wrinkler's reagents. The in-situ incubation was carried out for 3 hrs or 6 hrs. After the incubation period the bottles were retrieved and the dissolved oxygen was immediately fixed in dark and light bottle with Wrinkler's reagents.

Plate 4.5: Implanting In-situ Experimental Setup (Floating Platform) for Primary Productivity

After the incubation period, dissolved oxygen was determined up to three decimal points in all bottles using Winkler's titrimetric method. The calculations were performed through following steps.

a) *Net Oxygen Production mg/L*: Subtracting 'Initial Dissolved Oxygen' from 'Final Dissolved Oxygen (Light Bottle).'

b) *Oxygen Consumed by Community Respiration mg/L*: Subtracting 'Final Dissolved Oxygen (Dark Bottle)' from 'Initial Dissolved Oxygen.'

c) *Total Dissolved Oxygen Production mg/L*: Adding 'Oxygen Consumed by Community Respiration' to 'Net Oxygen Production.'

d) *Total Carbon Production mg C/mg O_2/L* : Multiplying 'Total Dissolved Oxygen Production' by 0.375 mg C/mg O_2

e) *Carbon Production Rate mg C/mg O_2/L/hr* : Dividing 'Total Carbon Production' by 'Incubation Time'

f) *Total Daily Productivity g C/m^3/d*: Multiplying 'Carbon Production Rate' by 1,000 to convert liters to cubic meters, then dividing by 1,000 to convert mg to grams, then is multiplying by 24 to convert hours to days.

g) *Photosynthesis Respiration Ratio* : Dividing gross primary production by respiration

Thus, total oxygen produced is calculated by adding the oxygen consumed in the dark bottles to the oxygen produced in the corresponding light bottles. Total oxygen production was used to calculate gross primary production by multiplying its value with the conversion factor 0.375 (Odum, 1956 and Ocean, 2016). The Primary Production values of the wetland thus obtained are expressed in g C/m^3/d (Selvaraj, 2005, Ocean, 2016 and Sarma, 2016). Various field and experimental observations were noted **(Annexure IX)**. It is to be noted that the parameters of Primary Productivity and Photosynthesis / Respiration Ratio were not in the initial

study plan but as the need was felt and also it was found to be interesting and hence decided to perform this in-situ experiments.

4.6 Sediment Assessment

Chemical and Physical analysis of wetland sediment can be used as a tool for the monitoring of nutrient levels or pollutants status and cycling in a wetland ecosystem. In order to make a valid comparison among sampling locations, consistent sampling technique were maintained. Thol wetland generally exhibits shallow sediment sampling scenarios and as the surface sediment contains the most recent information about the state of pollution (Zwart & Trivedi, 1994), Sediment samples were collected in a polythene bag by scooping the upper 3-4 cm with a plastic scoop. Care was taken when the scoop was raised through the water column during retrieval to minimize the loss of extremely fine material. Care was also taken not to walk on the sediment to be sampled and to remain back (downstream) and work with an extended hands. Five small separate subsamples from the same location were collected and made to single composite sample to minimize the natural variability (GEMI, 2016, Zwart & Trivedi, 1994 and **Plate 4.6**).

Plate 4.6: Bottom Sediment Sampling and Indicative Site Conditions

Soil texture was assessed by taking a small mass of soil in hand. The sediment samples were analyzed for parameters like Colour, pH, Conductivity, Total Kjeldahl Nitrogen, Available Nitrogen, Available Phosphorous, Organic Carbon, Organic Matter and Bulk Density.