Soil Sampling and Analysis

Soil samples were collected from each study site from the surface upto 30 cm. depth at monthly intervals (from July 2005 to June 2006). Soil samples were mixed and grinded in a mortar and passed through a 2 mm sieve and were stored in polythene bags for physical and chemical analysis. Soil samples collected in different months were analyzed for various physico-chemical parameters but data have been presented season-wise i.e. rainy season (July to October), Winter season (November to February) and summer season (March to June).

Mechanical properties

Soil texture refers to weight proportion in percentage of different size particles, such as coarse sand, fine sand, silt and clay.

The method of separating the soil particles into groups is known as mechanical analysis, i.e., percentage ratio of different size particles was done by Robinson pipette method (Piper (1966)).

Colour- The Colour of dry and wet soil was determined by comparing with Munsell's soil colour chart Munsell (1905).

Soil moisture- Soil moisture is expressed in percentage. A weighed quantity fresh soil sample was dried in electric oven at 105 °C till it attained a constant weight. The loss in weight denoted the amount of moisture present, which is expressed as percent dry weight of soil.
Water holding capacity

The water holding capacity is the maximum capacity of soil to hold moisture. It was determined using perforated brass cups as described by Piper (1966).

Porosity

Soil porosity is the percentage in terrestrial space or pores in a soil bulk that contains air and moisture. Total pore space was determined by using the following formula-

\[
\text{Porosity (\%)} = \frac{(Wt/\text{Vol})}{2.65} \times 100
\]

Soil pH

The pH is defined as the negative logarithm of hydrogen ion activity or concentration. A soil suspension having soil and water proportion of 1:5 was prepared in distilled water and its pH value was recorded electrometrically (pH-meter Elicomake, Model L112, with combined glass electrode Jackson (1967).

Organic carbon

The method suggested by Walkley and Black (1947) based upon the oxidation of carbon by nascent oxygen, liberated from K₂Cr₂O₇ in the presence of sulphuric acid was applied for determination of organic matter (Piper, 1966). Percentage of carbon was determined by using diphenylamine as indicator and titrating with ferrous ammonium sulphate solution in burette.

\[
\text{Percentage of carbon (\%C)} = \frac{V_1 - V_2}{W} \times 0.003 \times 100
\]

where,

- \(V_1 = \text{ml of 0.1 N K₂Cr₂O₇}\)
- \(V_2 = \text{ml of 1 N ferrous ammonium sulphate used}\)
- \(W = \text{weight of soil sample used}\)
- \(\% \text{ of organic carbon} = \%C \times 1.724\)
Nitrogen

The nitrogen was analyzed by micro-kjeldahl method Mishra, (1968) by digesting 10g powdered and sieved soil sample in 20ml concentrated H₂SO₄ using catalyst mixture (Copper sulphate + Mercuric oxide + Selenium powder + Sodium Sulphate) and distilling in a Markham unit. The results obtained are expressed in terms of N mg/g soil.

Phosphorus

5 g soil sample were used for determination of available Phosphorus by chlorostannous reduced molybdophosphoric blue colour in hydrochloric acid system (Jackson, 1967). The intensity of blue colour is directly proportional to Phosphorus content, which was determined spectrocolourimetrically at 660mm. the amount of Phosphorus in the aliquot was calculated with the help of standard curve. The results obtained are expressed in µg/g soil.

Exchangeable Cations (Potassium, Calcium and Sodium)

The exchangeable cations were extracted by leading the soil with neutral ammonium acetate solution. The leachate was used for the estimation of potassium, calcium, and sodium. These cations were estimated by the flame photometer using different filters Jackson (1967) and were applied same formula as described in water analysis of this chapter.

Heavy metals analysis in soil samples

Heavy metals like Cu, Ni, Cr and Mn were analyzed with the help of Atomic Absorption Spectrophotometer using Perkin-Elmer 5000 model with automatic burner control. Soil samples were taken and warmed gently by dissolving in conc. HNO₃ and filtered with what man filter paper No. 42 to make aliquot.
Water Sampling and Analysis

Water samples collected at three different study sites of Jargo River at monthly intervals in the first week of each month from July 2005 to June 2006, between 8 a.m. to 11 a.m. Water sample were collected in ten replicates from each of the site in clear plastic containers, using standard methods of collection (APHA 1985). Water samples were brought to the laboratory and kept in preservation at 4°C for further analysis of various physico-chemical parameters i.e. alkalinity, nitrate, phosphate, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical Oxygen demand (COD), calcium and potassium. The temperature, transparency and pH were analysed at the site. Water samples collected at monthly intervals were analyzed for various physico-chemical parameters but data has been calculated and presented in season wise i.e. rainy (July to October), winter (November to February) and summer (March to June).

Analytical methods

The analysis of the collected samples of different sites were studied by the methods described in standard methods for the analysis of water and waste water (APHA 1985), American water works Association (AWWA) and water pollution control Federation (WPCF).

Temperature

Temperature was measured at the site using a Celsius thermometer.

Hydrogen ion concentration

An electronic pH meter (ck-701) to the accuracy of 0.05 was used for the measurement of pH. The pH meter was standardized with stock buffers before each reading.
Transparency

Transparency was measured at the site with the help of Secchi disc of 20 cm diameter. This method was derived by an Italian scientist, A. Sechhi in 1865 (Reid and Wood, 1976). Transparency was estimated by the following formula

\[ T = \frac{A + B}{2} \]

Where,

- \( T \) = Transparency
- \( A \) = Water depth (cm) from the surface at which the disc disappears from the view in descent
- \( B \) = Water depth (cm) at which the disc reappears in ascent.

The distance between \( A \) and \( B \) is known as transparency coefficient (Reid and Wood, 1976).

Total dissolved solids

The dissolved solid is defined as solids capable of passing a glass fibre filter and dried to constant weight of 105°C. 100 ml of well mixed water sample is filtered through a standard glass fibre filter disc. The filtrate is allowed to evaporate and then dried to constant weight at 105°C cooled in a dessicator and weighed in mg/L.

Alkalinity

Take 50 ml of water sample. Add 2-3 drops of phenolphthalein indicator, if the solution remains colourless, the phenolphthalein alkalinity was zero. If pink colour appears, titrate the solution with 0.02 \( \text{NH}_2\text{SO}_4 \) until the first permanent pink colour disappears. Disappearance of pink colour indicates phenolphthalein alkalinity. Further, add 2-3 drops methyl orange indicator to the solution in which phenolphthalein alkalinity was determined. Titrate with 0.02 \( \text{NH}_2\text{SO}_4 \) acid to the
proper equivalence point. The colour changes from yellow to pink. Reappearance of pink colour indicates total alkalinity.

Volume of sample taken = 50 ml
Volume of H$_2$SO$_4$ used with phenolphthalein indicator = u ml
Volume of H$_2$SO$_4$ used with methyl orange indicator = v ml
Total volume of H$_2$SO$_4$ used with both indicator = u + v ml

Phenolphthalein alkalinity = \( \frac{u \times N \times 1000 \times 50}{\text{ml sample}} \)

Total alkalinity (as CaCO$_3$ mg/L) = \( \frac{(u + v) \times N \times 1000 \times 50}{\text{ml sample}} \)

N = Normality of H$_2$SO$_4$

Carbonate alkalinity = 2 \times \text{phenolphthalein alkalinity}

Bicarbonate alkalinity = Alkalinity - Carbonate alkalinity

**Dissolved oxygen (DO)**

Winkler's modified azide method was used to measure the dissolved oxygen content in water sample. DO of water samples is measured by precipitating as manganic basic oxide which is dissolved by concentrated sulphuric acid forming manganic sulphate. It immediately reacts with potassium iodide, already present, Liberating iodine which was determined by titrating with sodium thiosulphate (0.025 N) using starch as an indicator. The chemical reactions are as follows:-

\[
\begin{align*}
\text{MnSO}_4 + 2\text{KOH} &= \text{Mn(OH)}_2 + \text{K}_2\text{SO}_4 \\
2\text{Mn(OH)}_2 + \text{O}_2 &= 2\text{MnO(OH)}_2 \\
&\quad \text{(Manganic basic oxide)} \\
\text{MnO(OH)}_2 + \text{H}_2\text{SO}_4 &= \text{Mn(SO}_4)_2 + 3\text{H}_2\text{O} \\
&\quad \text{(Manganic Sulphate)} \\
\text{Mn(SO}_4)_2 + 2\text{KI} &= \text{MnSO}_4 + \text{K}_2\text{SO}_4 + \text{I}_2 \\
&\quad \text{(Iodine)}
\end{align*}
\]
The quantity of iodine liberated during this reaction is equivalent to the quantity of oxygen content of the water sample. The DO was calculated by the following formula.

$$\text{DO} = \frac{V \times N \times 8 \times 1000}{\text{ml. of sample}} \text{ (mg/l)}$$

Where, V and N are the volume and normality of the titrant respectively.

**Chloride**

Chloride is one of the major inorganic anions in water and waste water. It occurs mutually in all types of waters. However in natural fresh waters its concentration remains low and is generally less than that of sulphate and bicarbonate. The most important source of chloride in the water is the discharge of domestic and industrial effluent. It was measured by titration using silver nitrate as titrant and potassium chromate as indicator. Chloride was calculated by the following formula-

$$\text{Chloride (mg/l)} = \frac{A - B \times N \times 35450}{\text{ml. of the sample}}$$

Where,

- A = ml. of titrant use for sample
- B = ml. of titrant used for blank
- N = Normality of silver nitrate

**Potassium and Calcium**

Potassium and calcium were analyzed by using Systronic type Flame photometer at wavelength of 769 nm, in which characteristic light is produced due to excitation of electron when the sample having potassium (K) and calcium (Ca) was sprayed into the flame. The intensity of this radiation is directly proportional to the concentration of the element present.
Phosphate

Phosphate was analyzed by stannous chloride method. In the water sample (50ml), ammonium molybdate solution (4 ml) and stannous chloride solution in glycerol (10 drops) were added.

Development of blue colour show the presence of phosphate –P. The intensity of blue colour was measured at 680 nm by using colourimeter were compared with standard graph to obtain the phosphate- P content in the water sample.

Biochemical oxygen demand (BOD)

Initial DO was analyzed at the site by Automatic Oxygen Analyser and other bottles were incubated in BOD incubator at 20°C for 5 days and then analyzed for DO. The BOD value was calculated by the following formula-

\[
BOD = \frac{DO \text{ (initial)} - DO \text{ (after 5 days incubation)}}{\text{Decimal fraction of dilution}}
\]

BOD is the amount of dissolved oxygen required in mg/l for stabilizing the biodegradable organic matter. By microorganism of the water under aerobic conditions in the stated time.

Chemical oxygen demand (COD)

Chemical oxygen demand (COD) analyzed by dichromate reflux method. In which a known volume of water sample were refluxed with a known volume of potassium dichromate at concentrated sulphuric acid for two hours. The remaining amount of potassium dichromate after completing reflux was titrated with ferrous ammonium sulphate using ferron indicator. The COD calculated by the following formula-

\[
COD (\text{mg/l}) = \frac{(a - b) \times n \times 8000}{\text{ml. of sample}}
\]
Where,

\[ a = \text{ml of ferrous ammonium sulphate used for blank} \]
\[ b = \text{ml of ferrous ammonium sulphate used for the water sample} \]
\[ N = \text{Normality of ferrous ammonium sulphate}. \]

**Nitrate**

Nitrate was analyzed by the phenol disulphonic acid method. In which the steam dried water sample were dissolved in phenol disulphonic acid (2ml). The alkaline medium was made by adding ammonium hydroxide (10ml.). The development of yellow colour denoted the presence of NO₃. The intensity of colour is proportional to the amount of NO₃-N present in water sample which was measured by colourimeter at 410nm in terms of optical density. The final calculation was made with the help of standard graph-

\[ \text{Nitrate} = \frac{\text{Reading of Standard} \times 1000}{\text{ml of sample}} \text{ mg/l} \]

**Heavy metal's analysis in water samples**

Heavy metals are usually present in trace amounts in river waters. In the present investigation only four heavy metals *viz.* copper, chromium, nickel and manganese have been taken in account. For the analysis of heavy metals the sample were acidified with concentrated nitric acid (8 ml/l) to make pH of the solution around 2.0 and boiled for 10 minutes, then filtered when a clear solution was obtained. The filtrate was concentrated and made up to a volume of 100ml. Jargo river water may have some considerably different surface tension characteristics than the standard ones. The nabulization efficiency may be different between the sample and the standard. To compensate for nabulization effect, it was necessary to match the major matrix components of the sample and the standard. Thus there are three major interference i.e. matrix, chemical and ionization.
In present investigation atomic absorption spectroscopy method was used, because it is most widely used method for trace metal analysis. Atomic absorption spectroscopy method has high attainable sensitivity for a wide range of elements and high selectivity for the analyte element sought. It is also low in cost from other techniques except from colourimetric. However, few problems are encountered in the determination of trace concentrations of element in dilute aqueous solutions by atomic absorption spectroscopy.

The heavy metal content of the sample was analyzed by flame atomic absorption spectrophotometer using Perkin-Elmer 5000 model with automatic burner control. All furnace injections were made with micro-pipettes.

**Phytosociological Studies**

The phytosociological studies of aquatic macrophytes were done at all the three study sites, and 25×25 cm sized quadrates were used for the study. The quadrates were placed at intervals of five meters along the line transect. The species present in quadrate were noted and their numbers were counted individually / tiller of each species. For the basal area the measurement in diameter of the individual/ tiller was considered for each species at the point of emergence. From these collected data of macrophytes the frequency, relative frequency, density, relative density, dominance, relative dominance and importance value index (IVI) were determined using different formula.

**Frequency**

The term frequency refers to the degree of dispersion of individual species in an area and is usually expressed in terms of percentage occurrence. It can be defined as the chance or probability of an individual of a given species to be present in a randomly placed quadrate.
Frequency = \frac{\text{Number of quadrates in which the sp. occurred}}{\text{Total no. of quadrates studied}} \times 100

Relative Frequency = \frac{\text{Number of occurrence of a species}}{\text{Number of occurrence of all teh species}} \times 100

**Density**

This term is also an expression of the numerical strength of a species where the total no. of individuals of each species is divided by the total number of quadrates studied and expressed in numbers per square meter. Whereas relative density is an expression of numerical strength of a species in relation to the total number of individuals of all species in an area.

Density = \frac{\text{Total number of individuals of a species in all the quadrates}}{\text{Total number of quadrates sampled}} \times 100

Relative density = \frac{\text{Number of individuals of the species in all the quadrates}}{\text{Number of individuals of all the species in all quadrates}} \times 100

**Dominance**

This is an expression of the area covered or occupied by different species and is usually given as percentage. The cover is of great ecological significance because although the frequency and density of trees or shrubs may be lower than those of smaller plants, yet the dominating influence of tree may be grater in the community because of their more extensive canopy coverage. In grassland species the total coverage of ground by stems and leaves are called herbage cover, of the cover can be expressed in terms of percentage of area covered and can be determined through actual measurements by quadrate methods of sampling whereas the relative dominance is coverage value of species with respect to the sum of coverage of the rest of the species in the area.

\text{Basel area} = \pi r^2

= 3.14 r^2 (\pi= 22/7)
Where,

\[ r = \text{Radius of the stem at the point of emergence} \]

\[ \text{Dominance} = \text{Density} \times \text{Basel area} \]

Relative Dominance = \( \frac{\text{Total basal area of the species in all the quadrates}}{\text{Total basal area of all the species in all the quadrates}} \times 100 \)

**Important Value Index**

In the community, the quantitative value of each of the frequency, density, abundance and cover has its own importance but the total picture of ecological importance cannot be obtained by any one of these. For instance, frequency gives an idea as to how a species is dispersed in the area but we do not get any idea about its number or the area covered. Density on the other hand gives the numerical strength and nothing about the spread or cover. Dominance gives the numerical strength and nothing about the spread or cover. Dominance gives the basal cover only.

Therefore, in order to get the overall picture of ecological importance of a species with respect to the community structures, the percentage value of the relative frequency, relative density and relative dominance are collected together and this value out of 300 is called the Importance Value Index or IVI of the species. Usually after the quantitative estimation of relative values of density, dominance and frequency, the species are listed in order of decreasing importance. The IVI as such gives the total picture of sociological structure of a species in a community but it does not give the dimension or share of relative value of frequency, density and dominance.

\[ \text{Importance Value Index} = \text{Relative Frequency} + \text{Relative Density} + \text{Relative Dominance} \]
Standing crop biomass

Standing crop biomass was measured by harvest method (Odum, 1956). Selected macrophytes were collected by the use of quadrates of 25×25 cm in size. The samples of macrophytes were collected at a regular interval of 30 days in each of the month. The collection was made in three replicate at the second week of each month from July 2005 to June 2006. The macrophytes were washed carefully to remove soil. Then the collected plants were put in polyethylene bags. The plant sample were put in oven at 80°C to 48 hours and then weighed.

Net Primary Productivity

Net primary productivity was calculated from the biomass value at a particular time period and it expressed in gm$^{-2}$ da$^{-1}$ Briggs et al. (1920a, b).

\[
\text{NPP (gm}^{-2}\text{ da}^{-1}) = \frac{W_1 - W_2}{t_1 - t_2}
\]

Where, \(W_1\) & \(W_2\) are the dry weight of total plant at time \(t_1\) & \(t_2\) respectively.

Determination of Caloric Value

The caloric values were determined from the samples collected in different month (from July 2005 to June 2006) from three study sites. The plant samples were kept in the following categories from each study sites.

Above ground parts - \(Hydrilla\ verticillata, Ludwigia\ parviflora, Marsilea\ quadrifolia, Ipomoea\ aquatica\)

Below ground parts - \(Hydrilla\ verticillata, Ludwigia\ parviflora, Marsilea\ quadrifolia, Ipomoea\ aquatica\)
In the present study the oven dried plant materials were again kept in oven at 80°C for 24 hours. After drying the plant materials were powdered in grinding machine and were sieved with 40 mesh brass sieve. Before subjecting to a caloric analysis the plant material from each category was compressed into pellet (about 0.8 g) and made moisture free in desiccator.

For the combustion processes, Parr Oxygen Bomb Calorimeter Model 1341 was used. In each of combustion process, 10cm Parr nickel- chromium fuse wire was used.

**Heat of combustion of plant material**

The pellet was ignited in the calorimeter in the presence of oxygen essential for the combustion (15 to 20 atmospheric pressures). The volume of water taken inside the water bucket that surrounds the bomb shell was kept constant throughout in all the combustions. The caloric value of each plant sample was estimated based on the mean value of three or sometimes at least two replicates. The gross heat of combustion or the energy content per gram of the sample on dry weight basis was calculated as follows (Parr Instrument Company, Manual 130, 1968).

\[
\text{Gross heat of Combustion } H_g = \frac{t_w - e_1 - e_2 - e_3}{m}
\]

Where,

- \( t \) = Rise in temperature (°C)
- \( w \) = Water equivalent (cal/°C)
- \( e_1 \) = Heat of the formation of HNO₃ (cal.)
- \( e_2 \) = Heat of the formation of H₂SO₄ (cal.)
- \( e_3 \) = Heat of the Combustion of fuse wire (cal.)
- \( m \) = Weight of the sample (g)
Water equivalent of the calorimeter

The energy equivalent of the bomb shell (water equivalent) was determined by igniting benzoic acid pellet and the caloric value was estimated by the following formula.

\[ W = \frac{H_m + e_1 + e_3}{t} \]

Where,
- \( W \) = Water equivalent of calorimeter
- \( H \) = Heat of combustion of benzoic acid (6318 cal/g)
- \( m \) = Weight of benzoic acid (g)
- \( e_1 \) = Heat of the formation of HNO₃ (cal.)
- \( e_3 \) = Heat of the combustion of fuse wire (2-3 cal/cm)
- \( t \) = Rise in temperature (°C)

Fuse wire correction

Each combustion is initiated by passing electric current energizing through nickel chromium fuse wire, heating it to incandescence and thereby igniting the sample. The heat released is proportionate to the length of wire between the electrodes. The correction factor used for fuse wire is 2-3 cal/cm (Parr Inst. Co., Manual 130, 1968). 10 cm. of fuse wire was used in all estimation and hence correction of 23 calories was made.

Acid correction

Another minor source of error is the formation of acids, primarily nitric and sulphuric acid following combustion of organic compounds under pressure. The sulphur correction is made on the assumption that this element is completely converted into H₂SO₄ with a higher release of heat than would occur if it was simply oxidize to SO₂, as would occur at normal atmospheric pressures.
Formation of nitric acid also occurs under conditions prevailing in a bomb. An acid correction was estimated by assuming all acid was HNO₃, as amount of sulphur in plant material was insignificant. About 5ml of water was poured into the bottom of the bomb before combustion and later on after burning of the pellet this solution was titrated against 0.07 N sodium carbonate using 1-2 drops of methyl red as an indicator. At the above-mentioned normality of sodium carbonate 1ml titrate is equivalent to 1 calorie. The correction of acid was subtracted from the calculated calorific value.

**Biochemical analysis of macrophytes**

The oven dried plant samples were powdered in a mortar & passed through a 44 mesh sieve (0.353 mm, pore size) for the analysis of concentration of nitrogen, Phosphorus, potassium, calcium and sodium and heavy metals. Monthly collected plant samples were analyzed but data of different parameters have been presented season wise.

**Chlorophyll**

0.5 gm fresh leaf sample was chopped in 25 ml 80% acetone (acetone: water, 4:1 v/v). Tightly plugged flasks were refrigerated for 24 hrs. Finally volume was maintained 40 ml by 80% acetone and a pinch of MgCO₃ to buffer the extracting medium. Extraction was carried out in dark to avoid photo oxidation of pigments. Extract was filtered and centrifuged at 3000 rpm for 15 min. Optical densities of the solution were measured at 480, 510, 645 and 663 mm wavelengths. Pigment content was computed by the following formula given by Maclachlan and Zalic (1963) for chlorophyll.

\[
\text{Chlorophyll} = \frac{12.3D_{663} - 0.86D_{645}}{1000 \times W} \times V
\]

Where,

\[
W = \text{Weight of leaf (g)}
\]
D = Optical density  
V = Volume of sample (ml)

Nitrogen

The nitrogen concentration in four selected macrophytes was determined by micro-Kjeldahl method described by Jackson (1967). The oven dried powdered samples of plant (0.5 g) were digested in 10 ml concentrated H$_2$SO$_4$ using catalyst mixture (copper sulphate + potassium sulphate + selenium powder) for about 4-5 hours. After digestion the solution was extracted with distilled water and the volume was made up 100 ml. The extract was distilled with 40% NaOH solution in a Markham micro Kjeldahl distillation unit. The distillate was collected in 4% boric acid and 2 drops of mixed indicator (0.5% of bromocresol + 0.1% methyl orange). The ammonia absorbed boric acid was titrated against N/28 H$_2$SO$_4$ and the percentage of total nitrogen was calculated by using the formula.

\[
\text{Percentage nitrogen} = \frac{T - B \times N \times 10 \times 1.4}{S}
\]

where,  
T = Volume of H$_2$SO$_4$ used in sample titration, ml  
B = Volume of H$_2$SO$_4$ used in blank titration, ml  
N = Normality of standard H$_2$SO$_4$  
S = Weight of plant material, gm

Ashing Procedure

The wet ashing technique was adopted for the estimation of phosphorus, potassium and sodium. Plant material (0.2 to 0.5 g) was taken in a Kjeldahl flask and it was digested with ternary acid mixture (HNO$_3$ + H$_2$SO$_4$ + HClO$_4$ of ratio 10:1:4). The digestion was continued until the major portion of the acid mixture got volatilized and became colourless. The solution was extracted with distilled
water and volume was made up 100 ml. The extract solution was used for the above estimation.

**Phosphorus**

An aliquot of the extract solution was taken in 50 ml volumetric flask and pH was adjusted at 3 with the help of Na$_2$CO$_3$ and H$_2$SO$_4$ using dinitrophenol indicator. To the solution was added sulphotomolybdcic acid solution and molybdophosphoric acid blue colour was developed after adding a few drops of chlorostanous acid in the test solution and within the specified time, transmission was read in a colourimeter at 660 mµ. The concentration of the test solution was determined by referring their percentage transmission to the calibration curve prepared for the standard solution of potassium hydrogen phosphate (KH$_2$PO$_4$) of different concentrations (Jackson, 1967).

**Potassium and Sodium**

Potassium and sodium was estimated by Flame Photometer using the extract prepared in ternary acid. Standard solutions of higher and lower concentration were prepared for KCl and Na$_2$CO$_3$ as described by Jackson (1967). The concentration of test solution were calculated by comparative method using formula as described in water analysis of this chapter.

**Heavy metals**

The analysis of heavy metals in selected macrophytes were done by atomic absorption spectrophotometry using aliquot of the extract solution obtained by wet ashing technique.