Methods

Monitoring may be defined as the surveillance carried out to determine trends in relation per-determined standards. The monitoring is essential for the successful implementation of environmental legislation to ensure that the criteria and standards set by regulatory agencies (PCB) are maintained on a continuous basis. Environmental monitoring is an important tool in the process of follow up assessment in pollution control program ETA. The environmental monitoring must have a purpose and function for which assessment objectives is to be determined before the onset of monitoring program.

The present study consists information regarding sites of study area with respect to sampling and monitoring, Field survey was carried out & various physico-chemical parameters as well as soil samples were also analyzed. The study covers various laboratory analysis parts according to methodology mentioned.

Ambient Air Quality Monitoring

The study of ambient air quality monitoring in and around the periphery of Jhalana Hills was conducted during the period of October to December. The Ambient air quality was monitored in Jhalana Hills and around 2.0 km radius. A circle of 2.0 km radius includes 7 areas for air sample collection. Samples were collected from the site and brought to
the laboratory for analysis of different parameters with the use of sophisticated equipment available in laboratory. Methods for RSPM, SPM, SO₂ and NO₂ were adopted from the monitoring and testing manual of envirotech model no. APM 460 Based on CSIR/NEERI technology.

**SPM (Suspended Particulate Matter) and RSPM (Respirable Suspended Particulate Matter)**

Respiratory dust Sampler model No. APM 460 is one of common instruments used for the collection of SPM in recent years. Stoke's law offer the basic approach to collection by this technique. RDS is vacuum cleaner type motor that is used to draw a sample through a filter area. In the sampler ambient air laden with suspended particulates enters the system through the inlet pipe. As the air passes through the cyclone, coarse, non-respirable dust is separated from the air stream by centrifugal forces acting on the solid particulates. These separated particulated fall through the cyclone's conical hopper and is collected in the sampling bottle at its bottom. The fine dust forming the respirable fraction of the total suspended particulate passes through the cyclone and is carried by the air stream to the filter paper (size:8”x 10”) clamped between the top
Methods

cover and filter adapter assembly. The RSPM is retained by tire filter and the earner air exhausted from the system through the blower.

\[
\text{RSPM (µg/m}^3\text{)} = \frac{[\text{FW}_2 - \text{FW}_1]}{\text{A.V}} \times 10^6
\]

Where:

\(\text{FW}_2\) = Final Weight of filter Paper

\(\text{FW}_1\) = Initial Weight of Filter Paper

\(\text{A.V.}\) = Average Air Flow rate (m\(^3\))

\(10^6\) = For conversion of g to µg.

\[
\text{A.V.} = \frac{Q_2 + Q_1}{2} \times T
\]

\(Q_1\) = Initial flow rate (m\(^3\)/min)

\(Q_2\) = Final flow rate (m\(^3\)/min)

\(T\) = Sampling time (min)

\[
\text{NSPM (g/m}^3\text{)} = \frac{[\text{DC}_2 - \text{DC}_1]}{\text{A.V}} \times 06
\]
Methods

Where-

DC2 = Final Dust Collector Weight
DC1 = Initial Dust Collector Weight

A.V. = Average Air Flow rate (m$^3$)

$\text{A.V.} = \frac{Q2 + Q1 \times T}{2}$

$Q1 = \text{Initial flow rate (m3/min)}$

$Q2 = \text{Final flow rate (m3/min)}$

$T = \text{Sampling time (min)}$

TSPM = RSPM + NSPM

Analysis of SO$_2$

Oxides of sulphur are produced due to combustion of fossil fuels: particularly coal in power plants, which generally has 1-3% SO$_x$ are also produced during petroleum refining and metal smelting. Sulphur dioxide (SO$_2$) is itself a poison, but it can also form sulphuric acid (H$_2$SO$_4$). SO$_2$ is quickly absorbed and acts in the upper respiratory tract, reacts with cellular constituent chemicals such as enzymes. Sulphuric acid, which
SO₂ forms, lower pH and thus impairs enzyme functions and destroys various functional molecules. Chronic pollution aggravates lung diseases, chronic bronchitis pulmonary fibrosis, are some of the most common symptoms of SO₂ pollution (Schwartz, 1994).

Modified West-Geake method (1968) is adopted for the determination of SO₂. Sulphur dioxide from air is absorbed in Potassium Tetrachloramercurate (TCM). Solution (absorbent) is taken in the impizer tubes. A Dichoro Sulphite Mercurate (DCSN) complex which resists oxidation by the oxygen in the air is formed. Once formed this complex is stable to strong oxidants such as ozone and oxides of nitrogen and therefore, the absorber solution may be stored for some time prior to analysis. The complex is made to react with pararosaline and for formaldehyde to form the intensity colored pararosaline methylsulphonic acid. The absorbance of the solution is measured by means of a suitable spectrophotometer.

Procedure

Sulphur dioxide (SO₂) sampling was done after time interval of 8 hrs in 24 hrs. 30 ml TCN solution was added to the impinzer into the sampling system. Samples were collected in the black plaststic bottle after 8 hrs and the volume of air sampler was determined by multiplying the
flow rate by the time in minutes and the atmospheric pressure and
temperature was recorded at the end of sampling.

10 ml sample was taken in a 25 ml volumetric flask from impinzer
and 1.0 ml 0.06% sulphamic acid was added and allowed to react 10 min
to destroy the nitrite resulting from oxides of nitrogen. 2ml of
formaldehyde solution and 2.0 ml Pararosaniline solution was added in
the each volumetric flask and shaken thoroughly.

The flask was allowed to stand for 30 min for development of red-
violet color. The same procedure was applied to prepare blank by treating
10.0 ml unexposed absorbent solution, and measured transmittance of
560nm with the reagent blank as reference.

1. **Absorbing Reagents = Absorbing Reagents 0.04 m Potassium
tetraclromercurate (TCM) -**

   10.86 g mercuric chloride, 0.066 g EDTA and 6.0 g potassium
Chloride or sodium chloride 4.86 gm was dissolved in water and brought
to the mark in 1 liter volumetric flask.

2. **Reagents for Analysis =**

   - **Sulphamic acid (0.6%) -** 0.6 g sulphamic acid dissolved in 100 ml
distilled water.
Methods

- **Formaldehyde (0.2%)** - 5 ml Formaldehyde solution (Freshly prepared) (36-38%) was diluted to 1 litre with distilled water.

- **Purified Pararosaniline Stock Solution (0.2% nominal)**

  **Pararosaniline Stock Solution** - 0.5 g of specially purified pararosaniline (PRA) dissolved in 100 ml distilled water, kept for 2 days and filtered the solution. The solution is stable for 3 months if stored in a refrigerator.

  **Pararosaniline Working Solution** - 10 ml of stock PRA taken in 250 ml volumetric flask. 15 ml concentration HCL added and made up to volume with distilled water.

  $\text{SO}_2 \mu g/m^3$ was calculated according to the following formula-

  \[
  \text{SO}_2 (\mu g\text{SO}_2/m^3) = \frac{\mu g\text{SO}_2}{\text{Volume of air samples}} \times 10^6
  \]

  Volume of Air Sample = Flow rate of air x Time of sampling

- **Analysis of NO$_2$**

  Modified Jacob & Hochheiseri (Na-Aresenite) is adopted for the determination of NO$_2$. Ambient Nitrogen dioxide (NO$_2$) is collected by bubbling air through a solution of sodium hydroxide and sodium arsenite.
Methods

The concentration of nitrite ion (NO2) produced during sampling is determined calorimetrically by reacting the nitrite ion with phosphoric acid, sulfanilamide and N-(I-Naphthye)-Ethylene Diamine Dihydrochloride (NEDA) and measured the absorbance of the highly colored azodye at 540 mm.

Procedure

10 ml of the collected absorbing reagent sample solution was taken into a test tube. 1 ml of H2O2, 10 ml of Sulphanilamide solution and 1.4 ml of NEDA solution was added with thorough mixing and made up to 50 ml distilled water. A blank was prepared in the same manner using 10 ml if by unexposed absorbing reagent. After 10 min colour of development interval was measured and the absorbance was recorded at 540 mm against the blank. Samples with an absorbance greater than 1.0 must be reanalyzed after diluting an aliquot of the collected samples with an equal quantity of unexposed absorbing reagent.

Absorbing Reagents

4.0g of sodium hydroxide and 1.0 g of sodium arsenate dissolved in distilled water, and diluted to 1,000 ml.
Methods

Reagents for Analysis

**Sulfanilamide Solution** - Dissolved 20 g of Sulfanilamide in 700 ml distilled water. 50 ml of 85% phosphoric acid added with mixing and diluted to 1,000 ml. This solution is stable for one month, if refrigerated.

**NEDA Solution** - Dissolved 0.5 g of NEDA in 500 ml of distilled water. This Solution is stable for month, if refrigerated and protected from light.

**Hydrogen Peroxide Solution** - Diluted 0.2 ml of 30% hydrogen peroxide to 250 ml with distilled water. This solution may be used one month, if refrigerated and protected from light.

\[
\text{NO}_2 (\mu g\text{NO}_2/m^3) = \frac{\mu g\text{NO}_2/ml \text{ (measured)}}{50} \times 0.85 \times V
\]

Where-

50 = Volume of absorbing reagent in ml

V = Volume of Sample in m\(^3\)

0.85 = factor for collection efficiency

**Noise**

Noise is defined as unwanted sound which interferes with speech communication and causes annoyance, distracts from work, disturbs
Methods

Sleep, thus deteriorate quality of human environment therefore noise pollution survey has been carried out by model No.1900, Noise levels was measured at different location within the 10 Km radius of study area to establish present scenario. The criteria given area in equivalent sound level dB (A) leq. This measure is defined as the average A-weighted Mean Energy of the noise level over the measurement period.

Meteorology

Metrology plays a vital role in affecting the dispersion of pollutants, once discharged into the atmosphere. Since metrological factors show wide fluctuations with time, meaningful interpretation can be drawn only from long term reliable data. Such source of data is the Indian Meteorological Department (IMD) which maintains a network of meteorological stations at several important locations. All the metrological data were collected from the nearest IMD station to the study area. The nearest IMD station is situated at Bhudhsingpura sanganer. The metrological parameters recorded from this station were Temperature, Humidity, Rainfall, Wind Speed, Wind Direction, Visibility and Cloud Cover recorded.
Methods

Ground Water Analysis

The present study was conducted during the period of March to May. The samples of 10 areas were collected from Jhalana Hills in a circle of 2 km radius. Samples were collected in clean polythene bottles from Tube Well and Hand Pumps and analyzed for different parameters (APHA, AWWA, WPCF, 1985).

pH (Potential Hydrogenii)

pH is the negative log\(_{10}\) of the Hydrogen ion concentration in solution. pH of water samples were analyzed by digital pH meter (Century CP 901).

Reagents:

Standard buffer solution of pH 4.00, 7.00 and 9.2 buffer tablets were dissolved in distilled water and diluted to 100 ml.

Procedure

Standardize the instrument with electrode immersed in a buffer solution. Electrode was removed from buffer rinsed and blotted and then immersed in a second buffer. For the sample analysis, electrode was placed in the sample and the reading was noted on the meter.
Methods

Electrical Conductivity

Electrical conductance is the ability of a substance to conduct the electric current. Electrical conductivity of the sample was determined with the help of digital conductivity meter (Model No. 161 E).

Total Dissolved Solids (TDS)

Total dissolved solids are determined as the residue left after evaporation of the filtered sample. The total dissolved solid in drinking water reveals the saline behaviour of the water which indicates the organic pollution level of water. Total dissolved solids of the sample were determined with the help of water analysis kit (Model No.161 E).

Total Alkalinity

Total alkalinity is the measure of the capacity of the water to neutralize a strong acid.

Reagents

1. Hydrochloric acid (0.1 N) - 12 N concentrated HCl was diluted (sp. gr. = 1.18) to 12 times (8.34 --> 4100 ml) to prepare 1.0N HCl.

Further it was diluted to make 0.1 N HCl (100-1000 ml) and was standardized against sodium carbonate, (Na₂CO₃) solution.
Methods

I. Methyl orange indicator (0.0.54%)

II. Phenolphthalein indicator

Procedure

50 ml. of sample was taken in a conical flask and 2-3 drops of phenolphthalein indicator was added. If the solution remains colourless, phenolphthalein alkalinity is absent. If the colour changes to pink after addition of phenolphthalein, it was further titrated with 0.1 N HCl until the colour disappeared at end point. This is phenolphthalein alkalinity. Total alkalinity was determined by adding 2-3 drops of methyl orange to the same sample and titration was further continued until the yellow colour changes to pink at end point. This is total alkalinity.

\[
PA \text{ as } \text{CaCO}_3 (\text{mg/l}) = \frac{A \times \text{(Normality of HCl)} \times 1000 \times 50}{\text{ml. of sample (Volume)}}
\]

\[
TA \text{ as } \text{CaCO}_3 (\text{mg/l}) = \frac{B \times \text{(Normality of HCl)} \times 1000 \times 50}{\text{ml. of sample (Volume)}}
\]

Where,

\[A = \text{ml. of HCl used with only phenolphthalein}\]

\[B = \text{ml. of total HCl used with phenolphthalein and methyl orange}\]
PA = Phenolphthalein alkalinity

TA = Total Alkalinity

**Chloride**

Silver nitrate reacts with chloride to form very slightly soluble white precipitate of AgCl. At the end point when all the chlorides get precipitated free silver ions react with chromate to form silver chromate of redish brown colour. High content of chloride gives salty taste to water.

**Reagents**

1. AgNO$_3$ (0.02 N) - 3.400 g of dried AgNO$_2$ (A.R.) was dissolved in distilled water to make 1 litre of solution and kept in a dark bottle.

2. Potassium Chromate (5%) - 5 g of K$_2$Cr$_2$O$_4$ was dissolved in 100 ml of distilled water.

**Procedure**

50 ml of sample was taken in a conical flask and 2 ml of K$_2$Cr$_2$O$_4$ solution was added. This solution was titrated against 0.2 N silver nitrate until a persistent brick red end point appeared.  

Chloride (mg/l) = \(\frac{(ml \times N) \times 1000 \times 35.5}{ml \ of \ sample}\)
**Methods**

**Total Hardness**

Hardness is generally caused by the calcium and magnesium ions present in water. Calcium and magnesium form a complex of wine red colour with Eriochrome Black T at pH of 10.0 ± 0.1. The EDTA has got a stronger affinity towards Ca$^{+2}$ and Mg$^{+2}$ and therefore by addition of EDTA, the former complex is broken down and a new complex of blue colour is formed.

**Reagents**

1. **EDTA solution (0.01 M)** - 3.723 g of disodium salt of EDTA was dissolved in distilled water to prepare 1 litre of solution and stored in polyethylene bottle.

2. **Buffer solution** - (a) 16.9 g Ammonium chloride (NH$_4$Cl) was dissolved in 143 ml of concentrated ammonium hydroxide (NH$_4$OH). (b) 1.179 g of disodium EDTA and 0.780 g of MgSO$_4$.7H$_2$O were dissolved in 50 ml distilled water. Both (a & b) solutions were mixed and diluted to 250 ml with distilled water.

3. **Eriochrome Black T (Solochromc Black T) indicator** - 0.40 g of Eriochrome Black T was mixed with 100 g NaCl (A.R.) and grinded.
Methods

4. Sodium sulphide solution- 5.0 g of Na₂S.9H₂O or 3.7 g Na₂S.5H₂O was dissolved in 100 ml of distilled water. Bottle was tightly closed to prevent oxidation.

Procedure

50 ml. of water sample was taken in a conical flask and 1 ml of buffer solution and a pinch of Eriochrome Black T indicator was added, the solution turned wine red. The solution was titrated against EDTA solution until the wine red colour changes to blue at the end point.

Hardness as (mg/l) CaCO₃ = \( \frac{\text{ml of EDTA used} \times 1000}{\text{ml of sample}} \)

Calcium

EDTA is having a higher affinity towards calcium, the former complex is broken down and a new complex is formed. However EDTA has a property to combine with calcium.

Reagents

1. EDTA solution (0.01 M) - 3.723 g of disodium salt of EDTA was dissolved in distilled water to prepare 1 litre of solution and stored in polyethylene bottle.
Methods

2. Sodium Hydroxide, 1N- 40 g of NaOH was dissolved in distilled water and diluted to 1 litre.

3. Murexide indicator - 0.2 g of Ammonium purpurate was added to 100 g of NaCl (A.R.) and grinded.

Procedure

50 ml of sample was taken in a conical flask and 2 ml of NaOH solution was added. Then one pinch of murexide indicator was added. The solution turned pink. Solution was titrated against EDTA until the pink colour changed to purple.

Calcium (mg/1) = \frac{X \times 400.8}{\text{ml of sample}}

Where, X = Volume of EDTA used

Magnesium

Calcium and magnesium form a complex of wine red colour with Eriochrome Black T at pH 10.0. The EDTA has got a stronger affinity for Ca\(^{2+}\) and Mg\(^{2+}\); the former complex is broken down and a new complex of blue colour is formed. The value of Mg\(^{2+}\) can be obtained by subtracting the value of calcium from the total of Ca\(^{2+}\) + Mg\(^{2+}\).
Methods

(a) $\text{Mg}^{2+}, \text{mg/L} = \frac{Y-X \times 400.8}{\text{Volume of sample} \times 1.645}$

Where $Y = \text{EDTA used in Hardness determination}$

$X = \text{EDTA used in Calcium determination for the same volume of the sample}$

(b) $\text{Mg}^{2+}, \text{mg/L} = \text{Total hardness (mg/l CaCO}_3\text{)} - \text{Calcium hardness (as mg/L CaCO}_3\text{)} \times 0.244$

Where, Calcium hardness (as mg/l CaCO$_3$) = Ca, mg/L x 2.497

(Fluoride)

Fluoride concentration in water samples were analyzed with help of Orion Research Ion analyzer model 407 A, fluoride ion selective electrode. The fluoride ion selective electrode was used to measure the concentration of fluoride in aqueous sample by the use of appropriate calibration curve. The electrode does not respond to bound or complexed fluoride therefore a buffer solution (TISAB) of high total ionic strength is added to the samples.

Reagents

1. Stock fluoride solution-
Methods

- 221.0 mg anhydrous NaF dissolved in distilled water and diluted to 1000 ml (1 ml = 100 µg F)

2. Standard fluoride solution

- 20 ppm standard stock solution was diluted 5 times with distilled water to obtain 1 ml = 20 µgF.
- 2 ppm standard 20 ppm standard solution was diluted 10 times with distilled water to obtain 1 ml = 0.2 µgF

3. Total Ionic Strenght Adjustment Buffer (TISAB)

Approximately 500 ml distilled water placed in a 1 litre beaker 57 ml glacial acetic acid, 58 g NaCl and 4.0 g of 1,2-cyclohexylene diamine tetraacetic acid (CDTA) was added. It was stirred to dissolve beaker was placed in a cool water bath and slowly 6N NaOH was added stirring continuously until pH is fixed and made up the volume up to the mark.

Procedure

- The electrode slope was checked with the ion meter (54-60 mV).
- 10 ml of each 0.2 ppm, 2 ppm and 20 ppm fluoride standards were taken and 10 ml of TISAB was added and then the instrument was calibrated.
- After calibration 10 ml of sample was transferred to a beaker and 10 ml of TISAB was added.
Methods

Procedure

Standardize the instrument with electrode immersed in a buffer solution. Electrode was removed from buffer rinsed and blotted and then immersed in second buffer. For the sample analysis, electrode was placed in the sample and the reading was noted on the meter.

To determine the pH at the moisture saturation percentage of soil, 20g of soil was taken in a 150ml beaker to which 10ml distilled water was added. Stirred about half an hour at regular intervals and the pH was recorded by immersing the electrode in the solution. After pH determination alternatively the clear extract can be used for EC determination.

Electrical Conductivity (EC)

EC of the soil sample was determined by digital conductivity meter model no 161 E. Thus the measurement of EC can be directly related to the soluble salts concentration of the soil at any temperature.

To determine EC, 20g of soil was shaken mechanically for half an hour with 100 of distilled water in a 150ml beaker and note the reading in the meter.
Methods

Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS) were determined by same soil digital meter model no 161 E and the reading was taken directly from the display unit in mg/l.

Water Holding Capacity (WHC)

Method prescribed by U.S. Salinity Laboratory Staff (1954) was adopted for determination of WHC. The Water Holding Capacity of Storage Capacity of soil of Field Capacity is the extent to which it can hold capillary water. For determination of WHC 100 g air dried soil, was weighed. The soil was poured in a funnel which is felted with filter paper. This funnel was placed on measuring cylinder. Then 100 ml water was poured in to the funnel and the WHC of the soil was calculated by following formula.

\[
\text{WHC} \% = \frac{\text{Total volume of water} - \text{Volume of percolated water} \times 100}{\text{Total Volume of Water}}
\]

Total Organic Content (TOC)

The organic matter present in the soil is digested with excess of potassium dichromate and sulphuric acid and the residual unutilized
dichromate is then titrated with ferrous ammonium sulphate. Walkley and black rapid titration method was used for determination of TOC.

Oven dried or freeze dried soil sample was taken and passed through 0.5 mm sieve and 10g was placed at bottom of a dry 500ml conical flask. Added 10ml 1N K$_2$Cr$_2$O$_7$ solution and 20 ml conc. H$_2$SO$_4$ and mixed by gentle swirling. The flask was allowed to stand for 30 mm. Thereafter 200ml of distilled water was added. 10ml phosphoric acid followed by 1 ml of diphenylamine indicator was incorporated. The contents were titrated with 0.4 N ferrous ammonium sulphate; at the end point colour changed to brilliant green. Simultaneously, blank solution was taken with same quantity of the chemicals but without soil.

**Reagent**

1. **Potassium Dichromate Solution, 1N**

Dissolved 49.04 g of K$_2$Cr$_2$O$_7$ in distilled water to prepare 1 litre of solution.

2. **Ferrous Ammonium Sulphate 0.4 N**

Dissolved 156.86 g Fe (NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O in distilled water, by adding 14 ml conc. H$_2$SO$_4$ to prepare 1 litre of solution.
3. Diphenylamine indicator

Dissolved with care, 0.5 g diphenylamine in a mixture of 20 ml distilled water and 100 ml conc. Sulphuric acid.

4. Sulphuric Acid

\[ \text{H}_2\text{SO}_4 \text{ (sp. Gr. 1.84)} \]

5. Phosphoric acid

\[ \text{H}_3\text{PO}_4, \text{ Conc. (sp.gr.1.71)} \]

\[ \% \ C = 3.951 \times (1 - \frac{T}{S}) \]

\[ \text{g} \]

\[ \% \text{ Organic Matter} = \%C \times 1.724 \]

\[ \text{g} = \text{Weight of Sample in gm} \]

\[ \text{S} = \text{ml Ferrous Solution with Blank Titration} \]

\[ \text{T} = \text{ml of Ferrous Solution with Sample Titration} \]

Analysis of Flora

The living system better provides the correct scenario of environmental quality of the area. An extensive study like listing of present flora along with ethno-medicinal value of Jhalana Hills was conducted.
Methods

All plant specimens were collected during the maturity stage with the help of forest guards and herbarium assistant so as to ascertain the correct identification of plants and also to obtain information on their habit and habitat. Samples of plants were collected for scientific identification and herbarium preparation following standard procedures (Jain and Rao, 1977). The plants were identified with the help of deposited herbarium in University of Rajasthan, Department of Botany, Jaipur (Rajasthan) and scientific names of plant species were checked from flora and books.

Moreover, leaves of different selected plants viz Azadirachta indica (Neem), Polyalthia longifolia (Ashok), Ficus religiosa (Pipal), Bougainvillea and Thevetia peruviana (pili kaner) were also collected from randomly selected area to assess the impact of pollution on chlorophyll contents of the flora in and around the area.

Chlorophyll

The chlorophyll content of the leaves was estimated by Arnon's method. The plant material (1g fresh weight) was ground in pestle-mortar with a little sand and extracted in 80% (v.v.) aqueous acetone. The extract was centrifuged at 2000 rpm. The solution was made up to a know volume. The absorbance of the clear solution noted at 645nm and
663nm using a spectrophotometer. The amount of chlorophyll was calculated by using the following formula (Arnon 1949).

\[
\text{Total Chlorophyll (mg/g)} = \frac{20.2 \times A_{645} + 8.02 \times A_{663}}{a \times 1000 \times W} \times V
\]

\[
\text{Chlorophyll a (mg/g)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645}}{a \times 1000 \times W} \times V
\]

\[
\text{Chlorophyll b (mg/g)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663}}{a \times 1000 \times W} \times V
\]

Where

\( A \) = Absorbance at 645 and 663 mm

\( a \) = Length of light Path in the Cuvette

\( V \) = Volume of the extract in Milliliter

\( W \) = Fresh Weight of the Sample in gram