CHAPTER 3

3. MATERIALS AND METHODS

The present research work is in the field of industrial pollution, which is a current burning topic. The control of the pollution is imperative to improve the human health and to clean the environment. The prime aim of this research is to investigate the harmful pollutants in effluents, soil, trees and their treatment to reduce the pollution. The present study gives detailed information about the pollution sources and their hazardous impacts on the aquatic life, animals and human beings.

3.1 Description of sampling site

Alanganallur is situated at 10.07° North latitude, 78.05° East longitude and 203 meters elevation above the sea level. Alanganallur is a panchayat town in Madurai district in the state of Tamil Nadu, India. It is headquarters for the Alanganallur taluk and revenue block. Alanganallur is a small city in India, having about 11400 inhabitants. The world famous Jallikattu (similar to bull fight) is conducted here during the Pongal festival season, which occurs during the middle of January (usually 14th). Many people from in and around India come here during this festival to visit the Jallikattu. There is a Sugar factory located within 4 km from Alanganallur (figure 3.1.1). Agriculture is carried out in vast areas around this place. Rice, sugar cane, coconut and plantains are the major crops. Climate is moderate with no extremes. This place is well irrigated by periyar sub canal. Its meteorological conditions have yearly average, relative humidity 45%, rainfall 85.76 cm, minimum temperature is 26°C and maximum 36°C.
Figure 3.1.1 Map of Alanganallur indicating location of sampling sites.
3.2 Description of sugar industry

Industrialization is an important tool for the development of any nation. Consequently, the industrial activity has expanded so much all over the world. Today, it has become a matter of major concern in the deterioration of the environment (Tiwari et al, 1993). With the rapid growth of industries (sugar, paper, tannery, textile, sago and dye industries) in the country, pollution of natural water by industrial waste water has increased tremendously (Amathussalam et al, 2002).

Among them, sugar industry plays a major role in producing a higher amount of water pollution because they contain large quantities of chemical elements. The sugar industry is the second largest agro processing industry after textile in India. A typical sugar industry produces a large amount of by-products such as bagasse (the fibre residue of sugar cane), press mud (filter cake), molasses and distillery spent wash. Press mud contains about 70% organic matter and 29% minerals.

The National Co-op sugar mill is one of the old and big industries of this region. It is located about 18 kms from Madurai city (figure 3.2.1). The effluent mainly disturbs soil micro and macronutrients like Nitrogen, Phosphorus, Potassium, Copper, Zinc, Iron and magnesium which are very much important for green revolution. In case of contaminated soils they are having lower and higher amounts of nutrients which are unsuitable for plant cultivation. The physico-chemical analysis of sugar mill effluent affected soil revealed the presence of higher amount of minerals, toxic pollutants and soil organic matter (Baskaran et al, 2009).

The sugar mill effluent contain higher amounts of total hardness, total dissolved solids, biochemical oxygen demand and chemical oxygen. The effluent not only affects the plant growth
Figure 3.2.1 Sugar industry at Alanganallur
but also deteriorate the soil properties when used for irrigation (Maliwal et al, 2004). In addition to that, some traceable amount of heavy metals such as Zinc, Copper and lead were also present in the effluent (Borale et al, 2004).

Therefore it is essential to evaluate the impact of sugar mill effluent on the soil quality in the surrounding areas. The present study was undertaken to analyze the effects of effluents on soil and to carry out the correlation coefficient and regression analysis of soil physico-chemical parameters.

3.3 General aspects of sampling

The objective of sampling is to obtain a sample that is large enough to represent the whole bulk of material but, small enough to be transported easily to the lab. If a sample does not accurately represent the bulk from which it is drawn, the analysis however carefully conducted is bound to yield inaccurate results. The sampling techniques are generally different for liquid and solid samples (Vogel, 1989).

3.4 Soil analysis

3.4.1 Aim and Scope

The problem of environmental pollution on account of essential industrial growth is due to the problem of disposal of industrial waste whether solid, liquid or gaseous products. Polluted water, in addition to other effects directly affects soil not only in industrial areas but also in agricultural fields and river beds, thereby creating secondary source of pollution (Kisku et al., 2000, Barman et al., 2000). Various industries have been continuously adding a lot of waste water containing high level of nutrients, heavy metals and hazardous substances to the cultivable land
(Chandra et al., 2004, Malla and Mohanty, 2005, Malaviya and Rathore, 2007). These effluents not only increase the nutrient level but also exceed tolerance limits and cause toxicity (Mishra et al., 1999).

The effluent from sugar industry mainly disturbs soil micro and macronutrients like Nitrogen, Phosphorus, Potassium, Copper, Zinc, Iron and magnesium which are very much important for green revolution. In case of contaminated soils they are having lower and higher amounts of nutrients which are unsuitable for plant cultivation. The physico-chemical analysis of sugar mill effluent affected soil revealed the presence of higher amount of minerals, toxic pollutants and soil organic matter (Baskaran et al., 2009). In this regard, research work was initiated to assess the effect of sugar industry effluent on soil spread over an area of radius of 2 Km from the industry. The soil physico-chemical parameters such as pH, electrical conductivity, Nitrogen, Phosphorus, Potassium, Iron, Manganese, Zinc and Copper are monitored at residential and three polluted sites and at two different depths respectively.

3.4.2 Motivation

The sugar mill effluent contain higher amounts of total hardness, total dissolved solids, biological oxygen demand and chemical oxygen. The effluent not only affects the plant growth but also deteriorate the soil properties when used for irrigation (Maliwal et al., 2004). In addition to that, some traceable amount of heavy metals such as Zinc, Copper and lead were also present in the effluent (Borale and Patil, 2004). Therefore it is essential to evaluate the impact of sugar mill effluent on the soil quality in the surrounding areas.
3.4.3 Research Objectives

A research work is to be initiated to assess the effect of sugar industry effluent on soil spread over an area of radius of 2 Km from the mill and also at two different seasons.

i) The soil physico-chemical parameters such as pH, electrical conductivity, Nitrogen, Phosphorus, Potassium, Iron, Manganese, Copper and Zinc are monitored at residential and three polluted sites (site 1- between 0.5 and 1.5 Km from the sugar mill, Mettuppatti area- site 2 and 3 respectively) and at two depths (surface soil of depth 0-10 cm and sub-surface soil of depth 10-20 cm) and the results are statistically analyzed. The emphasis was given more to the heavy metal content in the soil horizons. Hence the heavy metal concentrations were determined at depths 0 cm (A), 5 cm (B) and 10 cm (C) respectively.

ii) The metal accumulating tree species will be recommended to reduce heavy metal pollution near sugar mill.

3.4.4 Work Plan

To study pollution induced in the soil of the industrial site by the effluents, the salient points were considered,

i) Extensive literature survey was carried out in order to update the current status of the problem in India.

ii) Survey of the selected industrial site was carried out to build a database regarding the relevant particulars of industry under investigation and the ecology of the vicinity of the industrial areas.
iii) The procedures were optimized for the application of Atomic Absorption Spectrometer (AAS).

iv) The contamination of the soil is to be assessed on the basis of geoaccumulation index, contamination factor, degree of contamination, metal contamination index and pollution load index.

v) Normalized scatter coefficient, Enrichment factor, principal component analysis (PCA) and cluster analysis studies will be carried out to analyze the heavy metals present in the soil.

vi) The results have been represented using Heavy Metal Index, Average of pollution index, vector modulus of pollution index, Nemerow pollution index, Pollution Load index, Metal pollution index, metal enrichment index and Potential ecological risk index.

3.4.5 Working Strategy

The proposed work has been performed with the help of available/ existing facilities such as Atomic Absorption Spectrometer, Hot cell and Analytical facilities (pH meter, Conductivity meter, TDS meter, and Turbidity meter).

3.4.6 Sampling

The soil samples collected should be representative of the area to which they belong. The method of soil sampling used and the amount of soil to be taken mainly depends on the kind of sample, the time available and the nature of the soil (Radojevic and Baskkin, 1999).
The soil samples were collected from two different depths A (0-10 cm), and B (10-20 cm) for a period between October 2010 and March 2011 (winter and summer) and soil physico-chemical parameters such as pH, electrical conductivity, Nitrogen, Phosphorus, Potassium, Iron, Manganese, Zinc and Copper were monitored.

In the present study, stratified regular sampling method was adopted for soil sample collection as in geo-assessment of the variables estimated; the stratified regular sampling is more suitable because this kind of sampling draws homogenous error (Burges and Webster, 1980; Burges et al., 1981). For this purpose the grid map of the study area has been used to know the distribution of heavy metal concentration in the whole region by stratifying the region into a regular-sized grid cells, each grid cell is further divided into many smaller subcells for a period between October 2010 and March 2011 (winter and summer). Five sampling points in a grid of 0.5×1 km at each sampling station (4 at the corners and one at the centre of the grid) were selected and composite samples consisting of three sub-samples were collected from the top (0 to 10 cm) layer of the soil using plastic spatula after removing the debris, rock pieces and physical contaminants. In order to have the background concentration values of the heavy metal elements, three soil samples were collected, each from 100 cm below ground level, which are least affected by the sugar mill. The samples were placed in the clean polythene bags, which were brought to the laboratory.

3.4.7 Sample Preservation

Sample preservation techniques such as addition of chemicals, pH control (for metal analysis the pH of the samples should be less than 2) and refrigeration (temperature up to 4° C), were utilized. These steps were significant to attain the following targets, i) To minimize the
biological activities, ii) To retard hydrolysis of chemical compounds and complexes, iii) To reduce the volatility of the constituents, iv) To stabilize the desired parameters for long period before analysis.

3.4.8 Sample treatment and analysis

In the present study, samples were brought to the laboratory where they were air dried and mixed thoroughly to obtain the representative samples. Soon after drying the debris and other objects were hand picked up and the sample was ground in a mortar to break up the aggregates or lumps, taking care not to break actual soil particles. Soil samples were then passed through a 2 mm sieve in order to collect granulometric fraction. Since trace metals are often found mainly in clay and silt fractions of soil and hence the size fraction <63 μm is most commonly in the recommended size. For this purpose the granulometric fraction was added with the dispersing agent and after shaking the sand fraction was separated from the clay and silt with <63 μm sieve (wet sieving) and was used to measure the concentration of the heavy metals Fe, Mn, Zn and Cu from all the samples collected.

Figure 3.4.6.1 Soil sampling in the industrial area
For this purpose the clay and silt fraction were digested by acids to get the solution by taking 5 g of sample into a 300 ml polypropylene wide-mouthed jar and distilled water was added to make a total 200 ml. Then it was acidified with 10 ml HF, 5 ml HClO₄, 2.5 ml HCl and 2.5 ml HNO₃ in order to completely digest the soil. This jar was shaken on an orbital shaker for 16 hours at 200-220 rpm before being filtered through whatman filter paper (No. 42) into acid washed bottles. The solution was stored and heavy metal contents were analyzed by Atomic Absorption Spectrophotometer as per the method recommended by Committee of Soil Standard Methods for Analyses and Measurement (1986).

3.4.9 Determination of pH and electrical conductivity

The pH of all samples was measured by using a portable, dual powered pH meter, HANNA Membrane pH meter, model HI 8314, which was pre-calibrated by using standard buffer solutions of pH 4.0, 7.0 and 9.0. For the determination of pH values, 20 g of fresh soil was taken in a clean and dry 100 ml beaker. Then 50 ml distilled water was added to the beaker and were thoroughly stirred with a glass rod for half an hour. pH of the suspension was determined (Gupta and Rorison, 1974). After each measurement, the electrode of the pH meter was washed with distilled water and cleaned with a piece of tissue paper.

The electrical conductivity of the effluent samples was measured in situ using pre-calibrated HANNA conductivity meter model HI 8314. Before each measurement, the conductivity cell was washed several times with the waste liquid under test and the measurement was taken at ambient temperature following the procedural instructions given by the manufacturer. The soil was mixed with deionized water at a soil: water ratio of 1:5.
conductivity of the soil samples was determined using the water-extract of the soil samples (Osaigbovo et al., 2006).

3.4.10 Determination of Nitrogen by Kjeldahl method

10 g of soil (which has been passed through a 20 mesh sieve) was weighed and transferred into an 800 ml Kjeldahl flask. 50 ml of sulphuric-salicylic acid mixture was added to the flask and swirled to bring the sample quickly into intimate contact. It was allowed to stand overnight. 5 g of sodium thiosulphate was added and heated gently for about 5 minutes to avoid frothing. The flask was cooled and 10 g of the sulphate mixture was added. It was digested on the Kjeldahl apparatus gradually raising the temperature until the digest becomes clear. It was cooled and 300 ml of distilled water was added and mixed. 100 ml of concentrated sodium hydroxide was added slowly by letting it run down the neck and settle in the bottom of the flask. Large piece of mossy zinc and a spoon of glass beads were added. The flask was connected to the distillation unit, shaked by swirling, heated and distilled into an Erlenmeyer flask containing 50 ml of 4 percent boric acid solution. 10 drops of bromocresol green-methyl red indicator was added and titrated with 0.05 M standard sulphuric acid solution to the first faint pink. Blank prepared in the same manner was titrated but without adding a soil sample (Bremer and Mulvaney, 1982).

\[
\text{Kjeldahl N} = \frac{(T - B) \times M \times 2.8}{S}
\]

T = ml of standard acid with sample titration, B = ml of standard acid with blank titration , M = molarity of sulphuric acid, S = weight of soil sample in g.
3.4.11 Determination of Phosphorus by Olsen’s method

5.0 g soil sample was weighed in 250 ml Erlenmeyer flask. 100 ml of NaHCO₃ was added. It was kept in a mechanical shaker for 30 minutes. The suspension was filtered through Whatman No. 40 filter paper into clean and dry 125 ml Erlenmeyer flask. First 5 to 10 ml of filtrate was discarded as it was turbid. 10 ml of filtrate was transferred to a 50 ml volumetric flask; acidified to pH 5 by adding 1ml of 2.5 M H₂SO₄. It was swirled carefully in the beginning, then vigorously to remove residual carbonates. It was made up to 40 ml with distilled water and 8 ml of the ammonium molybdate – ascorbic acid solution was added. The volume was made to 50 ml, mixed well and let stand for 10 minutes. The absorbance was read at 882 nm on the spectrophotometer. The colour is stable for 24 hours and maximum intensity is obtained in 10 minutes. P concentration of the sample was determined from a calibration curve relating the readings of absorption units to concentration in µg P/ml. For the preparation of the standard curve, 0, 2, 5, 10, 15 and 20 ml of 5 µg P/ml standard stock solution was added to a series of labelled 50 ml volumetric flasks followed by 10 ml of the NaHCO₃ extracting solution, 1 ml of 2.5 M H₂SO₄ (Bray and Kurtz, 1945).

\[
P \, \mu g/g \, \text{of the soil} = \frac{P \, \mu g/ml \times 50 \, ml \times 100 \, ml}{10 \, ml \times 5 \, g \, \text{soil}}
\]

3.4.12 Determination of Potassium

5 g of soil was weighed accurately and transferred into a 50 ml centrifuge tube. 20 ml of 1.0 M ammonium acetate solution was added to the tube; stoppered and kept in a reciprocal shaker for 5 minutes. Centrifuged at 2000 rpm for 5 minutes or until the supernatant is clear. The
supernatant was decanted into a 100 ml volumetric flask and the steps were repeated three more times. The supernatant was made up to 100 ml by adding ammonium acetate solution. A series of working K standard solutions was prepared in the range of 0 – 2 meq/l of K from stock solution of 0.02 M KCl already prepared. For better results, LiCl was added in each standard to yield a final concentration of about 5 meq/l of LiCl. K concentration in the extract was determined by flame photometer (Gupta and Rorison, 1974).

\[
\text{meq of K/100 g soil} = \frac{\text{Reading (meq/l) \times 100 ml \times 100 g}}{1000 \text{ ml Wt. of soil (g)}}
\]

\[
= \frac{R \times 10}{\text{Wt. of soil (g)}}
\]

3.4.13 Metal Analysis

3.4.13.1 Quantification of Atomic Absorption Spectrophotometer

The standard calibration method was adopted for quantification of results. Standard solutions were prepared in appropriate range (1-6 ppm) and their absorptions were recorded on the instrument. New calibration line was drawn every time before running the samples on AAS system. Triplicate samples were run to ensure the precision of quantitative results. The blanks prepared under identical analytical conditions but without the sample were routinely used to estimate the background reagent metal levels. Standard analytical conditions established for AAS analysis on Shimadzu Atomic Absorption Spectrophotometer (Model AA-2380) (figure 3.4.13.1) was given in table 3.4.13.1.
Table 3.4.13.1 Standard analytical conditions established for A.A.S analysis on Shimadzu Atomic Absorption Spectrophotometer (Model AA-2380)

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>HC Lamp Current (mA)</th>
<th>Slit width (nm)</th>
<th>Type of flame</th>
<th>Fuel flow rate (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>12.5</td>
<td>0.2</td>
<td>Air-C₂H₂</td>
<td>2.3</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>5</td>
<td>0.4</td>
<td>Air-C₂H₂</td>
<td>1.9</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>4</td>
<td>0.5</td>
<td>Air-C₂H₂</td>
<td>7.5</td>
</tr>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>3</td>
<td>0.5</td>
<td>Air-C₂H₂</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Figure 3.4.13.1 Illustration of Atomic Absorption Spectroscopy
3.4.14 Statistical Analysis

Mean value of the parameters are calculated to find out a typical representative of all the observations of a parameter. The mean value of parameter is given by Trivedy and Goel (1986). Statistical software SPSS (Statistical package for Social Sciences, Version 7.5) was used to compute the correlation (r values) for all possible correlations among soil parameters. The software is used to calculate the regression parameters a and b of the straight line $Y = a + bX$ by applying the well-known method of least squares (Gupta, 1974, Wonacott and Wonacott, 1981) to fit the experimental data to give straight line.

3.4.15 Index of geoaccumulation ($I_{\text{geo}}$)

A common approach to estimate the enrichment of metal concentrations above background or baseline concentrations is to calculate the geoaccumulation index ($I_{\text{geo}}$) (Muller, 1969). The method assesses the degree of metal pollution in terms of seven enrichment classes based on the increasing numerical values of the index. Index of geoaccumulation ($I_{\text{geo}}$) as proposed by Muller (1979) has also been widely used to evaluate the degree of metal contamination in terrestrial, aquatic as well as marine environments (Sahu and Bhosale, 1991; Sutherland, 2000). It is expressed as

$$I_{\text{geo}} = \log_2 \frac{C_n}{1.5 B_n}$$

Where $C_n$ is the concentration of the element in the enriched samples, and the $B_n$ is the background or pristine value of the element. The factor 1.5 is introduced to minimize the effect of possible variations in the background values which may be attributed to lithologic variations in the sediments (Stoffers et al. 1986). Therefore, if the concentration of element in a sample be
five times greater than the concentration of it in the background the sample is extremely polluted. Muller proposed the following descriptive classes for increasing $I_{\text{geo}}$ values in table 3.4.15.1. However, $I_{\text{geo}}$ of 6 is said to be indicative of 100-fold enrichment of a metal with respect to the background value.

**Table 3.4.15.1 Classes of the geoaccumulation index ($I_{\text{geo}}$)**

<table>
<thead>
<tr>
<th>Geoaccumulation index ($I_{\text{geo}}$)</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{geo}} \leq 0$</td>
<td>Practically uncontaminated</td>
</tr>
<tr>
<td>$0 &lt; I_{\text{geo}} &lt; 1$</td>
<td>Uncontaminated to moderately contaminated</td>
</tr>
<tr>
<td>$1 &lt; I_{\text{geo}} &lt; 2$</td>
<td>Moderately contaminated</td>
</tr>
<tr>
<td>$2 &lt; I_{\text{geo}} &lt; 3$</td>
<td>Moderately to heavily contaminated</td>
</tr>
<tr>
<td>$3 &lt; I_{\text{geo}} &lt; 4$</td>
<td>Heavily contaminated</td>
</tr>
<tr>
<td>$4 &lt; I_{\text{geo}} &lt; 5$</td>
<td>Heavily to extremely contaminated</td>
</tr>
</tbody>
</table>

3.4.16 Anthropogenic contamination factor (CF) and degree of contamination ($C_{\text{deg}}$)

These parameters quantify the degree of contamination as single-metal index (CF) and as overall degree of contamination ($C_{\text{deg}}$). The measure is relative to either average crustal composition of the respective metal or to a measured background value from a geologically pristine/uncontaminated area.

$$\text{CF} = \frac{C_m}{B_m}$$

$$C_{\text{deg}} = \sum \left( \frac{C_m}{B_m} \right)_i$$
Where \( i \) represents the respective metals (that is Fe, Mn, Zn and Cu), \( C_m \) is the measured concentration in soil while \( B_m \) is the background (adjacent forest) concentration value of metal (m) within the area of study. For the \( C_{deg} \), Hakanson recognized four descriptive classes (Hakanson, 1980) for contamination factor and degree of contamination which are tabulated in table 3.4.16.1 and 3.4.16.2.

**Table 3.4.16.1 Categories of contamination factor**

<table>
<thead>
<tr>
<th>Contamination factor</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>( CF &lt; 1 )</td>
<td>Low contamination factor indicating low contamination</td>
</tr>
<tr>
<td>( 1 &lt; CF &lt; 3 )</td>
<td>Moderate contamination factor</td>
</tr>
<tr>
<td>( 3 &lt; CF &lt; 6 )</td>
<td>Considerable contamination factor</td>
</tr>
<tr>
<td>( 6 &lt; CF )</td>
<td>Very high contamination factor</td>
</tr>
</tbody>
</table>

**Table 3.4.16.2 Degree of contamination**

<table>
<thead>
<tr>
<th>Degree of contamination</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{deg} &lt; 8 )</td>
<td>Low degree of contamination</td>
</tr>
<tr>
<td>( 81 &lt; C_{deg} &lt; 16 )</td>
<td>Moderate degree of contamination</td>
</tr>
<tr>
<td>( 16 &lt; C_{deg} &lt; 32 )</td>
<td>Considerable degree of contamination</td>
</tr>
<tr>
<td>( 32 &lt; C_{deg} )</td>
<td>Very high degree of contamination</td>
</tr>
</tbody>
</table>
**3.4.17 Element contamination index (ECI) and metal contamination index (MCI)**

Element contamination index (ECI) and overall metal contamination index (MCI) are expressions of single metal contamination within a sample or combined metal contamination for a sample relative to the background values of the respective metal and are expressed as:

\[
ECI = \left( \frac{C_m - B_m}{B_m} \right)
\]

\[
MCI = \sum \left( \frac{C_m - B_m}{B_m} \right)_i
\]

Where, \(i\), \(C_m\) and \(B_m\) are as defined above. According to Meybeck et al (2004), MCI was designed to describe general trace elements contamination on a scale shown in table 3.4.17.1.

**Table 3.4.17.1 Categories of Metal contamination index**

<table>
<thead>
<tr>
<th>Metal contamination index</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCI &lt; 5</td>
<td>Very low contamination</td>
</tr>
<tr>
<td>25 &lt; MCI &lt; 50</td>
<td>High contamination</td>
</tr>
<tr>
<td>50 &lt; MCI &lt; 100</td>
<td>Very high contamination</td>
</tr>
<tr>
<td>MCI &gt; 100</td>
<td>Extremely high contamination</td>
</tr>
</tbody>
</table>

**3.4.18 Pollution load index (PLI)**

Pollution load index for a particular site has been evaluated following the method proposed by Tomilson et al. (1980). This parameter is expressed as

\[
PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{1/n}
\]

Where \(n\) is the number of metals and \(CF\) is the contamination factor. The categories of contamination described using pollution load index were given in table 3.4.18.1.
Table 3.4.18.1 Categories of Pollution Load Index (PLI)

<table>
<thead>
<tr>
<th>Pollution Load Index</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLI = 0</td>
<td>Perfection</td>
</tr>
<tr>
<td>PLI = 1</td>
<td>Baseline level of pollutants</td>
</tr>
<tr>
<td>PLI &gt; 1</td>
<td>Progressive deterioration of the site</td>
</tr>
</tbody>
</table>

3.4.19 Enrichment factor

A common approach for estimating the anthropogenic impact on sediments is to calculate a normalized enrichment factor (EF) for metal concentrations above uncontaminated background levels (Salomons and Forstner, 1984; Dickinson et al., 1996; Hornung et al., 1989; Hernandez et al., 2003). The EF method normalizes the measured heavy metal, trace elements, rare earth elements and actinides content with respect to a sample reference metal such as Fe, Sc or Al (Ravichandran et al., 1995). The Enrichment Factor (EF) is a ratio of the concentrations of the heavy metals in the soil samples to the corresponding concentration of natural local background concentration. EF is calculated with the help of the formula given by Subramanian et al (1998).

\[
EF = \frac{\text{Value of a given metal concentration found on soil (ppm)}}{\text{Natural local background concentration of the metal (ppm)}}
\]

Five contamination categories are recognized on the basis of the enrichment factor (Sutherland, 2000) which is tabulated in table 3.4.19.1.
Table 3.4.19.1 Categories of enrichment factor

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Enrichment factor</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 2</td>
<td>Depletion to minimal enrichment</td>
</tr>
<tr>
<td>2</td>
<td>2 – 5</td>
<td>Moderate enrichment</td>
</tr>
<tr>
<td>3</td>
<td>5 – 20</td>
<td>Significant enrichment</td>
</tr>
<tr>
<td>4</td>
<td>20 – 40</td>
<td>Very high enrichment</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 40</td>
<td>Extremely high enrichment</td>
</tr>
</tbody>
</table>

3.4.20 Normalized Scatter Coefficient (NSC)

Normalized scatter coefficient (NSC) has been calculated to assess the temporal variability of the heavy metals in the soils. It helps us to understand the increasing or decreasing concentration of heavy metals in the soils with the passage of time which is independent of the past focusing only at the period of study. The NSC was calculated with the following formula (Sayadi and Sayyed, 2011).

\[
\text{NSC} = \frac{\text{Concentration in the last sampling} - \text{Concentration in first sampling}}{\text{Concentration in the last sampling} + \text{Concentration in first sampling}} \times 100
\]

The NSC values +100% indicates absolute increase while -100% means absolute decrease. The value of 0% can be regarded for no change in the parameters under consideration.

3.4.21 Heavy Metal Index (HMI)

Soil analysis was carried out in and around sugar mill at nine different sampling sites which are categorized as control site (S2, S6, S7, S8 and S9) and polluted sites (S1, S3, S4 and
S5. Sites S2, S6, S7, S8 and S9 were far away from sugar mill and sites S1, S3, S4 and S5 were near sugar mill. For the soil, Heavy Metal Pollution Index (HMI) was determined, which is the mathematical function for indicating total heavy metal pollution of the site, according to the summation equation proposed by Herzig (1993).

\[
\text{Heavy Metal Index} = \sum_{i=1}^{n} \text{LC}_i
\]

where \( \text{LC}_i \) represents load class that is the load category value of the \( i^{th} \) heavy metal and ‘n’ is the number of heavy metals used for computing HMI.

The numerical load class is hard to pick up at a glance and hence converted into dots representing proportionally increasing load representative supply (LRS). The LRS values for each metal were obtained by dividing their range values into six equidistant categories. This graphic conversion of heavy metal pollution into a pictograph enables a quick overview and allows simple direct comparisons of elements amongst the study site and background values. Similarly, a six-level verbal evaluation called the “predicate” of the load representative supply (LRSP) is used to facilitate the verbal evaluation. Furthermore, HMI is divided into six equidistant load categories and provided with pictographs and predicates which assist in the characterization of study sites from least to the heaviest pollution levels (table 3.4.21.1).
Table 3.4.21.1 Heavy Metal Index (HMI) categories, pictographs and predicates

<table>
<thead>
<tr>
<th>HMI load categories</th>
<th>Pictographs</th>
<th>Predicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7.0</td>
<td>.</td>
<td>Very low</td>
</tr>
<tr>
<td>7.01-14.00</td>
<td>•</td>
<td>Low</td>
</tr>
<tr>
<td>14.01-21.00</td>
<td>◆</td>
<td>Medium</td>
</tr>
<tr>
<td>21.01-28.00</td>
<td>◆</td>
<td>High</td>
</tr>
<tr>
<td>28.01-35.00</td>
<td>◆</td>
<td>Very high</td>
</tr>
<tr>
<td>&gt; 35.0</td>
<td>◆</td>
<td>Critically high</td>
</tr>
</tbody>
</table>

3.4.22 Pollution Indices

Caeiro et al., (2005) analyzed the pollution indices to assess heavy metal contamination and classified them into two types: (i) contamination indices and (ii) ecological risk indices.

3.4.22.1 Integrated indices

Integrated indices are indicators used to calculate more than one metal contamination, which were based on the single indices. Each kind of integrated index might be composed by the above single indices separately. According to algorithm, eight integrated methods were illustrated as following.

3.4.22.2 Average of Pollution index

An average of pollution index \( \text{PI}_{\text{Avg}} \) can be defined as

\[
\text{PI}_{\text{Avg}} = \frac{1}{m} \sum_{i=1}^{m} P_i
\]
where \( P_i \) is the single pollution index of heavy metal \( i \), and \( m \) is the count of the heavy metal species. This kind of pollution index was used by Bhattacharya et al., (2006). A \( P_{\text{Avg}} \) value of \( >1.0 \) indicates low quality soil because of contamination.

### 3.4.22.3 Vector modulus of pollution index

A vector modulus of pollution index (\( P_{\text{vectorM}} \)) can be defined as

\[
P_{\text{vectorM}} = \left( \frac{1}{m} \sum_{i=1}^{m} P_i^2 \right)^{\frac{1}{2}}
\]

where \( P_i \) is the single pollution index of heavy metal \( i \) and \( m \) is the count of the heavy metal species.

### 3.4.22.4 Nemerow pollution index

A Nemerow pollution index (\( P_{\text{Nemerow}} \)) was applied to assess the quality of soil environment widely (Cheng et al., 2007) and was defined as

\[
P_{\text{Nemerow}} = \left( \frac{1}{m} \sum_{i=1}^{m} P_i^2 + P_{\text{imax}}^2 \right)^{\frac{1}{2}}
\]

where \( P_i \) is the single pollution index of heavy metal \( i \); \( P_{\text{imax}} \) is the maximum value of the single pollution indices of all heavy metals, and \( m \) is the count of the heavy metal species. The quality of soil environment was classified into five grades from Nemerow pollution index: \( P_{\text{Nemerow}} < 0.7 \), safety domain; \( 0.7 \leq P_{\text{Nemerow}} < 1.0 \), precaution domain; \( 1.0 \leq P_{\text{Nemerow}} < 2.0 \), slightly polluted domain; \( 2.0 \leq P_{\text{Nemerow}} < 3.0 \), moderately polluted domain; and \( P_{\text{Nemerow}} > 3.0 \), seriously polluted domain by Cheng et al., (2007).
3.4.22.5 Pollution index

Johansson and Johnsson (1976) and Ott (1978) developed pollution index (contamination index) which was given by the formula

\[
\text{PI} = \sum_{i=1}^{n} W_i C_i
\]

where \( W_i \) is the weight for pollution variable \( i \); \( C_i \) the highest concentration of pollution variable \( i \) reported in a location of interest. For each pollutant \( i \), the weight was based on the reciprocal of the median of observed concentrations.

This index allows the identification of priority contaminations sites for implementation of decontamination action. It requires several measurements in the same sampling location. No threshold classification from unpolluted to high pollution.

3.4.22.6 Pollution Load index

Wilson and Jeffrey (1987) framed the pollution load index (ecological risk index) as follows:

\[
\text{PLI} = \text{antilog}_{10} \left( \frac{1 - C - B}{T - B} \right)
\]

where \( B \) is the baseline value—not contaminated; \( T \) the threshold, minimum concentrations associated with degradation or changes in the quality of the estuarine system. Wilson and Jeffrey (1987) defined \( B \) and \( T \) for the different contaminants; \( C \) the concentration of the pollutant. For each place the PLI calculation takes into account all the \( n \) contaminants:
\[ PLI = (PLI_1, PLI_2, \ldots, PLI_n)^{1/n} \]

Varies from 10 (unpolluted) to 0 (highly polluted).

**3.4.22.7 Metal pollution index**

Metal pollution index was given by Usero et al., (1996) which was categorized as contamination index.

\[ MPI = (M_1, M_2, \ldots, M_n)^{1/n} \]

where \( M_n \) is the concentration of metal \( n \) expressed in mg/kg of dry weight.

**3.4.22.8 Metal enrichment index**

Riba et al., (2002) developed the metal enrichment index (contamination index) as follows:

\[ MEI = \frac{C_i - C_0}{C_0} \]

where \( C_i \) is the total concentration of each metal \( i \); \( C_0 \) the heavy metal background level established for the ecosystem studied.

**3.4.22.9 Potential ecological risk index**

Potential ecological risk index (ecological risk index) was framed by Riba et al., (2002a) as given below:

\[ ERF = \frac{C_i - C_{SQV}}{C_{SQV}} \]
where $C_i$ is the total concentration of each metal $i$ measured; $C_{SQV}$ the highest concentration of the heavy metal non-associated with biological effects (chemical concentration associated with adverse effects); polluted stations have values equal to or greater than 1.

Five classes of terminologies were suitable to describe the degree of contamination. The five classes of contamination degrees and their terminologies for soils were tabulated in Table 3.4.22.9.1.

Table 3.4.22.9.1 Terminologies for contamination classes on integrated indices

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Values</th>
<th>Contamination classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Unpolluted</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Low polluted</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Moderately polluted</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Strongly polluted</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Extremely polluted</td>
</tr>
</tbody>
</table>

When the $PI_{Avg}$ and $PI_{vector M}$ are used with the $\Sigma w_i=1$ condition, terminologies can also be used like single indices. $\Sigma Tr$ or $\Pi Tr$ would be used for the integrated indices based on the single index.

3.4.22.10 Index comparison

Contamination indices measure the contamination or enrichment levels and ecological risk indices; evaluate the potential for observing adverse biological effects. Each index was scored from 1 (lowest performance) to 3 (highest performance) for every criterion presented.
above, and a total performance score was summarized for all the indexes used (table 3.4.22.10.1).

**Table 3.4.22.10.1 Score of the metal assessment indices, based on several criteria**

<table>
<thead>
<tr>
<th></th>
<th>Contamination indices</th>
<th>Ecological risk indice (PLI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPI</td>
<td>I</td>
</tr>
<tr>
<td>Simplicity</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Representative</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Credibility</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Comparability</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity and robustness</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Acceptable levels of uncertainty</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>

In each management unit the indices were calculated using the median values of chemical concentration in all the locations belonging to each management area. This mode was also used where the index was nominal. These measures of the central tendency were used instead of an arithmetic mean as the objective of the analysis is to show the main trend in the index values for each management area. Moreover, the arithmetic mean should only be used for normal distributions and should not be used in the present of outliers (Wheater and Cook, 2002).

For MPI and PLI a geometric increment was employed which was divided into four classes. MPI used a classification from clean to highly contaminated (as it is only a contamination index); for PLI a classification from unimpacted to highly polluted was given, according to the index author’s classification.
In an overall comparison of the contamination and ecological risk indices the MPI and PLI indices have the highest performance scores, according to the indicator criteria MPI due to its simplicity and PLI due to its simplicity, representative, comparability, sensitivity and robustness. It has the lowest performance score since it needs reference site values. It may give imprecise values because of the undue influence of one of the measurements used in the final composite values (DelValls et al., 1998). It has no threshold for maximum pollution and does not allow comparison between ecosystems.

3.4.23 Overview of Principal Component Analysis (PCA)

Principal component analysis (PCA) can be considered as the mother of all methods in multivariate data analysis. The aim of PCA is dimension reduction and PCA is the most frequently applied method for computing linear latent variables (components). PCA can be seen as a method to compute a new coordinate system formed by the latent variables, which is orthogonal, and where only the most informative dimensions are used. Latent variables from PCA optimally represent the distances between the objects in the high-dimensional variable space. Here the distance of objects is considered as an inverse similarity of the objects. PCA considers all variables and accommodates the total data structure; it is a method for exploratory data analysis (unsupervised learning) and can be applied to practical any X-matrix; no y-data (properties) are considered and therefore not necessary (Varmuza and Filzmoser 2008).

In the present study, estimates are obtained for the initial factors from principal component analysis (PCA). PCA, a type of multivariate analysis, has been widely employed in soil and sediment pollution studies. The most commonly PCA type producing more interpretable components is the varimax rotation, which is applied in the current study. PCA enables a
reduction in data and description of a given multidimensional system by means of a small number of new variables.

PCA with varimax normalized rotation was applied to data set. The varimax rotation is the most commonly used rotational strategy and maximizes the sum of these variances for all the factors. The aim of rotational algorithms is to become clear pattern of loadings, that is, factors that are clearly marked by high loadings for some variables and low loadings for others. In this respect, loadings > 0.71 are typically regarded as excellent and < 0.32 very poor.

3.4.24 Cluster Analysis

In contrast to principal component analysis, cluster analysis does not reduce the number of characters, but stepwise reduces the number of objects by placing them into groups. An agglomerative clustering method starts with as many clusters as there are objects, and then sequentially joins objects (or clusters), on the basis of their similarity, to form new clusters. This process continues until one big cluster is obtained that contains all objects. The result of this process is usually depicted as a dendrogram, in which the sequential union of clusters, together with the similarity value leading to this union, is depicted.

Component analysis was applied to data set using the Ward's method combined with 1-Pearson r for clustering of the metals. Ward's method is distinct from all the other methods because an analysis of variance approach is used to evaluate the distances between clusters. The objective of cluster analysis is to group objects into clusters such that objects within one cluster share more in common with one another than they do with the objects of other clusters. Component analysis was also applied to evaluate similarity of sampling stations with respect to
metal concentrations in street dust. Euclidian distance was calculated as measures of similarity and the single linkage was used to link clusters.

3.5 Effluent Analysis

3.5.1 Aim and Scope

There are about 369 sugar factories located in India (Manohar Rao, 1987). During 1991-92, 134 million tonne of cane was crushed by 392 sugar factories out of 239 million tonne of cane produced from an area of 3.8 million hectare in India (Anonymous, 1993). During sugar production, more than 4.02 million tonne press mud was produced in 1991-92 (Jambhekar, 1992) and the annual production of press mud was estimated to be five million tonne. This will create environmental pollution to the environment.

There are thirty eight sugar mills in Tamil Nadu of which sixteen mills are in cooperative sector, three mills are in public sector and nineteen mills are in the private sector (Bakkialalakshmi and Vinodhini, 2008). Sugar industry offers employment potential and contributes substantially to economic development. Apart from sugar and alcohol, these factories generate many by-products and waste materials. For example, large amount of organic and inorganic chemicals are being generated (Rajukkannu and Manickam, 1997). The discharge of effluent will create pollution to the environment. Therefore it is essential to evaluate the impact of sugar mill effluent on the surrounding areas. The present study was undertaken to analyze the effects of effluent.
3.5.2 Motivation

The studies of Dasarath et al (2005) on effluents generated from Nizam Deccan Sugar factory at Bodhan, Nizamabad, District of Andhra Pradesh, India revealed that electrical conductivity found in between 1557-13050 µmhos/cm and higher BOD values. Studies of Nomulwar et al (2005) on the sugar factory effluents revealed that most of the parameters such as color, odor, total dissolved solids, chemical oxygen demand, total alkalinity, pH, temperature, phosphate and sulphate have exceeded ISI limits. The effluents contain high amount of total hardness, total dissolved solids, biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

3.5.3 Research Objectives

Following are the three main objectives for the study of industrial effluents with reference to the pollution, i) Identification of the pollution sources, ii) Estimation of the industrial toxic levels and iii) Treatment of the industrial effluents.

3.5.4 Work Plan

To study pollution induced in and around the sugar industry by the effluents, the salient points were considered,

i) Extensive literature survey was carried out in order to update the current status of pollution problem posed by sugar industry in India.

ii) Effluent from sugar industry was collected for the determination of toxic inorganic elements contaminating the ecosystem of the industrial cities.
iii) The parameters like Sodium Absorption Ratio (SAR), Kelleys Ratio (KR), Percent Sodium (PS), Magnesium Ratio (MR) and Residual Sodium Carbonate (RSC) were calculated.

iv) Wilcox ratio to represent the effluent quality was calculated for different months.


vi) Statistical analysis was carried out.

vii) On the basis of the data obtained and its interpretation, procedures were developed to decontaminate the effluents from the toxic inorganic elements and to make them immobilize.

3.5.5 Working Strategy

The proposed work has been performed with the help of available/ existing facilities such as pH meter, Conductivity meter, TDS meter, Turbidity meter, Spectrophotometer and Colorimeter.

3.5.6 Sampling

The effluent sampling point should be the same as that specified in the NPDES discharge permit (USEPA, 1988). The effluent samples for the present study was collected every month from October 2010 to March 2011 from the effluent discharge stream of sugar mill. On the day of sampling, the samples were collected in two litre polythene can, once in four hour for 24 hour
and mixed in equal proportions to get uniform homogeneous samples (Rainwater et al., 1960). These samples were used for analysis of water quality parameters such as colour, odour, turbidity, oil and grease (OG), total dissolved solids, electrical conductivity (EC), pH, temperature, total solids (TS), total suspended solids (TSS), chloride, sulphate, Sodium, Calcium, Magnesium, Iron, carbonate and bicarbonate, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), total acidity and dissolved phosphate.

Figure 3.5.6.1 Effluent sampling in sugar industry

3.5.7 Samples Preservation

Temperature changes quickly; pH may change significantly in a matter of minutes. Microbiological activity may affect the nitrate-nitrite ammonia content, phenol or BOD concentration, or the reduction of sulfate. Color, odor, and turbidity may increase, decrease, or change in quality (APHA, 2005). Hence it becomes important to preserve the samples. The bottles for sample preservation were thoroughly cleaned by rinsing with 8M Nitric acid solution followed by washing it with distilled water and finally with double distilled water. Then, the bottles were rinsed thrice with the effluent samples and the effluent samples were stored in a
refrigerator at temperature approximately 4ºC, after adding the necessary preservatives, APHA (1985). This is essential for retarding biological action, hydrolysis of chemical compounds and complexes and reduction of volatility of constituents. For COD, Sulphuric acid was added to bring pH to 2, for phosphates 20 mg of mercuric chloride was added and refrigerated. The mixed, homogeneous effluents were taken out from the refrigerator only at the time of analysis.

3.5.8 Sample treatment and analysis

Samples collected for off-site toxicity testing are to be chilled to 0-6°C during or immediately after collection, and shipped iced to the performing laboratory. Sufficient ice should be placed with the sample in the shipping container to ensure that ice will still be present when the sample arrives at the laboratory and is unpacked. Insulating material should not be placed between the ice and the sample in the shipping container unless required to prevent breakage of glass sample containers.

Water temperature should be maintained within the limits specified for each test. The temperature of test solutions must be measured by placing the thermometer or probe directly into the test solutions, or by placing the thermometer in equivalent volumes of water in surrogate vessels positioned at appropriate locations among the test vessels. Temperature should be recorded continuously in at least one test vessel for the duration of each test. Test solution temperatures should be maintained within the limits specified for each test. DO concentration and pH should be checked at the beginning of each test and daily throughout the test period.

The DO concentration in the samples should be near saturation prior to use. Aeration may be used to bring the DO and other gases into equilibrium with air, minimize oxygen demand, and
stabilize the pH. However, aeration during collection, transfer, and preparation of samples should be minimized to reduce the loss of volatile chemicals.

In the present study, samples were used for analysis of water quality parameters such as color, odor, turbidity, oil and grease (OG), total dissolved solids, electrical conductivity, pH, temperature, total solids, total suspended solids, chloride, sulphate, Sodium, Calcium, Magnesium, Iron, carbonate and bicarbonate, BOD, COD, DO, total acidity and dissolved phosphate.

3.5.8.1 Determination of physico-chemical parameters of the effluent

3.5.8.1.1 Determination of Color

The presence of different substances imparts color to the industrial effluent. Color is measured using the standardized method of comparison with a color scale, the Hazen scale (NF-T90.034). The latter is determined using various dilutions of a potassium chloroplatinate solution using colorimeter (Jhakrani et al., 2009).

![Colorimeters Used in Color Measurement](image)

3.5.8.1.2 Determination of Odor

The odor of fresh wastewater is usually not offensive, but a variety of odorous compounds are released when wastewater is decomposed biologically under anaerobic conditions. The principal odorous compound is hydrogen sulphide (the smell of rotten eggs).
Other compounds, such as indol, skatol, cadaverin and mercaptan, formed under anaerobic conditions or present in the effluents of pulp and paper mills (hydrogen sulphide, mercaptan and dimethylsulphide), may also cause a rather offensive odor. Odor is measured by successive dilutions of the sample with odor-free water until the odor is no longer detectable.

3.5.8.1.3 Determination of Turbidity

Clear water may appear cleaner than turbid water, but it is not necessarily healthier. Turbidity is measured in nephelometric turbidity units (NTU) or formazin turbidity units (FTU), depending on the method and equipment used.

The nephelometer was turned on. It was standardized using the 0.02 NTU reference standard. The sample was allowed to come to room temperature before analysis. The sample was mixed thoroughly to disperse the solids. The sample was agitated gently to resuspend any heavier particles without introducing air bubbles. The cuvette was filled approximately to half (12 mm) of the top with a sample aliquot directly from the sample bottle. The cap on the cuvette was placed and carefully cleaned of any condensation from the outside of the cuvette with a lint free wiper such as Kim wipes. (Condensation may be prevented by coating the outside of the cuvette with a small amount of silicon oil). The sample cuvette was placed into the well and aligned with the locator pin on the optical well and the NTU reading was taken directly from the display. The appropriate display range was selected for best resolution. For example, for an expected turbidity of 30-50 NTU, the display of 0-100 NTU was selected. The turbidity was read within 3-5 seconds.
3.5.8.1.4 Determination of Oil and Grease by Partition Gravimetric Method

One litre of a representative sample was collected in a wide mouth glass bottle that has been rinsed with the solvent (trichlorotrifluoroethane). It was acidified to pH 2 by adding concentrated hydrochloric acid. A separate sample for oil and grease determination was collected, since loss of grease will occur on sampling equipment if composite sampling was done. Individual sample collected at prescribed time intervals should be analyzed separately to obtain average concentration over an extended period.

The acidified sample was added to a separating funnel. The sample bottle was rinsed carefully with 30 ml of trichlorotrifluoroethane and the solvent washings were added to the separating funnel. It was shaken vigorously for about 2 minutes. The solvent layer was drained through a funnel containing solvent moistened filter paper into a clean, tared distillation flask. If a clear solvent layer was not obtained, 1 g of sodium sulphate (Na₂SO₄) crystals was added to the filter paper cone and emulsified solvent was slowly drained on to the crystals. It was extracted two more times with 30 ml of solvent each time, but first the sample container was rinsed with the solvent. The extracts were collected in a tared distillation flask and filter paper was washed with an additional 10 to 20 ml of the solvent. Solvent from distillation flask was distilled over a
water bath at 70°C. The residue was transferred quantitatively using a minimum quantity of solvent into a clean, tared, dried beaker. The beaker was placed on water bath for 15 minutes at 70°C till all the solvents were evaporated off. The beaker was cooled in a dessicator for 30 minutes and weighed.

Oil and grease, mg/l = M/V X 1000

where M = mass, in mg, of the residue, V = volume, in ml, of the sample taken.

3.5.8.1.5 Determination of Temperature

The temperature of wastewater will vary from season to season and also with geographic location. In cold regions the temperature will vary from about 7 to 18°C, while in warmer regions the temperatures vary from 13 to 24 °C. The limnological characteristics of the water body depend upon solar radiations hence Temperature was recorded by a good thermometer with 0°C to 60°C range and having a least count of about 1°C. The Temperature of sample was measured at the time of sampling on the site.

Figure 3.5.8.1.5.1 Determination of temperature of the effluent

3.5.8.1.6 Determination of Total solid, total dissolved solid and total suspended solid

Total Solids (TS) are the total of all solids in a water sample. They include the total suspended solids and total dissolved solids. Total Suspended Solids (TSS) are the amount of
filterable solids in a water sample. Samples are filtered through a glass fiber filter. The filters are dried and weighed to determine the amount of total suspended solids in mg/l of sample. Total Dissolved Solids (TDS) are those solids that pass through a filter with a pore size of 2.0 micron or smaller. They are said to be non-filterable. After filtration the filtrate (liquid) is dried and the remaining residue is weighed and calculated as mg/l of Total Dissolved Solids. The total solids (TS) contents of wastewater are used in the design and process control of wastewater treatment facilities. Total dissolved solids (TDS) are used to evaluate the suitability of water for both domestic supplies and industrial purposes.

3.5.8.1.6.1 Total Solids (TS)

A clear dry glass beaker (which was kept at 103°C in an oven for 1 hour) of 150ml capacity was taken and appropriate identification mark was put on it. The beaker was weighed and the weight was noted. 100ml of the thoroughly mixed sample, measured by the measuring cylinder, was poured in the beaker. The beaker was placed in an oven maintained at 103°C for 24 hours. After 24 hours, the beaker was cooled and weighed. The weight of solids in the beaker was found by subtracting the weight of the clean beaker determined. The total solid (TS) was calculated as follows:

\[ \text{Total solids, TS (mg/l)} = \frac{\text{mg of solids in the beaker} \times 1000}{\text{volume of sample}} \]

3.5.8.1.6.2 Total Dissolved Solids (TDS)

100 ml of sample was taken and filtered through a double layered filter paper and the filtrate was collected in a beaker. The beaker was placed in an oven maintained at 103°C for 24 hours. After 24 hours, the beaker was cooled and weighed. The weight of solids in the beaker
was found by subtracting the weight of the clean beaker determined and the dissolved solid contents was calculated as follows:

Total Dissolved Solids, TDS (mg/l) = mg of solids in the beaker x 1000/ (volume of sample)

3.5.8.1.6.3 Total Suspended Solids (TSS)

Total Suspended Solids was calculated as follows:

Total Suspended Solids, TSS (mg/l) = TS (mg/l) – TDS (mg/l)

Figure 3.5.8.1.6.1 Determination of total solid, total dissolved and suspended solids

3.5.8.1.7 Determination of Electrical Conductivity

In order to determine the conductivity of a solution, a standard solution for the calibration of conductivity meter is prepared by weighing out accurately 5.1 g of KCl and diluting to one litre with pure water in a litre volumetric flask. A 10 mL portion of this solution is diluted to one litre with distilled water in a volumetric flask. It is a working standard with conductivity of 100 µS cm⁻¹. Then a conductivity cell is suspended in the solution holding it approximately 1.5 cm above the bottom of the beaker so that it is not in contact with any of the beaker walls. The conductivity reading is adjusted to 100 µS cm⁻¹ (Frank et al., 1974).
3.5.8.1.8 Determination of pH

pH is a term used universally to express the intensity of the acid or alkaline condition of a solution. The pH meter (Cyber Scan 1000, Eutech, Singapore) was standardized according to the manufacturer’s instructions. A standard buffer solution with a pH value close to that of the water to be tested was selected. The temperature control was set to the temperature of the buffer. The meter was set to the pH of the buffer at that temperature. The electrode response was checked by measuring a second standard buffer solution of different pH. The electrode was washed thoroughly first with distilled water and then with the sample. The temperature control was set to the temperature of the sample. Electrodes were immersed in the sample and pH was recorded after stabilizing the system.
3.5.8.1.9 Determination of Chloride

Sample (10 ml) was taken in conical flask and 2-3 drops of potassium chromate solution added. It was titrated with 0.1N AgNO₃ till the color change from yellow to brick red, the reading was noted from burette.

\[
\text{Chloride (mg/l)} = \frac{N \times M \times 35450}{V}
\]

Where \(N\) = Normality of titrant (0.1 N), \(M\) = Mean of three readings, \(V\) = Volume of sample ml, 35450 = Standard value of equation.

3.5.8.1.10 Determination of Sulphate

Suitable volume of sample was taken and diluted to 100 mL into a 250 mL Erlenmeyer flask. 20 mL of buffer solution was added and mixed well. The flask was constantly stirred with the help of stirrer. One spatula of BaCl₂ crystals were added with stirring. The stirring was continued for one minute after addition of BaCl₂. The suspension was poured into an absorption cell of photometer and turbidity was measured at 5 ± 0.5 min. To correct for sample colour and turbidity, a blank was run to which BaCl₂ was not added (Rossum and Villarruz, 1961).

\[\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg (SO}_4^{2-}) \times 1000}{\text{mL of sample}}\]

Figure 3.5.8.1.10.1 Photometer to determine sulphate
3.5.8.1.11 Determination of Calcium

Calcium can be determined by EDTA titrimetric method. In this method, the pH of the sample is made sufficient high (12-13) to precipitate Magnesium as Hydroxide and Calcium only is allowed to react with EDTA in the presence of a Murexide indicator.

\[
\mathrm{Mg}^{2+} + 2 \text{NaOH} \rightarrow \mathrm{Mg(OH)}_2 + 2 \text{Na}^+
\]

\[
\mathrm{Ca}^{2+} + 2 \text{EDTA} \rightarrow \mathrm{Ca(EDTA)}_2 + 2 \text{Na}^+
\]

Calcium and Magnesium form a complex of wine red colour with Erichrome Black-T at high pH. The EDTA has got stronger affinity for Ca\(^{++}\) and Mg\(^{++}\) can be obtained by subtracting the value of Calcium from the total of Ca\(^{++}\) and Mg\(^{++}\).

Figure 3.5.8.11.1 Determination of Calcium and Magnesium using EDTA titration method

3.5.8.11.1 Total hardness

25 or 50 mL well mixed sample was taken in porcelain dish or conical flask. 1-2 mL of buffer solution was added followed by 1 mL inhibitor. A pinch of Erichrome black T was added and titrated with standard EDTA (0.01M) till wine red color changes to blue, the volume of EDTA required (A) was noted. A reagent blank was run and the volume of EDTA (B) was noted.

Volume of EDTA required by sample was calculated, \( C = (A-B) \).
For natural waters of low hardness, a larger sample volume that is 100-1000 mL was taken for titration and proportionately larger amounts of buffer, inhibitor and indicator were added. Standard EDTA titrant was added slowly from a micro burette and a blank was run using redistilled, deionised water of the same volume as sample. Blank correction was applied for computing the results.

### 3.5.8.1.11.2 Calcium hardness

25 or 50 mL sample was taken in a porcelain dish. 1mL NaOH was added to raise pH to 12.0 and a pinch of Murexide indicator was added. It was titrated immediately with EDTA till pink colour changes to purple. The volume of EDTA required (A) was noted. A reagent blank was run. The mL of EDTA required (B) was noted and kept aside to compare end points of sample titrations.

The volume of EDTA required by sample was calculated, \( C = A - B \).

The EDTA (0.1M) solution was standardized following the procedure of calcium hardness from 1 to 4, using standard calcium solution.

Titrations are best conducted at or near normal room temperatures. The colour change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition presents a problem in hot water. The pH specified in the recommended procedure may result in \( \text{CaCO}_3 \). Although the titrant can redissolve such precipitates slowly, a drafting end point often will yield low results. A time of 5 min of the overall procedure minimises the tendency for \( \text{CaCO}_3 \) to precipitate. The sample was diluted with distilled water to reduce \( \text{CaCO}_3 \) concentration. If precipitation occurs at the dilution of 1+1, the following modifications were
used because too small a volume contributes a systematic error due to the burette-reading error (Goetz and Smith, 1959).

Total hardness as CaCO$_3$ mg/L = \( C \times D \times 1000 / \text{mL sample} \)

where, \( C \) = volume of EDTA required by sample, \( D \) = mg CaCO$_3$ equivalent to 1mL EDTA titrant

Calcium hardness CaCO$_3$ as mg/L = \( C_1 \times D \times 1000 / \text{mL sample} \)

where \( C \) = volume of EDTA used by sample, \( D \) = mg CaCO$_3$ equivalent to 1 mL EDTA titrant.

Magnesium hardness = Total hardness as CaCO$_3$,

mg/L – Calcium hardness as CaCO$_3$, mg/L

**Alkaline (Carbonate) hardness and non-alkaline (non-carbonate) hardness**

These types of hardness can be calculated from total hardness and total alkalinity as, i) If total hardness as CaCO$_3$ > total alkalinity as CaCO$_3$, then, Alkaline hardness = Total alkalinity, ii) Non-alkaline hardness = Total hardness – Total alkalinity. iii) If total hardness as CaCO$_3$ < total alkalinity as CaCO$_3$, then, Alkaline hardness = Total hardness, iv) Nonalkaline hardness = Nil

**3.5.8.1.12 Determination of Carbonate**

The sample was prepared and titrated to the end-point pH without recording intermediate pH values and without undue delay. As the end point is approached smaller additions of acid was
made till the pH equilibrium was reached. Alkalinity was determined using the formula as given below,

\[
\text{Alkalinity mg CaCO}_3/ \text{L} = \frac{A \times t \times 1000}{\text{mL sample}}
\]

where \(A\) = Standard acid used, \(t\) = titer of standard acid, mg CaCO\(_3\)/mL

Figure 3.5.8.1.12 Determination of Carbonate using titrimetric method

3.5.8.1.13 Determination of Biochemical Oxygen Demand

Biochemical Oxygen Demand (BOD) is the measure of the degradable organic material present in a water sample and can be defined as the amount of Oxygen required by the Micro Organisms in stabilizing the Biologically Degradable Organic matter under aerobic conditions.

Guideline BOD values for classification of raw untreated water is given below (Table 3.5.8.1.13).

The quality of effluent can be easily assessed by its biological oxygen demand (BOD) and chemical oxygen demand (COD). Effluent quality in term of BOD and COD as given by Metcalf and Eddy (1979) is presented in table 3.5.8.1.13.1.
Table 3.5.8.1.13 Guideline BOD values for classification of raw untreated water

<table>
<thead>
<tr>
<th>Quality class</th>
<th>Designated best use</th>
<th>BOD value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Drinking water source without conventional treatment but with chlorination</td>
<td>2 or less</td>
<td>Could cause problems in treatment, larger CL₂ demand and residual taste/odour problem.</td>
</tr>
<tr>
<td>B</td>
<td>Drinking water source with conventional treatment</td>
<td>3 or less</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5.8.13.1 Effluent characterization in terms of BOD and COD

<table>
<thead>
<tr>
<th>Effluent Characterization</th>
<th>BOD (mg/L)</th>
<th>COD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak</td>
<td>&lt; 200</td>
<td>&lt; 400</td>
</tr>
<tr>
<td>Medium</td>
<td>350</td>
<td>700</td>
</tr>
<tr>
<td>Strong</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Very strong</td>
<td>&gt; 750</td>
<td>&gt; 1500</td>
</tr>
</tbody>
</table>

Sample collection, preservation and storage

Grab or composite samples were collected. The composite samples were kept at or below 4°C during compositing. Samples for BOD may degrade significantly during storage. Reduction of BOD was minimized by analyzing samples promptly or by cooling it to near freezing temperature during storage. The maximum holding time recommended between collection and analysis is 48 hours. Chilled samples were warmed to 20–27°C ± 3°C before analysis.

Dilution water was prepared in a glass container by bubbling compressed air in distilled water for about 30 minutes. 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride, ferric chloride, and sewage water solutions were added for each litre of dilution water and mixed thoroughly. The sample was neutralized to pH around 7.0 by using 1 N NaOH or H₂SO₄. Since the dissolved oxygen (DO) in the sample was likely to be exhausted, it was usually necessary to prepare a suitable dilution of the sample according to the expected BOD range. Dilutions were prepared in the bucket and the content was mixed thoroughly. Three sets of the BOD bottles were filled. One set of the bottles was kept in the BOD incubator at 27°C for five
days, and the DO content in another set was determined immediately. DO content was determined in the sample bottles, immediately after completion of five days. Similarly for blank, three BOD bottles were taken for dilution water. The DO content was determined and the other was incubated with the sample to determine DO after five days.

**Dilution technique**

Dilution that result in a residual DO of at least 1 mg/l and a DO uptake of at least 2 mg/l after 5 days incubation produce the most reliable results. Several dilutions of prepared samples were made to obtain DO uptake in this range. A more rapid analysis, such as COD, may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, the following dilutions were used: 0-1%, for strong industrial wastes, 1-5 % for raw and settled waste water, 5-25% for biologically treated effluent, 25-100% for polluted river waters (table 3.5.8.1.13.2).

BOD of the sample was calculated as follows:

a. When dilution water is not seeded

$$\text{BOD as } O_2 \text{ mg/L} = \frac{(D_1 - D_2) \times 100}{\% \text{ dilution}}$$

\[D_1 = \text{DO of sample immediately after preparation, mg/L}\]

\[D_2 = \text{DO of sample after incubation period, mg/L}\]
Table 3.5.8.1.13.2 Dilutions for various ranges of BOD in the samples

<table>
<thead>
<tr>
<th>Range of BOD mg/l O₂</th>
<th>Dilution (%)</th>
<th>Sample volume in 1 litre of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>No dilution</td>
<td>1000</td>
</tr>
<tr>
<td>4-12</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>10-30</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>20-60</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>40-120</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>100-300</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>200-600</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>400-1200</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>1000-3000</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>2000-6000</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Above 6000</td>
<td>0.05</td>
<td>0.5</td>
</tr>
</tbody>
</table>

b. When dilution is seeded

\[ \text{BOD O}_2 \text{ mg/L} = \frac{(\text{D}_1 - \text{D}_2) - (\text{B}_1 - \text{B}_2) \times 100}{100 \times \% \text{ dilution}} \]

c. When material is added to sample or to seed control

\[ \text{BOD O}_2 \text{ mg/L} = \frac{(\text{D}_1 - \text{D}_2) - (\text{B'}_1 \times \text{B'}_2) \times \text{F} \times 100}{100 \times \% \text{ dilution}} \]

where, \( \text{D}_1 \) = DO of sample immediately after preparation, mg/L, \( \text{D}_2 \) = DO of sample after incubation period, mg/L, \( \text{B}_1 \) = DO of blank (seeded dilution water) before incubation, mg/L, \( \text{B}_2 \) = DO of blank (seeded dilution water) after incubation, mg/L, \( \text{F} \) = ratio of seed in diluted sample to seed in seed control (Vol. of seed in diluted sample / Vol. of seed in seed control), \( \text{B'}_1 \) = DO of seed control before incubation, mg/L, \( \text{B'}_2 \) = DO of seed control after incubation, mg/L
### 3.5.8.1.14 Determination of Chemical Oxygen Demand

![Figure 3.5.8.14 COD Digestion Apparatus Model 2015D](image)

50 ml of sample (for samples with COD of greater than 900 mg O$_2$/L, smaller sample portion was diluted to 50 ml) was placed in 500 ml refluxing flask. 1 g of HgSO$_4$, several glass beads was added and 5 ml of sulphuric acid reagent was added very slowly, with mixing to dissolve HgSO$_4$. It was cooled while mixing to avoid possible loss of volatile materials. 25 ml, 0.0147 M K$_2$Cr$_2$O$_7$ solution was added and mixed. The flask was attached to condenser and cooling water was turned on. Remaining sulphuric acid reagent (70 ml) was added through open end of condenser. Stirring was continued, while adding sulphuric acid reagent.

\[
\text{COD, mg O}_2/\text{L} = \frac{(A - B) \times M \times 8000}{\text{sample}}
\]

Where: $A$ = ml FAS used for blank, $B$ = ml FAS used for sample, $M$ = molarity of FAS

### 3.5.8.1.15 Determination of Dissolved Oxygen (DO)

![Figure 3.5.8.15 Determination of Dissolved Oxygen (DO) using Winkler’s method](image)
The sample was collected in 250 ml Winkler bottles at the sampling site and fixed by adding 2 ml of MnSO$_4$ reagent and 2 ml of alkali iodide-azide reagent. The bottle was closed tightly, sealed and mixed thoroughly. The sample was transferred to laboratory. In laboratory the bottles were opened and the supernatant liquid was carefully removed (30-40 ml) without disturbing the precipitate. 2.0 ml of ortho-phosphoric acid was added and the solution was kept in darkness for 5-10 minutes, then 2-3 drops of starch indicator, was added and titrated with standard sodium thiosulphate solution 0.1N. At the end point, the blue colour disappeared (Chemistry for Environmental Engineering).

\[
\text{DO mg/ l} = \frac{M \times N \times 8000}{V}
\]

Where: \(M\) = Mean of three readings, \(N\) = Normality, \(V\) = Volume of sample, 8000 = standard value of equation

3.5.8.1.16 Determination of total acidity

25 mL of sample is pipetted into Erlenmeyer flask. If free residual chlorine is present, 0.05 mL (1 drop) of 0.1 N thiosulphate solution is added. 2 drops of methyl orange indicator is added. These contents were titrated against 0.02 N hydroxide solutions. The volume of titrant at end point is noted when colour change from orange red to yellow. Then two drops of phenolphthalein indicator is added and titration continued till a pink colour just develops. The volumes of the titrant used were noted down.

\[
\text{Acidity in mg/ L as CaCO}_3 = \frac{A \times B \times 50000}{V}
\]
where \( A = \text{mL of NaOH titrant}, \ B = \text{Normality of NaOH}, \ V = \text{mL of the sample}, \)

Total acidity = mineral acidity + CO\(_2\) acidity

**Figure 3.5.8.1.16 Determination of total acidity using titration method**

### 3.5.8.1.17 Determination of Iron

In 100 mL volumetric flasks 2 mL of (1+1) HNO\(_3\) and 10 mL of 1+1 HCl were added and diluted to 100 mL with water. Before preparing mixed standards, each stock solution was analysed separately to determine possible spectral interference or the presence of impurities. When preparing mixed standards care was taken that the elements are compatible and stable. Mixed standard solutions were stored in an FEP fluorocarbon (Teflon) or unused polyethylene bottle. Calibration standards were verified initially using the quality control standard; monitored weekly for stability.

The samples were analysed using calibration blank. This permits a check of the sample preparation regents and procedures for contamination. Samples were analysed, alternatively with analyses of calibration blank. It was rinsed for at least 60 seconds with dilute acid between samples and blanks. After introducing each sample or blank, the system was let to equilibrate before starting signal integration. Each analysis of the calibration blank was examined to verify
that no carryover memory effect has occurred. If carryover is observed, rinsing was repeated until proper blank values are obtained. Appropriate dilutions of the sample were made to determine concentrations beyond the linear calibration (table 3.5.8.1.17.1).

Table 3.5.8.1.17.1 Suggested wavelength, detection limit and upper limit concentration for Iron

<table>
<thead>
<tr>
<th>Element</th>
<th>Suggested Wavelength nm</th>
<th>Estimate Detection Level µg/L</th>
<th>Alternate Wavelength nm</th>
<th>Calibration Concentration mg/L</th>
<th>Upper Limit Concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>259.94</td>
<td>7</td>
<td>238.20</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

3.5.8.1.18 Determination of Sodium by Flame emission photometric method

Trace amounts of sodium can be determined by flame emission photometry at the wavelength of 589 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices such as prisms or gratings. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at 589 nm is approximately proportional to the concentration of the element. If alignment of the wavelength dial with the prism is not precise in the available photometer, the exact wavelength setting, which may be slightly more or less than 589 nm, can be determined from the maximum needle deflection and then used for the emission measurements. The calibration curve may be linear but has a tendency to levels off at higher concentrations (Gilbert et al., 1950).

\[ \text{mg Na/L} = (\text{mg Na/L in portion}) \times D \]

Where: \( D = \text{dilution ratio} \) \( D = \text{mL sample} + \text{mL water} / \text{mL sample} \).
3.5.8.1.19 Determination of Dissolved Phosphate

Phosphorous occurs in natural waters and in wastewater almost solely in the form of various types of phosphates. These forms are commonly classified into orthophosphates and total phosphates. These may occur in the soluble form, in particles of detritus or in the bodies of aquatic organisms. The various forms of phosphates find their way into wastewater, effluents and polluted water from a variety of sources.
100 mL well mixed sample was taken in 150 mL conical flask. One drop of phenolphthalein indicator was added. If red colour develops, sulphuric acid solution was added dropwise to just discharge the colour. 1 mL sulphuric acid solution was added in excess. It was boiled gently for at least 90 minutes by adding distilled water to keep the volume between 25 and 50 mL. The solution was cooled; one drop of phenolphthalein indicator was added and neutralized to a faint pink colour with hydroxide solution. The concentration of phosphate was read from the calibration curve.

3.5.8.1.20 Determination of Sodium Absorption Ratio (SAR), Kelleys Ratio (KR), Percent Sodium (PS), Magnesium Ratio (MR) and Residual Sodium Carbonate (RSC)

The formula for calculating Sodium Absorption Ratio (SAR) was

$$\text{SAR} = \frac{\text{Na}^+}{[(\text{Ca}^{2+} + \text{Mg}^{2+})/2]^{1/2}}$$

Kelleys Ratio (KR) can be calculated using the formula

$$\text{KR} = \frac{\text{Na}^+}{\text{Ca}^{2+} + \text{Mg}^{2+}}$$

The formula for calculating Percent Sodium (PS), Magnesium Ratio (MR) (Kannan et al., 2003) and Residual Sodium Carbonate (RSC) (Machiraju et al., 2009) were given by

$$\text{PS} = 100 \times \frac{(\text{Na}^+ + \text{K}^+)}{\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+}$$

$$\text{MR} = 100 \times \frac{\text{Mg}^{2+}}{\text{Ca}^{2+} + \text{Mg}^{2+}}$$

$$\text{RSC} = [\text{CO}_3^{2-} + \text{HCO}_3^-] - [\text{Ca}^{2+} + \text{Mg}^{2+}]$$

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3.5.8.1.21 Determination of Wilcox Ratio

The formula for calculating Wilcox Ratio (Wilcox, 1948) was

\[
WR = \left( \frac{Na^+}{Ca^{2+} + Mg^{2+} + Na^+ + K^+} \right)
\]

3.5.8.1.22 Calculation of Water Quality Index

For the calculation of water quality index (Pradhan et al., 2000) the following equations have been used in different steps:

**Step 1:**

Quality rating, \( q_n \) = 100 \( \times \) \(
\frac{V_n - V_i}{V_s - V_i}
\)

Where, \( V_n \) – Actual amount present in polluted water on nth parameter, \( V_i \) – The ideal value of parameter, \( V_i = 0 \) for the suitable water except pH and DO which is 7.0 mg/l and 14.6 mg/l, respectively, \( V_s \) – standard value of the parameter.

**Step 2:**

Unit weight (\( W_n \)) for various parameters is inversely proportional to the recommended standard (\( S_n \)) for the corresponding parameter.

\[
W_{n1} = \frac{K}{S_{n1}}
\]

Where \( K = \frac{1}{1/ (s1)^{1/2} + 1/ (s2)^{1/2} + \ldots + 1/ (s_n)^{1/2}}
\)

\( \sum W = 1 \), Considered here.

n=1
Step 3:

Sub indices (SI) = \( (q_n)^{W_n} \)

Step 4:

The overall water quality index was calculated taking the geometric mean of these indices (SI)n

\[
WQI = \Sigma (SI)_n = \Sigma (q_n)^{W_n}
\]

\[
WQI = \text{Anti Log}_{10} \left[ \Sigma W_n \text{ Log}_{10} q_n \right]
\]

Water quality parameters, their ICMR, WHO STANDARDS and assigned unit weights were tabulated in table 3.5.8.1.22.1.

Sanchez et al (2006) calculated the objective water quality index using the following equation:

\[
WQI = k \frac{\sum_{i=1}^{n} C_i P_i}{\sum_{i=1}^{n} P_i}
\]

where \( n \) is the total number of parameters, \( C_i \) is the value assigned to parameter \( i \) after normalization and \( P_i \) is the relative weight assigned to each parameter. \( P_i \) value range from 1 to 4, with 4 assigned to a parameter that is most important for aquatic life preservation (DO) and value of 1 assigned to the parameter that has a smaller impact (chloride). \( k \) is the objective constant and is equal to 1.
Table 3.5.8.1.22.1 Water quality parameters, their ICMR, WHO STANDARDS and assigned unit weights

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>ICMR/WHO/CPHEEO/IS standards</th>
<th>Unit weights $W_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity</td>
<td>10</td>
<td>0.01654</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>500</td>
<td>0.00033</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>100</td>
<td>0.00165</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>0.5</td>
<td>0.3308</td>
</tr>
<tr>
<td>pH</td>
<td>7.5-8.5</td>
<td>0.0221</td>
</tr>
<tr>
<td>Chloride</td>
<td>250</td>
<td>0.00066</td>
</tr>
<tr>
<td>Sulphate</td>
<td>400</td>
<td>0.00041</td>
</tr>
<tr>
<td>Calcium</td>
<td>75</td>
<td>0.0022</td>
</tr>
<tr>
<td>Magnesium</td>
<td>150</td>
<td>0.0011</td>
</tr>
<tr>
<td>BOD</td>
<td>30</td>
<td>0.0055</td>
</tr>
<tr>
<td>COD</td>
<td>250</td>
<td>0.00066</td>
</tr>
<tr>
<td>DO</td>
<td>5</td>
<td>0.0331</td>
</tr>
<tr>
<td>Iron</td>
<td>0.3</td>
<td>0.5513</td>
</tr>
<tr>
<td>Sodium</td>
<td>200</td>
<td>0.00083</td>
</tr>
<tr>
<td>Phosphate</td>
<td>5</td>
<td>0.0331</td>
</tr>
</tbody>
</table>

The WQI status was given in table 3.5.8.1.22.2

Table 3.5.8.1.22.2 Water Quality Index and its status

<table>
<thead>
<tr>
<th>WQI</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 25</td>
<td>Excellent</td>
</tr>
<tr>
<td>26 – 50</td>
<td>Good</td>
</tr>
<tr>
<td>51 – 75</td>
<td>Poor</td>
</tr>
<tr>
<td>76 – 100</td>
<td>Very Poor</td>
</tr>
<tr>
<td>100 and above</td>
<td>Unsuitable for drinking, propagation of wildlife, fish culture and irrigation</td>
</tr>
</tbody>
</table>

The water quality classification system adopted here is proposed by Kannel et al (2007); Jonnalagadda and Mhere (2001), Dojlido et al (1994). According to which, WQI in the range of
0–25 is very bad, 26 – 50 is bad, 51 – 70 is medium, 71 – 90 is good and 91 – 100 is excellent. Table 4.2.1.9 gives the different parameters that were used in the evaluation process, as well as their relative weights and the normalization factors. These values were adopted from various literatures, Pesce and Wunderlin (2000); Cude (2001); Debels et al (2005); Sanchez et al (2006); Kannel et al (2007).

3.5.8.1.23 Determination of Heber Water Quality Index (HWQI-1)

The percent saturation of dissolved oxygen present in water at a given temperature was determined through the use of percent saturation chart. A graph was plotted between dissolved oxygen and temperature in parallel axis. The line joining the opposite ends of the axes were marked which represent the percent of saturation. A straight line was drawn between the water temperature of the sample site and the dissolved oxygen in mg/l. The percent saturation at the intercept on the sloping scale was recorded. Example: Let the DO value was 8 mg/l and the measured water temperature was 15ºC. Drawing a straight line between two values cuts the percent saturation axis at 80 %. It means that about 80% of dissolved oxygen remained and 20 % was used by something present in that water (Figure 3.5.8.1.23).

Procedure for calculating the overall Heber Water Quality Index (HWQI-1)

The Heber Water Quality (HWQI-1) for the sampling station was formulated after the seven quality tests are completed and the results of each test recorded (Rajendran and Mansiya, 2011).

Procedure for determining Q-value

The water quality scientists drew a graph between the levels of water quality ranging from 0 (worst) to 100 (best) from the raw data (pH values 2-12). The curves drawn were then averaged to obtain a weighting curve for each parameter. The test value was plotted at the
bottom of the respective weighing curve chart and a vertical line was drawn until it intersects the weighing curve line. From the point of intersection, a horizontal line was drawn to the "Y" axis. The point of intersection on the y-axis provides Q-value for that particular parameter. The Q-value was recorded in the HWQI table.

The weighing curve value (that is, Q-value) for each test was multiplied by the weighing factor (Brown et al., 1970; Mitchell and Stapp, 2000) listed in the table for a particular test. The value was tabulated in the total column of HWQI table. The weighing factor for the parameters indicates its significant contribution to overall water quality determination. For example, from the WQI chart, dissolved oxygen (DO) with a weighing factor of 0.133 is considered a more important test than solids at 0.09 in overall water quality determination (Figure 3.5.8.1.23.1).

The resulted Q-value was then multiplied by the appropriate weighing factor. The overall water quality index (WQI) for the sampling station during different months was determined by adding the total of the seven test results.

Figure 3.5.8.1.23 % saturation chart for dissolved oxygen  Figure 3.5.8.1.23.1 DO % Saturation Chart
Table 3.5.8.1.23 Variable used in WQI calculation, scores of normalization and relative weights

<table>
<thead>
<tr>
<th>Variable</th>
<th>R</th>
<th>W</th>
<th>Normalization factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>21/16</td>
<td>22/15</td>
<td>24/14</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>7-8</td>
<td>7-8.5</td>
</tr>
<tr>
<td>EC mmhos/cm</td>
<td>&lt;750</td>
<td>&lt;1000</td>
<td>&lt;1250</td>
</tr>
<tr>
<td>DO mg/l</td>
<td>&gt;=7</td>
<td>7.5</td>
<td>&gt;6.5</td>
</tr>
<tr>
<td>TDS mg/l</td>
<td>&lt;100</td>
<td>&lt;500</td>
<td>&lt;750</td>
</tr>
<tr>
<td>TSS mg/l</td>
<td>&lt;20</td>
<td>&lt;40</td>
<td>&lt;60</td>
</tr>
<tr>
<td>Ca mg/l</td>
<td>&lt;10</td>
<td>&lt;50</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Mg mg/l</td>
<td>&lt;10</td>
<td>&lt;25</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Cl mg/l</td>
<td>&lt;25</td>
<td>&lt;50</td>
<td>&lt;100</td>
</tr>
<tr>
<td>SO₄²⁻ mg/l</td>
<td>&lt;25</td>
<td>&lt;50</td>
<td>&lt;100</td>
</tr>
<tr>
<td>DP mg/l</td>
<td>&lt;0.025</td>
<td>&lt;0.05</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>BOD mg/l</td>
<td>&lt;0.5</td>
<td>&lt;2</td>
<td>&lt;3</td>
</tr>
<tr>
<td>COD mg/l</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>
Heber Water Quality rating was given in table 3.5.8.1.23.1

Table 3.5.8.1.23.1 HWQI-1 Quality Rating

<table>
<thead>
<tr>
<th>Range</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>90-100</td>
<td>Excellent</td>
</tr>
<tr>
<td>70-90</td>
<td>Good</td>
</tr>
<tr>
<td>50-70</td>
<td>Medium</td>
</tr>
<tr>
<td>25-50</td>
<td>Bad</td>
</tr>
<tr>
<td>0-25</td>
<td>Very Bad</td>
</tr>
</tbody>
</table>

3.5.8.1.24 Multivariate statistical analysis between COD and BOD

The COD and BOD of the effluents were recorded from October to March. Mean Relative Quadratic Error was defined as

\[
\text{MRQE} = \left( \frac{\sum (\text{BOD}_{\text{Exp}} - \text{BOD}_{\text{Cal}})}{n-1} \right)^{\frac{1}{2}}
\]

where \( n \) is the number of data points.

Mean value of the parameters are calculated to find out a typical representative of all the observations of a parameter. The mean value of parameter is given by Trivedy and Goel (1986).

\[
\text{Mean (} \bar{x} \text{)} = \frac{\sum x}{n}
\]

Range signifies the fluctuation of parameters and is given by

\[
\text{Range} = \text{Maximum-Minimum}
\]

Standard deviation, which shows the degree of spread of a normal curve of distribution and is determined by using the relationship given below:
Standard deviation = \left\{ \frac{\sum (x-\bar{x})^2}{[n-1]} \right\}^{\frac{1}{2}}

Statistical software SPSS (Statistical package for Social Sciences, Version 7.5) is used to compute the correlation (r values) between COD and BOD. The software is used to calculate the regression parameters a and b of the straight line \( Y = a + b \ X \) by applying the well-known method of least squares (Gupta, 1974, Wonnacott and Wonnacot, 1981) to fit the experimental data to give straight line. Correlation is merely a tool of ascertaining the degree of linear dependence between parameters. Regression analysis tries to find out the average relationship between parameters. It refers to the method by which estimates are made of the values of one or more other parameters.

The correlation coefficient between the variables x and y (Trivedy and Goel, 1984) is

\[ r = \frac{\Sigma xy - \bar{x} \Sigma y}{\left[ (\Sigma x^2 - (\bar{x}) \Sigma x)(\Sigma y^2 - (\bar{y}) \Sigma y) \right]^{\frac{1}{2}}} \]

Regression analysis tries to find out the average relationship between parameters. It refers to the method by which estimates are made out of the values of one or more other parameters. The following forms of relationship are considered for the software development. The values of empirical parameters a and b were calculated with the help of equations.

\[ b = \frac{\Sigma xy - \bar{x} \Sigma x}{\Sigma x^2 - \bar{x} \Sigma x} \]

\[ a = \bar{y} - bx \]
Keeping the above observations in mind, linear and exponential relationships are proposed, i) \( y = a + bx \), ii) \( y = ax^2 + bx + c \), iii) \( y = ax^3 + bx^2 + cx + d \) and iv) \( y = a (x^b) \).

3.6 Plants as biomonitors

3.6.1. Aim and Scope

Sugar industries rank second amongst mavar agro based industries in India. Sugar industry is seasonal in nature and operates only for 120 to 200 days in a year (early November to April). A significant large amount of waste is generated during the manufacture of sugar and contains a high amount of production load particularly in items of suspended solids, organic matters, press-mud, and bagasses and air pollution (Bevan, 1971, Hendrickson et.al.1971).

Gaseous emissions such as \( \text{CO}_x \), \( \text{SO}_x \), and \( \text{NO}_x \), were reported both from process and fired equipment from sugar industry (Khwaja and Quraishi, 2003). Air pollutants can directly affect plants via leaves or indirectly via soil acidification (Steubing et al, 1989). When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Dohmen et al, 1990). Plants provide an enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollutant level in the air environment (Escobedo et al, 2008), with a various extent for different species (Hove et al, 1999).

Some plants thrive in environments that others would find toxic, these plants can clean-up various sources of manmade pollution; both organic (petrochemical) and inorganic (heavy metal toxins). Trees remove a significant amount of pollution from the atmosphere as part of their normal functioning. They directly increase the quality of the air in the city and its surrounding area and should be considered an integral part of any comprehensive plan aimed at improving overall air quality (Abida Begum et al, 2009).
Trees provide a large leaf surface onto which particles are deposited and gases are removed. Pollution is removed by nearly all parts of a tree; the soil, roots and vegetative portions of the tree species. Trees respire and exchange gases through stomates, or holes, on their leaves; these gases include those necessary for the tree’s functioning as well as other gaseous air pollutants. Once inside the leaf, gases diffuse into the spaces between the cells of the leaf to be absorbed by water films or chemically altered by plant tissues. Trees also reduce air pollution by intercepting airborne particles and retaining them on the leaf surface, called dry deposition. Some can be absorbed by the leaf surface itself, although most remain on the plant surface (Joshi et al, 2008).

Leaf surfaces are most efficient at removing pollutants that are water soluble including sulfur dioxide, nitrogen dioxide and ozone. Pollutants travel through plants by translocation via the xylem and phloem. Chemical pollutants absorbed by the leaves are translocated to the root areas where they can be broken down by microbes in the soil and pollutants absorbed by the roots can be broken down and translocated to the leaves where they are released into the atmosphere (Abida Begum et al, 2010).

Previous studies also showed the impact of air pollution on ascorbic acid content (Hoque et al., 2007), chlorophyll content (Flowers et al., 2007), leaf extract pH (Klumpp et al., 2000) and relative water content (Rao, 1979). These separate parameters gave conflicting results for same species (Han et al., 1995). However, the air pollution tolerance index (APTI) based on all four parameters has been used for identifying tolerance levels of plant species (Singh and Rao, 1983; Singh et al., 1991). Several contributors agree that air pollutants effect plant growth adversely (Sodhi, 2005; Henry and Heinke, 2005; Rao, 2006; Bhatia 2006). Air pollution tolerance index is used by landscapers to select plant species tolerance to air pollution (Yan-Ju, 2008).
The present study was carried out for the selection of plant species which can be grown around industrial/urban areas in India. Plants differ considerably with reference to their responses towards pollutants, some being highly sensitive and others hardy and tolerant. On the basis of the APTI and some relevant biological and socioeconomic characters, the anticipated performance index (API) of various plant species was determined for green belt development.

### 3.6.2 Motivation

Higher plants (herbs, shrubs and trees) are often used for the following-up of changes in air quality and the extent of the impact of air pollution (Falla et al. 2000), leading to an enormous amount of published biomonitoring studies; only a fraction of these studies, using higher plant species as bioaccumulator and/or impact biomonitor/bioindicator are given in table 3.6.2. The tobacco cultivar Bel-W3 proved indeed to be very suitable for biomonitoring the ambient O\(_3\) concentration (Falla et al. 2000, Kafiatullah et al. 2012); Tradescantia pallida is susceptible to the effects of traffic pollution (Crispim et al. 2012); coniferous trees have been used since 1980 to highlight the pollution impact of SO\(_2\) and O\(_3\), based on growth variation and chlorosis (Manninen and Huttunen 1995) and the leaf area of higher plants exposed to air pollutants can be reduced by the inhibition of leaf formation, reduced leaf expansion and accelerated leaf abscission (Kozlowski et al. 1991).

For many decades, plants have been used for monitoring visible injury (necrosis and/or chlorosis), since foliar symptoms are rather specific for some air pollutants. For example, in 1999 a European Network for the Assessment of Air Quality by the use of bioindicator plants (EuroBionet) was set up for monitoring air quality and promoting environmental awareness. Visible damage of plants, such as tobacco (Nicotiana tabaccum), poplar (Populus nigra), Italian
rye grass (Lolium multiflorum) and curly kale (Brassica oleracea) was used to monitor O₃, heavy metals, polycyclic aromatic hydrocarbons and sulphurous compounds.

Most of the apparatus to measure photosynthesis, chlorophyll fluorescence and non-destructive measurements of chlorophyll, is constructed for plant leaves and much information is available on the effects of atmospheric pollutants on plant metabolism (De Temmerman et al., 2004). However, higher plants are unlikely to be the best accumulative biomonitor/bioindicator for air pollutants, when compared to mosses and lichens, due to the presence of a cuticle and stomata in the tissues of higher plants, which makes them less permeable than mosses to air pollutants (Aboal et al., 2004).

3.6.3 Research Objectives

The aim of the work was to gain insight into the impact of ambient air quality on leaf characteristics of trees, in order to assess the potential of using trees in biomonitoring studies. The study was focused on i) The impact of ambient air emissions from sugar industry on tree leaves of Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica and Pongamia pinnata. ii) To assess the potential of Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica and Pongamia pinnata as a passive biomonitor to obtain information about the ambient pollutant concentration, and to quantify the response of its leaf characteristics (morphological, anatomical, physiological).
Table 3.6.2 Impact biomonitors/bioindicators and accumulation biomonitors/bioindicators with their response to several air pollutants (O$_3$, NH$_3$, VOC, SO$_2$, NO$_2$, hydrogen fluoride (HF), black carbon (BC)), ‘+’ indicates an increase, ‘-’ indicates a decrease and 0 indicates no change of the plant characteristic

<table>
<thead>
<tr>
<th>Species</th>
<th>Air pollutant</th>
<th>Parameter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betula pendula</td>
<td>SO$_2$</td>
<td>fluctuating asymmetry</td>
<td>+ Kozlov et al. (1996)</td>
</tr>
<tr>
<td>Caesalpinia echinata</td>
<td>O$_3$</td>
<td>chlorophyll fluorescence</td>
<td>- Moraes et al. (2002)</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>net photosynthesis</td>
<td>- Tiwari et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>stomatal conductance</td>
<td>- Tiwari et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>phenol content</td>
<td>+ Tiwari et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>peroxidase activity</td>
<td>+ Tiwari et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>O$_3$</td>
<td>drop contact angle</td>
<td>0 Paoletti et al., (2007)</td>
</tr>
<tr>
<td></td>
<td>O$_3$</td>
<td>stomatal density</td>
<td>+ Paoletti et al., (2007)</td>
</tr>
<tr>
<td>Ficus microcarpa</td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>peroxidase activity</td>
<td>+ Li (2003)</td>
</tr>
<tr>
<td></td>
<td>SO$_2$, NO$_2$, O$_3$</td>
<td>superoxide dismutase activity</td>
<td>0 Li (2003)</td>
</tr>
<tr>
<td>Molinia caerulea</td>
<td>VOC</td>
<td>drop contact angle</td>
<td>0 Cape et al. (2003)</td>
</tr>
<tr>
<td>Nicotiana tabaccum</td>
<td>O$_3$</td>
<td>necrosis</td>
<td>+ Nali and Lorenzini (2007)</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>NH$_3$</td>
<td>glutamine synthetase activity</td>
<td>+ Pearson and Soares (1998)</td>
</tr>
<tr>
<td></td>
<td>O$_3$</td>
<td>net photosynthesis</td>
<td>- Schenone et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>O$_3$</td>
<td>stomatal conductance</td>
<td>- Schenone et al. (1994)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>SO$_2$</td>
<td>drop contact angle</td>
<td>Cape et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>O$_3$</td>
<td>drop contact angle</td>
<td>0 Cape et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>NO$_x$</td>
<td>drop contact angle</td>
<td>- Viskari et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>NO$_x$</td>
<td>stomatal conductance</td>
<td>+ Viskari et al. (2000)</td>
</tr>
<tr>
<td>Species</td>
<td>NO\textsubscript{x}, BC</td>
<td>BC epicuticular wax amount</td>
<td>- Viskari et al. (2000)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>S heavy metals</td>
<td>fluctuating asymmetry</td>
<td>+ Kozlov and Niemala (1999)</td>
</tr>
<tr>
<td></td>
<td>NH\textsubscript{3}</td>
<td>chlorophyll fluorescence</td>
<td>+ Black et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{2}</td>
<td>fluctuating asymmetry</td>
<td>0 Kozlov et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{2}</td>
<td>drop contact angle</td>
<td>- Cape et al. (1983)</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>urban versus rural</td>
<td>stomatal density</td>
<td>Kardel et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>urban versus rural</td>
<td>stomatal pore surface</td>
<td>- Kardel et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>urban versus rural</td>
<td>stomatal conductance</td>
<td>- Kardel et al. (2010)</td>
</tr>
<tr>
<td>Pongamia pinnata</td>
<td>SO\textsubscript{2}, NO\textsubscript{2}</td>
<td>total carbohydrate</td>
<td>- Bamniya et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{2}, NO\textsubscript{2}</td>
<td>total protein</td>
<td>- Bamniya et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{2}, NO\textsubscript{2}</td>
<td>chlorophyll content</td>
<td>- Bamniya et al. (2012)</td>
</tr>
<tr>
<td>Populus nigra</td>
<td>O\textsubscript{3}</td>
<td>chlorosis</td>
<td>+ Novak et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{3}</td>
<td>drop contact angle</td>
<td>- Schreuder et al. (2001)</td>
</tr>
<tr>
<td>Populus x euramericana</td>
<td>O\textsubscript{3}</td>
<td>tannins content</td>
<td>+ Giacomo et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>O\textsubscript{3}</td>
<td>amount chloroplasts</td>
<td>- Giacomo et al. (2010)</td>
</tr>
<tr>
<td>Robinia pseudoacacia</td>
<td>NO\textsubscript{2}, O\textsubscript{3}</td>
<td>mesophyll thickness</td>
<td>+ Rashidi et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{2}, O\textsubscript{3}</td>
<td>stomatal density</td>
<td>+ Rashidi et al. (2012)</td>
</tr>
<tr>
<td>Salix borealis</td>
<td>SO\textsubscript{2}, heavy metals</td>
<td>fluctuating asymmetry</td>
<td>0 Zvereva et al. (1997)</td>
</tr>
<tr>
<td>Taraxacum officinale</td>
<td>VOC</td>
<td>chlorophyll a</td>
<td>+ Cape et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{2}</td>
<td>stomatal pore surface</td>
<td>- Balasooriya et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{2}</td>
<td>stomatal density</td>
<td>+ Balasooriya et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{2}</td>
<td>δ13 C</td>
<td>- Balasooriya et al. (2008)</td>
</tr>
<tr>
<td>Trifolium pratense</td>
<td>VOC</td>
<td>drop contact angle</td>
<td>0 Cape et al. (2003)</td>
</tr>
<tr>
<td>Quercus robur</td>
<td>trace elements</td>
<td></td>
<td>- Aboal et al. (2004)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>Metals</td>
<td></td>
<td>+ Monaci et al. (2000)</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td>NH\textsubscript{3}</td>
<td></td>
<td>- Leith et al. (2009)</td>
</tr>
<tr>
<td>Salix alba</td>
<td>heavy metals</td>
<td></td>
<td>+ Vasheggy et al. (2005)</td>
</tr>
</tbody>
</table>
3.6.4 Work plan

Air pollutants have been abundantly associated with many adverse ecological effects, such as vitality losses, decreasing species diversity and shifts in community composition (Spellerberg, 1998). To protect vegetation, air quality limit values were established for the most important pollutants and concentrations of these pollutants are measured by air quality monitoring stations using physico-chemical methods. However, the knowledge of the effect of mixtures of air pollutants is not sufficient to determine from physico-chemical measurements alone the cumulative, antagonistic or synergistic effect of air pollution on a plant (Fuhrer et al., 1997). Consequently, a rising interest in biomonitoring, which gives a more realistic assessment of the impact of air quality on ecosystems, is observed (Falla et al. 2000). As plants are immobile and more sensitive in terms of physiological reaction to the common air pollutants than humans and animals, they better reflect local conditions (Raz et al. 2011).

Biomonitoring can be performed through analyses on the vegetation already present in a given study area (so-called passive biomonitoring), or carried out with selected test plants introduced at the study site (active biomonitoring) (Nali and Lorenzini, 2007). Several active biomonitoring studies have been carried out under controlled circumstances in open top chambers or greenhouses (Broadmeadow and Jackson 2000, Monaci et al. 2000, Novak et al. 2003) and under field conditions (in the vicinity of point sources) (Calzoni et al., 2007, Franzaring et al., 2007, Rey-Asensio and Carballeira, 2007) to investigate the effect of (extremely high concentrations of) various (mixtures of) air pollutants on plants. The work plan was:
i) Air pollution tolerance index (APTI) was used to assess the tolerance/resistance power of plants against air pollution.

ii) By combining the resultant APTI values with some relevant biological and socio-economic characters (plant habit, canopy structure, type of plant, laminar structure and economic value), the API was calculated for different species.

iii) To determine the effect of air pollution exposure and damage on the chlorophyll, pheophytin, carotenoid, porphyrins, chlorophyllide, and less polar caroteoid content of five tree species growing near sugar mill and residential area.

iv) Chlorophyll a, chlorophyll b and carotenoids of five tree species growing near sugar mill were determined using various solvents for extracting various pigments and were examined comparatively.

v) Linear regression analysis was performed between independent variables such as Chlorophyll, pH, RWC, ascorbic acid and dependent variable such as APTI by using XL STAT (Version 10) software.

3.6.5 Working Strategy

UV spectrophotometer, pH meter and homogenizer were used to perform the proposed work.

3.6.6 Sampling

Plant characteristics, mainly traditional fitness components such as growth and total biomass production, are frequently used in air quality studies (Woodbury and Laurence 1994,
Figure 3.6.6 a) Sampling of leaves near sugar industry
Figure 3.6.6 b) Leaves of *Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica* and *Pongamia pinnata*
Sant Anna-Santos et al. 2006). For example, Lovett et al. (2009) showed that O$_3$ affects the cell membrane functioning, leading to reduction in photosynthesis and thus slower tree growth. Unfortunately, growth and biomass production determination is destructive and time consuming. Instead, morphological (specific leaf area (SLA)), physiological (chlorophyll fluorescence, stomatal resistance (RS), anatomical (leaf wettability) and biochemical (chlorophyll content, malondialdehyde) leaf characteristics can be used as rapid, non-destructive, diagnostic monitoring tools.

The research work was mainly confined near sugar factory. Leaves of five tree species such as Ficus benghalensis, Delonix regia, Ficus religiosa, Azadirachta indica and Pongamia pinnata were collected from polluted area and residential area. The screening and selection of the tree species was partly based on literature survey of similar work and guidelines of Central Pollution Control Board. The five leaf samples were collected at the lower most position of canopy at a height of 6-7ft from the ground surface.

3.6.7 Sample Preservation

Samples were cleaned with distilled water and then refrigerated (22ºC) under suitable condition for biochemical analysis.

3.6.8 Sample treatment and analysis

Leaves of *Ficus benghalensis*, *Delonix regia*, *Ficus religiosa*, *Azadirachta indica* and *Pongamia pinnata* tree species were washed thoroughly with tap water for about 8–10 times. It was again washed thoroughly with distilled water for about two times to remove all minor traces of clay, silt, sand or any other contamination. It was dried in an incubator (BE-200, Memmert,
Germany), at 50 –59 ºC for about 24 hours. After complete dryness, the sample was crushed in grinder (Moulinex)), into fine powder. Homogenization of each sample was done to get uniform results. The powder was then collected in proper sized plastic containers with screw caps.

3.6.9 Determination of biochemical indicators

3.6.9.1 Estimation of pH of leaf

20 grams of air-dried leaf samples were passed through 2 mm sieve and the contents were transferred into a clean 100 ml beaker. It was made up to 50 ml using distilled water. A glass rod was used to stir the contents intermittently. It was allowed to stand for half an hour.

The electrodes were washed with distilled water and wiped off with a piece of filter paper. The leaf solution was stirred again for taking reading. The electrodes were dipped into the leaf solution and the function switch was adjusted to the particular pH range. The pH reading of the supernatant solution and the suspension were recorded.

3.6.9.2 Estimation of percent relative water content of leaf

A weighing bottle with its stopper was placed in an electric oven at 150˚c for about fifteen minutes. The moisture bottle was removed and the stopper was replaced. The moisture bottle was kept in cool dessicator and the leaf sample was transferred to the bottle and the stopper was fixed. It was quickly weighed. The stopper was removed and the bottle was kept in dessicator. The weight was taken. The loss in weight was expressed in percentage.

\[
\text{Weight of moisture bottle alone} = A \text{ gram}
\]

\[
\text{Weight of moisture bottle alone + sample} = B \text{ gram}
\]
Weight of plant sample alone = (B-A) gram

Weight of moisture bottle + sample after drying in the oven = C gram

Weight of water content in the sample = (B-C) gram

Percentage of water content in the sample = (B-C)/(B-A) × 100

3.6.9.3 Estimation of ascorbic acid

10 grams of the leaf sample was ground with glass pestle mortar and macerated well with four percent oxalic acid. The contents were transferred into 100 ml volumetric flask by filtering through muslin cloth.

The extraction was repeated with four percent oxalic acid for 3 to 4 times. The solution was made up with four percent oxalic acid. 10 ml of this solution was pipette out into a 250 ml conical flask and 10 ml of four percent oxalic acid was added. The solution was titrated against 0.02 percent dye solution taken in a burette. The end point was appearance of permanent pale pink colour.

Preparation of standard ascorbic acid solution

100 mg of pure ascorbic acid was transferred into 100 ml volumetric flask and made up to the mark using four percent oxalic acid. It forms an ascorbic acid solution of one mg/mlitre. Flask and made up to the volume using four percent oxalic acid. 10 ml of this solution was pipetted out in to a 100 ml standard flask and made up to the volume using four percent oxalic acid. It provides a concentration of 0.1 mg ascorbic acid/ml, 10 ml of this solution was pipette out in to a 250 ml conical flask and 10 ml of 4 percent oxalic acid was added to it. It was titrated
against 0.02 percent dye solution taken in the burette. The titrated value gave the quality of 0.02 percent dye solution to 1 mg of ascorbic acid. The end point was the appearance of permanent pale pink colour. From the titre values of the sample material, the ascorbic acid content of the sample material was calculated by using the formula suggested by Keller and Schwager (1977).

Volume of 0.02 percent solution used for 10 ml

Of 0.01 mg/ml ascorbic acid standard solution = Vml

V ml of 0.02 percent dye = 1 mg of ascorbic acid

Therefore 1 ml of dye = 1/v mg of ascorbic acid

Volume of dye solution used for sample titration= A ml

Therefore A ml of dye = 1/v × A mg ascorbic acid

Ascorbic acid content of the sample = A × 1× 100/V × 10

3.6.9.4 Estimation of total chlorophyll

One gram of fresh leaves were taken in a porcelain mortar and macerated with 80 percent acetone and centrifuged at 3000 RPM for 10 minutes till the chlorophyll were extracted into the solvent. The supernatant was collected and made up to 10 ml using 80 percent acetone. The optical density of the extract was measured at 645, 663 and 652 nm, using 80 percent acetone as blank. Total chlorophyll content was determined using the formula.

Total chlorophyll content = Optical density value at 652 nm × 1000 ×V (mg1g) / 34.5 × 1000 × W

Where V = Final volume of acetone extract, W = Fresh weight in gram.
3.6.9.5 Calculation of Air Pollution Tolerance Index (APTI) of plants

Air pollution tolerance index (APTI) was proposed by Singh and Rao, (1983) to assess the tolerance/resistance power of plants against air pollution. The air pollution tolerance index was calculated using the formula (Singh and Rao, 1983):

\[
\text{APTI} = \frac{A(T + P) + R}{10}
\]

Where A = Ascorbic Acid (mg/g), T = Total Chlorophyll (mg/g - f.w), P = pH of the leaf extract, R = Relative water content of leaf (%). Based on the development and evaluation of APTI values among the samples they were categorized into four groups as given in table 3.6.9.5.1.

Table 3.6.9.5.1 Categories of tree species based on APTI

<table>
<thead>
<tr>
<th>APTI value</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-100</td>
<td>Tolerant</td>
</tr>
<tr>
<td>29-17</td>
<td>Intermediate</td>
</tr>
<tr>
<td>16-1</td>
<td>Sensitive</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>Very sensitive</td>
</tr>
</tbody>
</table>
3.6.9.6 Calculation of Anticipated Pollution Index (API)

By combining the resultant APTI values with some relevant biological and socio-economic characters (plant habit, canopy structure, type of plant, laminar structure and economic value), the API was calculated for different species. Based on these characters, different grades (+ or -) are allotted to plants. Different plants are scored according to their grades (Dali Mondal et al, 2011). The criteria used for calculating the API of different plant species are given in tables 3.6.9.6.1 and 3.6.9.6.2.

Table 3.6.9.6.1 Anticipated Performance Index (API) of plant species

<table>
<thead>
<tr>
<th>Grade</th>
<th>Score (%)</th>
<th>Assessment category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Up to 30</td>
<td>Not recommended</td>
</tr>
<tr>
<td>1</td>
<td>31-40</td>
<td>Very poor</td>
</tr>
<tr>
<td>2</td>
<td>41-50</td>
<td>Poor</td>
</tr>
<tr>
<td>3</td>
<td>51-60</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>61-70</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>71-80</td>
<td>Very good</td>
</tr>
<tr>
<td>6</td>
<td>81-90</td>
<td>Excellent</td>
</tr>
<tr>
<td>7</td>
<td>91-100</td>
<td>Best</td>
</tr>
</tbody>
</table>
### Table 3.6.9.6.2 Gradation of plant species on the basis of Air Pollution Tolerance Index

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Grading character</th>
<th>Pattern Assessment</th>
<th>Grade allotted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tolerance</td>
<td>Air Pollution Tolerance Index (APTI)</td>
<td>12.0-16.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.1-20.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.1-24.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.1-28.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.1-32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32.1-36.0</td>
</tr>
<tr>
<td>2</td>
<td>Biological and Socio-Economic</td>
<td>Plant Habitat</td>
<td>Small</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Large</td>
</tr>
<tr>
<td></td>
<td>Canopy Structure</td>
<td>Sparse/Irregular/Globular</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spreading crown/open/semi dense</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spreading dense</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Type of plant</td>
<td>Deciduous</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Evergreen</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Size</td>
<td>Small</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Texture</td>
<td>Smooth coriaceous</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Laminar structure</td>
<td>Hardness</td>
<td>Delineate Hardy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Economic value</td>
<td>Less than three uses</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Three or four uses</td>
</tr>
</tbody>
</table>
3.6.10 Determination of Chlorophyll and its derivatives

Chlorophyll a, b and Carotenoid:

The preweighed samples of five tree species were dissolved in 80% acetone (20 ml per each gram) and grained using mortar and pestle and then homogenized using a homogenizer at 1000 rpm for about 5 minutes. Then the samples were filtered using cheese cloth. The extracts obtained were centrifuged at 5000 rpm for about 10 minutes. The supernatants were separated and absorbances were read at 200 – 700 nm on UV spectrophotometer (figure 3.6.10.1). The experiment was repeated thrice for statistical analysis. The content of chlorophyll a, b and carotenoid were calculated using the equations of Porr et al (1989) and Holm (1954) respectively.

\[
\text{Chl a} = 12.25 A_{663.6} - 2.55 A_{646} \text{ (µg/ml)}
\]

\[
\text{Chl b} = 20.31 A_{646.6} - 4.91 A_{663.6} \text{ (µg/ml)}
\]

\[
\text{Car} = 4.69 A_{440.5} - 0.267 \text{Chl}_{a+b} \text{ (µg/ml)}
\]

Porphyrrins

The above acetone extract was mixed with equal amount of hexane and kept undisturbed till interface appears. The upper phase contains less polar compounds dissolved in hexane and the lower phase contains more polar compounds dissolved in acetone. The upper and lower fractions were separated and the lower fraction was used to measure the absorbance at 575, 590 and 628 nm. The equation of Khan et al (1976) was hired to determine the content of
protoporphyrin IX (PPIX), magnesium-protoporphyrin IX (MGPP) and protochlorophyllide (Pchlide).

\[
PPIX = 196.25 A_{575} - 46.6 A_{590} - 58.68 A_{628} \text{ (nmole)}
\]

\[
MGPP = 61.81 A_{590} - 23.77 A_{575} - 3.55 A_{628} \text{ (nmole)},
\]

\[
Pchlide = 42.59 A_{628} - 34.22 A_{575} - 7.25 A_{590} \text{ (nmole)}
\]

**Chlorophyllide (Chlide) a and b**

The lower acetone fraction was used to measure the absorbance at 667 and 650 nm. The Beer-Lambert law and method of McFeeters (1971) was used to calculate the content of chlorophyllide a and b.

\[
\text{Chlide } a = A_{667}/ 74.9 \text{ (mM)}
\]

\[
\text{Chlide } b = A_{650}/ 47.2 \text{ (mM)}
\]

**Pheophytin (Phe) a and b**

The upper hexane fraction was dried with nitrogen and dissolved in 80% acetone. The absorbance were measured at 665.4 and 663.4 nm, which were major absorption peaks of pheophytin a and b respectively. The content of pheophytin a and b were calculated using the equation of Lichtenthaler (1987).

\[
Phe a = 22.42 A_{665.4} - 6.81 A_{653.4} \text{ (µg/ml)},
\]

\[
Phe b = 40.17 A_{653.4} - 18.58 A_{665.4} \text{ (µg/ml)}
\]
Less polar carotenoid (LP Car)

The acetone fraction dissolved in HCl was used and the absorbance was measured at 470 nm. The content of less polar carotenoid was calculated using Lichtenthaler equation.

\[
LP \text{ Car} = \frac{(1000 \, A_{470} - 4.28 \, A_{665.4} - 4.78 \, A_{653.4})}{164} \, (\mu g/ml)
\]

Plant pigments are degraded into small molecules recycling into the nature after completing their mission. Eight steps are involved in the degradation pathway of chlorophyll to pheophorbide (figure 3.6.10.1).

![Degradation pathway of chlorophyll](image-url)
Figure 3.6.10.1 Determination of Chlorophyll and its derivatives
3.6.11 Determination of chlorophylls a, b and total carotenoids using various solvents

Extraction process

The preweighed samples of five tree species were put separately in acetone (80% and 100%), ethanol and ethyl acetate (20 ml per each gram) were grained using mortar and pestle and then homogenized using a homogenizer at 1000 rpm for about 5 minutes. Then the samples were filtered using cheese cloth. The extracts obtained were centrifuged at 5000 rpm for about 10 minutes. The supernatants were separated and absorbances were read at 400 – 700 nm on UV spectrophotometer. Maximum absorbance of chlorophyll a, is at 662 nm, chlorophyll b, is at 646 nm and for total carotenoid, 470 nm. The experiment was repeated thrice for statistical analysis. The amounts of pigments present in them were calculated according to the formula of Lichtentaler and Wellburn (1985) and tabulated in table 3.6.11.1.

Table 3.6.11.1 Formula used in the calculation of pigments

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Solvents</th>
<th>Formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80% Acetone</td>
<td>Chlorophyll a = 12.21 A$<em>{663}$ – 281 A$</em>{646}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorophyll b = 20.13 A$<em>{646}$ – 5.03 A$</em>{663}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carotenoids = 1000 A$_{470}$ – 3.27[Chl a] – 104 [Chl b]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total pigments = chlorophyll a + chlorophyll b + carotenoids</td>
</tr>
<tr>
<td>2</td>
<td>100% Acetone</td>
<td>Chlorophyll a = 11.75 A$<em>{662}$ – 2.350 A$</em>{645}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorophyll b = 18.61 A$<em>{645}$ – 3.960 A$</em>{662}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carotenoids = 1000 A$_{470}$ – 2.270 [Chl a] – 81.4 [Chl b]/230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total pigments = chlorophyll a + chlorophyll b + carotenoids</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>Chlorophyll a = 15.65 A$<em>{666}$ – 7.340 A$</em>{653}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorophyll b = 27.05 A$<em>{653}$ – 11.21 A$</em>{666}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carotenoids = 1000 A$_{470}$ – 2.860 [Chl a] – 85.9 [Chl b]/245</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total pigments = chlorophyll a + chlorophyll b + carotenoids</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>Chlorophyll a = 11.75 A$<em>{662}$ – 2.350 A$</em>{645}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorophyll b = 18.61 A$<em>{645}$ – 3.960 A$</em>{662}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carotenoids = 1000 A$_{470}$ – 2.270 [Chl a] – 81.4 [Chl b]/230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total pigments = chlorophyll a + chlorophyll b + carotenoids</td>
</tr>
</tbody>
</table>
3.7 SEM-EDAX characterization of deposited particles in soil and tree leaves

SEM is a powerful technique applied in micro-imaging of a variety of surfaces. This technique can be used in exploring the surface structure to determine particle size and texture on that surface. The surface of a solid sample is scanned in a raster pattern with a beam of energetic electrons. Several types of signals are produced from a surface in this process, including backscattered, secondary, and Auger electrons; X ray fluorescence photons; and other photons of various energies. All of these signals have been used for surface studies, but the two most common are (1) backscattered and secondary electrons, which serve as the basis of SEM, and (2) X ray emission, which is used in electron microprobe analysis (Skoog et al., 1971).

Figure 3.7.1 (Connor et al., 2003) shows the essential elements of SEM. The electron gun, fitted with a W, LaB₆ or Field Emission (FE) gun operates typically over the range 0.1-30 kV accelerating voltage. A condenser lens produces a demagnified image of the source, which in turn is imaged by the probe forming lens onto the specimen. The electron path and sample chamber are evacuated. Scanning coils deflect the probe over the rectangular raster, the size of which, relative to the display screen, determines the magnification. Detectors collect the emitted electron signals, which after suitable amplification can be used to modulate the intensity of the beam of the display video screen, which is rastered in synchronism with the probe.
EDAX is widely used technique to analyze the chemical components in a material under SEM. This method detects the X-rays produced as the result of the electron beam interactions with the sample. Mapping of the distribution of the different chemical elements constituting the specimen can be obtained. X-ray data is processed to obtain the percentage of each measured element present in the individual particles. The compositional and morphological data are then combined for exploratory data analysis.

SEM analysis of the soil sample

Scanning electron microscopy (SEM) is used to understand the morphology of the contaminated soil sample. The SEM analysis was carried out with the help of a computer controlled field emission.

SEM-EDS analysis of the plant samples

The leaves of *Azadirachta indica*, *Albizia lebbeck* and *Pongamia pinnata* were collected from sugar mill. The leaves are thoroughly washed with ample water to remove clay, sands,
dusts and associated algae. The cleaned leaves samples were shade dried and dried in oven at 60 °C for four hours to remove moisture content. Dried samples were ground into fine powder using agate mortar. These samples are used for the SEM-EDS (Scanning Electron Microscope–Energy Dispersive Spectrometer) analysis.

3.8 Effluent treatment

3.8.1. Aim and Scope

Om et al. (1994), while studying the combined effect of different concentrations of wastes of distillery and sugar mill, observed inhibition of seed germination, seedling growth and biomass in okra (*Abelmoschus esculentus* L.). Experiments conducted by Dutta and Boissya (1999) for studying the effect of low concentration of paper mill effluent on growth and field NPK contents in rice showed increase in growth and yield of crop. Kaushik et al. (1996) reported that low concentrations of sugar factory effluent had no effect on seed germination of *Triticum aestivum* L. Muhammad and Khan (1985) found that industrial effluent reduced the germination percentage of kidney bean (*Phaseolus aureus*) and ladyfinger (*Abelmoschus esculentus*). While working with *Cicer arietinum*, Dayama (1987) reported that even highly diluted industrial effluent (5% of industrial effluent) adversely reduced the seed germination.

Vegetables and pulses are of primary importance for meeting the food requirements of people world-wide, because they are the key source of essential components such as vitamins, minerals and dietary fibers (Ogle et al. 2001; Mukerji 2004) in an adequate and balanced human diet. Radish root is rich in ascorbic acid, folic acid, and potassium.

Keeping in view the importance of vegetables and pulses in human health, adverse effects of polluted water on their growth, it is essential to examine whether irrigation with treated
industrial effluents have a beneficial effects on vegetables and pulses. Thus, the present study was aimed to assess whether treated waste water could safely be used to irrigate crop plants.

3.8.2 Motivation

Karunyal et al., (1994) studied the effects of tannery effluent at different concentrations (25, 50, 75 and 100%) on seed germination of *Oryza sativa* and found that the germination was inhibited by 25 and 50% effluent and completely suppressed by 75 and 100% effluent. Application of spentwash with 50 times dilution in rice (CO43) resulted in normal yield (Rajannan et al., 1998). The maximum grain yield was recorded in rice variety ADT 42 due to 75 times diluted distillery spentwash treatments which was on par with 100 times diluted spentwash application (Chinnusamy et al., 2001).

Augusthy and Mani (2001) conducted physico-chemical analysis of the rubber factory effluents and revealed that high amounts of total suspended and dissolved solids, sulphate, phosphate, total nitrogen were also present in significant amounts. On evaluation of seed quality responses with *Vigna radiata* found that at higher concentration (above 50%) of effluent, the seed germination percentage was retarded. Diluted effluent (up to 50%) favoured seedling growth. Seedling length and number of lateral roots were increased by low concentrations of effluent.

Aliotta et al., (2002) revealed that olive Mill waste water had phytotoxic influence on wheat cv.Ofonto due to the polyphenoles and other unidentified substances. Kaushik *et al.* (2005) conducted laboratory experiments to study the effect of textile effluents at different concentrations in the range of 0-100% (untreated and treated) on seed germination (%), delay index (DI), plant shoot and root length, plant biomass, chlorophyll content and carotenoid of three different cultivars of wheat.
Rani and Alikhan (2007) studied the effect of treated distillery effluent on two cultivars of *Oryza sativa* L. Cv. Saka-4 and Pusa 44 after diluted with tap water that is 100, 50 and 25% in petriplates over the control. Singh et al., (2007) observed that the percentage germination and seedling vigour of rice and wheat decreased significantly with an increase in spentwash concentration.

A laboratory work was undertaken to assess the waste water quality parameters of treated distillery effluent and their effect of various concentrations like 0%, 25%, 50%, 75% and 100% on seeds germination, speed of germination, peak value and germination value of three selected seeds that is Wheat (*Triticum aestivum*), Pea(*Pisum sativum*) and Lady’s Finger (*Abelmoschus esculentus*), where the highvalue of T.S (4285), B.O.D (544.5) and C.O.D. (2433) indicates the high inorganic and organic load. Germination percentage decreases with increasing concentration of effluent in all the tested seeds, where as the germination speed, peak value and germination value increases from control to 25% and 50% concentration and decreases from 50% to 75% and 100% effluent (Pandey et al., 2007).

The higher concentration of mixed effluent was not advisable for irrigation purpose, however, it could be used for irrigation purpose after proper treatment and dilution (one part treated effluent and five parts of available irrigation water), as this dilution level was found growth and yield promotory (Kamlesh et al., 2007). Nagajyoti et al., (2008) grew *Arachis hypogaea* in pots for a period of 30 days where the soils were treated with different effluent concentrations (25, 50, 75 and 100%) collected near an industrial area and a control was carried out.
Dhanam and Arulbalachandran (2009) studied pollution by Briquetting and Carbonization plant effluent and its effect on five varieties of groundnut to evaluate its irrigation potential. The effluent is brownish black in colour. It contains higher amount of total nitrogen, metallic and non-metallic ions, sulphates, sodium, chloride, calcium and magnesium.

Abida et al., (2009) conducted growth room experiments to assess as to whether treated textile effluent could be safely used to irrigate some winter vegetables. Varying levels of treated and untreated textile effluents were applied to germinating seeds of some winter vegetables and their effect was evaluated on germination and early growth stage using seed germination, growth, and biochemical attributes. The wastewater of a tannery in Multan, Pakistan, was analyzed by Hussain et al., (2010). It was alkaline with high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values along with much higher concentrations of total settleable salts and suspended solids, sodium adsorption ratio and high amount of sodium having the water quality class $C_3S_1$.

### 3.8.3 Research Objectives

The following main objectives for the treatment of effluent are i) In view of shortage of water, sugar mill effluent could safely be used for irrigation to crops after proper processing, ii) A simple and cost effective treatment procedure for the removal of organic matter through the adsorption on tree leaves.

### 3.8.4 Work plan

For the treatment of the effluent, the salient points were considered, i) To perform pot experiment using various concentrations of treated effluents (10%, 25%, 50%, 75% and 100%)
and ii) To treat the sugar mill effluent using Mangifera indica leaf powder and to compare the suitability of different kinetic equations and to investigate the relationships between the parameters of the various equations.

3.8.5 Working Strategy

The proposed work has been performed with the help of available/ existing facilities such as UV spectrophotometer, pH meter, Conductivity meter, TDS meter and Turbidity meter.

3.8.6 Sampling

The effluent samples for this study was collected from the effluent discharge stream of sugar mill. On the day of sampling, the samples were collected in two litre polythene can, once in four hour for 24 hour and mixed in equal proportions to get uniform homogeneous samples. The sampled leaves were taken from the upper third of the crown. 10-20 grams of fresh matured leaves for each sampled age class were chosen.

3.8.7 Sample Preservation

For broad-leaved trees, the leaves were detached from the twigs (and for some species, the small leaves from the axis). The shoots of the current year and those of the second year are separated and preserved in separate bags. The samples were dried in a clean room and stored in a cool place in perforated polyethylene bags. These identifications were given on the outer side of the bag (directly on the bag by indelible ink, or by clasping a label on the bag). The identification was also given on the inner side of the bag on a paper label written with indelible ink. The label should be folded in order to avoid leaves contamination by contact with ink.
3.8.8 Sample treatment and analysis

It was not necessary to cut the petioles of the leaves. The samples shall be washed with distilled water. Oven drying was done at a maximum of 80°C, for at least for 24 hours. Dry samples should be ground in order to obtain a fine homogeneous powder. There will always remain some fibres, depending on the tree species; this was not a major inconvenience if they are small and if the powder was mixed carefully before taking samples for analysis.

3.8.9 Pot Experiment

In this experiment, 100 seeds of green gram (Vigna radiata) and radish (Raphanus sativus) seeds sown in each plastic pot (10 cm diameter and 10 cm height) filled with 1 kg ordinary river sand washed well first with tap water and then with distilled water so as to flush through all the salts previously present in the sand. The various concentrations of treated effluents (10%, 25%, 50%, 75% and 100%) were taken for the study in triplicate. This experiment prolonged for 14 days.

3.8.10 Estimation of chlorophyll and carotenoid

The preweighed samples were put in 80% acetone and grained using mortar and pestle and then homogenized using a homogenizer at 1000 rpm for about 5 minutes. Then the samples were filtered using cheese cloth. The extracts obtained were centrifuged at 5000 rpm for about 10 minutes. The supernatants were separated and absorbances were read at 400 – 700 nm on UV spectrophotometer. Maximum absorbance of chlorophyll a, is at 662 nm, chlorophyll b, is at 646 nm and for total carotenoid, 470 nm. The experiment was repeated thrice for statistical analysis. The amounts of pigments present in them were calculated according to the formula of Lichtentaler and Wellburn (1985)
Chlorophyll a = 12.21 $A_{663} - 281 A_{646}$

Chlorophyll b = 20.13 $A_{646} - 5.03 A_{663}$

Carotenoids = $1000 A_{470} - 3.27[\text{Chl a}] - 104 [\text{Chl b}]$

Total pigments = chlorophyll a + chlorophyll b + carotenoids

3.8.11 Statistical analysis of the data

The data recorded from both these experiments were subjected to two way analysis of variance (ANOVA) using computer software package Costat 6.3 (CoHort, Berkley, California, USA).

3.8.12 Adsorption

Adsorption techniques have gained favour recently due to their efficiency in the removal pollutants too stable for conventional methods. Adsorption produces a high quality product, and is a process, which is economically feasible (Choy, 2003). Also, adsorption process provides an attractive alternative treatment, especially if the adsorbent is inexpensive and readily available (Ozacar and Sengil, 2003). Furthermore this process has the edge on the other method due to its sludge free clean operation and complete removal of dyes even from dilute solution (Malik, 2003). Therefore one of the powerful treatment processes for the removal of dyes from water with a low cost is adsorption.

3.8.12.1 Principles of Adsorption

Adsorption is a surface phenomenon that can be defined as the increase in concentration of a particular component at the surface or interface between two phases. In any solid or liquid, atoms at the surface are subject to unbalanced forces of attraction normal to the surface plane. In
discussing the fundamentals of adsorption, it is useful to distinguish between physical adsorption, involving only relatively weak intermolecular forces, and chemisorption, which involves essentially the formation of a chemical bond between the sorbate molecule and the surface of the adsorbent. Although this distinction is conceptually useful, many cases are intermediate and it is not always possible to categorize a particular system unequivocally.

3.8.12.2 Theory of adsorption

Adsorption is a process that substance in solution accumulates on the surface of adsorbents. In the process, the dissolved substances (pollutants) are transferred from the liquid phase to the solid phase, and therefore removed from solution (Tchobanoglous et al., 2003). The adsorbate is the substance removed from solution and the adsorbent is the solid material on which adsorbate accumulates.

3.8.12.3 Sorption mechanisms

With different adsorbates and adsorbents, different mechanisms are involved in adsorption. Monolayer, multilayer, pore filling are the familiar mechanisms for adsorption (Figure 3.8.12.3.1). Besides, in the adsorption of organic compounds, another phenomenon of partitioning might be observed, especially when the adsorbent contains high content of natural organic matter (NOM) (Stefan, 2006).

![Figure 3.8.12.3.1 The sketch of adsorption mechanisms](image)

Figure 3.8.12.3.1 The sketch of adsorption mechanisms
3.8.12.4 Monolayer, multilayer and pore filling

In adsorption processes, the adsorbates can be bound to the solid surface by chemical bonding, Van der Waals force, and electrostatic force to form monolayer or multilayer on the surface of adsorbent.

In monolayer adsorption, all the adsorbates are directly adsorbed on the adsorbent surface. As assumed in Langmuir isotherm model, which is the typical isotherm for monolayer adsorption, the adsorbent has a homogeneous surface, all adsorption sites are energetically equivalent, and there are no interactions between the adsorbed molecules (Langmuir, 1918).

Different from the monolayer assumptions of Langmuir, another popular isotherm, Freundlich isotherm model, supposes a heterogeneous surface of adsorbents with various adsorption sites (Freundlich, 1907). Brunauer-Emmett-Teller (BET) isotherm, which is widely applied for gas-solid system, is built up on multilayer adsorption (Soto et al., 2011). In the multilayer adsorption, adsorption behavior happens not only on the surface of adsorbent itself, the adsorbed molecules on adsorbent surface can be the new ‘surface’ to bind other free adsorbates (Stefan, 2006).

3.8.12.5 Adsorption as a Treatment Process

Adsorption is a process by which material accumulates at the interface between two phases as liquid-liquid, gas-liquid or liquid-solid. The adsorbing phase is called the adsorbent, and any substance being adsorbed is termed as an adsorbate. Adsorption onto solid adsorbents has great environmental significance, since it can effectively remove pollutants from both aqueous and gaseous streams. Due to the high degree of purification that can be achieved, this process is often used at the end of a treatment sequence.
Adsorption is a commonly used method in water treatment and other separation processes. Among the other methods, adsorption is fast and simple in operation. The key factor for the adsorption process is the choice of adsorbent. A good quality adsorbent should have fast kinetics of interaction with the adsorbate, porous structure resulting in high surface area and high adsorption capacity.

3.8.12.6 Adsorbent

The *Mangifera indica* tree leaves were collected from Periyakulam, Tamilnadu, India. They were gathered from twigs into clean plastic bags. Washed with triple distilled water and laid flat on clean table to dry. Dry leaves were grounded with grinder. After grounded, the leaf particles were sieved and stored into plastic bag by size, and ready to use. 40 mesh size of *Mangifera indica* leaf particles (Figure 3.8.12.6.1 and Figure 3.8.12.6.2) were used as adsorbent for these studies.

3.8.12.7 Adsorption Experiment

Adsorption experiments were conducted by varying contact time. The experiments were carried out in 250 mL Erlenmeyer flasks and the total volume of the reaction mixture was kept at 100 mL. The pH of solution was maintained at a desired value by adding 0.1 M NaOH or HCl. The flasks were shaken for the required time period in a water bath shaker.

The kinetics study was carried out by agitating 250 mL flasks containing 2 g of *Mangifera indica* leaf powder and 100 mL lead solutions in water bath shaker. The mixture was agitated at 120 rpm at room temperature. The contact time was varied from 0 to eight hours. At predetermined time, the flasks were withdrawn from the shaker and the reaction mixtures were
filtered through Whatman filter paper No. 40. The isotherm study was performed. All the experiments were performed in duplicates. The filtrate samples were analyzed for COD and BOD. The percentage removal of organic matter from the waste water was calculated according to the following equation:

\[
\% \text{ Removal} = \frac{C_i - C_f}{C_i} \times 100
\]

where \( C_i \) and \( C_f \) are initial and final COD and BOD.

3.8.12.8 Adsorption Equilibrium Study

In general, an adsorption isotherm is an invaluable curve describing the phenomenon governing the retention (or release) or mobility of a substance from the aqueous porous media or aquatic environments to a solid-phase at a constant temperature and pH (Limousin et al., 2007, Allen et al., 2004). Adsorption equilibrium (the ratio between the adsorbed amount with the remaining in the solution) is established when an adsorbate containing phase has been contacted with the adsorbent for sufficient time, with its adsorbate concentration in the bulk solution is in a dynamic balance with the interface concentration (Kumar and Sivanesan, 2007; Ghiaci et al., 2004).

Typically, the mathematical correlation, which constitutes an important role towards the modeling analysis, operational design and applicable practice of the adsorption systems, is usually depicted by graphically expressing the solid-phase against its residual concentration (Neibi, 2008). Its physicochemical parameters together with the underlying thermodynamic assumptions provide an insight into the adsorption mechanism, surface properties as well as the degree of affinity of the adsorbents (Bulut, 2008).

Over the years, a wide variety of equilibrium isotherm models (Langmuir, Freundlich, Brunauer–Emmett–Teller, Redlich–Peterson, Dubinin–Radushkevich, Temkin, Toth, Koble–
Figure 3.8.12.6.1 *Mangifera indica* tree leaves

Figure 3.8.12.6.2 Powdered leaves of *Mangifera indica*
Corrigan, Sips, Khan, Hill, Flory-Huggins and Radke-Prausnitz isotherm), have been formulated in terms of three fundamental approaches (Malek and Farooq, 1996). Kinetic consideration is the first approach to be referred. Hereby, adsorption equilibrium is defined being a state of dynamic equilibrium, with both adsorption and desorption rates are equal (Langmuir, 1916). Whereas, thermodynamics, being a base of the second approach, can provide a framework of deriving numerous forms of adsorption isotherm models (De Boer, 1968; Myers and Prausnitz, 1965), and potential theory, as the third approach, usually conveys the main idea in the generation of characteristic curve (Dubinin, 1960). However, an interesting trend in the isotherm modeling is the derivation in more than one approach, thus directing to the difference in the physical interpretation of the model parameters (Ruthven, 1984).

An accuracy of an isotherm model is generally a function of the number of independent parameters, while its popularity in relation to the process application is an indicative of its mathematical simplicity (Malek and Farooq, 1996). Undoubtedly, linear regression analysis has frequently been employed in accessing the quality of fits and adsorption performance (Kundu and Gupta, 2006) primarily owing to its wide usefulness in a variety of adsorption data (Rivas et al., 2006) and partly reflecting the appealing simplicity of its equations (Ayoob and Gupta, 2008). However, during the last few years, a development interest in the utilization of nonlinear optimization modeling has been noted (Prasad and Srivastava, 2009). A number of researches have been advocated to investigate the applicability of linear or nonlinear isotherm models in describing the adsorption of dyes, heavy metals and organic pollutants onto activated carbons, zeolites, chitosans, bentonites, montmorillonites, kaolinites and a list of low-cost adsorbents.

In 1984, Harter (1984) had firstly examined Langmuir isotherm model in an ions adsorption system (adsorption of ion phosphate, zinc, and copper by soil). Without sufficient
ranges of adsorbate concentration, he emphasized that the estimation of maximum adsorption capacity could be quite misleading (in error by 50% or more), reducing the variability of its linearity. In 1988, Persoff and Thomas (1988) had proposed the use of nonlinear least-squares (NLLS) curve-fitting method for determination of the Michaelis–Menten and Langmuir adsorption isotherm constants (from the experimental data). From the application, they concluded that weighted NLLS yielded a more precise and accurate estimation. More recently, similar observations have been reported by several researchers (Jumasiah, 2005; Lai et al., 2008). The authors suggested that the linearized equations apparently generate real problems and errors arising from the complexities and complications for simultaneous transformation of data, leading to the violation of theories behind the isotherms.

It is important to evaluate the most appropriate correlations for equilibrium curves, to optimize the design of a sorption system. Langmuir, Freundlich and Tempkin isotherm models were used to describe the adsorption equilibrium.

3.8.12.8.1 Two parameter isotherms

3.8.12.8.1.1 Langmuir isotherm

This describes quantitatively the formation of a monolayer adsorbate on the outer surface of the adsorbent, and after that no further adsorption takes place. Thereby, the Langmuir represents the equilibrium distribution of metal ions between the solid and liquid phases (Vermeulan et al., 1966). The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. The model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of the surface. Based upon these assumptions, Langmuir represented the following equation:
\[
\frac{C_a}{Q_0} = \frac{Q_0 K_L C_e}{1 + K_L C_e}
\]

Langmuir adsorption parameters were determined by transforming the Langmuir equation into linear form.

\[
\frac{1}{C_a} = \frac{1}{Q_0} + \frac{1}{Q_0 K_L C_e}
\]

Where \(C_e\) = the equilibrium concentration of adsorbate (mg/L-1) \(C_a\) = the amount of metal adsorbed per gram of the adsorbent at equilibrium (mg/g). \(Q_0\) = maximum monolayer coverage capacity (mg/g) \(K_L\) = Langmuir isotherm constant (L/mg).

The values of \(Q_0\) and \(K_L\) were computed from the slope and intercept of the Langmuir plot of \(1/C_a\) versus \(1/C_e\).

### 3.8.12.8.1.2 Freundlich Isotherm

Freundlich isotherm (Freundlich, 1906) is the earliest known relationship describing the non-ideal and reversible adsorption, not restricted to the formation of monolayer. This empirical model can be applied to multilayer adsorption, with non-uniform distribution of adsorption heat and affinities over the heterogeneous surface (Adamson and Gast, 1997). Historically, it is developed for the adsorption of animal charcoal, demonstrating that the ratio of the adsorbate onto a given mass of adsorbent to the solute was not a constant at different solution concentrations (Ahmaruzzaman, 2008). In this perspective, the amount adsorbed is the summation of adsorption on all sites (each having bond energy), with the stronger binding sites are occupied first, until adsorption energy are exponentially decreased upon the completion of adsorption process (Zeldowitsch, 1934).

At present, Freundlich isotherm is widely applied in heterogeneous systems especially for organic compounds or highly interactive species on activated carbon and molecular sieves. The
slope ranges between 0 and 1 is a measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero. Whereas, a value below unity implies chemisorptions process where \(1/n\) above one is an indicative of cooperative adsorption (Haghseresht and Lu, 1998). Recently, Freundlich isotherm is criticized for its limitation of lacking a fundamental thermodynamic basis, not approaching the Henry’s law at vanishing concentrations (Ho et al., 2002).

The empirical Freundlich isotherm is based on the equilibrium relationship between heterogeneous surfaces. This isotherm is derived from the assumption that the adsorption sites are distributed exponentially with respect to the heat of adsorption. The logarithmic linear form of Freundlich isotherm may be represented as follows (Freundlich, 1907; Alagumuthu et al., 2010):

\[
\log C_a = \log K_f + \frac{1}{n_f} \log C_e
\]

where \(K_f\) (L/g) and \(1/n_f\) are the Freundlich constants, indicating the sorption capacity and sorption intensity, respectively.

### 3.8.12.8.1.3 Tempkin Isotherm

Tempkin isotherm is the early model describing the adsorption of hydrogen onto platinum electrodes within the acidic solutions. The isotherm (Tempkin and Pyzhev, 1940) contains a factor that explicitly taking into the account of adsorbent–adsorbate interactions. By ignoring the extremely low and large value of concentrations, the model assumes that heat of adsorption (function of temperature) of all molecules in the layer would decrease linearly rather than logarithmic with coverage (Aharoni and Ungarish, 1977). As implied in the equation, its derivation is characterized by a uniform distribution of binding energies (up to some maximum binding energy). Tempkin equation is excellent for predicting the gas phase equilibrium (when
organization in a tightly packed structure with identical orientation is not necessary), conversely complex adsorption systems including the liquid-phase adsorption isotherms are usually not appropriate to be represented (Kim et al., 2004).

Tempkin isotherm, assumes that the heat of adsorption decreases linearly with the coverage due to adsorbent-adsorbate interaction (Vijayaraghavan et al., 2006). The Tempkin isotherm has generally been applied in the following linear form (Tempkin and Pyozhev, 1940):

\[
Ca = B \ln A + B \ln C_e
\]

\[
B = \frac{RT}{b}
\]

where \( A \) (L/g) is Tempkin isotherm constant, \( b \) (J/mol) is a constant related to heat of sorption, \( R \) is the gas constant (8.314 J/mol K) and \( T \) the absolute temperature (K). A plot of \( C_a \) versus \( \ln C_e \) enables the determination of the isotherm constants \( A, b \) from the slope and intercept.

**3.8.12.9 Kinetic Models**

**3.8.12.9.1 Ordered Models**

The kinetics of adsorption at solid/liquid interfaces is of crucial importance in biological systems and for a variety of technological processes (Rudzinski and Plazinski, 2007). The overall sorption process may be controlled by film diffusion, intraparticle diffusion or sorption on the surface (Febrianto et al., 2009). In order to gain a better understanding of the biosorption process, various kinetic models are used to test experimental data. The pseudo first-order (Abdelwahab, 2007; Ngah and Hanafiah, 2008), pseudo second-order (Ho and MacKay, 1999;
Chojnacka, 2006; Pamukoglu and Kargi, 2006) and intraparticle diffusion (Gulnaz et al., 2005; Ncibi et al., 2008) models are used frequently. Modelling of kinetic data is fundamental for the industrial application of sorption because it enables comparison among different biosorbents under different operational conditions (Anirudhan and Radhakrishnan, 2008). Modelling offers useful information to gain insight into adsorption mechanisms and to design fixed-bed systems (Qiu et al., 2009; Farooq et al., 2010). It is important in optimizing operational conditions for pollutant removal from wastewater systems.

The prediction of the rate-limiting step is an important factor in the design of the adsorption process (Kalavathy et al., 2005). It is governed by the adsorption mechanism. For a solid–liquid sorption process, the solute transfer is usually characterized by external mass transfer (boundary layer diffusion), or intraparticle diffusion, or both. The mechanisms of adsorption can be analysed in three steps as follows (Kalavathy et al., 2005; Rudzinski and Plazinski, 2007): i) Transport of the solute from the bulk solution through the liquid film to the adsorbent exterior surface, ii) Transport of the adsorbate within the pores of the adsorbent (particle diffusion), iii) Adsorption of the adsorbate on the exterior surface of the adsorbent.

Generally, the last step is the equilibrium reaction and it is very rapid; the resistance is assumed to be negligible (Rudzinski and Plazinski, 2007). The slowest step determines the rate; the rate-controlling parameter can be distributed between intraparticle and film diffusion mechanisms (Yao et al., 2010).

First-order kinetic models often describe reactions at the particle/solution interface. Both single first-order and multi first-order reactions have been described by many investigators (Sparks, 1989; Scheidegger and Sparks, 1996; Sparks, 1995; Bunnett, 1986).
While pseudo first-order models have been used widely to describe the kinetics of chemical reactions on natural materials, a number of other simple kinetic models also have been employed. These include various ordered equations, such as zero-order, pseudo second-order, and fractional-order, and Elovich, power function or fractional power, and parabolic diffusion models. A brief discussion of some of these is given; the final forms of the equations are given in table 3.8.12.9.1.1.

Table 3.8.12.9.1.1 Linear Forms of Kinetic Equations

<table>
<thead>
<tr>
<th>Name of Kinetic modeling</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Order Model</td>
<td>$C_s = C_{so} - k_0 t$</td>
</tr>
<tr>
<td></td>
<td>$C_{so}$ - Initial substrate concentration, mg COD or BOD/L</td>
</tr>
<tr>
<td></td>
<td>$C_s$ – Substrate concentration, mg COD or BOD /L</td>
</tr>
<tr>
<td></td>
<td>$t$ - Degradation time, h</td>
</tr>
<tr>
<td></td>
<td>$k_0$ - Zero-order rate constant, h$^{-1}$</td>
</tr>
<tr>
<td>Pseudo first Order Model</td>
<td>$\ln \frac{C_s}{C_{so}} = -k_1 t$</td>
</tr>
<tr>
<td></td>
<td>$k_1$ – first order rate constant, h$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>(Durai et al., 2011)</td>
</tr>
<tr>
<td>Pseudo second Order Model</td>
<td>$\frac{1}{C_s} = \frac{1}{C_{so}} + \frac{k_2 t}{C_{so}}$</td>
</tr>
<tr>
<td></td>
<td>$k_2$ – second order rate constant, L mg$^{-1}$h$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>(Xu et al., 2006)</td>
</tr>
<tr>
<td>Simple Elovich’s Equation</td>
<td>$C_s = 1/\beta_s \ln (\alpha_s \beta_s) + 1/\beta_s \ln t$</td>
</tr>
<tr>
<td></td>
<td>$\alpha_s$ – initial COD or BOD reduction constant, $\beta_s$ – COD or BOD reduction rate constant</td>
</tr>
<tr>
<td></td>
<td>(Zeldowitsch, 1934)</td>
</tr>
<tr>
<td>Exponential Model</td>
<td>$C_s = at^b$</td>
</tr>
<tr>
<td></td>
<td>$a$ - COD or BOD reduction magnitude constant (L(mg-1h-1)b), $b$ – COD or BOD reduction rate constant</td>
</tr>
</tbody>
</table>

3.8.12.10 Adsorption Diffusion Models

It is generally known that a typical liquid/solid adsorption involves film diffusion, intraparticle diffusion, and mass action. For physical adsorption, mass action is a very rapid
process and can be negligible for kinetic study. Thus, the kinetic process of adsorption is always controlled by liquid film diffusion or intraparticle diffusion, that is, one of the processes should be the rate limiting step. Therefore, adsorption diffusion models are mainly constructed to describe the process of film diffusion and/or intraparticle diffusion.

3.8.12.10.1 Parabolic Diffusion Model

The typical parabolic diffusion equation is given by

$$C_s = C_{so} + kpt^{0.5}$$

where $k_p = \text{Rate constant for parabolic diffusion model.}$

3.8.12.11 Linear Regression Method

Coefficient of determination, which represents the percentage of variability in the dependent variable (the variance about the mean), is employed to analyze the fitting degree of isotherm and kinetic models with the experimental data (Karadag et al., 2007). Its value may vary from 0 to 1 (Kumar and Sivanesan, 2006). The determination coefficient ($R^2$) is defined as the ratio of explained variance to the total variance.
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