Chapter-II

MATERIALS AND METHODS

AMBIENT AIR QUALITY MONITORING

For ambient air quality monitoring at the various selected road sites, the air samples were collected and analysed using High Volume Air Sampler (Envirotech APM - 410). Gaseous pollutants are sampled intermittently for 8 hours, however High Volume Samplers are run for 24 hrs. at the sampling points, for the particulates present in the ambient air (Cadle, 1975 and Manahan, 1983).

(a) Dust fall rate

Dust fall rate is determined by collecting settleable dust in settling jar of known area by placing them at different sites for known period of time (Lyons and Scott, 1990 and Raju, 1997). Dust fall rate (DFR) is calculated by using the formula –

\[ DFR \ (g \ m^{-2} \ day^{-1}) = \frac{g \ particulates}{AC \times n} \]

where,

- \( AC \) = Cross-sectional area \((\pi r^2)\) of the jar mouth in m²
- \( n \) = Number of days for which the jar was exposed.

(b) Particulate Pollutants

Air-borne Total Suspended Particulate Matters (TSPM) are sampled through the glass fibre filter (Whatman EPM-2000) attached to the hopper of the High Volume Sample. The size cut of the sampler inlet is 25 x 20 cm² and particle filtering capacity ranged from 0.1 μm to 100 μm in diameter. Suction rate of
A sampler was around 1.5 m³ of air per minute, drawing a total volume of about 2,000 m³ air per sample (Katz, 1977).

Toxic ions as sulphate and nitrate, soot, dust particles, carbon particles etc. together constitute the Total Suspended Particulate Matter (TSPM), because they remain suspended in air. Calculation for suspended particulate matter (SPM) is made by the following formula:

\[
SPM \left( \mu g \ m^{-3} \right) = \frac{W_2 - W_1}{V} \times 10^6
\]

Where, \( w_2 \) = Weight of the filter paper after sampling (grams)
\( w_1 \) = Weight of the filter paper before sampling or fresh (grams)

(Note: Both \( w_1 \) and \( w_2 \) are to be measured after incubating 24 hours at 20 to 25°C).

\[ V = Q \cdot T \] (cubic meters)

Where, \( V \) = Volume of air sampled
\( Q \) = Average sampling rate (cubic metre per minute)
\( T \) = Sampling time (minutes)

\[ Q = \frac{Q_1 + Q_2}{2} \]

Where, \( Q_1 \) = Initial sampling rate indicated by the orifice metre at the start of sampling
\( Q_2 \) = Final sampling rate indicated by the orifice metre just before the end of sampling.

(c) Gaseous Pollutants

Gaseous pollutants such as \( SO_2 \) and \( NO_2 \) can be sampled by using the portable air sampler. For gaseous pollutants, analysis of the sample is more time consuming than sample collection. The minimum sampling time is 20 minutes per...
sample. At least 6 samples were be collected at an interval of 30 minutes between each sample. For sampling of the gases, such as SO$_2$ and NO$_2$, the probe is connected to impingers containing the absorbent liquid and SO$_2$ was analysed through the methods given by West and Gaeke (1956) and NO$_2$ through the method given by Natusch and Hopke, (1983) and modified by Jacob and Hochheischer (Merryman et al., 1973).

(i) Sulphur dioxide

SO$_2$ gas is collected in 0.1 m Sodium tetra chloromercurate (Na$_2$HgCl$_4$) solution, which was used as an absorbent. Further analysis of the gas is done following West and Gaeke (1956) method. When SO$_2$ from the air stream is absorbed in a sodium tetrachloromercurate solution, it forms a stable dichlorosulphitomercurate. The amount of SO$_2$ is then estimated by the colour produced, when P-rosaniline hydrochloride is added to the solution.

Reagents preparation

The reagent used for the analysis of SO$_2$ is prepared in the laboratory. To prepare absorbing solution (0.1 m solution tetrachloromercurate), we dissolve 27.2 gm (0.1 m) of mercuric chloride and 11.7 gm (0.2 m) of sodium chloride in 1 litre of distilled water. This solution was stored at room temperature for several months. For the preparation of P-Rosaniline, we will dissolve 0.20 gm of P-rosaniline hydrochloride in 100 ml of distilled water and filter after a duration of 48 hours. This solution is stable for at least 3 months, if stored in the dark and cool environment. Further, we will pipette 20 ml of this solution into a 100 ml of volumetric flask, will add 6 ml of concentrated hydrochloric acid. After allowing it to stand for 5 minutes, the solution is diluted to 100 ml with distilled water. Colour of the solution is pale yellow with a greenish tint.

To prepare the solution of Formaldehyde we will dissolve 640 mg of sodium metabisulphite (assay 65% as SO$_2$) in one litre of water. This will yield a
solution of approximately 0.40 mg ml\(^{-1}\) as SO\(_2\). This solution will be standardized by titration with standard 0.01N iodine with starch as indicator and will be adjusted to 0.0123 N. Attention must be given to prepare and standardise the solution freshly.

Making a thin paste of 1.25 gm of soluble starch in cool water and pouring it into the 500 ml of boiling water prepare's starch solution. We will boil the solution for a few minutes with continuous stirring. Then we will cool and store it in glass stoppered bottle.

**Analysis**

For analysis, sample collected in 10 ml of absorbing solution is taken and added to 1.0 ml of P-rosaniline solution, and the solution is mixed well. Further, we will treat 10 ml of unexposed sodium tetra chloromercurate solution in the same manner for use as the blank. If the collecting reagent remains exposed to the atmosphere during interval between sampling and analysis, the blank should also be exposed in same manner. After 20 minutes, we will read the absorbance at 560 \(\mu m\) in a spectrophotometer with the blank as reference.

To prepare calibration curve, we will pipette exactly 2 ml of standard sulphite solution into a 10 ml of volumetric flask and we will dilute it to the mark with absorbing reagent. This final solution contains 3.0 ml of SO\(_2\) per millilitre. We will add exactly 0.5, 1.0, 1.5 and 2.0 ml of the diluted standard sulphide solution to a series of 10 ml volumetric flasks and dilute to the marks with absorbing reagent. We will continue with the method given earlier. We will plot the absorbance (optical density) as the ordinate against the microlitres of SO\(_2\) per 10 ml of absorbing solution on rectangular co-ordinate paper. The slope of the straight-line best fitting to the data will provide the result. The concentration of SO\(_2\) is calculated as follows:
Concentration of SO$_2$ = ($\mu$g SO$_2$/ml x V)/V air

Where, $\mu$g SO$_2$/ml = Value from standard curve
V = Total volume of absorbing solution
V$_{air}$ = Volume of air in m$^3$ (flow rate x time)

With the above mentioned method, the concentration of SO$_2$ at various selected sites has been computed.

(ii) Nitrogen oxides

Sampling and analysis of NO$_2$ gas is based on the modified Jacob and Hochheiser method (Merryman et al. 1973). Nitrogen oxides as nitrogen dioxide are collected by bubbling air through a solution of sodium hydroxide to form a stable solution of nitrate. The nitrite ion produced during sampling is determined colorimetrically by reacting the exposed absorbing reagent with phosphoric acid, sulphanilamide and (1-napthyl) ethylene diamine dihydrochloride.

Reagent preparation

The reagents needed for the analysis of NO$_2$ are prepared in the laboratory. To prepare absorbing reagent, 4.0 gm of sodium hydroxide is dissolved in distilled water and diluted to get 1000 ml solution.

Further, to prepare sulphanilamide solution, 20.0 gm of sulphanilamide is dissolved in 700 ml of distilled water, and 50 ml of concentrated phosphoric acid is added (85%) with constant stirring and diluted to 1000 ml.

To prepare NEDA solution, 0.5 gm of N (1-napthyl) ethylenediamine dihydrochloride is dissolved in distilled water. This solution is stable for a month if refrigerated and protected from light.
To get hydrogen peroxide solution 0.2 ml of 30 percent hydrogen peroxide is dissolved in 250 ml distilled water. The solution may be used for a month if protected from light.

For preparing standard nitrite solution, we will dissolve sufficient desiccated sodium nitrite (assay of 97% or greater), diluted with distilled water to 1000 ml, so that a solution containing 1000 μg nitrogen dioxide per ml, is obtained. The amount of sodium nitrate is calculated as follows:

\[ G = \frac{1.500}{A} \times 100 \]

Where, \( G \) = Amount of sodium nitrite in gms

\( 1.500 \) = Gravimetric factor in converting NO\(_2\) into sodium nitrite, and

\( A \) = Assay, percent

**Analysis**

Sample is collected through sampling tray in 50 ml of absorbing reagent (Sodium hydroxide solution) kept in an impinger. For analysis, we will pipette out 10 ml of the collected sample into a test tube. Now 1.0 ml of hydrogen peroxide solution, 10.0 ml of sulphanilamide solution, and 1.4 ml of NEDA solution are added with thorough mixing after the addition of each reagent. We will prepare a blank in the same manner using 10 ml of absorbing reagent. After an interval of 10 minutes, colour develops and then measurement of absorbance at 540 μm against the blank is carried out. We will read μg nitrogen dioxide per ml from the standard curve.

To prepare the calibration curve, we will dilute 5.0 ml of the 1000 μg nitrogen dioxide per ml solution to 200 ml with absorbing reagent. This solution will contain 25 μg nitrogen dioxide per ml. We will pipette 1, 2, 5 and 15 ml of the
25 μg nitrogen dioxide per ml solution into 50, 50, 100 and 250 ml of volumetric flasks and dilute it to the mark with absorbing reagent. The solutions contain 0.50, 1.00, 1.25 and 1.50 μg nitrogen dioxide per ml respectively. We will plot absorbance versus μg nitrogen dioxide per ml. The concentration of nitrogen dioxide is calculated as follows:

Nitrogen dioxide in μg m⁻³

\[
\frac{\mu g \text{ NO}_2/\text{ml} \times V}{V_{\text{air}} \times 0.35}
\]

Where, μg NO₂ ml⁻¹ = Value from standard curve

\( V \) = Total volume of absorbing solution

\( V_{\text{air}} \) = Volume of air in m³ (flow rate × time)

0.35 = Overall average efficiency

**PHYTOSOCIOLOGICAL STUDY**

The special field of the study of communities with respect to their components, structure and classification, forms the basis of division of ecology called phytosociology. The study of Importance Value Index (IVI) help in establishing relative significance of different grass and need species naturally growing in an area, as it is affected by the ambient environment. Study of frequency, density and dominance is essential for IVI (Mishra, 1954).

**Frequency**

Frequency, the term introduced by Raunkiaer (1934) indicates the number of sampling units in which a given species occurs, and thus expresses the distribution or dispersion of various species in a community. Percentage frequency is calculated as follows –
Number of sampling units in which the species occurred

Frequency = \frac{\text{Number of sampling units in which the species occurred}}{\text{Total number of units studied}} \times 100

Frequency of a species in terms of its dispersion relative to that of all the rest of the species is called as relative frequency (R.F.) and is calculated as given below –

R.F. of a Species = \frac{\text{Number of occurrence of a species}}{\text{Number of occurrence of all species}} \times 100

**Density**

Density represents the numerical strength of species in the community, in the terms of the number of individuals per unit area. Density is calculated by the given formula –

\text{Density} = \frac{\text{Total number of a sp. in all quadrate}}{\text{Total number of quadrate sampled}} \times 100

Relative density (R.D.) is the study of numerical strength of a species in relation to total number of individuals of all species, and can be calculated as under –

R.D. of a Species = \frac{\text{Number of individuals of the species in all quadrats}}{\text{Number of individuals of all species in all quadrats}} \times 100

**Dominance**

Dominance which is also called as “Basal Cover” refers to the ground actually penetrated by the stem (Hanson and Churchill, 1961). Average basal area is calculated using the following formula and is converted into cm².

Average basal area = \pi r^2

Where \pi = 3.14 and \( r \) = radius of stem.

In herbs, radius is measured 2.5 cm above the ground or in the ground level, (Mishra, 1954).
The area of an average individual when multiplied by the density gives the Basal cover or dominance in \( \text{cm}^2 \text{ m}^{-2} \).

Relative dominance (R.D.) is coverage value of a species with respect to the sum of coverage of the rest of species in the area (and not with respect to ground area), and is calculated using the given formula –

\[
\text{R.D. of a Species} = \frac{\text{Total basal area of the species in all the quadrats}}{\text{Total basal area of all the species in all the quadrats}} \times 100
\]

**Importance Value Index**

In any community structure, the quantitative value of each of the frequency, density and dominance has its own importance, but the total picture of ecological importance can not be obtained by any of these. Therefore in order to have a really overall of ecological importance of a species with respect to the community structure, the value of relative frequency, relative density and relative dominance are added together, this value is out of 300 and is called importance value index or IVI of the species.

\[
\text{IVI} = \text{R.F.} + \text{R.D.} + \text{R.Do.}
\]

**PHYTOCHEMICAL ANALYSIS OF HERBS**

Phytochemical analysis of the herbs placed at the polluted and control sites were also done fortnightly. Leaves are the primary acceptor of pollutants (Devina et al., 1991 and Okano et al., 1986) so phytochemical analysis of the new leaves for various parameters was started in March 2005 and was continued till February 2006.

**Photosynthetic Pigments (Chlorophyll)**

0.5 g fresh leaf sample was chopped in 25 ml 80% acetone (acetone : water, 4 : 1 v/v). Tightly plugged flasks were refrigerated for 24 hrs. Finally volume was
maintained 40 ml by 80% acetone and a pinch of MgCO₃ to buffer the extracting medium. Extraction was carried out in dark to avoid photooxidation of pigments. Extract was filtered and centrifuged at 3000 g for 15 min. Optical densities of the solution were measured at 480, 510, 645 and 663 nm wave-lengths. Pigment content were computed by the following formulae given by Duxbury and Yentsch (1956) for carotenoids and Maclachlan and Zalik (1963) for chlorophyll ‘a’ and ‘b’.

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

\[
\text{Chlorophyll ‘b’} = \frac{19.3 D_{645} - 3.6 D_{663}}{1000 \times W} \times V
\]

\[
\text{Carotenoids} = \frac{7.6 D_{680} - 1.49 D_{370}}{1000 \times W} \times V
\]

where,

- \( W \) = Weight of leaf (g),
- \( D \) = Optical density,
- \( V \) = Volume of sample (ml).

**Sugar Content**

Sugar present in the form of glucose in the plant samples was determined by the phenol reagent method (Dubios et al., 1956).

**Ascorbic Acid**

Ascorbic acid content of leaf sample was determined spectrophotometrically. 500 mg of fresh leaf sample was homogenised in 20 ml. extracting medium, prepared by dissolving 500 mg oxalic acid and 75 mg sodium salt of EDTA in 100 ml. distilled water. Homogenised leaf sample was centrifuged for 15 minute at 6000 g. 1.0 ml homogenate was mixed thoroughly with 5.0 ml. Dichlorophenol indophenol (DCPIP) (20.0 µg/ml) with constant shaking. Now optical density of the pink coloured solution was measured at 520 nm (\( E_a \)). To this, one drop of 1% ascorbic acid solution was added to bleach the pink colour completely. Then optical density of the turbid solution (\( E_t \)) was measured at the
same wave-length. A calibration curve was plotted using ascorbic acid solution of varying strength (10-50 \( \mu \)g/m). Ascorbic acid content was calculated by the formula given by Keller and Schwager (1977).

\[
\text{Ascorbic acid (mg/g fresh wt.)} = \frac{(E_0 - E_s - E_t)V}{W \times 1000}
\]

where, \( E_0 \), \( E_s \) and \( E_t \) are optical densities of blank, plant sample and sample with ascorbic acid respectively.

\( V = \) Volume of extract,

\( W = \) Weight of the leaf sample (g).

**Nitrogen**

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

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

where, \( T = \) Volume of \( \text{H}_2\text{SO}_4 \) used in sample titration, ml

\( B = \) Volume of \( \text{H}_2\text{SO}_4 \) used in blank titration, ml

\( N = \) Normality of standard \( \text{H}_2\text{SO}_4 \)

\( S = \) Weight of plant material, gm
Ashing Procedure

The wet ashing technique was adopted for the estimation of phosphorus, potassium and sodium. Plant material (0.2 to 0.5 g) was taken in a Kjeldahl flask and it was digested with ternary acid mixture ($HNO_3 + H_2SO_4 + HCIO_4$ of ratio 10:1:4). The digestion was continued until the major portion of the acid mixture got volatilized and became colourless. The solution was extracted with distilled water and volume was made upto 100 ml. The extract solution was used for the above estimation.

Phosphorus

An aliquot of the extract solution was taken in 50 ml volumetric flask and pH was adjusted at 3 with the help of $Na_2CO_3$ and $H_2SO_4$ using dinitrophenol indicator. To this solution was added sulphomolybdic acid solution and molybdophosphoric acid, blue colour was developed after adding a few drops of chlorostanous acid in the test solution and within the specified time, transmission was read in a colorimeter at 660 m$m$. The concentration of the test solution was determined by referring their percentage transmission to the calibration curve prepared for the standard solution of potassium hydrogen phosphate ($KH_2PO_4$) of different concentrations (Jackson, 1967).

Potassium

Potassium was estimated by Flame Photometer using the extract prepared in ternary acid. Standard solutions of higher and lower concentration were prepared for KCl and $Na_2CO_3$ as described by Jackson (1967). The concentration of test solution were calculated by comparative method using formula as described in water analysis of this chapter.