



**MATERIALS**

**&**

**METHODS**

## CHAPTER- 3

# MATERIALS & METHODS

In the present research work material and different required methods were taken from standards given by APHA (American Public Health Association) and air quality manual of CPCB (Central Pollution Control Board) for better studies. The comparison of studied parameters were done with National Ambient Air Quality Standards 2009 prescribed by CPCB as follows:-

**NATIONAL AMBIENT AIR QUALITY STANDARDS 2009**

Pollutants	Time Weighted Average	Concentration in Ambient Air		Methods of Measurement
		Industrial, Residential, Other Areas	Ecologically Sensitive Areas (Notified by Central Government )	
Sulphur Dioxide (SO <sub>2</sub> ), µg/m <sup>3</sup>	Annual* 24 Hours **	50 80	20 80	-Improved West and Gaeke Method
Nitrogen Dioxide (NO <sub>2</sub> ), µg/m <sup>3</sup>	Annual* 24 Hours **	40 80	30 80	-Jacob & Hochheiser modified (NaOH-NaAsO <sub>2</sub> ) Method
Particulate Matter (Size less than 10 µm) Or PM <sub>10</sub> , µg/m <sup>3</sup>	Annual* 24 Hours **	40 60	40 60	- Gravimetric - TEOM - Beta attenuation
Ozone (O <sub>3</sub> ) µg/m <sup>3</sup>	8 Hours * 1 Hours **	100 180	100 180	-UV Photometric - Chemiluminescence -Chemical Method
Lead (Pb) µg/m <sup>3</sup>	Annual* 24 Hours **	0.50 1.0	0.50 1.0	- AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper - ED-XRF using Teflon filter
Carbon Monoxide(CO), Mg / m <sup>3</sup>	8 Hours ** 1 Hours **	02 04	02 04	-Non dispersive Infrared (NDIR) Spectroscopy
Ammonia (NH <sub>3</sub> ), µg/m <sup>3</sup>	Annual* 24 Hours **	100 400	100 400	- Chemiluminescence - Indolphenol method
Benzene(C <sub>6</sub> H <sub>6</sub> ), µg/m <sup>3</sup>		05	05	-Gas Chromatography (GC) based continuous analyzer -Adsorption and desorption followed by GC analysis
Benzene (a) Pyrene (BaP) Particulate phase only, ng/ m <sup>3</sup>	Annual*	01	01	-Solvent extraction followed by HPLC/GC analysis
Arsenic (As), ng/ m <sup>3</sup>	Annual*	06	06	AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper
Nickel (Ni), ng/ m <sup>3</sup>	Annual*	20	20	AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper

## **Experimental Design:**

The methodology of the proposed work is purely analytical and followed as per CPCB, MoEF Guideline for Ambient Air Quality Monitoring and American Public Health Association, procedure for analysis and testing of air pollutants (APHA). According to CPCB's, NAAQM guideline the most common air pollutants are RSPM, SO<sub>2</sub>, & NO<sub>x</sub>.

### **3.1. Ambient Air Quality Monitoring:**

Central Pollution Control Board initiated its own National Ambient Air Quality Monitoring (NAAQM) Programme in 1984. NAAQM programme, operated by CPCB, many State Boards have set up ambient air quality monitoring stations under their own programme known as State Ambient Air Quality Monitoring (SAAQM) programme. National Environmental Engineering Research Institute (NEERI) monitors ambient air quality at 30 stations covering 10 major cities.

### **3.2. Determination of Sulphur Dioxide in Air (Modified West Geake, 1956)**

Samples for SO<sub>2</sub> were collected with the help of high volume air sampler. Sulphur Dioxide from air is absorbed in a solution of Potassium tetrachloromercurate (TCM) solution was used as an absorbent for SO<sub>2</sub> following the Modified West Geake Method APHA (1977) as modified by CPCB (2013-2014). Samples were collected at the flow rate of one 1 lpm (litre per minutes) at 4 hrs. Intervals each for 24 hrs. Duration at a height 1 to 1.5 m. Sampling was done November, December and February for the year 2011-2012 and 2013-2014. Samples were analyzed using p-Rosaniline hydrochloride solution with the help of visible spectrophotometer at 560 nm wave length. Volume of air samples was calculation by multiplying the average flow rate and time. SO<sub>2</sub> Was calculated using values from standard graph as:

$$C (\text{SO}_2 \mu\text{g m}^{-3}) = \frac{(A_s - A_b) \times CF \times V_s}{V_a \times V_t}$$

Where

C SO<sub>2</sub> = Concentration of Sulphur dioxides,  $\mu\text{g m}^{-3}$

A<sub>s</sub> = Absorbance of sample

A<sub>b</sub> = Absorbance of reagent blank

C<sub>F</sub> = Calibration factor

- $V_a$  = Volume of air sampled,  $m^{-3}$   
 $V_s$  = Volume of air sample, ml  
 $V_t$  = Volume of aliquot taken for analysis, ml

Average of values for 24 hrs. was taken

### 3.3. Determination of Nitrogen oxide in the Atmosphere (Modified Jacobs-Hochheiser, 1958)

The nitrogen oxides were sampled with the help of high volume air sampler. 1N Sodium Hydroxide and Sodium Arsenite solution was used to absorb the NO<sub>x</sub> from ambient air as per APHA (1977) and as modified by CPCB (2013-2014). Sampling was done parallel to SO<sub>2</sub>. The sample was analyzed using NEDA (N- (1-naphthyl) ethylene diamine dihydrochloride) solution and absorbance was measured at 540 nm wavelength using visible spectrophotometer.

Calculation was done as:

$$C (\text{NO}_2 \mu\text{g m}^{-3}) = \frac{(A_s - A_b) \times CF \times V_s}{V_a \times V_t \times 0.82}$$

Where

- $C \text{ NO}_2$  = Concentration of Nitrogen oxides,  $\mu\text{g m}^{-3}$   
 $A_s$  = Absorbance of sample  
 $A_b$  = Absorbance of reagent blank  
 $CF$  = Calibration factor  
 $V_a$  = Volume of air sampled,  $m^{-3}$   
 $V_s$  = Volume of air sample, ml  
 $V_t$  = Volume of aliquot taken for analysis, ml  
 0.82 = Sampling efficiency

### 3.4. Determination of Respirable Suspended Particulate Matter (RSPM) by Gravimetric, Method

Air samples were collected for RSPM using high volume air sampler as per APHA (1977) and as modified by CPCB (2011, 2012, 2013, and 2014). The glass

fiber filter papers (8 X 10 in) 20.3 X 25.4 size were used to collect the samples after proper conditioning and identification. Electronic balance was used for weighing filter papers before and after the sampling. The volume of air sampled was calculated with the help of manometer attached with high volume air sample. Samples were collected by keeping HVS at the height of 1 to 1.5 m from ground level. Filter papers were brought to laboratory in polythene bag for further analysis.

Filter Media – A Glass fiber filter of 20.3 X 25.4 cm (8 X 10 in) size

### **Calculation:**

RSPM concentration was calculated as:

$$C \text{ PM}_{10} \text{ } \mu\text{g}/\text{m}^3 = \frac{(W_f - W_i) \times 10^6}{V}$$

Where,

$C \text{ PM}_{10}$  = Concentration of PM<sub>10</sub>,  $\mu\text{g}/\text{m}^3$

$W_i$  = Initial weight of filter in g

$W_f$  = Final weight of filter in g

$10^6$  = Conversion of g in  $\mu\text{g}$

$V$  = Volume of air sampled,  $\text{m}^3$

Average of 3 values for 24 hrs. was taken

### **3.5. Crop Quality Analysis:**

Biochemical parameters analysis of two crops of each; Kharif: Soybean, Maize & Rabi: Wheat, Gram.

**3.5.1. AIR Tolerance Index (APTI)** from leaves of crops includes: Two crops of each; Rabi: Wheat, Gram & Kharif : Soybean, Maize

#### **3.5.2. Estimation of protein content in seeds (Lowry *et al.*, 1951)**

1. Reagent A - 0.05 N NaOH.
2. Reagent B - 10% trichloro acetic acid.
3. Reagent C - 0.5% of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 1% solution of potassium tartarate.

4. Reagent D - 2% solution of  $\text{Na}_2\text{CO}_3$
5. Reagent E - (Carbonate Copper Solution) 100 ml of reagent D was mixed with one ml of each reagent C.
6. Reagent F - The folin-ciocalate reagent was diluted with double distilled water so as to make it 1N acid and store in refrigerator under complete darkness.

Protein content in the seed/grains was estimated by taking the 100 mg sample, crushed with 10 ml NaOH and centrifuged at 3,000 rpm for 10 min, 0.2 ml supernatant was collected in centrifuge tube and diluted with 2 ml of distilled water and 3 ml of 10 percent trichloro acetic acid (TCA) were added. The intensity of blue color was measured at 660 nm. Standard graph was prepared by dissolving crystalline bovine albumin.

### 3.5. 3. Estimation of total carbohydrates in seeds (Malhotra & Sarkar, 1979)

1. **Copper reagent A** - Dissolve 25 gm of sodium carbonate (anhydrous), 25 gm of Rochelle's salt (PST), and 20gm of sodium bicarbonate and 200gm of sodium sulphate in 800ml of distilled water and dilute it to one liter, filter and store.
2. **Copper reagent B** - 15% of  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$  containing 1 or 2 drops of conc.  $\text{H}_2\text{SO}_4$  per 100 ml.
3. 1N.  $\text{H}_2\text{SO}_4$
4. 1N NaOH
5. Arsenomolybdate

200 mg of dried samples was hydrolyzed with 5 ml of 1 N  $\text{H}_2\text{SO}_4$  at 50 °C for 30 minute. One ml. of extract was taken and to this one ml of copper reagent A & B was added in the ratio of 25:1 ml. Then it was boiled for 80min. and 1 ml. of arsenomolybdate reagent was added. Deep blue colour appears slowly. The volume was made upto 25 ml with distilled water and absorbance was read at 500nm against blank. The absorbance were calibrated from the graph prepared with glucose.

#### **4. Bio-monitoring Assessment Methods:**

**4.1. APTI** of crops and plants was calculated by using following formula:  $APTI = [AA (T + P) + R] / 10$ , where R = Relative water content in mg/g/fw, AA = Ascorbic acid in mg/g/fw, T = Total chlorophyll in mg/g/fw, and P = pH of leaf extract mg/g/fw, (Singh and Rao, 1983).

Classification of crop sensitive / tolerant to the air pollutant on the basis of APTI value is following

<b>S. No</b>	<b>APTI value</b>	<b>Response</b>
1	< 1	Very sensitive
2	10 < APTI < 16	Intermediate/ Mediocre
3	APTI 17	Resistent

**4.2. Photosynthetic pigment Analysis:** For pigment content analysis leaf samples were collected in early morning ( 07.00 to 09.00) hours and brought to the laboratory in polythene bags kept in ice box containing ice to nullify the adverse effects of high light intensity and temperature. During sampling, care being taken that plants of same spp. attaining almost equal height, with approximately similar trunk girth, were selected for sampling. The sampling was carried out from a height of 01 to 02 meter from the ground level.

The leaves were washed with distilled water and cut into small pieces (avoiding veins) and mixed properly. Three replicates, each of 100 mg fresh weight were for further analysis.

The leaves were washed with distilled water and cut into small pieces. 100 mg each fresh leaves were taken and grounded with 5 ml of 80% acetone in a mortar-pestle with a pinch of washed sand. The grounded samples were centrifuged, supernatant was collected and sediment was washed with 2 ml of 80 % acetone and again centrifuged for 3 minutes. The final volume of supernatant was made up to 10 ml by adding 80 % acetone. The samples were analyzed with the help of make visible spectrophotometer. Absorbance was read in 645 to 663 nm for chlorophyll (a) and (b) respectively and 480 and 510 nm for carotenoids. The concentration of chlorophyll

(a), (b) and carotenoid was calculated with the help of absorption coefficient of Arnon (1949). Following formulae were used:

Photosynthetic pigment (mg/g/fw) of leaves = Chl.a + Chl.b+ Carotenoid

$$\text{Chl.a (mg/g)} = 22.7 \times \text{OD } 663 - 2.69 \times \text{OD } 645,$$

$$\text{Chl.b (mg/g)} = 12.9 \times \text{OD } 645 - 4.68 \times \text{OD } 663$$

$$\text{Carotenoid (mg/g)} = 7.6 \times \text{OD } 480 - 1.49 \times \text{OD } 510$$

**4.3. Relative water content (RWC) :**  $\text{RWC} = [\text{FW}-\text{DW}] / (\text{TW}-\text{DW}) \times 100$ , where FW = fresh weight, DW= dry weight and TW= turgid weight.

Fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water over night, blotted dry and weighed to get turgid weight. Now the leaves were dried in an oven at 70 °C and reweighed to obtain the dry weight (Singh, 1977).

**4. 4. Leaf extract pH:** 5 g of the leaves were homogenized in 50 ml deionized water, and then filtered and the pH of filtered leaf extract was determined by digital pH meter (Singh and Rao, 1983).

**4. 5. Ascorbic acid content (AAC):** Ascorbic acid content was determined by taking 1 g of the fresh foliage in a test-tube and 4 ml oxalic acid - EDTA extracting solution was added. Then 1 ml of orthophosphoric acid and 1 ml 5 percent tetraoxosulphate (VI) was added to this mixture. Later 2 ml of ammonium molybdate was added followed by then 3 ml of water. The solution was then allowed to stand for 15 minutes. The absorbance was measured at 660-665 nm with a spectrophotometer (Bajaj and Kaur, 1981).

## **5. Heavy metal estimation:**

Thoroughly washed leaf sample were oven dried and powdered. This was Sieved through 100 mesh screen, and lead, Cadmium, Copper and Nickel. Estimation was done as per Perkin Eimer Manual (1981). 0.5 gram sample of dry powder was digested with 10 ml Con. HNO<sub>3</sub> and allowed to stand overnight. This was heated carefully on hot plate till initiation of red nitric acid fumes and then allowed to cool. Two ml of HClO<sub>4</sub> (70%) was added to it and heated until evaporation. Now dissolved



in 50 ml distilled water and filtered through what man No. 42 filter. The clear solution obtained was analyzed for lead, Cadmium, Copper and Nickel content by atomic absorption spectrophotometer.

## **6. Protein analysis in plant leaves :**

All the Experiments were performed in triplicate and mean values were considered for analysis.

The leaves samples were collected and then sub samples were prepared as described in photosynthesis pigment analysis. The sub samples of 100 mg fresh weight each were crushed in 3ml of 0.05N NaOH solution with a pinch of fine washed sand. Another 3ml of 0.05 N NaOH was used for rinsing. The homogenate was centrifuged to separate the fraction at 3000 RPM for five minutes and supernatant was collected. The sediment was washed twice with 1.5 ml. of 0.05N NaOH and supernatant were added to earlier one. Final volume of the was made up to 10 ml using 0.05 N NaOH.

The protein estimation was done following the method of Lowry *et al.*, (1951), with the help of following reagents:

Reagent A - 2% solution of  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH solutions.

Reagent B - 0.05%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1% Potassium tart rate solution.

Reagent C - Carbonate copper solution (50 ml of reagent A was mixed with 1ml of reagent).

Reagent D - Dilute Folin reagent.

In a centrifuged tube 0.4 ml of the extract was taken and diluted with 2 ml of distilled water and 3 ml of 10% Trichloro acetic acid (TCA) so as to precipitate protein part of the extract. The mixture was placed for 15 minut at room temperature and and then centrifuged for 10 minutes at about 4000 RPM. Sediment was collected. The sediment was dissolved in 0.4 ml of 1N NaOH. This sample was kept in oven at 70°C for 30 minutes. Then 2 ml of freshly prepared reagent C (carbonate copper solution) was added. After 10 minuts 0.2ml of reagent D (Folin ragent) was added

and mixed rapidly. After 30 minutes a blue colour developed which was read at 540 nm.

Standard solution of 20 to 200 $\mu$ g strength were prepared from stock solution. Optical density was read at 540 nm wavelength and standard curve was drawn. Values of protein in sample were obtained with the help of standard curve and calculated as mg per g fresh weight .

## REFERENCES

- Arnon D.I. (1949).** Coenzyme in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*. 24: 1 – 15.
- Bajaj K.L., Kaur G. (1981).** Spectrophotometric Determination of L- Ascorbic Acid in vegetable and fruits. *Analyst*. 106:117-120.
- IS 5182 Part 6** Methods for Measurement of Air Pollution: Oxides of Nitrogen.
- IS 5182 Part 2** Method of Measurement of Air Pollution: Sulphur Dioxide.
- IS 5182 Part 23** Method of Measurement of Air Pollution: Respirable Suspended Particulate Matter (PM<sub>10</sub>) cyclonic flow technique.
- Jacob M.B., Hochheiser S. (1958).** Continuous sampling and ultra-micro determination of nitrogen dioxide in air. *Analytical Chemistry*. 32: 426.
- Lowery O .H., Rosebrough N. J., Farr A. C., Randall R. J. (1951).** Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*. 193: 265-75.
- Malhotra S. S., Sarkar S. K. (1979).** Effect of sulphur dioxide on sugar and free amino acid content of pine seedlings. *Physiologia Plantrarum*. 47: 223-8.
- Method I.O.-2.1** Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM<sub>10</sub> Using High Volume (HV) Sampler.
- Method 501**, Air Sampling and Analysis, 3rd Edition, Lewis publishers Inc.
- Perkin Eimer Manual. (1981).** Manual for estimation of heavy metals by atomic absorption spectrophotometer.
- Singh. (1977).** Practical plant Physiological. *Kalyani Publishers*, New Delhi
- Singh S.K., Rao D.N. (1983).** Evaluation of plants for their tolerance to air pollution". In: *Proceedings Symposium on Air Pollution Control, Indian Association for Air Pollution Control, New Delhi India*. 1: 218-224.
- West P.W. Gaeke G.C. (1956).** Fixation of SO<sub>2</sub> as disulfitomercurate (II) and subsequent colorimetric determination. *Analytical Chemistry*, 28: 1816 – 9.