Chapter 2

# MATERIALS & METHODS

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## 2.1 STUDY AREA

Major portion of the Vadodara Urban Development Authority (VUDA) area is more or less plain comprising approximately 714.56 $Km^2$ with the M.S.University of Baroda campus in the centre. The area includes limits of Baroda Municipal Corporation (BMC) and 103 Villages of Baroda, Waghodia and Padra Taluka.

### 2.1.1. Location

Area under Vadodara Urban Development Authority is situated between 73° to 74° 10' E longitude 21° to 23° N latitude,30 m above mean sea level. Baroda is a beautiful and well planned city in central Gujarat,W.India. The river Vishwamitri originates from the hills of Pavagadh and flows north-south through the centre of Baroda. The river Mahi is on the north-west of Baroda and forms the western boundary of the area. The 'Mini' which originates from a tank near Samlaya in Savli taluka falls in to Mahi.

### 2.1.2 Climate

The climate of Baroda is markedly periodic and is characterised by a dry and increasingly hot summer from March to June, a dry and cold winter from November to February and a warm south-west monsoon from July to September. July & August receive higher rainfall. Climatic factors like temperature,relative humidity, wind speed, wind direction and rainfall were recorded during the year 1983-1987 from the meteorological observatory of the M.S.University of Baroda. The wind direction and pollution concentration were the main criteria used for establishing experimental zones. During monsoon mostly the winds are south-west. The pollutants of

the industries during this season are carried to northern or north-eastern direction (Fig. 1). During winter the wind is generally from north-east or north and the pollutants are carried towards south-west or south direction. The heat during summer (March - June) is intense. The temperature is rising as high as 39.5° during May and 12.1°C minimum temperature during December. The relative humidity is minimum (41%) during the summer months in March and maximum (85%) during the monsoon in the month of August. Ombrothermic curve was prepared from the data of temperature, rainfall and relative humidity (Fig.2).

### 2.1.3 Soil

The soil of the area under investigation is a vast alluvial deposit of black soil. North-western sector (of VUDA area)consisted of highly fertile land (Sabnis, 1967).

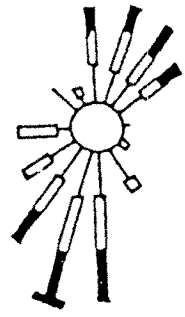
### 2.1.4 Vegetation

Vegetation around industries was heavily affected causing economic loss to the farmers. Farmers in general grow rice, millet and maize during monsson. After the harvest of monsoon crops farmers grow wheat, potato, cauliflower, cabbage etc. near the industrial complexes like IPCL, Refinery complex, Nandesari industrial estate, GSFC,GACL etc. showed damaging symptoms of air pollution. Mango, papaya chickoo,lemon and jamun trees suffered heavily,younger premature fruits often fall. Agriculture, due to air contamination was less productive.

### 2.1.5 Pollutants and their sources

Present invetigation was conducted in north-west region of VUDA area where the major polluting industries are

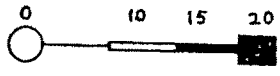
WINDROSE DIAGRAMS ( Fig. 1 )



ANNUAL

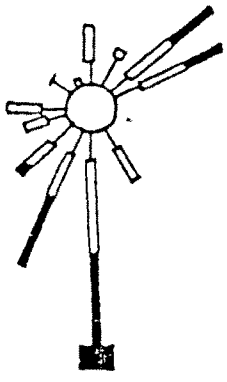
N

scale:
1 cm = 5 %

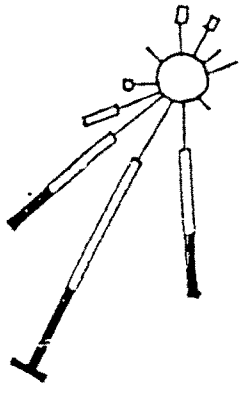



SEASONAL

summer



monsoon



winter

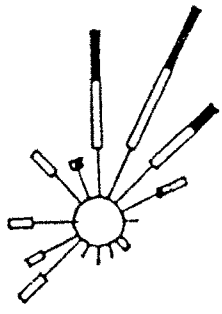
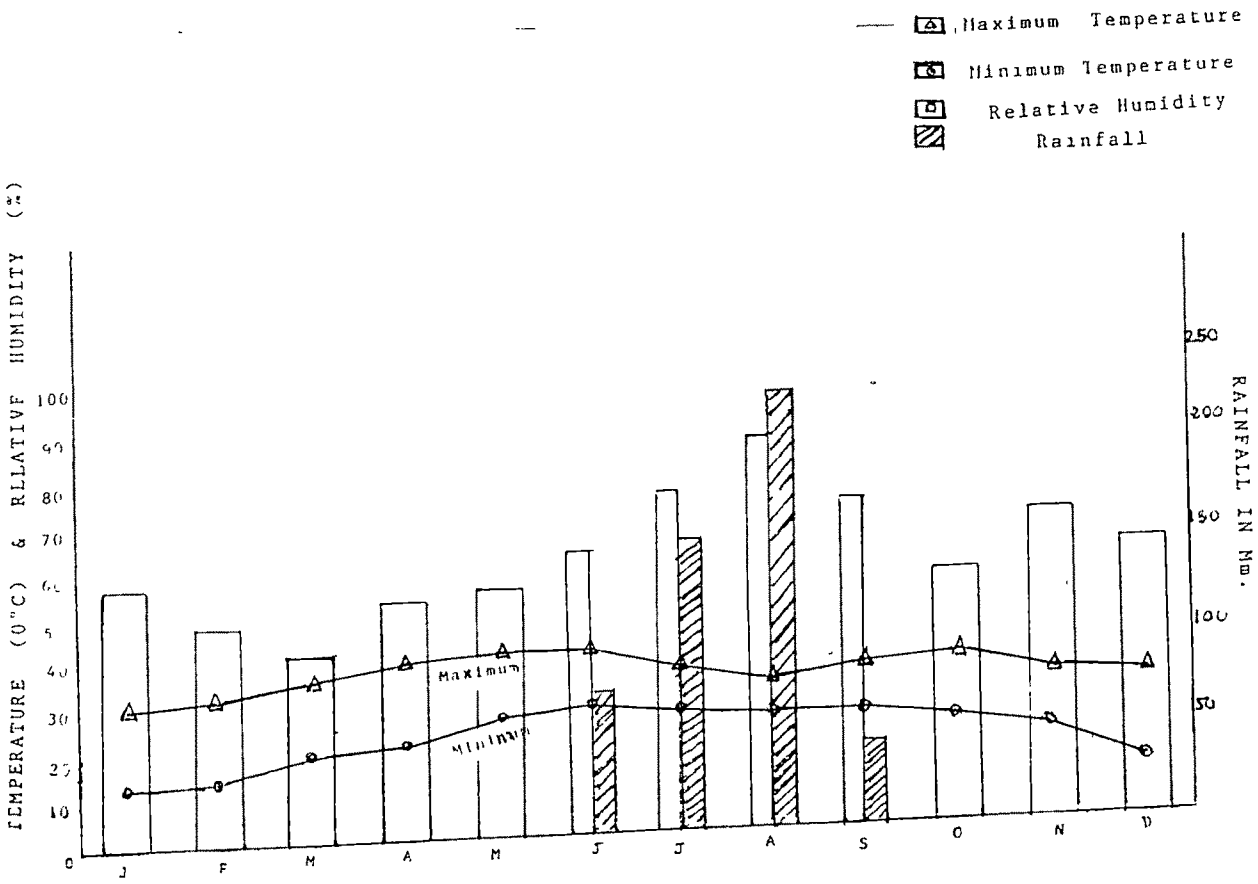


Fig 2 OMBROTHERMIC DIAGRAM FOR BARODA

(1983-1937 mean)



located. The major industries situated in this area are a Fertilizer factory, an Oil Refinery, a Petro-chemical complex, an Alkalies and chemical plants,an industrial estate at Nandesari having 369 small and medium scale chemical industries. The products and pollutants of these industries are given in table 4.

The National High-way No.8 with a heavy vehicular traffic passing through this area is an added source of autoexhaust pollution.

## 2.2 SELECTION OF ZONES

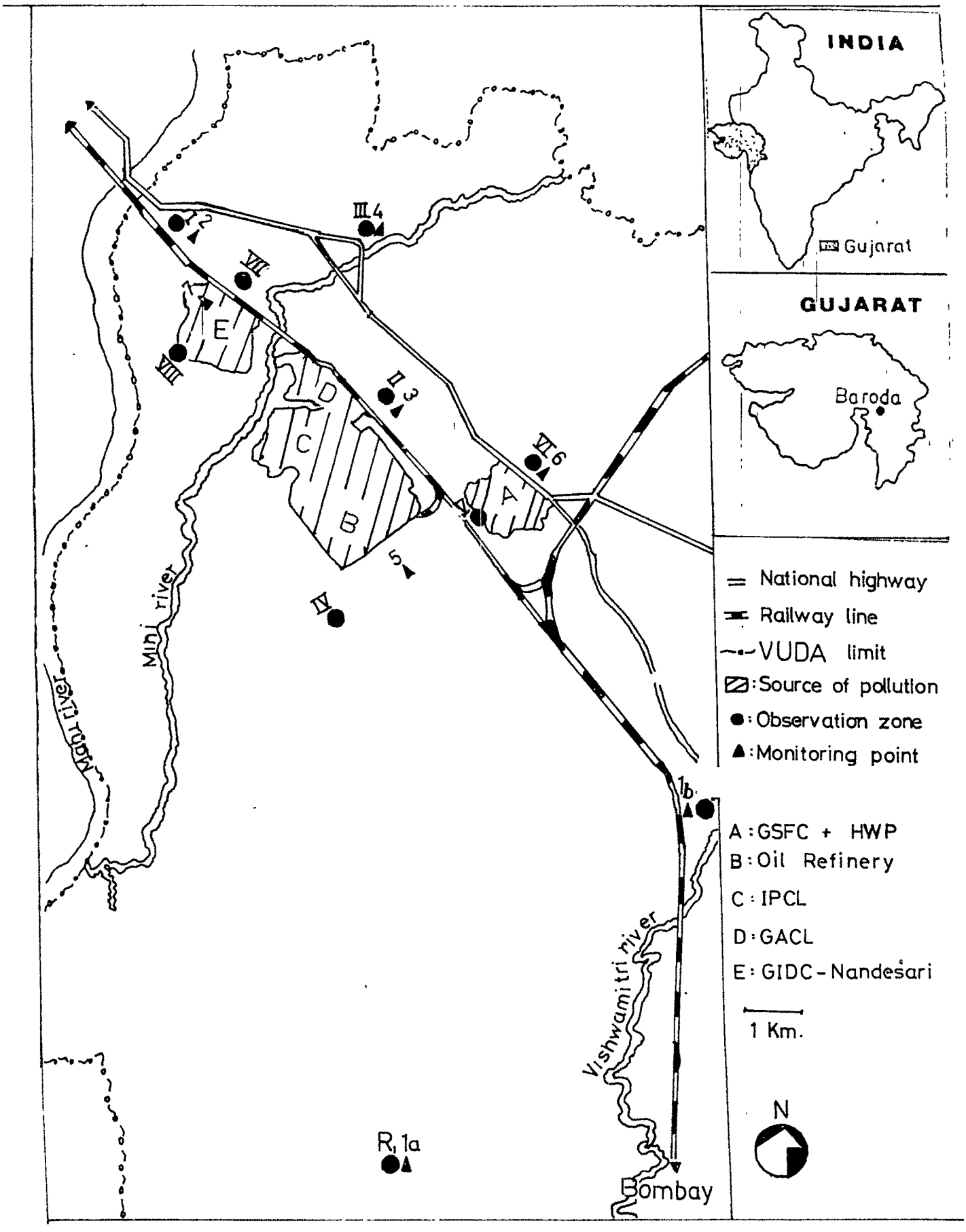
Zones were selected according to pollutants' concentration. National Institute of Occupational Health (NIOH) monitored ambient concentration of major air pollutants viz.,$SO_2$,$NO_x$ and SPM during 1982-1986 and method used by them are given below. This data and general survey of the vegetation were used in selection of zones at different places under VUDA. The experimental zones were classified as high, medium and low pollution zones. Zones were numbered from I to VIII on the basis of increasing concentration of pollutants. Zones I, II & III were considered as low, zone IV as medium and zones V, VI, VII & VIII were considered as high pollution zones. Two more zones $R_1$ & $R_2$ away from the pollution source were selected as reference zones (Fig.3).Every zone had separate monitoring point except zone IV & V and zone VII & VIII had common monitoring point i.e.5 and 7 respectively (Fig.3)

## 2.3 MONITORING OF MAJOR AIR POLLUTANTS AT SELECTED ZONES

The major air pollutants viz, $SO_2$, $NO_x$ & SPM were monitored once in a week for 24 hours at the interval of

Fig. 3 MAP OF THE STUDY AREA





eight hours. Based on this data, monthly and seasonal average and maximum & minimum concentration of pollutants were recorded. Certain peaks were missed in absence of continuous monitoring system. Different pollutants were monitored by using high volume sampler. Absorbing media was different for different pollutants. Air was allowed to pass continuously through impingers having specific absorbing media for a particular gas.

$SO_2$ was monitored by West & Gaeke method (1956). The ambient air was absorbed in potassium tetrachloromercurate (TCM). This solution was treated with sulphamic acid and formaldehyde. Pararosaniline reagent was used to develop the colour. Optical density of the treated samples was measured at 560 nm. The result ($SO_2$ concentration) obtained was in ppm which was then converted to $ug/m^3$ by using formula.

$$SO_2\ \mu g/m^3 = \frac{ppm \times 64 \times 10^6}{24470}$$

$SO_2$ concentration in $\mu g/m^3$ was recorded by using standard graph prepared with sodium metabisulphite.

The method for $NO_x$ monitoring was followed from the Indian Standard Institute 5182 Part VI (1975). The gas was absorbed in 0.IN sodium hydroxide solution. This solution was then treated with hydrogen peroxide, sulphanilamide and N-ethylene diamine dihydrochloride. The colour developed was read at 540 nm. Standard graph for $NO_x$ was prepared with sodium nitrite. Chlorine was monitored at zone II & VIII. Free chlorine was absorbed in the alkaline solution (NaOH) and the estimation was done by colorimetric O-tolidine method (Rand et al.,1977).

## 2.4 DESIGNING OF EXPERIMENTS

### 2.4.1 Field Survey

General field survey was done to assess the pollution impact on different cereal and cash crops viz., millet, paddy, maize,wheat, potato, pigeon pea & tobacco in different fields at different zones. The fields selected were at different distances from the main pollution source. Visual observation like damage in foliar tissues and reduction in yield were recorded for all the above crops. Detailed study of morphological parameters, growth analysis and yield was done for only three cereal crops viz., wheat, paddy & maize. Observations were recorded by random samplings of $1M^2$ plot in the crop field. Average of twentyfive sample plots for each crop at each zone was taken. The three crops investigated were:

1. Triticum aestivum Linn. (wheat)
2. Oryza sativa Linn. (paddy)
3. Zea mays Linn. (maize)

Crop survey was done at all the zones (I to VIII) and compared with reference zone $R_1$. At each zone ten fields were selected for each crop. Observations were recorded periodically (at 20 days interval) till harvesting. Parameters studied were plant height, total leafarea, visible symptoms, percentage leafarea damage, above ground biomass, relative growth rate (RGR),net assimilation rate (NAR) and yield.

### 2.4.2 Potted Plant Exposure Study

Of the various crops growing in the area selected for general crop survey to assess the pollution damage, the

following graminaceous crops were selected for detailed pot exposure study.

1. Triticum aestivum Linn. Var. J-24
   (Eng. Wheat, Vern. Ghaun)
2. Oryza sativa Linn. Var.Guj-17
   (Eng. Paddy, Vern. Danger)
3. Zea mays Linn.Var.American Sweet corn
   (Eng. Maize. Vern. Makai)

The certified seeds were procured from agriculture University. Perforated polythene bags (35 x 30 cms) filled with garden soil rich in organic manure were transported to all the experimental zones. Ten bags for each crop were kept at each zone in wirenet fenced cages. Fifteen seeds were sown in each pot on the same day at all the places. Water treatment was similar at all the zones from a common source (University campus). All other cultural practices except the air were uniformly maintained for all the bags at all the zones. Seven days after the date of sowing, thinning was done and five plants were allowed to grow in each bag. Observations for each parameters were recorded at a regular interval of 20 days upto the crop maturity by removing one plant from each pot.

Morphological and growth parameters recorded were root length, shoot length, number of leaves, total leafarea, leafarea damage (injury index), above ground biomass, relative growth rate (RGR) and net assimilation rate (NAR) etc.

Biochemical parameters like chlorophyll, soluble sugars, reducing sugars, sulphur, protein, ascorbic acid,and chloride content in foliar tissues were estimated.. The yield was recorded at the harvest.

### 2.4.3 Artificial Fumigation Study

The study was conducted in simulated conditions in a fumigation chamber. Sulphur dioxide has a more vulnerable history as an air pollutant than any other chemical, hence $SO_2$ gas was selected for the present artificial fumigation study. Wheat and paddy crops were fumigated in $1M^3$ fumigation chamber and maize was fumigated in 3 $M^3$ fumigation chamber. Plants were exposed to $SO_2$ by continuous flow method. $SO_2$ gas (100%) from cylinder, after diluting with atmospheric air by air blower and adjusting the air flow with rotameters, was passed through fumigation chamber. The concentration of $SO_2$ within the chamber was maintained by adjusting air flow and was periodically monitored by absorbing the air from the outlet of the chamber in sodium tetra chloromercurate solution (West and Gaeke,1956). Fan inside the chamber was switched on to facilitate uniform mixing of the gas. To minimise the temperature rise inside the chamber, exposures were carried out between 7-30 to 10-30 A.M. During exposure time, temperature inside the chamber was higher by 1°C and ± 5% change in relative humidity as compared to the ambient conditions.

Two sets of five replicates (bags) were prepared following similar procedure as for pot exposure study. One set was exposed to sulphur dioxide (E) and another set was kept in chamber but unexposed (UE) to sulphur dioxide and was considered as reference. The plants (E) were exposed to $SO_2$ for two hours on alternate days till their maturity.

All the parameters recorded were similar as in pot exposure study.

### 2.4.4 Mitigation of Pollution Damage

**a. Under simulated conditions**

Eight sets each of five replicates were prepared similar to the pot exposure study. Ascorbic acid aqueous solutions were prepared of 0.005 M, 0.0075 M, 0.01 M (Molar) concentration. Two sets of three treatments each (treated and treated & exposed) were taken for each concentration ($T_1, T_2, T_3$, and $T_1E, T_2E, T_3E$).Total six sets were prepapred for treated plants (3x2). The ascorbic acid solutions were foliarly sprayed on the plants for all the six sets starting from 20 days to its maturity at 100 days. Total five sprays were given at regular interval of 20 days. One set from each treatment was exposed for two hours on alternate days to 0.2 ppm sulphur dioxide.Remaining two sets out of eight sets were kept as reference sets (UTUE & UTE). One set was exposed to 0.2 ppm sulphur dioxide, the other set was kept as unexposed.

Visual observations, foliar damage, morphological, biochemical and yield parameters were recorded similar to the pot exposure study.

Percentage recovery in different parameters in treated & exposed plants ($T_1E, T_2E$ & $T_3E$) were calculated with reference to untreated and exposed (UTE) plants. Treated and unexposed plants ($T_1, T_2$ & $T_3$) were used to know the difference from normal plants (UTUE).

**Code for treatment**

| Treated & Exposed | Treated & Unexposed | Concentration of Ascorbic acid | Amount sprayed gm m -2 |
|---|---|---|---|
| $T_1E$ | $T_1UE$ | 0.005 M | 0.18 |
| $T_2E$ | $T_2UE$ | 0.0075 M | 0.27 |
| $T_3E$ | $T_3UE$ | 0.01 M | 0.36 |

UTE = Untreated & Exposed

UTUE = Untreated & Unexposed

### 2.4.4 b. Under field conditions

Cereal crops grown by farmers in the fields (viz.wheat (winter),paddy & maize (monsoon) crops) were selected near a fertilizer complex at a distance of 0.5 to 0.6 km from the source. In each field 21 plots of $1M^2$ were prepared Seven sets of three replicates each were designated. Three sets were of ascorbic acid treatment and other three sets were treated with urea solution. One set was kept as untreated and exposed to ambient air,it was considered as reference. These sets were labelled as $A_1$, $A_2$, $A_3$ & $U_1$, $U_2$, $U_3$ and C. Ascorbic acid solutions of 0.005M, 0.01M & 0.02M and urea solutions of 0.16M, 0.32M & 0.64M were prepared. These aqueous solutions of different concentrations were foliarly sprayed on all the three field grown cereal crops. Reference plants were sprayed with distilled water only.

Growth parameters and biochemical parameters were recorded similar to pot exposure study. Final yield was recorded at harvest. Percentage recovery was calculated for each parameter over reference plants. Cost benefit ratio was calculated from the yield data.

Code for treatment

| Code | Chemical concentration | Amount sprayed |
|---|---|---|
| $U_1$ | 0.16 M Urea | 1.80 gm $m^2$ |
| $U_2$ | 0.32 M Urea | 3.60 gm $m^2$ |
| $U_3$ | 0.64 M Urea | 7.20 gm $m^2$ |
| $A_1$ | 0.005 M Ascorbic acid | 0.18 gm $m^2$ |
| $A_2$ | 0.01 M Ascorbic acid | 0.36 gm $m^2$ |
| $A_3$ | 0.02 M Ascorbic acid | 0.72 gm $m^2$ |
| C | Untreated with any chemical | |

## 2.5 METHODS USED FOR DATA COLLECTION OF VARIOUS PARAMETERS

### 2.5.1 Morphological & Growth Parameters

Various parameters viz root length, shoot length, number of leaves, total leafarea, leafarea damage were determined and injury index of crops was calculated in field and artificial fumigation experiments.

$$\text{Injury index ( \% )} = \frac{\text{Damaged leaf area}}{\text{Total leaf area}} \times 100$$

Injury index was not recorded at maturity stage as normal maturity symptoms were seen. Above ground biomass was recorded as gm/plant dry weight.

Growth analysis for relative growth rate (RGR) and net assimilation rate (NAR) was calculated for growth parameters by using the following formula

$$\text{RGR}_{\text{mg/gm/day}} = \frac{1}{W} \cdot \frac{dW}{dt} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

$$\text{NAR}_{\text{mg/cm}^2\text{/day}} = \frac{1}{F} \cdot \frac{dW}{dt} = \frac{W_2 - W_1}{T_2 - T_1} \left(\ln F_2 - \ln F_1\right)$$

Where

$W_1$ = Initial dry weight of plant
$W_2$ = Final dry weight of plant
$F_1$ = Initial leafarea
$F_2$ = Final leafarea
$T_1$ = Time of initial observation
$T_2$ = Time of final observation
$W$ = Increase in dry weight $(W_2-W_1)$
$F$ = Increase in leafarea $(F_2-F_1)$
$dW/dt$ = Slope of total dry weight curve over time.

Weight of 1000 seeds was recorded on dry wt. basis. The average weight of grains for $1M^2$ was recorded and based upon it yield was calculated and expressed as $kg/100M^2$. The method adopted for studying yield was similar for all the three crops. Percentage reduction in yield was recorded at all the zones over reference zone.

**Foliar epidermal study**

Leaves of wheat and paddy at 80 days age were collected from high pollution zones (V & VIII) and reference

zone ($R_2$) and were immediately fixed in FAA (Formaline 40%, Glacial Aeetic acid and Alcohol 50%, 5:5:90) and brought to the laboratory. Epidermal peels were taken manually and after staining in saffranin were observed under microscope. Number of epidermal cells and stomata per unit area were counted. Stomatal index was calculated using the following formula:

$$\text{Stomatal Index} = \frac{S}{S + E} \times 100$$

S = Number of stomata per unit area

E = Number of epidermal cells per unit area

### 2.5.2 Biochemical Parameters

The biochemical parameters analysed in foliar tissues of plants of wheat, paddy & maize were chlorophyll ascorbic acid, proteins, total soluble sugars, reducing sugars, sulphur & chloride.

#### 2.5.2.1 Chlorophyll

The chlorophyll content was estimated by following Holden's (1965) method. 100 mg of fresh leaf samples was crushed in a pre-chilled mortar and pestle with 80% cold acetone. A pinch of calcium carbonate was added to prevent chlorophyll degradation during estimation. Glass powder was added for easy maceration. The extract was centrifuged at 3000 rpm for 15 minutes. The supernatant was collected. The process was repeated till the residue became colourless. The collected supernatant was diluted upto required constant volume (25 ml). The samples were kept in dark to avoid chlorophyll degradation. Optical density of the extract with reference to blank was measured at different wavelengths.

The Chlorophyll - a and Chlorophyll - b contents were determined by Maclachlan and Zalik's (1963) formulae.

$$\text{Chlorophyll a mg/gm fresh wt.} = \frac{(12.3 \times OD_{663}) - (0.86 \times OD_{645})\ XV}{d \times 1000 \times w}$$

$$\text{Chlorophyll b mg/gm fresh wt.} = \frac{(19.3 \times OD_{645}) - (3.6 \times OD_{663})\ XV}{d \times 1000 \times w}$$

where

V = Total volume of supernetant (ml)
d = Distance travelled by light (cm)
W = Weight of the sample (gms)
OD = Optical density at particular wavelength

### 2.5.2.2 Ascorbic acid

It was estimated by Schaffert and Kingsley's (1955) method. 100 mg of fresh leaf sample was ground with 0.5% oxalic acid and centrifuged at 3000 rpm for five minutes. Supernatant was taken and definite volume (25 ml) was made. To this activated charcoal was added for conversion of ascorbic acid into dehydro ascorbic acid. 2,4, Dinitro phenyl hydrazine, was used for colour development which was measured at 515 nm. The total ascorbic acid content was calculated from the standard graph plotted for AR ascorbic acid.

### 2.5.2.3 Protein

It was estimated by following Hartree's (1972)method. 100 mg fresh leaf sample was ground in 0.05 N NaOH. Folin and Ciocalteu reagent was used for colour

development. The optical density was measured at 650 nm. The standard graph was plotted with crystalline Bovine albumin.

### 2.5.2.4 Sugars

The extraction was done by the method of Mc Cready et al.(1950). To 100 mg dry leaf sample boiling ethanol and water (1:1 v/v) were added. After centrifugation at 5000 rpm for 15 minutes, the supernatant was collected. Process was repeated for five times. Collected supernatant was evaporated and dissolved in water. Estimation of total soluble sugars and reducing sugars were done from this.

#### i Soluble sugar

The estimation was done by Yemn & Will's (1954) method using anthrone reagent. The colour developed was read at 620 nm. The standard graph was prepared with glucose.

#### ii Reducing Sugar

Estimation was done by Mc Cready et al., method (1950). Sample was analysed by using 2 ml of 0.4% potassium ferricynide (prepared freshly) and 1 ml of carbonate cyanide (stock solution was prepared by dissolving 0.1500 gm of sodium cyanide and 8 gm of anhydrous sodium carbonate in 500 ml distilled water) sample was read at 520 nm. The OD was compared with standard curve prepared with glucose.

### 2.5.2.5 Sulphur

It was determined by Garrido's method (1964). Total sulphur content in dry foliar powder was determined colorimetrically. 100 mg dried leaf sample was digested with mixture of nitric acid and perchloric acid (3:2). The

turbidity was determined at 420 nm, after addition of Barium chloride & tween-eighty reagent . Standard graph was plotted with ammonium sulphate.

### 2.5.2.6 Chloride

It was determined by Humphrie's (1956) method. Dry leaf samples were fused with calcium oxide and then ashed at 500°C. Double titration with silver nitrate and standard ammonium thiocyanate was done to estimate chloride content using the following formula.

1 ml of 0.1N $AgNO_3$ = 3.55 mg Chlorine