Chapter 2

MATERIALS & METHODS

2.1 STUDY AREA

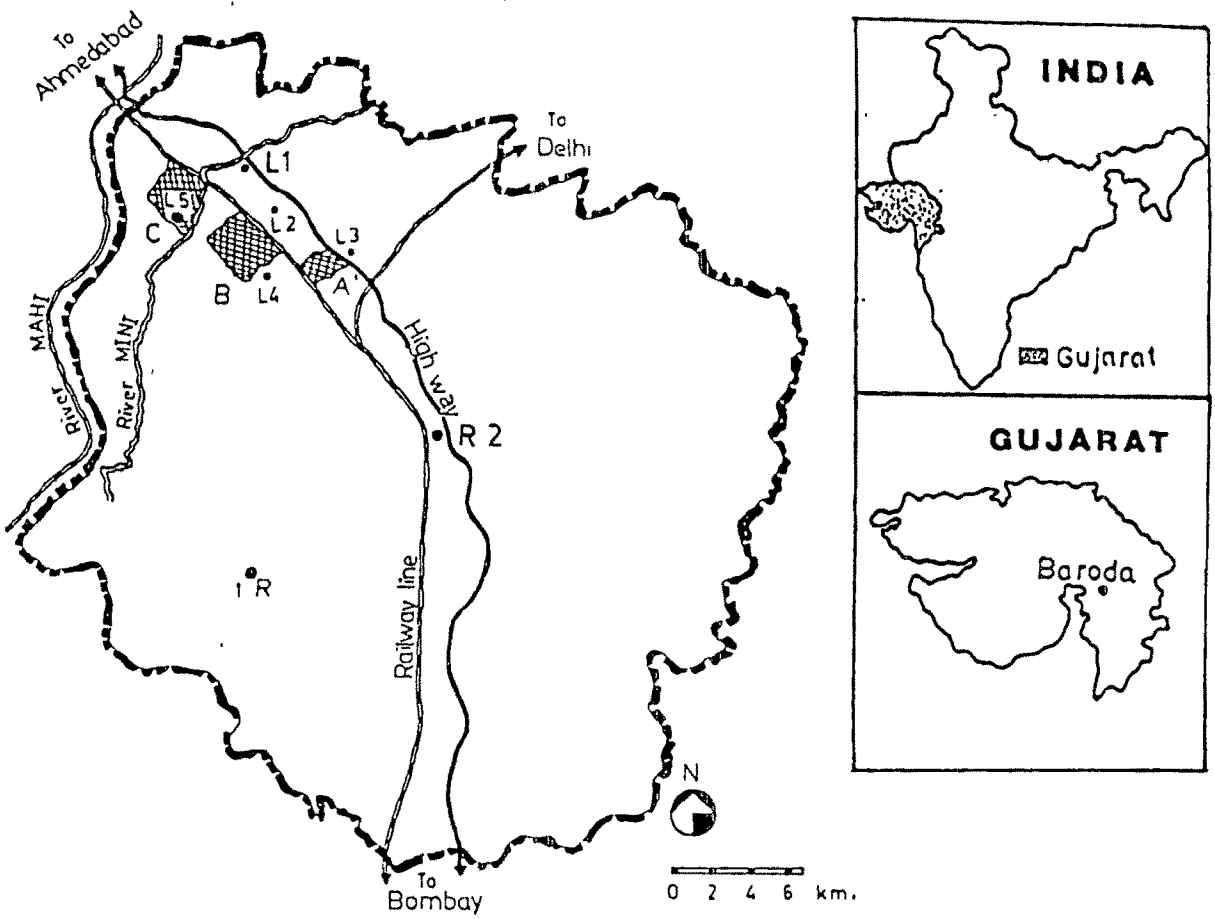
Vadodara Urban Development Area (Gujarat, India) situated between 73° to 74° 10' E longitude, 21° to 23° N latitude, 400 Km north of Bombay and 30 m above mean sea level was taken as the study area for this investigation. The area is more or less plain. General soil types are black soil and red loam and the area is very fertile for agricultural use. Soil characters are almost same at all the localities. Excepting for changes in wind direction and speed, other meteorological factors remained the same.

2.2 POLLUTANTS, THEIR SOURCES AND LOCALITIES

After the discovery of natural gas in the surrounding area, it has witnessed rapid industrialization. The principal industries are an oil refinery, a petrochemical complex, a fertilizer complex, an alkalies and chemicals plant, a phenolics unit, a heavy water plant and a glass factory. The Nandesari industrial estate situated in the north-west direction of Vadodara, comprises 369 small and medium-scale chemical industries in an area of $\simeq$ 5 Km^2. Majority of these units manufacture acids, various petrochemical products and some metallurgical components. Because of the high density of industries, the area has become a major polluted locality. Major industries, their products and pollutants emitted are given in the following pages. The National High-way No. 8 with a heavy vehicular traffic also passes through the study area causing damage to vegetation (Krishnayya & Bedi, 1986[a]).

Major pollutants in the study area are sulphur dioxide, oxides of nitrogen, carbon monoxide, carbon dioxide, hydrocarbons, ammonia, suspended particulate matter (SPM) etc. Localized pollutants are ethylene, chloride and various other acidic vapours. SO_2, NO_X and SPM were regularly monitored (weekly once for 24 hours, at three 8 hourly intervals) at

Map 1: Study area showing localities sources of pollution
(Vadodara urban development area) Western India.



R - Reference
L - Locality

Sources of pollution
A - Fertilizer complex
B - Petrochemicals and Refinery complex
C - Medium & Small-scale Chemical industries

different localities by National Institute of Occupational Health. From this data annual mean concentrations with minimum and maximum recordings were obtained. Seasonal pollutant concentrations (for summer, monsoon and winter) were also calculated. Based on this data localities having different pollutant concentrations were chosen from the study area (map 1). Two reference areas having smaller concentration of pollutants were taken for comparison. Meteorological parameters such as minimum and maximum temperatures, relative humidity (mean monthly observations from 1983 to 1986) were obtained from the Meteorological observatory, M.S. University, Baroda. Based on the wind speed and direction, windrose diagrams were made.

2.3 GENERAL VEGETATION SURVEY

To study the general impact of air pollution on vegetation, a locality having maximum concentration of recorded pollutants was selected. Five quadrats of 4.0 x 4.0 m size were laid at each point on both windward and leeward directions at a distance of 1.25, 1.75, 2.50 and 3.00 Km from pollution source till the species distribution remained constant and/or almost equal to the reference. Distribution of various herb species was recorded. A reference locality having smaller concentration of pollutants was taken for comparison. Study was carried out during winter in the months of January-February. Throughout the gradient edaphic features remained the same.

Visible damages such as chlorosis and necrosis were recorded based on the pictorial atlas of Malhotra & Blauel (1980). Visible symptoms that appeared on tree species were also recorded.

2.4 GROWTH PARAMETERS STUDIED FOR THE TREE SPECIES

From the observations of the general survey, three

different tree species, Azadirachta indica Juss., Moringa pterygosperma Goert., and Tamarindus indica Linn., were selected for studying their differential response at different polluted localities. Of the three species taken, Moringa is a fast growing species followed by Azadirachta and Tamarindus. Tamarindus is an evergreen species while the other two are deciduous.

From the localities chosen, equal number of different tree species were selected. Visible symptoms such as chlorosis, necrosis, were qualitatively and semi-quantitatively recorded. Growth parameters such as height of the tree, circumference at breast height (CBH), canopy cover, % leaflessness and damaged leaf area were recorded. Based on the principle that instrument which can measure an angle of 45° can be used to measure height (Michael, 1984), set-square (equilateral) was taken for measuring the height of the tree. Point having 45° was kept near the observer's eye. The base of the triangle was kept parallel to the ground surface. Two points of the lateral side of the triangle and the tip of the tree canopy were adjusted by moving forward and backward till all the three points align on a straight line. The distance between the observer and the tree was measured. To this, height of the observer upto eye level was added to get the height of the tree. CBH was measured by using a standard Freeman's metre tape.

The spread of the canopy cover on windward and leeward directions was measured. Percentage leaflessness was a visual observation taking the leaf cover of the reference tree as 100%. Definite number of leaves were taken, leaf area and leaf area damaged were measured using graph paper and the % leaf area damaged to the leaf area measured was calculated. During the reproductive phase, % flowering and fruiting were semi-quantitatively estimated. In each tree, five branches of equal size were randomly selected and the number of flowers and fruits were counted (Krishnayya & Bedi, 1988[a]). The

reference reading was taken as 100% and the percentage decrease in other localities was calculated in comparison with the reference. All the values are expressed in percentages only.

2.5 BIOCHEMICAL ESTIMATIONS

To study the subtle effects of pollutants biochemical parameters such as chlorophyll content, total proteins, ascorbic acid, total sulphydryl groups (TSH), glutathione (GSH), peroxidase and acid phosphatase were estimated. Foliar samples from different localities were collected, brought to the laboratory, washed under running tap water and were subjected to analyses immediately.

2.5.1 Chlorophyll estimation

Leaf samples were weighed (100 mg), ground in 80% chilled acetone (Holden, 1965). A pinch of calcium carbonate was added to prevent the pigment degradation during extraction. Absorbance was measured at 645, 652 and 663 nm to calculate chlorophyll a, chlorophyll b and total chlorophyll (Maclachlan & Zalik, 1963).

2.5.2 Total proteins

Known amount of leaf tissue was taken, ground in chilled 0.95% potassium chloride. From the extract 1 ml was taken and 2 ml of chilled 10% trichloroacetic acid was added. Sample was centrifuged and the precipitate was dissolved in 1 N sodium hydroxide. This was kept in water-bath at 100°C for 8 min. After cooling, the samples were centrifuged, suitable aliquots were taken and protein content was estimated (Lowry et al., 1951).

2.5.3 Ascorbic acid

Leaf samples were extracted in 5% metaphosphoric and

10% glacial acetic acid solution and centrifuged. Suitable aliquots of supernetant were taken and ascorbic acid was colorimetrically determined using dinitrophenyl hydrazine as the colour developing reagent (Roe, 1954).

For total sulphydryl groups, glutathione, peroxidase and acid phosphatase, experimentation was carried out under cold room conditions.

2.5.4 Total sulphydryl groups (TSH)

Leaf tissues were extracted in 0.02 M EDTA. TSH was estimated using Ellman's reagent (Jozef & Lindsay, 1968).

2.5.5 Glutathione (GSH)

Foliar samples were ground in 25% metaphosphoric acid and centrifuged. Suitable aliquots were taken from the supernetant and GSH was estimated following Alloxan 305 method (Patterson & Lazarow, 1955).

2.5.6 Peroxidase and Acid phosphatase

Leaf tissues were extracted in 95% potassium chloride and centrifuged at 15,000 g for 15 min. Suitable aliquots were taken from supernetant. Peroxidase was estimated using hydrogen peroxide as the substrate (Shanon et al., 1966). Acid phosphatase was estimated using polynitrophenyl phosphate as the substrate (Bergmeyer, 1974).

2.5.7 Sulphur

Washed leaf samples were oven-dried at 80°C for 48 h, powdered and passed through a 100 micron sieve. Known amounts of the powdered samples were taken and digested in an acid mixture (nitric acid and perchloric acid 3 : 2). Sulphur was

estimated using barium chloride-tween 80 as the turbidometric reagent (Garrido, 1964).

All the estimations were done monthly twice for 12 months. Afterwards the observations were carried out randomly to check consistency of the earlier results. These are not mentioned.

2.6 POTTED PLANT EXPOSURE STUDY

Saplings of the three tree species viz. Azadirachta indica Juss., (2 year - old), Moringa pterygosperma Goert., and Tamarindus indica Linn., (6 month old) were procured from nursery of the State Forest Department. They were transferred into (35 x 40 cm) perforated polythene bags, filled with humus-rich garden soil and were conditioned in the university arboratum. Saplings of the species were of different heights, 30.4 to 44.0 cms in Azadirachta, 10.0 to 15.0 cm in Moringa and 25.4 to 35.5 cm in Tamarindus. Equal number of saplings (15) for each species were randomly selected and transferred during mid August to different localities (Map 1). Saplings were protected in wire-net enclosed areas. Saplings were also kept in the University Botanical Garden and it was taken as reference locality, having smaller concentrations of pollutants.

Regular watering (thrice in a week) and other cultural practices such as nutrient supply were the same for all pots in all the localities. Growth parameters such as sapling height, number of leaves/plant and leaf area/leaf were measured at a regular interval of 30 days upto June. As all the three species taken have compound leaves, average leaf area/leaf was calculated from average number of leaflets/leaf and average leaflet area measured by using graph paper. Visible symptoms such as chlorosis, necrosis and premature leaf fall were qualitatively observed. At the end of June, foliar samples from all the localities were collected and oven-dried at 80°C

for 48 h. Later sulphur was estimated in the dried and powdered samples.

2.7 ARTIFICIAL FUMIGATION EXPOSURES

To study the individual effects of pollutants tree saplings were exposed to a known concentration of sulphur dioxide for a definite time under simulated conditions. Tree saplings used in this study were from the same lot procured from the State Forest Department nursery.

2.7.1 Fumigation exposure procedure

Saplings were exposed to relatively high amounts of SO_2 (0.2 ppm) for short durations to see their response to peak concentrations which occur occasionally at industrial areas. Three sets of 5 saplings for each individual tree species were taken. One set in each species was taken as reference, one set was exposed to 0.2 ppm SO_2 and the third set was treated with 100 $\mu g\ ml^{-1}$ ascorbic acid spray and exposed to 0.2 ppm SO_2. Fumigation was done in acrylic chambers (3 m^3). The chamber received air at a rate sufficient to provide one air change per minute. A metered quantity of SO_2 from a cylinder was added to one of the air streams to achieve the desired concentration. SO_2 concentration inside the chamber was monitored throughout the exposure period (West & Gaeke, 1956). Exposure was done for 2 h per day, 6 days in a week. This was continued for 6 months. Ascorbic acid was sprayed twice a week. Spraying was done by using an atomizer till all the leaves of treated saplings became wet (50 - 60 ml approx.). Treatment was given after the fumigation. During exposure time, temperature inside the chamber was more by 1°C and ± 5% change in relative humidity was seen as compared to ambient conditions.

2.7.2 Choice of ascorbic acid concentration

In a preliminary study saplings were exposed daily to 0.2 ppm SO_2 for 2 h. Saplings were sprayed with different concentrations of ascrobic acid. Later, protein content in the foliar samples was estimated to see the recovery rate as compared to exposed untreated ones. As the ascorbic acid concentration increased the recovery rate also increased, but the increase was directly proportional upto 100 $\mu g\ ml^{-1}$ solution. Afterwards the increase in recovery was very less as compared to the ascorbic acid concentration. Hence, 100 $\mu g\ ml^{-1}$ concentration was chosen as the suitable one for mitigating the SO_2 effect to a certain extent. Difference between treated reference and untreated reference was small (1 - 2%). So only untreated reference set was maintained.

2.7.3 Morphological & Biochemical observations

Growth parameters such as shoot length, number of leaves/ plant and leaf area/leaf were measured. All the above mentioned biochemical parameters were also estimated. Observations were made at a regular interval of 30 days and were continued for 6 months.

2.8 ANATOMICAL OBSERVATIONS

2.8.1 Stomata

Leaf samples were collected from 3 different localities. From the collected leaf samples small pieces were cut, equidistant from the midrib. They were mounted on stubs keeping adaxial side top. Samples were coated with gold/palladium and later scanned with Cambridge stereoscan S_4^{10} scanning electron microscope.

2.8.2 Ultrastructure

Two sets of Azadirachta indica Juss., saplings were exposed to 0.2 ppm SO_2. One set was treated with ascorbic acid as mentioned earlier. A reference set was taken for comparison. Exposure was carried out for 10 days. The objective of this study was to see the role of ascorbic acid in lessening the adverse effects of SO_2 at ultrastructural level. Leaf samples were fixed in 6% glutaraldehyde in 0.2 M cacodylate buffer pH 7.2 for 2 h at 4°C. After three washings in buffer the samples were post-fixed in 2% osmium tetroxide in the same buffer for 10 h at 4°C, washed in buffer 4 times and dehydrated through a graded series of acetone. The tissues were finally embedded in resin (Spurr, 1969). Ultrathin sections were cut with a diamond knife on Jeol Jem ultramicrotome, stained with uranyl acetate followed by lead citrate. Sections were examined with Jeol Jem 100 X transmission electron microscope.

TABLE - 2 : MAJOR INDUSTRIES AND POLLUTANTS OF THE STUDY AREA

Industry	Raw Material	Products	Pollutants
Oil Refinery	Crude Oil	C_1 - C_4 Hydrocarbons (gas fuel) Gasolene (Aviation turbine fuel) Naphtha Heavy Kerosene Fuel Kerosene	Ethylene and other hydrocarbons, SO_2, CO
Petrochemical Complex	Naphtha, Superior Kerosene (from Refinery) Methanol, Cl_2, NH_3, Alcohols H_2SO_4 (used for processing of Naphtha at different plants)	Orthoxylene, Mixed xylene, Paraxylene Dimethyl tetraphthalate, Ethylene dichloride, Indothene Polyethylene glycol, ethylene glycol Ethylene oxide acrylonitrile, Acrylic fibre, Vinyl chloride, Indovin PVC Hydrocyanic acid Benzene etc.	SO_2, CO_2, CO, NO_X mixture of malodorous gases including H_2S and hydrocarbons, Cl_2 SPM (Carbon)
Fertilizer complex		Diammonium phosphate, Ammonium sulphate, Urea, Caprolectum, Liquid ammonia	SO_2, NO_X, NH_3, HF, SPM
Phenolics industry		Formaldehyde, Phenolic resins	
Alkalies and Chemicals Industry		Caustic soda, liquid chlorine, Mercury, Hydrochloric acid, Sodium Cyanide	Cl_2, Hg

contd....

Table - 2 (contd...)

Industry	Raw Material	Products	Pollutants
GIDC Estate's (Nandesari) Medium scale Industries			
Deepak Nitrite Ltd.		Sodiun nitrite, Nitric acid etc.	NO_X
Ashok Organic Industries Ltd.		Ethylene diamine tetra-acetic acid (EDTA) and its salts Acetic acid, HCl, Methyl -dichloro acetate	Cl_2
Benzo Chemicals Combines		Benzyl chloride, Acetyl chloride, Ammonium chloride	Cl_2
Apex Chemicals		Paranitrochlorobenzene, Sodium hydrogen sulphide	Cl_2, H_2S