MATERIALS AND METHODS

SELECTION OF SPECIES AND SITES:

Two deciduous tree species Dalbergia sissoo Roxb. (Fabaceae), Holoptelea integrifolia (Roxb.) Planch.(Ulmaceae) and an evergreen tree Syzygium cumini (Linn.) Skeels. (Myrtaceae) were selected for the present study. The test site selected for pollution affected trees was close to Gujarat State Fertilizer Complex(GSFC), Baroda. All the trees selected were growing within half a kilometer range of the factory. The giant fertilizer complex products are diammonium phosphate, ammonium sulfate, urea, caprolactum and liquid ammonia etc,. The fertilizer complex air emission is a combination of various pollutants viz., sulfur dioxide, nitrogen oxide, ammonia, hydrogen flouride and suspended particulate matter. The quality of ambient air around GSFC was monitored by Central Pollution Control Board and its composition and concentrations for the year 1990 are as follows:

AMBIENT AIR QUALITY MONITORING DATA-1990. GSFC, BARODA.(in g/m^3).

SO_2	NO_x	NH_3	SPM
41	24	04	346

Wind direction for the major part of year was south-west and Dalbergia and Syzygium were on wind ward side. In the monsoon period (June to September, 1990) the direction of wind was North -east, during which the Holoptelea trees were exposed by the pollutants. It was observed that in the vicinity of GSFC consid-

erable foliar damage caused to the vegetation. The tree species were also exposed by auto exhaust, as the National Highway No.8 passes along the site, besides the air emission of GSFC.

The sites selected for the three tree species around in relatively less polluted were :

i) Kayavarohan-38 Km away (towards South) from the pollution source for Syzygium

ii) Dhakor road- 35 Km away (towards North) from the pollution source for Dalbergia

iii) Maharaja Sayajirao University campus about 9 km away (South West) from the pollution source for Holoptelea.

The selection of the above mentioned three tree species was primarily made on the basis of their timber value and comparable age of trees, sufficient number of tree species, similarity in topography, soil type and other climatic factors at both the sites, in addition to the visual damage observations at the polluted site. Maximising homogeneity between sites will minimise the variability arising from site specific factors and thereby facilitate the assessment of air pollution effects on radial growth (Fox et al., 1986).

SAMPLE COLLECTION PROCEDURE:

Cambial tissue together with the inner phloem and outer xylem was removed from the main trunk of the trees at a height of about 1.5 Meter from the ground using chisel, hammer and grafting knife. The samples removed were about 60mm long, 40mm wide and deep enough to include both the current season's growth and the

whole of the previous season's annual ring. Monthly samples from each site were taken in the first week of every month from January 1990 to December 1990. After each collection, the wounds were covered with sealing wax to prevent the entry of pathogens. Simultaneously, small pieces of 2-3 year old branches measuring one Cm diameter were also collected. Soon after their collection, the samples were fixed in F.A.A.(Berlyn and Miksche, 1976) and aspirated shortly afterwards in the laboratory. The sampled blocks were then cut into pieces approximately 20x20x20 mm with a small hacksaw and after a week in FAA, the pieces were transferred to 70% ethanol in a air tight containers for preservation. At the time of each sampling the phenological details of each species were recorded. The meteorological data on maximum and minimum temperatures, sunshine hours, rain fall and relative humidity of the year 1990 were collected from the observatories of soil conservation research center, Vasad and M.S. University of Baroda.

MICROTOMY AND STAINING:

Small blocks of samples comprising phloem, cambium and xylem of the main stem were microtomed at 15-20µm thickness in transverse, tangential and radial longitudinal planes from two different blocks using Leitz sliding microtome. The sections were taken only in transverse plane for the young branches. The sections were arranged in series on slide flooded with 70% ethanol and then tied to the slide with a fine cotton thread.

The sections were stained in Tannic acid-Ferric Chloride-

Lacmoid combination (Cheadle et al., 1953) or Toludine blue (O'Brien et al., 1964) or Tannic acid-Ferric chloride-Saffranin (Jensen, 1962) or Tannic acid-Ferric chloride (Foster, 1934). After staining, the sections were dehydrated through ethanol-xylene series and mounted in DPX.

MACERATION:

Xylem portion of one mm width from cambial zone was used to macerate fibres and vessel members. Maceration was done with Jeffrey's fluid (Berlyn and Miksche, 1976) or combination of Hydrogen peroxide and Acetic acid (1:1). After thorough washing, the macerated elements were stained in saffranin or toluidine blue before mounting in glycerine jelly.

MEASUREMENTS :

Cambial activity was determined by counting the number of undifferentiated layers lying between the xylem and phloem from transverse sections. Radial enlargement was the basis for distinguishing the cambial zone cells from their immediate derivatives during active period of cambium. The differentiating xylem and phloem cells were determined by their radial enlargement and thin cell walls. The length and width of fusiform cambial cells, tangential diameter of cambial ray cells, ray height, width and the number of rays passing through one cm tangential width of cambium and the dimensions of vessel elements and xylem fibres were measured with an occular micrometer scale arranged in a Carl Zeiss microscope. The width of the xylem growth ring was measured directly from wood blocks with the help of Wild-Leitz

stereo zoom microscope. The number of vessels per $0.5mm^2$ in transverse sections of xylem was counted on Metzer projection microscope. For each parameter hundred measurements were selected at random and they were statistically analysed to determine the mean and standard deviation.

Drawings:

The dimensional details and meteorological data were either represented in histograms or plotted on graphs. Schematic diagrams were drawn illustrating the seasonal variation in the mean number of cell layers in the cambial zone in the main stem and young branch and differentiating xylem and phloem elements in the main stem.

HISTOCHEMICAL TECHNIQUES:

Radial longitudinal sections were used for histochemical studies. Starch was localised by I KI (Johanssen, 1940) and total I_2 insoluble polysaccharides were stained by periodic acid-Schiff's reagent (Feder and O'Brien, 1968). Accumulation of starch grains in axial and ray parenchyma of phloem and xylem of the main stem and young branch and in fusiform cambial cells and cambial ray cells from the main stem was measured with the following scale (modified) given by Essiamah and Eschrich, 1985.

_/ = starch present

X = starch absent

\- = tissues to less than 1/4 filled starch

\+ = tissues to 1/4 filled starch

O = tissues to 1/3 filled starch

● = tissues to 1/2 filled starch

●● = tissues to 3/4 filled starch

●●● = tissues completely filled with starch

Mercuric bromophenol blue (Mazia et al., 1953) or Coomassie brilliant blue R 250 (Eklavya, 1979) were employed to localise proteins. For control, tissues were pre-treated with sodium nitrite and acetic acid prior to the staining. For the detection of lipids Sudan black-B stain was used (Jensen, 1962). For control, tissues were treated with ether-ethanol at 60 ^{o}C and washed in running water prior to the staining. Phenolic contents were identified with Nitroso reaction (Reeve, 1951).

SOIL ANALYSIS:

To compare the characters of the soil of pollution site and pollution free sites, soil samples were obtained from each site of sample collection. Soil sample was collected from two feet below the outer layer and packed in polythene bags. pH, conductivity, Nitrogen, phosphate and potash of soil were analysed by soil-chemistry division, G.S.F.C. The data obtained from the soil analysis are given below :

	G.S.F.C.	DHAKOR Rd.	KAYAVAROHAN	M.S.U.
Nitrogen	0.53	0.88	0.66	0.43
Phosphorous	45.60	43.50	20.40	20.40
Potash	150.00	110.00	212.50	80.00
PH	7.80	7.90	8.40	8.30
EC conductivity	0.12	0.12	0.32	0.32

PHOTOGRAPHY:

Photomicrographs were taken from stained sections on a Zeiss photo microscope using ORWO NP 55 negative film. To examine the lignified walls of xylem, unstained sections were observed and photographed under polarized light with Leitz laborlux 12 Pol or Zeiss Axiomat and epiflourescence light with Zeiss flourescence microscope. The filters BP 546 450-490, FT 580 FT-510, LP 590 LP-520 were used with the flourescence microscope. For the xylem growth rings wood blocks were directly photographed using Wild-Leitz stereomicroscope. The positive prints were taken on Sterling normal glossy and resin coated paper.