mean minimum of 14.8° C in January and the two rainy seasons (June to September and October to November) coming one after the other but with opposite wind regimes, corresponding to the south-west and north-east monsoons. The marked thunderstorm with occasional hail storms and squalls in April-May and September-October are also typical. The other important features are the predominant low clouding and more or less steady temperatures with small diurnal variation during the monsoon season, the early morning dew and, mist or fog during October to February. The climatological data for the study period are presented in Table - 2.1

2.4 Vegetation

The topographical and climatic features of the city are favourable for the growth of a variety of herbs, shrubs and trees. The vegetation is regarded as deciduous jungle type wherein some of the cultivated species have become naturalized. During the major part of the year, the vegetation is active and a few species remain dormant for a short period in April and May. As the monsoon advances, the ground vegetation becomes dominant, completely covered by annual weeds that continue to flower till late January.

2.5 Vehicular Data

The traffic in the city is very much on the increase in view of immigration and the growth of trade and commerce. The total number of registered vehicles in the city as on November 1996 were 9,84,000 with a five yearly growth rate of 174% over 1991 (Fig. 1).

2.6 Ambient Air Quality

The rapid proliferation of vehicles, especially two wheelers is expected to have an adverse effect on the quality of air. To study the impact of vehicular pollution on air quality, the south Zonal office of the Central Pollution Control Board has been monitoring air quality at three stations viz. Ananda Rao Circle (100 metre away from study site namely Bangalore Bus Station), AMCO Batteries on Mysore Road and near Graphite India in Krishnarajapuram. The data on two major pollutants sulphur-dioxide (SO2) and oxides of nitrogen (NOx) for the study period are presented in Table 2.2.
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3.1 Description of Experimental Sites

The sites selected for the present study have no pollution emitting sources in the close vicinity other than automobile emission (Fig. 2). They include Jnanabharathi Campus (S I) - a control site situated 12 km away from the busy traffic area with a meagre movement of < 300 v/day. The soil and plant materials for the control sets were collected from the interior places of the campus keeping in mind the possible interference of pollutants. Among the polluted sites, Vijayanagar (S II) is a major residential area with a traffic density of 21,500 per day. Site III is Bangalore Bus Station, situated in the heart of the city with an approximate traffic density of 30,000 vehicles each day. The diesel driven city transport buses are the predominant vehicles here. The fourth site is Mahatma Gandhi Road (S IV) with a traffic density of about 39,000 a day, most of them are petrol driven two wheelers and cars as the entry of heavy vehicles is prohibited on this road. The fifth site is Yeshwanthapura (S V), a busiest traffic centre with high commercial activities. Buses, inter-state trucks, cars and two wheelers dominate this road with an average of 48,000 vehicles per day.

3.2 Plants selected for the Study

Besides the naturally growing herbs and shrubs, several ornamental plants and avenue trees have been planted along the roadways. The following plant species, selected for the investigation, were identified using the Flora of Hassan District (Saldanha and Nicolson, 1976). Identifications were further confirmed with those in the Herbarium of the Department of Botany Bangalore University.

Ageratum conyzoides L. Asteraceae
Alternanthera pungens H.B. and K. Amaranthaceae
Amaranthus spinosus L. Amaranthaceae
Bauhinia purpurea L. Caesalpiniaceae
Bidens biternata (Lour.) Merr. and Sherff. Asteraceae
Bougainvillea spectabilis L.
Chloris barbata Sw.
Cynodon dactylon (L.) Pers.
Euphorbia hirta L.
Gomphrena celosioides Mart.
Lagascea mollis Cav.
Parthenium hysterophorus L.
Plumbago zeylanica L.
Polyalthia longifolia (Sonn.) Thu.
Pongamia pinnata (L.) Pierre.
Portulaca oleracea L.
Santalum album L.
Tridax procumbens L.
Vernonia cinerea (L.) Less.
Vinca rosea L.
(Syn. Catharanthus roseus (L.) G. Don.).

Nyctaginaceae
Poaceae
Poaceae
Euphorbiaceae
Amaranthaceae
Asteraceae
Asteraceae
Plumbaginaceae
Annonaceae
Papilionaceae
Portulacaceae
Santalaceae
Asteraceae
Asteraceae
Apocynaceae

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The criteria adopted for the selection are:

i. To have representatives from among the different growth forms *viz.*, herbs, shrubs and trees.

ii. Emphasis on herbs owing to their likely good responses to pollutants.

iii. To have members of both monocots and dicots.

iv. Close proximity to roadways and because,
   a) In contrast to lower plants, higher plants generally have a well developed conducting system which perhaps supports an effective translocation of pollutants
   b) The proportion of the pollutants either deposited or intercepted on the larger leaf area is likely to promote plant - pollutant interactions.

v. The native plants growing in the polluted and cleaner habitats are likely to provide valuable information on pollution tolerance and adaptability.

3.3 Sampling

The analysis for heavy metals in plants and supporting soil were carried out from October 1994 to April 1996 covering six seasons (Rainy season - Oct 1994 and Sep 1995; Winter - Feb 1996 and Jan 1996; Summer - April 1995 and May 1996).

The parameters such as pigments, sulphates, ascorbic acid oxidase and peroxidase activities were analysed for rainy season (Oct 1994), winter (Feb 1995) and summer season (April 1995). The proline content in plants was estimated during Oct 1995, Feb and Nov 1996. The mobility of metals in relation to soil characteristics was assessed for two seasons *viz.*, rainy (Oct 1995) and winter (Jan 1996). The epidermal studies (Nov 1995), nitrite content and nitrite reductase activities in plants (Oct 1996) and, heavy metal analysis in unwashed leaf samples are one time studies (Oct 1995).
3.4 Analytical Methods

3.4.1 Soil Parameters

a) Sediment analysis

To determine the mobility of metals along the vertical gradient in soil, ten core samples were collected between 0-2, 2-5, 5-10, 10-15, 15-20 and 20-30 cm depths using a core extractor of 2.5 cm diameter. The samples were collected in polythene bags, labeled and transported to laboratory. All the samples were mixed to obtain composite samples of respective depths, air dried for three days and sieved through 2mm nylon mesh.

The pH was determined in soil suspensions (Soil : Water, 1:5 w/v) using Systronics model 361 pH meter. The organic carbon content was determined by Walkley and Black titration method, and nitrogen by microkjeldahl technique (Trivedi et al., 1987). The electrical conductivity, potassium and phosphorus were determined following the Standard Methods (Jackson, 1973).

b) Heavy metals

Besides sediment samples, twelve surface soil samples (0-2 cm) were also collected from each site, at random, to cover the entire sampling area and composite samples were prepared. Large stones and plant materials were removed, dried for three days at 60°C and sieved through 60 nylon mesh.

Digestion of 0.1g sample was carried out with 10cm³ of 1:1 mixture of concentrated nitric acid and hydrofluoric acid contained in 50cm³ polypropylene squat beakers (Ward et al., 1977). The solutions were taken to dryness over a water bath and the residues were redissolved in 2 M hydrochloric acid. The solution was filtered through Whatman No. 42 filter paper and washed repeatedly using glass distilled water to a final volume of 25 ml.
3.4.2 Plant Parameters

a) Heavy metals

The analysis for heavy metals in plants was carried out by collecting plant samples within three metres from the edge of the road with maximum collection from the road divider that supported good growth of plants. The root and leaf samples were separately washed in slowly running tap water for 3 min, treated with 0.1 N HCl for 30 sec, followed by repeated washing with glass distilled water. Samples were also wiped with cloth and brush wherever necessary to remove the adhered particles. In one sample during Oct 1995 (no rains occurred for twenty days before sampling) leaves were divided into washed and unwashed portions to estimate difference in the extent of surface adsorption. All the samples were oven dried at 200° C for 24 hours, powdered using glass mortar and pestle.

To samples weighing 1 g, 15 ml of concentrated nitric acid and 5 ml of perchloric acid were added, set aside overnight and heated on sand bath until the solution evaporated to about 3 ml (Djingova et al., 1991). The contents were redissolved in 5 ml of 50% (v/v) hydrochloric acid, filtered in Whatman filter No. 42, and final volume of 25 ml was made after washing with glass distilled water (Beavington, 1975).

Both the soil and plant solutions were analysed in Varian Techron AA-30 Atomic Absorption Spectrophotometer using acidified aqueous standard solutions. All concentrations are expressed in µg/g dry weight.

b) Epidermal studies

For selected plants, epidermal peels were taken manually from either side of the fresh leaves, stained in Iodine-Phenol-KI reagent (Heath, 1947) and observed under light microscope. Frequencies of stomata, epidermal cells, abnormal guard cells were recorded and stomatal index calculated. Stomatal length and breadth were also measured from the peels using the method of Hill (1980). The viability of guard cells, subsidiary cells and epidermal cells was estimated using 0.1% neutral red dye (Black and
Black, 1979). The data presented are from a minimum of thirty microscopic fields (0.125 mm$^2$) from around fifteen peels of different leaves.

Stomatal index (SI) was calculated using the formula (Salisbury, 1927).

$$SI = \frac{S}{(E+S)} \times 100$$

Where $S = \text{number of stomates per microscopic field (0.125mm}^2\text{), and}$ $E = \text{number of epidermal cells in the same area.}$

c) Pigments

The pigment extracts were prepared by macerating 1g of fresh leaf in 90% (v/v) chilled acetone and little magnesium carbonate. Extracts were centrifuged at 2500 rpm for 10 min to 10,000 rpm for 15 min depending on the nature of extract. All operations were carried out under dim light.

i. Chlorophyll

The concentration of chlorophyll 'a', 'b' and total chlorophyll were determined by measuring the absorbance at 645, 663 and 750 nm, after diluting aliquot to 80% acetone extract, using UV - visible Spectrophotometer (Systronics - 108). The formula of Arnon (1949) was used for calculations.

$$\text{Chl 'a' mgg}^{-1} = 12.7 (A_{663}) - 2.69 (A_{645}) \times \frac{V}{1000} \times W$$
$$\text{Chl 'b' mgg}^{-1} = 22.9 (A_{645}) - 4.68 (A_{663}) \times \frac{V}{1000} \times W$$
$$\text{total Chl mgg}^{-1} = 20.2 (A_{645}) + 8.02 (A_{663}) \times \frac{V}{1000} \times W$$

Where $A$ is the absorbance at 663, 645 nm minus the absorbance at 750 nm.

ii. Phaeophytin

The phaeophytin contents were determined by measuring the absorbance at 665 and 750nm before and after acidification. Calculations were done using the formula of Wetzel and Westlake (1974).
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mg phaeophytin / sample = 11.9 (v/l) (1.7Da) · Chl a

Where Da = absorbance at 665 nm minus 750 nm after acidification
v = volume of the extract in ml
l = length of the light path in cm.

iii. Carotenoids

The carotenoids were determined after recording absorbance at 480nm and calculations were made using the formula of Strickland and Parson (1968).

Carotenoids, mg/l = v x OD 480 x 40

Where v = volume of extract in ml

All the concentrations are expressed in mg/g fresh weight.

d) Determination of sulphate-sulphur

The sulphate-sulphur content in plants were determined by the method of Wimberley (1968). One gram of dried and sieved plant material in 50 ml of distilled water was shaken for 30 min after adding 1.5 g charcoal. The extract was filtered through Whatman No. 2 filter paper. Five ml of aliquot and 0.5 ml of HCl (36%) was diluted to 25 ml and 0.25 g of Barium chloride crystals were added. Absorbance of the extract was taken at 480 nm after two min. The sulphate-sulphur concentrations were determined using a standard curve and the results expressed in percentage of sulphate-sulphur per gram plant material.

e) Proline estimation

The proline content was estimated by the method of Bates (1973). For this purpose fully expanded disease free leaves were collected, washed in distilled water and blotted.

About 0.5 g material was homogenised in 10 ml of 3% aqueous sulphosalysilic acid and filtered through Whatman No.2 filter paper. Extraction was repeated and filtrates were pooled. Two ml of filtrate was

1 Chlorophyll 'a' was determined separately by using Talling and Driver's (1963) formula since the specific absorption coefficient 11.9 is for 90% acetone at 665nm.
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reacted with 2 ml of freshly prepared acid - ninhydrin (1.25 g in 30 ml warm glacial acetic acid and 20 ml of 6 M phosphoric acid), and 2 ml of glacial acetic acid in test tubes for 1 hour at 100°C on a water bath. The tubes were transferred to ice bath to terminate the reaction. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 30 seconds. The chromophore containing toluene was aspirated from the aqueous phase, and the absorbance read at 520 nm at room temperature using toluene blank.

The concentrations were determined from a standard curve and calculated on a fresh weight basis.

\[
\text{Proline, } \mu \text{ moles/g} = \frac{\mu g \text{ proline/ml x ml toluene}}{115.5} \times \frac{5}{\text{g sample}}
\]

Where 115.5 is the molecular weight of proline.

f) Determination of Ascorbic acid

Ascorbic acid contents were determined by the method of Aberg (1958). One gram of fresh leaves was homogenised in 4 ml of freshly prepared oxalic acid (0.4% w/v), filtered through 2 layers of nylon cloth and centrifuged at 1000 x g for 20 min. The final volume was made upto 10 ml using oxalic acid.

Five ml of extract in a white porcelain dish was titrated against standardised 2-6 dichloro-phenol-indophenol (50 mg in 250 ml water and 42 mg NaHCO3) to pink end point which persisted for at least 15 seconds. Five ml of standard ascorbic acid (0.20 mg/ml) was also titrated similarly. Ascorbic acid content was calculated using the formula,

\[
\text{Ascorbic acid, } \text{mg g}^{-1} = I \times S \times D/A \times 1/W
\]

Where

- \( I \) = ml of Indophenol reagent used in the titration
- \( S \) = mg of ascorbic acid reacting with 1 ml of Indophenol
- \( D \) = volume of the extract in ml
- \( A \) = aliquot titrated in ml
- \( W \) = weight of sample in g.
g) Ascorbic acid oxidase activity

This was assayed following the method of Oberbacher and Vines (1963). Fresh leaves were macerated with five parts of (w/v) 0.1M phosphate buffer (pH 6.5) in a cold mortar and pestle. The homogenate was centrifuged at 3000 x g for 15 min. The supernatant was used as enzyme source. All operations were carried out at below 4°C.

The enzyme assay mixture contained, 3 ml of 0.1 M phosphate buffer (pH 5.6), 0.5m moles of ascorbic acid and 0.1 ml of enzyme extract. The decrease in absorbance peak of the acid due to oxidation by ascorbic acid oxidase was followed at 265 nm at an interval of 30 sec for 5 mins. The buffer and boiled enzyme extracts served as blank. The enzyme activity is expressed as change in absorbance per minute.

h) Peroxidase activity

One gram of freshly harvested leaves were homogenised in 3ml of 0.1M phosphate buffer (pH 7.0) and centrifuged at 18000 x g for 15 min at 4°C. The assay mixture consisted of 3 ml buffer, 0.05 ml guaiacol (240 mg in 100 ml d. water) and 0.03 ml of 12.3 mM hydrogen peroxide (Putter, 1974). The peroxidase reaction was initiated by adding 0.1ml of filtrate and the absorbance read at 436 nm (after an initial absorbance rise of 0.05) to note the time required in minutes (Δt) to increase the absorbance by 0.1.

\[
\text{Enzyme activity units/L} = \frac{3.18 \times 0.01 \times 1000}{6.39 \times 1 \times \Delta t \times 0.01}
\]

i) Nitrite (NO\textsubscript{2}) and nitrite reductase (NiR) activity

The nitrite content and nitrite reductase activities were assayed by collecting leaves of uniform size at around 17.00 hours (day) and next day at 5.00 hours (early morning) from the same plant. The washed leaves were blotted, weighed, cut into small pieces and ground in a cold mortar and pestle with 3 ml grinding medium, in each case, composed of 0.1 M potassium phosphate buffer (pH 7.5), 10\textsuperscript{-3} M cysteine and 10\textsuperscript{-3} EDTA. The homogenate was squeezed through nylon cloth and the filtrate was
centrifuged at 18000 x g for 20 min. All operations were carried out below 4°C. Nitrite content and NiR activities were determined as follows.

- Nitrite
  One ml aliquot of the supernatant was combined with 1.5 ml of 1% (w/v) sulfanilamide in 1.5N HCl and 1.5 ml of 0.02% (w/v) N-(1-naphthyl) ethylene diamine dihydrochloride. After 20 min the mixture was centrifuged at 18000 x g for 5 min and the absorbance of the supernatant was measured at 540 nm. Nitrite concentration was determined using a standard graph prepared by analytical grade sodium nitrite (Yoneyama et al., 1979).

- Nitrite reductase (NiR) activity
  The NiR activity was assayed principally by the method of Ida and Morita (1973). The assay mixture contained 1.0 ml of 0.1 M tricine - K OH buffer (pH 7.5), 0.2 ml of 5mM NaNO₂, 0.1 ml of 20mM methyl viologen and 0.1 ml of enzyme extract in a final volume of 2.0 ml. The reaction mixture was incubated for 15 min at 30°C, after adding 0.2M sodium bicarbonate to start the reaction. The reaction was stopped by shaking vigorously with a flush mixer until the blue colour of reduced methyl viologen disappeared completely. An aliquot of 0.1 ml was withdrawn into 1.0ml 1% (w/v) sulphanilamide in 1.5 N HCl. One ml of 0.02% (w/v) N - (1-Naphthyl) ethylene diamine dihydrochloride was added, and the volume made to 5 ml with water. After 20 min the absorbance was determined at 540 nm.

  The non-enzymatic loss of nitrite was determined with control assay mixture without the enzyme. The enzyme dependent disappearance of the substrate was estimated by subtracting the amount of nitrite which disappeared in the control assay. The enzyme activity is expressed in μmoles nitrite reduced per hour g⁻¹ fresh weight.
3.5 Computation of Indices

3.5.1 Heavy Metal Index (HMI)

The heavy metal indices were computed to characterise the study sites for heavy metal pollution load by means of extensive analyses using the method of Herzig (1993). The mathematical function used for the calculation is

\[
\text{Heavy Metal Index} = \sum_{i=1}^{n} LC_i
\]

Where \( i = 1 \)

Load class ; load category value of elements (see section 4.2)

\( n = \) heavy metals used.

3.5.2 Accumulation Index (AI)

Heavy metal accumulation index was calculated by the method of Bjerre and Schierup (1985).

\[
AI = \frac{\text{mean metal concentration in plant}}{\text{mean metal concentration in soil}}
\]

3.5.3 Enrichment Factor (EF)

Heavy metal enrichment factor in soil and plants were determined by the formula of Djingova et al. (1993).

\[
EF = \frac{\text{Conc. of element in polluted samples}}{\text{Conc. of element in background samples}}
\]

3.5.4 Air Pollution Tolerance Index (APTI)

Air pollution tolerance index was calculated using the described procedures (Dutta and Sinha Ray, 1995). The parameters considered are total
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chlorophyll, ascorbic acid content, pH of the leaf extract\(^1\) and relative water content of leaves\(^2\).

\[
\text{APTI} = \frac{A(T+P)+R}{10}
\]

Where

- \(A\) = ascorbic acid in leaf, mg g\(^{-1}\) dry weight
- \(T\) = total chlorophyll, mg g\(^{-1}\) dry weight
- \(P\) = pH of leaf extract
- \(R\) = relative water content in per cent.

The results are expressed as APTI per cent reduction/stimulation over control.

3.6 Statistical Analyses

The impact of traffic density on various parameters (variables) was determined on the basis of statistical principles. The strength of the relationship between pairs of variables are estimated with the help of coefficient of correlation (r).

The variability of metal concentration in soil and plants as a quadratic function of traffic density was established through non-linear regression (R\(^2\)) analysis (Draper and Smith, 1991).

The spatial (depth) dependence of heavy metals in soil with organic carbon and pH is explained by semi-variance which is the measure of the similarity (on average basis) among different depths (Burgess and Webster, 1980). The more alike are the depths, the smaller is the semi-variance and vice-versa.

The source of variance due to the site, plant, and site-plant effects were tested by ANOVA and comparisons between pairs of sites, plant and site-plant interaction effects were established using critical difference (CD).

\(^1\) The pH of the fresh leaves were determined by homogenising 5g in 25ml distilled water.

\(^2\) The Relative Water Content (RWC) was calculated using the formula of Singh (1977)

\[
\text{RWC} (\%) = \frac{(F-D/T-D)}{100}, \text{where } F = \text{the fresh weight of leaf, } D = \text{dry weight, } T = \text{turgid weight (dipped in distilled water for 24 hrs.)}
\]
The student's t-test was performed to compare the epidermal variations in plant species between pairs of sites having variable degree of vehicular density. The effective degrees of freedom was calculated theoretically for testing the significance level (Steel and Torrie, 1980).

To identify the plant responses to vehicular pollution Cumulative Variable Index (CVI) analysis (Barreto et al., 1988) for heavy metals, variables other than heavy metals and both in combination was carried out. This method generated an index by integrating several quantitative characteristics in numeric form. These are provided as standardised targets and intensities as a function of mean and standard deviation from a total number of variables. Simple correlation coefficient is also included.

The species are ranked such a way that, higher the heavy metal accumulation least is the index value and vice versa. Variables such as chlorophyll 'a', 'b', carotenoids and ascorbic acid were given negative weightage since they showed inverse relationship with traffic density. The index arranged in ascending order is divided into three equidistant categories as 'high', 'moderate' and 'low' response categories.

The statistical analysis were performed using statistical presentation software system.