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BIOCHEMICAL AND ULTRASTRUCTURAL CHANGES IN PLANT FOLIAGE EXPOSED TO AUTO-POLLUTION

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Abstract. Auto-pollution is the by-product of our mechanized mobility, which adversely affects both plant and human life. However, plants growing in the urban locations provide a great respite to us from the brunt of auto-pollution by absorbing the pollutants at their foliar surface. Foliar surface configuration and biochemical changes in two selected plant species, namely *Ficus religiosa* L. and *Thevetia nerifolia* L., growing at IT crossing (highly polluted sites), Picup bhawan crossing (moderately polluted site) and Kukrail Forest Picnic Spot (Low polluted site) were investigated. It was observed that auto-exhaust pollution showed marked alterations in photosynthetic pigments, protein and cysteine contents and also in leaf area and foliar surface architecture of plants growing at HP site as compared to LP site. The changes in the foliar configuration reveal that these plants can be used as biomarkers of auto-pollution.

Keywords: auto pollution, biochemical changes, ultrastructural changes, *Thevetia nerifolia*, *Ficus religiosa*, biomarkers

1. Introduction

Notwithstanding, automobiles are the lifeline for the city dwellers, but also serve as one of the major sources of pollution in Indian metro cities and big towns. In Lucknow city alone, the vehicle number has frighteningly augmented many folds (presently over 0.6 million) during past one decade, which has compounded the health problems of the citizens. The reasons for high incidence of mortality and morbidity associated with auto-pollution are many; firstly, the city has more population of two wheelers than 3- and 4-wheelers, which being largely 2-stroke, belch out more pollutants due to incomplete internal combustion, as compared to 4 wheelers; secondly, the pollutants from the auto-exhaust are released at very low height from the ground and hence not diffused into the larger sphere of the environment; thirdly, the dilution of auto-emission is impended by the high-rise buildings along the roads. In such situations, the pollution level increases alarmingly, particularly in the winter season, in morning or evening hours, due to atmospheric inversion and hence, the city dwellers have to suffer from chronic diseases like asthma, lung cancer, bronchitis, kidney failure etc. To reduce the ill effects of automobile pollution, first we have to find out a solution at the source to contain emission of pollutants from the auto-exhaust. The new automobile technology, based on alternative fuels other than gasoline or diesel like CNG, propane or LPG, is most welcome, as these fuels emit less pollutants than conventional fuels and therefore, treated as environmentally acceptable fuels. On the other hand, for the pollutants already discharged to the atmosphere, the avenue trees have to be planted along the roadsides, which can serve as sinks for the air pollutants. Some plant species have been identified to be able to absorb, detoxify and tolerate high level of pollution (Nivane *et al.*, 2001). However, this noble service to mankind is not rendered by plants without any cost, but they have to suffer from the damage caused by air pollution, as they are exposed round the clock. As a consequence, they develop foliar injury symptoms specific to particular air pollutant or mixture and modify their metabolism and leaf architecture to adapt to new environment. Thus, the plants can be used as both passive bio-monitors and bio-mitigators in the urban environment to indicate the environmental quality and to attenuate the pollution level in a locality (Beckett *et al.*, 1998; Cox, 2003). In view of plant's role in indication and abatement of air pollution, this study was carried out to assess the impact of automobile pollution on the plant metabolism and foliar surface configuration of plants growing along the roadsides in the urban locality in order to identify their role as the biomarkers of air pollution.

2. Materials and Methods

The Lucknow city is situated between 26°52′N latitude and 80°56′E longitude and 120 m above the sea level in the central plain of the Indian subcontinent. It is the capital of Uttar Pradesh, one of the largest and highly populous states of India. The city is spread over an area of 79 km^2 and has a population of more than 1.7 million. It has distinct tropical climate with a marked monsoonal effect. The year is divided into three distinct seasons i.e. summer (March to June), rainy (July to October) and winter (November to February). The temperature ranges from a minimum of 5 °C in winter to a maximum of 47 °C in summer. The mean average relative humidity is 60% and rainfall 1006.8 mm.

In order to assess the effect of automobile exhaust pollution on the biochemical and surface ultrastructural changes of the plant foliage, three sites i.e. Kukrail forest picnic spot (LP), Picup bhawan crossing (MP) and IT crossing (HP) were selected on the basis of low, moderate and very high traffic density to serve as non-polluted, moderately polluted and highly polluted sites. At these sites, because of being residential areas, there were no other pollution causing sources i.e. either fugitive or anthropogenic. On these sites, air monitoring of levels of SO_2, NO_2, O_3, SPM and heavy metals was also carried out to evaluate the actual air quality status.

A high volume sampler (Envirotech make-APM 460) was used for monitoring of SPM (Suspended Particulate Matter) and for gaseous pollutants; an attachment device (APM 411) with the high volume sampler was used to bubble air at the flow rate of 1.5 ml/min in glass impingers filled with 25 ml of different absorbing solutions for monitoring of different gaseous pollutants i.e. potassium tetrachloro mercurate (TCM) for SO_2, sodium hydroxide-sodium arsenite solution for NO_2, and potassium iodide solution for O_3. The samples were brought to the laboratory and analysed within the reasonable time, following the standard methods for SO_2 (West and Gaeke, 1956), NO_2 (Jacobs and Hochheiser, 1958) and O_3 (Byers and Saltzman, 1958). Heavy metals in the air were analysed with an Atomic Absorption Spectrophotometer (make AAS, GBC Avanta Σ). All the measurements were carried out twice a month at all the sites throughout the year and here we are presenting seasonal mean values of each parameter.

In order to collect the plant samples, a plant survey was made at three selected sites i.e. HP site, MP site and LP site and two common plants of about same age i.e. *Ficus religiosa* – a tree and *Thevetia nerifolia*–a shrub were selected from these sites for the foliar biochemical and surface configuration studies. About 100 mature leaves from each plant species from the road facing branches were plucked between 11–12 AM through random selection. These leaves were placed into polythene bags, brought to the laboratory and preserved at $4 \pm 0.5\,^{\circ}C$ till analysis. The samples were analyzed for different parameters within 24 h of their harvesting.

After extracting in 80% chilled acetone, the chlorophyll *a* and *b* ($mg\,g^{-1}$ fw) were estimated, using the colorimetric method described by Arnon (1949), while carotenoid content ($mg\,g^{-1}$ fw) was determined, following the method of Duxbury and Yentsch (1956). Protein content ($mg\,g^{-1}$ fw) was determined by the method outlined by Lowry *et al.* (1951). For leaf area measurement, leaf area meter (Make Delta T Devices, UK) was used. Cysteine (nmole g^{-1} fw) was estimated, following the method outlined by Gaitonde (1967). Heavy metals ($\mu g\,g^{-1}$ dw) in the plant foliage were analyzed by using a GBC Avanta Σ Atomic Absorption Spectrophotometer (AAS), following the standard protocol of APHA (1998). The standard reference materials (BNDs) were used for the calibration of AAS.

In order to carry out Scanning Electron Microscopic studies (SEM), leaf samples of about same age were plucked from the branches of these plants facing the roadside and were washed thoroughly and repeatedly with tap water, followed by deionized water using camel hairbrush to eliminate all loose dust from the leaf surface. Then the pieces of $0.3\,cm^2$ of these samples were fixed in 2.5% of gluteraldehyde only under field condition to avoid any post plucking damage or changes. Afterwards, these samples were brought to the laboratory and dehydrated in ethanol series (30–100%), and further drying was carried out in a critical point drier (Tousimis, Samdri PVT3, Sweden) with liquid CO_2 at 1072-psi pressure and $31.4\,^{\circ}C$ temperature. Materials were then coated with thin conductive film of gold about 200 Å in thickness in an ion sputter coater (JFC 1100) and then examined under scanning electron microscope (Philips XL-20) and photographed.

The data collected during this study were analyzed statistically following standard methods outlined by Gomez and Gomez (1984).

3. Results and Discussion

Ambient air quality monitoring in different localities of Lucknow city indicated that IT crossing (HP) was highly charged with pollutants coming out of automobile exhaust (SO_2, 41.92 μg m^{-3}, NO_2, 38.24 μg m^{-3}, O_3, 38.24 μg m^{-3} and SPM, 995.00 μg m^{-3}), followed by Picup bhawan crossing (MP) (SO_2, 38.48 μg m^{-3}, NO_2, 21.66 μg m^{-3}, O_3, 27.56 μg m^{-3} and SPM, 427.70 μg m^{-3}) in the descending order, while Kukrail forest picnic spot (LP) showed very low levels of pollution load (NO_2, 4.30 μg m^{-3}, O_3, 20.00 μg m^{-3} and SPM, 150.00 μg m^{-3}). However, SO_2 level could not be detected at this site (Table I). It was observed that at all the sites, levels of gaseous pollutants (SO_2, NO_2 and O_3) were below the threshold limits, while the SPM level was alarmingly very high at two sites, barring Kukrail picnic spot, where SPM was found below the threshold limits and was, therefore, considered as low polluted site (LP).

The ambient concentration of these pollutants showed a positive correlation with the traffic density at all the sites. As shown in Table II, at HP site, the vehicle numbers were 4552 vehicles h^{-1} for petrol and 2171 vehicles h^{-1} for diesel, while at LP site, these numbers were only 42 vehicles h^{-1} for petrol and 10 vehicles h^{-1} for diesel. However at MP site, diesel and petrol vehicular population was numbered as 2289 vehicles h^{-1} and 362 vehicles h^{-1}, respectively.

As the SPM contains many trace elements, they were analyzed by atomic absorption spectrophotometer to assess their ambient load. The trace element concentration in SPM at different sites is presented in Table I. The trend of different

TABLE I
Ambient air quality at low, moderately and highly polluted sites

	Pollutants (μg m^{-3})[d]			
Sites	SO_2	NO_2	O_3	SPM
LP[a]	BDL[e]	4.30 ± 0.32	20.00 ± 1.17	150.00 ± 11.1
MP[b]	38.48 ± 3.5	21.66 ± 2.2	27.56 ± 1.9	427.70 ± 38.5
HP[c]	41.92 ± 2.86	38.24 ± 2.16	38.24 ± 1.33	995.00 ± 64.5
	Metals (μg m^{-3})			
	Fe	Cu	Zn	Ni
LP	18.96 ± 0.92	0.87 ± 0.06	0.46 ± 0.04	BDL
MP	39.24 ± 2.14	2.34 ± 0.18	2.47 ± 0.12	0.02 ± 0.001
HP	52.76 ± 5.10	6.27 ± 0.21	3.13 ± 0.30	0.02 ± 0.001

[a] Low polluted.

[b] Moderately polluted.

[c] Highly polluted.

[d] Values are mean ± SD, $n = 10$.

[e] Below detectable limit.

TABLE II
Vehicular traffic density at low, moderately and highly polluted sites

	Traffic density (vehices hour^{-1})[d]		
Sites	Petrol	Diesel	Total
Lp[a]	42 ± 4	10 ± 2	52 ± 8
MP[b]	2289 ± 72	362 ± 22	2652 ± 92
HP[c]	4552 ± 31	2171 ± 16	6723 ± 55

[a] Low polluted.
[b] Moderately polluted.
[c] Highly polluted.
[d] Values are mean ± SD, $n = 10$.

metals in the SPM was in the order of Fe $>$ Cu $>$ Zn $>$ Ni. All these metals showed a positive correlation with the traffic density, as their concentration increased with the augmenting traffic load. A maximum value of ambient Fe (52.76 μg m^{-3}) was observed at HP site, followed by MP site (39.24 μg m^{-3}) and minimum (18.96 μg m^{-3}) at LP site. As far as concentration of other metals (Cu, Zn and Ni) was concerned, they also showed the similar trend. The concentrations of Cu, Zn and Ni were observed as 6.27, 3.13 and 0.02 μg m^{-3} at the HP site and 2.34, 2.47 and 0.02 μg m^{-3} at MP site, respectively, while at the LP site, Cu and Zn showed their concentrations as low as 0.87 and 0.46 μg m^{-3}, respectively. However, Ni was below detection limit at this site.

Increasing levels of SPM in cities above the threshold limits are of alarming concern, as the incidence of automobile emission related diseases like bronchial asthma, blood pressure, cancer etc. has increased manifolds among the city residents. Emission of auto-exhaust-borne heavy metals may be associated with the following circumstances; i) a higher rate of abrasion in vehicular engines and greater emission of particulates including dust by slow running vehicles over the fast running vehicles, ii) additional pollution produced by the vehicles halting at traffic signals and iii) less favourable condition of ventilation and dispersion of pollutants in street surrounded by high rise buildings. But we are not concerned only with the amount of SPM, but the elemental composition of atmospheric particulate matter has also received increasing attention in recent years. All the metals that could be ascribed to traffic, have been found at remarkably high levels in petrol and diesel exhausts (Que Hee, 1994). Tyre wear is an important source of Zn, brake releases particles of Cu while vehicle detachment and fluid leakage are the sources of Fe and Zn (Ball *et al.*, 1991). There are generally two different pathways of the entry of particulate matter in to earth's atmosphere; one from auto-exhaust and another from earth's crust. For long time, they remain suspended in the atmosphere to serve as media for the transfer or (re-) distribution of metals across various environmental reservoirs on the earth's system. They get eventually removed from the earth's system by either dry or wet deposition. Hence being constituent of SPM, the atmospheric cycle of most metals is tightly sub-ordinate to the fate of suspended PMs regardless of their origin (Kim *et al.*, 2002). Although a number of research groups around the world (KretzsChamar *et al.*, 1980; Carreras and Pignata, 2001) have generated adequate information about the distribution of trace elements in the environment, but there is hardly any information available for the urban areas of India.

Plants on exposure to auto-exhaust pollution underwent several biochemical and ultra structural changes, which could be also manifested in morphological changes on prolonged exposure.

The photosynthetic pigments are the most liable to be damaged by auto-pollution. Figure 1A reflects the values of chlorophyll *a* in the foliar tissues of the plants growing along roadsides at selected sites. It was noticed that chl *a* content decreased with increasing pollution load. In the case of *Thevetia nerifolia*, the chl *a* content at LP site was 1.91 mg g^{-1}, which declined to 1.32 mg g^{-1} (31%) at MP site and 0.98 mg g^{-1} (49%) at HP site, while in *F. religiosa*, chl *a* content, which was recorded 2.26 mg g^{-1}at LP site, reduced to 1.94 and 1.16 mg g^{-1} at MP and HP sites, showing a reductions of 14% and 49%, respectively. A wide variation in chlorophyll b content was also observed in the foliar tissue of plants growing at different sites in relation to the intensity of urban pollution due to automobiles. As evident from Figure 1, the chl *b* content in *T. nerifolia* was observed as 1.14 mg g^{-1} at LP site, 0.80 mg g^{-1} at MP site and 0.82 mg g^{-1} at HP site, exhibiting a maximum reduction of 49% at HP site as compared to LP site. However, in *F. religiosa*, it varied between a minimum of 0.40 mg g^{-1} at HP site and a maximum of 1.12 mg g^{-1} at LP site. When percent reduction in chl *b* was calculated, it was found 6% at MP site and 64% at HP site, as compared to LP site. These data suggest that, as compared to *T. nerifolia*, *F. religiosa* showed greater tolerance with regard to chlorophyll at MP site, but at higher concentration of pollutants i.e. at HP site, failed to maintain it. Chlorophyll is said to be an index of productivity, hence any alteration in chlorophyll concentration may change the morphological, physiological and biochemical behaviour of the plant. Air pollution-induced degradation in photosynthetic pigments was also observed by a number of workers (Bansal, 1988; Singh *et al.*, 1990). According to Rao and LeBlanc (1966), at cell sap pH between 2 and 3.5, takes place displacement of Mg^{2+} from the chlorophyll molecule by H^+, which is generated due to the splitting of H_2SO_3 into SO_3^{-2} and H^+. This leads to the formation of a chlorophyll degradation product, phaeophytin, which, no longer, serves to trap the solar energy for photosynthesis. According to Yu (1988), at pH above 3.5, HSO_3^-, which is formed during SO_2 metabolization, generates superoxide radical (O_2^-) which causes the oxidation of carotenoids and in the absence of carotenoid protection, oxidation of chlorophyll molecule occurs, resulting in the reduction of photosynthetic ability of the plant. In general, higher plant species synthesize more of the chl *a* than chl *b* and a ratio of chl *a* to chl *b* provides a measure of tolerance index of a tree. High value of this ratio indicates better tolerance to air pollution and *vice-versa* (Kondo *et al.*, 1980).

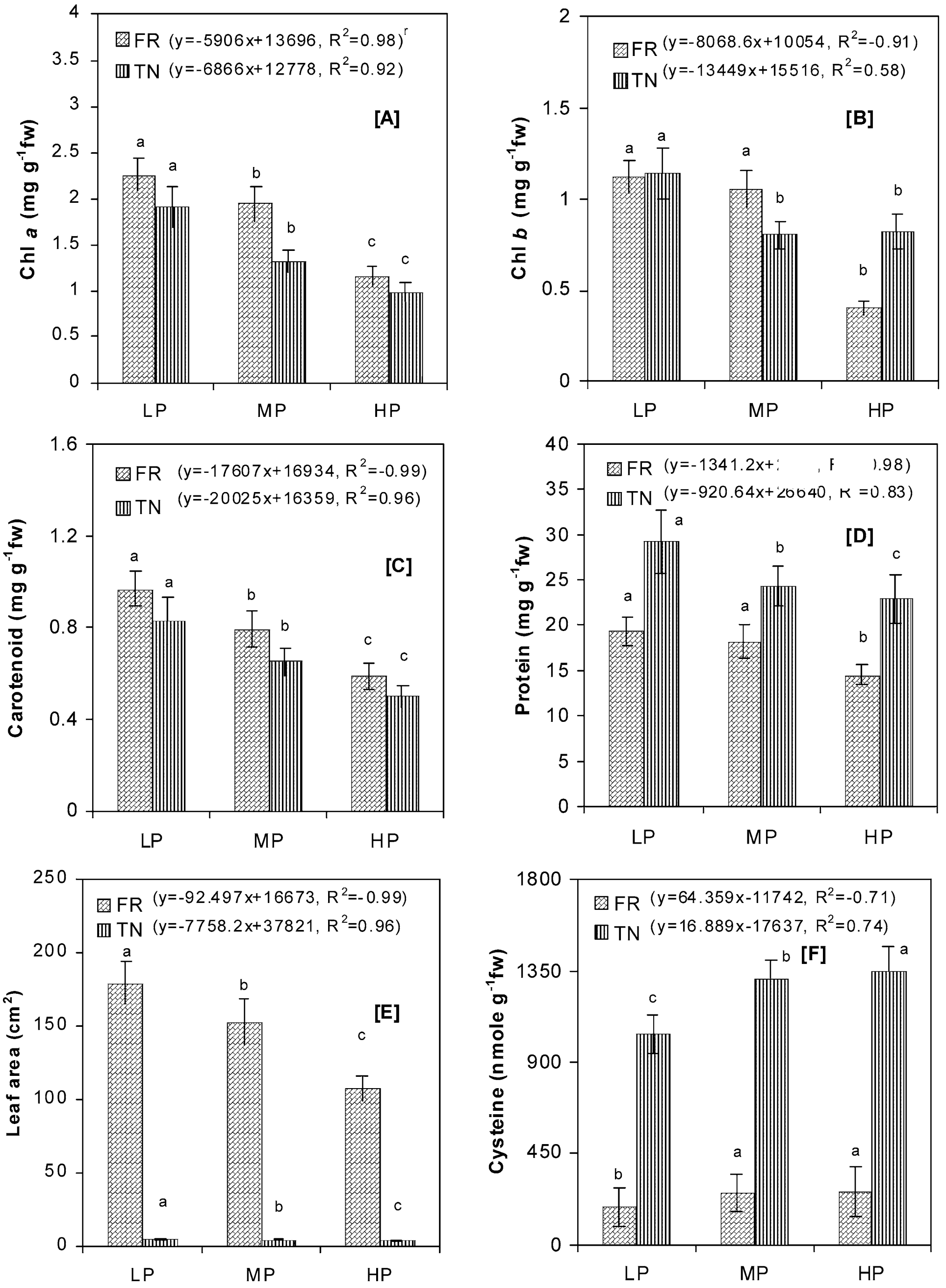

Figure 1. Dynamics of foliar parameters of *Ficus religiosa* (FR) and *Thevetia nerifolia* (TN) plants growing at highly polluted (HP) site as compared to low polluted (LP) site. r; correlation between foliar parameters and vehicular traffic density for particular plant species (values are mean ± SD, $n = 10$). Different superscripts on bars showed significant ($P < 0.05$) difference between the means according to DMRT).

Carotenoids, which act as antioxidant and also protect chlorophyll from photooxidation, was also estimated and data were presented in Figure 1C. It was observed that carotenoid content, like other photosynthetic pigments, decreased with increasing pollution load in the environment. In *T. nerifolia*, the carotenoid content which was recorded 0.83 mg g^{-1} at LP site, got reduced to 0.65 mg g^{-1} at MP site and 0.50 mg g^{-1} at HP site, showing reductions of 22% and 40%, respectively. However, *F. religiosa* showed reductions of 19% and 39% in the carotenoid content, at MP (0.79 mg g^{-1}) and HP (0.59 mg g^{-1}) sites, respectively, as compared to LP site (0.97 mg g^{-1}). Many workers have reported the loss of carotenoid pigments caused by various pollutants (Young *et al.*, 1988; Williams and Banerjee, 1995). Within the chloroplasts, carotenoids play an important function, particularly as photoprotective agents. But under stress condition, normal protective process may become overloaded and hence cellular destruction including pigment degradation occurs (Senser *et al.*, 1990). Oxidation of carotenoids takes place through light-catalyzed reactions resulting in the formation of epoxide, which is further reduced in dark by an enzyme-catalyzed reaction (Calvin, 1955). Krinsky (1966) has confirmed the existence of such epoxide cycles and its role in protection of chlorophylls against photo-oxidation.

Like photosynthetic pigments, a decline in the foliar protein content was also observed at the polluted sites (Figure 1D). *F. religiosa* showed a maximum reduction of 25% in the foliar protein content at HP site, in comparison to LP site. Foliar protein contents in *F. religiosa* were estimated to be 19.37, 18.18 and 14.61 mg g^{-1} at LP, MP and HP sites, respectively. In the same fashion, in *T. nerifolia*, the values of protein content were found as 29.27 mg g^{-1} at LP site, 24.40 mg g^{-1} at MP site and 22.90 mg g^{-1} at HP site, showing percent reduction of 17% and 22% at MP and HP sites, respectively, as compared to control site. Kumar and Dubey (1998) have also concluded that pollutants coming out of auto-exhaust may cause inhibitory effect on protein synthesis. The reduction in the foliar protein content was probably due to either breaking down of existing protein and/or reduced *de novo* synthesis of protein (Iqbal *et al.*, 2000). Reduction in protein content could also be attributed to decreased photosynthesis (Constantinidou and Kozlowski, 1979; Singh *et al.*, 1988).

As the leaves are the synthesizing organs of the plant, they are liable to be damaged first on the prolonged exposure to auto-exhaust pollution. The leaf area of *F. religiosa* declined from 179.09 cm^2 at LP site to 152.55 cm^2 at MP site and 107.22 cm^2 at HP site, showing reduction of 15% and 40%, respectively, while in *T. nerifolia*, a maximum reduction of 17% was observed at HP site, with respect to LP site, with its values as 4.91, 4.44 and 4.06 cm^2 at LP, MP and HP sites, respectively. (Figure 1E). Our results were in conformity with the findings of Agrawal *et al.* (1991) and Salgare and Thorat (1995). Lima *et al.* (2000) also observed a reduction in the leaf area of *Phaseolus vulgaris* in the environs of Bahia city of Brazil. She was of the view that leaf area reduction indicated the inability of a species to activate physiological protection mechanisms. On the other hand, Sharma *et al.* (1980) stated that reduced leaf area in a polluted environment was not only an indication of retarded growth, but possibly, also a morphological adaptation to highly polluted environment, because with the smaller leaf size, the lesser will be the absorption of obnoxious gas. Iqbal *et al.* (2000) observed both positive and negative trend of leaf area changes in plants growing in polluted environment in their investigation.

In contrast to above parameters, a marked increase in foliar cysteine content of the selected plant species was observed at the HP site as compared to low polluted (LP) site (Figure 1F). The cysteine content in the plant foliage of *F. religiosa* enhanced from 180.60 nmole g^{-1} at LP site to 260.18 nmole g^{-1} at HP site, showing a maximum enhancement of 44%. However, at MP site, an augmentation of 40% was observed with its value as 253.02 nmole g^{-1} as compared to LP site. Similarly in the case of *T. nerifolia*, 1033.80 nmole g^{-1} foliar cysteine content at LP site increased to 1308.80 nmole g^{-1} at MP site and 1348.38 nmole g^{-1} at HP site, showing percent induction of 27% and 30%, respectively. A higher enhancement in foliar cysteine content, at MP site and HP site, in the F. religiosa reflects its ability to metabolize SO_2 pollutant absorbed from the environment. DeKok (1989) and Tausz *et al.* (1998) have also observed the similar trend of increased cysteine content in plants exposed to air pollutants, particularly SO_2. Cysteine works as a buffering agent of SO_2 in the plant cell under the air pollution stress, as its –SH group containing amino acid is functional in the pH range of 5–8 (Smith and Raven, 1979). According to DeKok *et al.* (2000), the cysteine produced by the sulfate reductive pathway may be stored in the vacuole or transported out of the cell. Excess cysteine, however, is degraded to release H_2O, while a part of it may be oxidized back to sulfate and the cycle is replicated.

Estimation of Iron (Fe), Copper (Cu) and Zinc (Zn) accumulation by the plant foliage, growing at selected sites, showed marked alterations in their concentrations (Table III). In general, it was noted that in the foliage of both the plants, metal accumulation was maximum at the HP site as compared to the LP site. Foliage of *F. religiosa* showed a maximum accumulation of Fe i.e. 1221.75 $\mu g\, g^{-1}$ at HP site and lowest i.e. 192.25 at LP site, while *T. nerifolia* accumulated a maximum of 174.50 Fe at HP site and a minimum of 45.50 at LP site. In the same fashion, *F. religiosa* accumulated the maximum (146.00 $\mu g\, g^{-1}$) amount of Zn at HP site and minimum (24.00 $\mu g\, g^{-1}$) at LP site, whereas *T. nerifolia* accumulated 15.00 $\mu g\, g^{-1}$ and 6.25 $\mu g\, g^{-1}$ Zn at HP and LP site, respectively. However, as far as accumulation of Cu was concerned, it was found maximum (55.50 $\mu g\, g^{-1}$) in *T. nerifolia* growing at HP site, while same plant growing at LP site, showed an accumulation of 3.50 $\mu g\, g^{-1}$ Cu only. Like wise, *F. religiosa* accumulated 14.75 and 2.50 $\mu g\, g^{-1}$ Cu at HP and LP sites, respectively. Higher plants act as biomonitors of aerial heavy metal contamination because of their bioaccumulative properties. Higher plants not only intercept metals from atmospheric deposition, but also accumulate them from the soil. Air-borne heavy metals, when deposited on soil, are taken up by the plants via their root system and translocated to other parts of the plant (Shparyk and Parpan, 1990). Particle deposition on leaf surface may be affected by a number of factors including particle size and mass, wind velocity, leaf orientation, size, moisture level

TABLE III

Accumulation of heavy metals by selected plants species growing at low moderately and highly polluted sites

Metals	Plants[d]					
	Ficus religiosa			*Thevetia nerifolia*		
	LP[a]	MP[b]	HP[c]	LP	MP	HP
Fe (μg g^{-1} d.w.)	192.25 ± 7.84	748.25 ± 6.94	1221.75 ± 82.7	45.50 ± 3.21	146.00 ± 8.42	174.50 ± 12.3
Zn (μg g^{-1} d.w.)	24.00 ± 1.29	79.25 ± 4.68	146.00 ± 10.3	6.25 ± 0.52	12.75 ± 1.10	15.00 ± 1.32
Cu (μg g^{-1} d.w.)	2.50 ± 0.11	14.75 ± 1.35	16.00 ± 1.63	3.50 ± 0.31	37.00 ± 4.32	55.50 ± 2.35

[a] Low polluted, [b] Moderately polluted, [c] Highly polluted, [d] Values are mean ± SD, $n = 10$.

and surface characteristics (Bache *et al.*, 1991). The deposited particles may be washed by the rain into the soil, re-suspended or retained on the plant foliage. The degree of retention is influenced by the weather conditions, nature of pollutant, plant surface characteristics and particle size (Harrison and Chirgawi, 1989). In an investigation on Cd, Cu, Ni and Pb uptake from the air and soil by *Achillea millefolium* (milfoil) and *Hordeum vulgare* (barley), Pilegaard and Johnsen (1984) concluded that Cu and Pb accumulations were correlated with aerial deposition, but not with soil concentrations. In contrast, Ni and Cd contents in plants were correlated with their contents in soil. Metal accumulation in plants can reflect the relative extent of the burden and its dispersal in the environment (Monaci *et al.*, 2000; Shparyk and Parpan, 2004). Sawidis *et al.* (1995) have observed a distance-dependent decrease in foliar metal content, indicating the role of source in metal contamination. Alfani *et al.* (1996) observed that metal concentration was significantly higher in leaves from the roadside plants. They also observed a positive correlation between leaf deposition and leaf metal accumulation. Therefore, plants growing along the roadsides may also work as phytoremediator of air-borne metals released from the automobiles.

A significant correlation was found between changes in different foliar parameters and the status of ambient air quality at different selected sites as reflected in Table IV.

Plant leaves are the primary receptors for both gaseous and particulate pollutants of the atmosphere. Hence, they have to bear the brunt of constant exposure to

TABLE IV

A correlation between ambient air quality and foliar parameters of selected plant species

		Pollutants			
Foliar parameters	Plant species	SO_2	NO_2	O_3	SPM
Chlorophyll a	*F. religiosa*	−0.77[b]	−0.97[a]	−0.99[a]	−1.00[a]
	T. nerifolia	−0.96[a]	−0.99[a]	−0.97[a]	−0.94[a]
Chlorophyll b	*F. religiosa*	−0.63[NS]	−0.90[a]	−0.94[a]	−0.97[a]
	T. nerifolia	−0.99[a]	−0.85[b]	−0.78[b]	−0.72[b]
Carotenoid	*F. religiosa*	−0.89[a]	−1.00[a]	−1.00[a]	−0.99[a]
	T. nerifolia	−0.92[a]	−1.00[a]	−0.99[a]	−0.97[a]
Protein	*F. religiosa*	−0.74[b]	−0.96[a]	−0.98[a]	−1.00[a]
	T. nerifolia	−0.99[a]	−0.96[a]	−0.92[a]	−1.00[a]
Leaf area	*F. religiosa*	−0.83[b]	−0.99[a]	−1.00[a]	−1.00[a]
	T. nerifolia	−0.93[a]	−1.00[a]	−0.99[a]	−0.97[a]
Cysteine	*F. religiosa*	1.00[a]	0.91[a]	0.86[b]	0.80[b]
	T. nerifolia	1.00[a]	0.91[a]	0.87[b]	0.82[b]

[a] Significant at 1% level.

[b] Significant at 5% level.

NS: not significant.

TABLE V

Stomatal variations in *Ficus religiosa* and *Thevetia nerifolia* species growing at low and highly polluted sites

		Stomatal variations[c]		
Plants	Sites	Size (μ)	Frequency (per mm^2)	
Ficus religiosa	LP[a]	7 × 20	Range	355–400
			Mean	378 ± 31.81
	HP[b]	28 × 15	Range	88–222
			Mean	155 ± 14.75
Thevetia nerifolia	LP	30 × 16.6	Range	266–400
			Mean	333 ± 24.75
	HP	20 × 13.3	Range	400–578
			Mean	489 ± 45.86

[a] Low polluted.

[b] Highly polluted.

[c] Values are mean ± SD, $n = 10$.

phytotoxic pollutants. Before these pollutants enter the leaf tissues, they interact with foliar surface and modify its configuration, which might be visible under SEM. When the leaf samples of these plants were examined under SEM, the following observations were recorded.

As far as *F. religiosa* was concerned, there was well-defined granular epicuticular wax on the foliar surface of healthy population, but withered and distorted wax was observed on the population collected from HP site. In polluted populations, the dust particles coming out of automobile exhaust were embedded into the epicuticular wax and thus formed a crust on the foliar surface (Figure 2a).

An uneven epicuticular surface was observed in the control population, while in the polluted population, the cuticular surface was even and almost flat. As far as stomata were concerned, in control population, stomata were slightly sunken because of elevated outer stomatal edges and were widely open (Figure 2b). While in polluted condition, stomata were severely damaged. The stomata were raised slightly to its normal level. Stomatal openings were occluded by the particles on the leaves of polluted site. It was also observed that cuticle was ruptured at the edge of stomata to form crypt like structure (Figure 2c). A reduction in stomatal frequency and an increase in stomatal size were also observed on the leaf surface of the plants growing at HP site as compared to the control site (Table V).

When SEM micrographs of *Thevetia nerifolia* growing at LP and HP sites were compared, it was found that epicuticular wax was granular in normal, while it was withered and disturbed in the polluted population. An apparent disturbance in the ornamentation of cuticle was observed in the leaves collected from HP site (Figure 3a). The rugged pattern of the cuticle was deformed and altered considerably (Figure 3b,c).

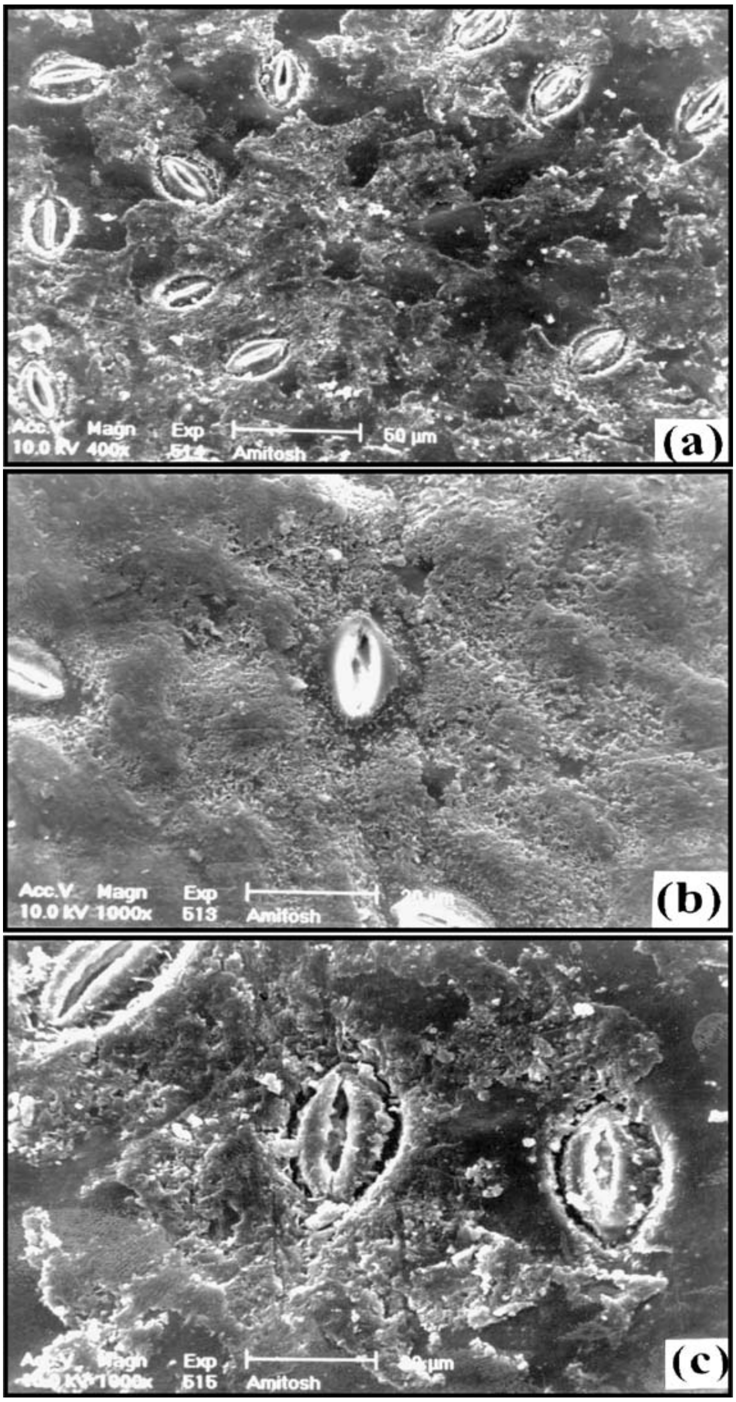

Figure 2. Scanning electron micrographs of *Ficus religiosa*; (a) HP site, general surface; (b) LP site, magnified; (c) HP site, magnified.

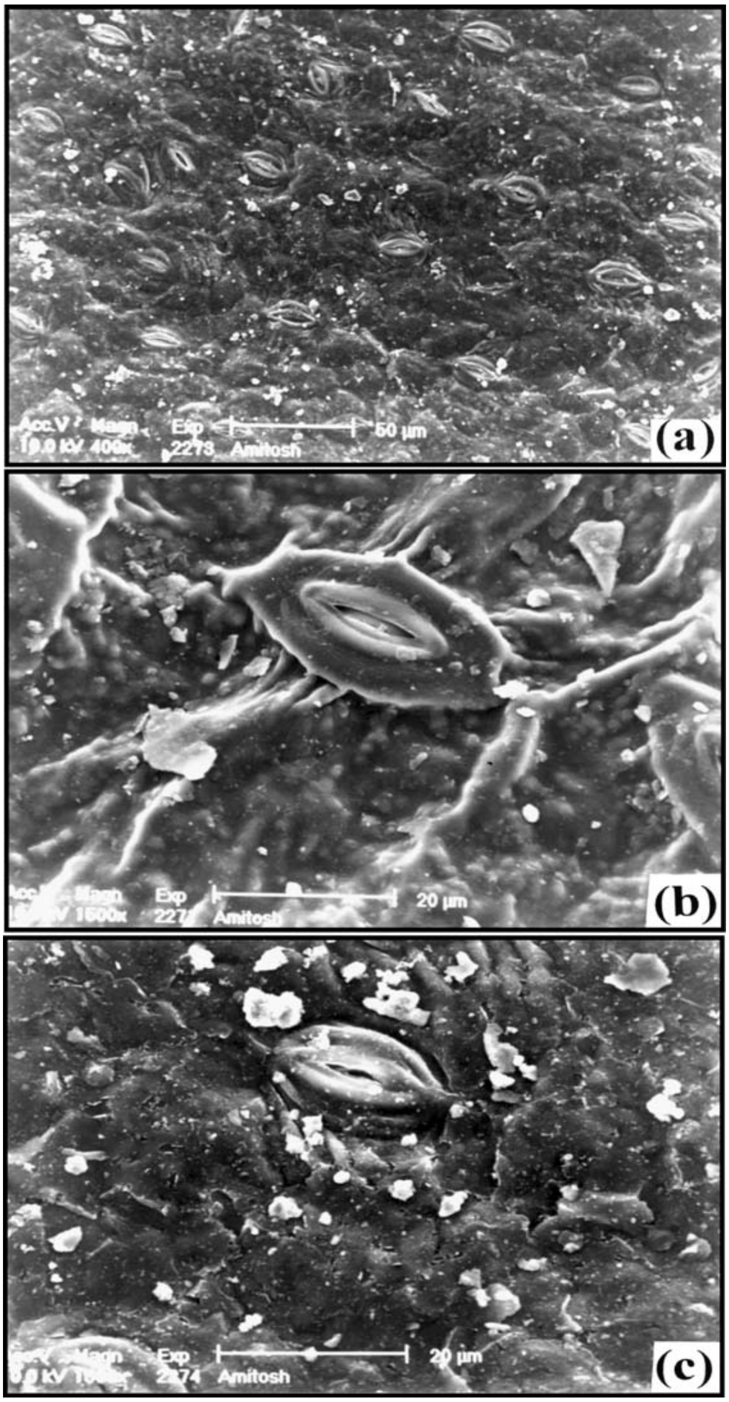

Figure 3. Scanning electron micrographs of *Thevetia nerifolia*; (a) HP site, general surface; (b) LP site, magnified; (c) HP site, magnified.

Stomatal size was decreased to nearly 70%, while stomatal frequency was increased to about 50% in polluted population (Table V) as compared to healthier one. Encrypted and slightly sunken stomata were observed on the leaves of the plants growing in the polluted environment (Figure 3c). Cuticular cracking at the outer edge of stomata was also observed in the polluted population. Thus, there were conspicuous alterations in the pattern of foliar surface configuration in leaves exposed to auto-pollution with respect to healthier ones.

Plant cuticles, which are the main barriers between the interior and the outer environment of the leaf, remain in continuous contact with air pollutants. Therefore, any specific or predictable alteration in cuticle due to air pollutants could serve as diagnostic marker of air pollution exposure. Deformities of cuticle may be linked to the lost turgidity and the dehydration of the epidermal cells (Eveling, 1984).

The state of epicuticular wax is important, because reduces the contact between pollutants and the leaf surface. Although it is normally crystalline and soft, it can be altered by the impact of ambient pollutants (Baker and Hunt, 1986). In our study, the deformation of epicuticular wax was probably due to the presence of SO_2 in the ambient air, which might solubilize wax changing the crystalline structure of the epicuticular wax (Lendzian, 1984).

Further, low dose of SO_2 may induce stomatal opening, while stomatal closure is facilitated by high dose of SO_2 (Noland and Kozlowski, 1979). Stomatal closure at high SO_2 dose may be associated with accumulation of CO_2 in the sub-stomatal cavities due to decline in photosynthesis. It has been reported that air pollutants increase cell permeability by damaging the membrane integrity (Keller, 1986), more so in the case of sensitive species (Forooq and Beg, 1980). Pollutant-induced increased cell permeability may cause a loss of water from the guard cell to make them flaccid, which results in stomatal closure. Further, encrustation or dust deposition on leaf cuticle due to particulate penetration in to epicuticular wax may reduce the intensity of the incident light hampering photosynthesis which may, further, lead to an accumulation of CO_2 in to the sub-stomatal cavities and hence stomatal closure.

4. Conclusion

Significant changes were observed both in the biochemical and foliar surface ultra structural configuration of both *Ficus religiosa* and *Thevetia nerifolia* plants growing at HP site in comparison to MP and LP sites. However, despite these changes, plants were thriving well at the polluted environment. Therefore, these plant species may be used as biomarkers and mitigators of pollutants coming out of the automobile exhaust.

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