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Air pollution impact on micromorphological and biochemical response of *Tabernaemontana divaricata* L. (Gentianales: Apocynaceae) and *Hamelia patens* Jacq. (Gentianales: Rubiaceae)

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Abstract. In the present investigation an attempt was been made to assess the air pollution effects on micromorphological and biochemical parameters of *Tabernaemontana divaricata* (Gentianales: Apocynaceae) and *Hamelia patensi* (Gentianales: Rubiaceae). In polluted area the number of stomata and clogged stomata were found to be higher then control, where as the number of unclogged stomata are found to be very less in the control site. The stomatal breadth and pore length were found to be decreased in polluted area in both the plants when compared to control. The number of subsidiary cells, trichome length values was found to be less then control plants. However trichomes are absent in *T. divaricata*. The stomatal index was found to be higher in both the plants is when compared to control. The chlorophyll a, chlorophyll b, and total chlorophyll content were found to be maximum in control samples when compare to polluted samples. But ascorbic acid, relative water content, pH and Air Pollution Tolerance Index (APTI) were found to be maximum in polluted samples when compare to control. Based on the present study both the plant species were categorized as tolerant to air pollution.

Keywords: Pollution, Air Pollution Tolerance Index, APTI, Micromorphology, Stomata.

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Introduction

Urban air pollution is a serious problem in both developing and developed countries. Vehicles exhaust emissions are a dominant feature of urban environments and are widely believed to have detrimental effects on plants (Honour et al., 2009). The booming vehicular pollution is a global phenomenon which has completely transformed the socio-economic scenario in urban areas all over the world. Though the number of vehicles in India may be less in comparison to those in more advanced nations, the environmental pollution is quite formidable due to predominance of old and poorly maintained vehicles, and narrow, uneven and bumpy roads. Carbon monoxide, hydrocarbons (benzene, methane and ethylene), oxides of nitrogen (NO_x), sulphur dioxide (SO_2), lead (Pb) and other suspended particulate matter (SPM) like smoke, metal and inert dust are the major pollutants emitted by the automobile vehicles (Ho and Tai, 1988). All these pollutants are released at the ground level and their upward movement is restricted due to tall buildings and congested thorough fares. Therefore, high build up of pollutants frequently occur causing adverse effects on the environmental quality of urban areas. The gasoline passenger cars and two and three wheelers are the principal source of carbon monoxide (CO), the two - and three - wheelers also contribute about 70% of the total hydrocarbon emissions.

The diesel vehicles are principle contributors of soot particles (Pundir, 1994).

Air pollution directly affects plants via leaves or indirectly via soil acidification. When exposed to air borne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Liu and Ding, 2008). The atmospheric SO_2 adversely affects various morphological and physiological characteristics of plants. High soil moisture and high relative humidity aggravated SO_2 injury in plants (Tankha and Gupta, 1992). Pollutants can cause leaf injury, stomatal damage, premature senescence, decreased photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari et al., 2006). Air pollution stress leads to stomatal closure, which reduces CO_2 availability in leaves and inhibits carbon fixation. Net photosynthetic rate is a commonly used indicator of impact of increased air pollutants on tree growth (Woo et al., 2007). The interaction between plants and different types of pollutants were influence of environmental pollution focus on physiological and ultrastructural aspects (Psaras and Christodoulakis, 1987; Velikova et al., 2000).

The use of plants as monitors of Air pollution has long been established as plants are the initial acceptors of air pollutants. They act as the scavengers for many air borne particulates in the atmosphere (Joshi and Swami, 2007). Air pollution has the potential to reduce yield and the nutritional quality of crop plants (Jager et al., 1993). Thus plants play a vital role as indicators of pollution. The emission of air pollutants from the various industrial and social activities has a large impact upon the vegetation growing in these areas. The main sectors emitting air pollutants are road transport, power and heat production sectors and industry.

Therefore, the present work is designed for the study of air pollution effect on leaf characteristics of two plant species (*Tabernaemontana divaricata* L. and *Hamelia patens* Jacq.) in Mysore, Karnataka State, India.

Materials and methods

Leaves of *T. divaricata* and *H. patens* were collected from Chikkahalli and K. R. Circle which served as control and polluted area respectively. K. R. Circle which is located in the central part of Mysore city is one of the busiest areas of the city with a very high traffic density. Chikkahalli which is 10 km away from the city served as control area as it had negligible traffic.

Micromorphological studies

Leaf samples collected from both polluted and control areas were processed following the method of Ahmed and Yunus (1974). The matured leaf samples were cut into small bits and taken in separate test tubes. 30% nitric acid solution was added to each test tube. The mixture was boiled in a water bath for 3 h till the leaf bits became transparent. The leaf bits were washed in distilled water and stained with 2% safranin. Excess stain was removed by washing with distilled water and mounted using gelatin jelly. A small amount of the jelly was taken on a slide and gently warmed using Bunsen burner. The leaf bits were placed to the jelly and a coverslip was mounted over it and observed under light microscope. Micromorphological characteristics such as frequency of stomata, trichomes, size of the stomata, stomatal index and trichome length were studied in 10 microscopic fields selected at random from each side and measured using ocular micrometer. Photomicrographs were taken with a digital camera at different magnifications.

Biochemical studies

Leaf samples were washed and collected in polythene bags until further process. Fresh leaves were used for the estimation of chlorophyll a, chlorophyll b, total chlorophyll, and total ascorbic content.

Chlorophyll estimation: The chlorophyll content of leaves was estimated following the procedure described by Arnon (1949). The volume of solvent extraction was made up to 100 mL by adding 80% acetone. The absorbance was

measured by using spectrophotometer at 645 nm, and 663 nm.

Ascorbic acid content: The total ascorbic content was estimated using the method of Behrens and Madere (1994). 0.2 mL of supernatant was taken and 1 mL of 5% TCA was added and mixed well. 1 mL of 2% DNPH reagent was added to this mixture and incubated on a water bath for 15 min to get the precipitate. 7 mL of 80% H_2SO_4 was added and the absorbance was measured at 540 nm spectrophotometrically. The total ascorbic acid content was determined with standard curve by using ascorbic acid.

Relative water content

Relative water content of leaf sample was calculated by the method described by the Singh and Rao (1986). The relative water content of leaf sample was estimated as percentage moisture on over dry wt. basis.

$$\text{Relative water content} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

pH

pH of aqueous leaf abstract was determined using digital pHmeter.

APTI

Air Pollution Tolerance Index (APTI) was determined by calculating the ascorbic acid content, total chlorophyll content, pH of leaf extract and relative water content of leaf. APTI was calculated by the method described by Singh and Rao (1983):

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

Where:

A = Ascorbic acid (mg g^{-1} FW);

T = Total chlorophyll (mg g^{-1} FW);

P = Leaf extract pH; and

R = Relative water content (%) of the leaves.

Results

The two shrub species i.e. *T. divaricata* and *H. patens* growing at K. R. Circle, showed decrease in leaf size and deposition of dust on the leaves, when compared to control. The leaves of *T. divaricata* appeared pale green in colour. It was more pronounced in *T. divaricatum* compared to *H. patens*. Changes in the micromorphological characteristics such as stomatal number, subsidiary cell number, stomatal pore length and breadth, stomatal index, trichome number, length and breadth in leaf samples from control and polluted areas is presented in Table 1.

The stomatal number increased in the leaf samples of both the species growing at polluted areas. The stomatal pore size was found to be reduced in polluted sample (Figure 1, C and D) when compared to control (Figure 1, A and B). The stomatal number increased to a mean value of 50.9 and 61.9 in polluted leaf samples of *T. divaricata* and *H. patens*, respectively. The length and breadth of stomatal pore however was reduced in the leaf samples of both the species (4.3 and 0.13, and 3.4 and 0.3, respectively) growing at polluted area (Figure 1). Most of the stomata in leaf samples of both the species from polluted area were found to be clogged (Figure 2, C and D). The subsidiary cells were found to be decreased in number in both the species growing at polluted area (7.3 and 6.7) compared to control area (9.2 and 7.3). While an increase in the stomatal index was observed in both the plants. Trichomes were observed only in *H. patens* of the present investigation. A threefold decrease in the length of the trichomes and was observed in leaves from polluted area (Figure 3 C and D (compared to control area while the breadth of the trichomes increased in leaves growing at polluted area and compared to leaves from control area (Figure 3 A and B). The number of trichomes in leaves growing at polluted site was found to be less when compared to leaf samples from control site Figure 3 A and B.

Biochemical parameters chlorophyll a, b, and total chlorophyll, ascorbic acid content, relative water content and pH were presented in Table 2. There was no significant variation in the chlorophyll a content in the leaves of *T. divaricata* growing in K. R. Circle compared to leaves of control area; while it showed only a slight reduction in the leaves of *H. patens* from 0.63 to 0.43 mg/g. Chlorophyll b and

Table 1. Micromorphological characteristics of *T. divaricata* and *H. patens* from polluted and control site.

Plant name		Number of stomata	Clogged stomata	Stomata pore size length	Stomata pore size breadth	Subsidiary cells	Stomatal index	Trichome number	Trichome length	Trichome breadth
T. divaricata	C	32.7±1.4^b	0.92±0.1^b	4.6±0.1^a	1.5±0.1^a	9.2±0.2^a	15.67±0.1^b	-----	-----	-----
	P	50.9±0.2^a	43.9±1.0^a	4.3±0.9^b	0.13±0.05^b	7.3±0.15^b	17.89±0.1^a	-----	-----	-----
H. patens	C	55.2±1.0^b	0.89±0.1^b	4.2±0.1^a	0.93±0.05^a	7.3±0.1^a	18.67±1.1^b	75.33±2.5^a	20.2±0.1^a	1.5±0.1^b
	P	61.9±2.0^a	59.4±0.2^a	3.4±0.05^b	0.3±0.1^b	6.7±0.1^b	20.3±0.5^a	24.33±2.0^b	6.1±0.1^b	2.6±0.1^a

Mean ± SD followed by same superscript are not statistically significant between the concentrations when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level. C = Control; P = Polluted.

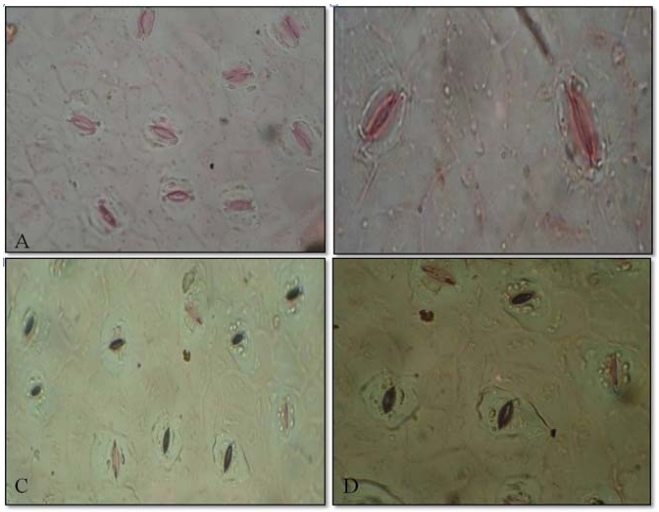

Figure 1. Stomata in leaf samples *T. divaricata* and *H. patens* from control and polluted areas. Note reduction in stomatal pore size in polluted leaf. (A) *T. divaricata* from control area; (B) *H. patens* from control area; (C) *T. divaricata* from polluted area reduced pore length; and (D) *H. patens* from polluted area reduced pore length.

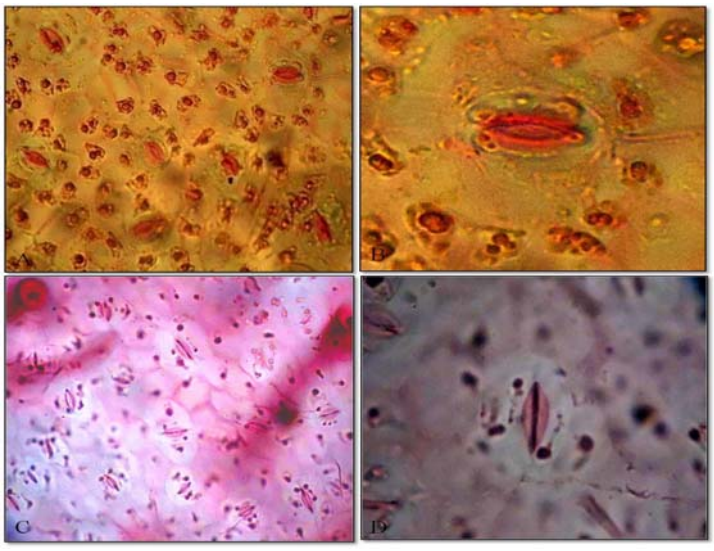

Figure 2. Stomata in leaf samples *T. divaricata* and *H. patens* from control and polluted areas. Note clogged stomata in leaf sample from polluted area. (A) *T. divaricata* from control area unclogged stomata; (B) *H. patens* from control area unclogged stomata; (C) *T. divaricata* from polluted area clogged stomata; and (D) *H. patens* from polluted area clogged stomata.

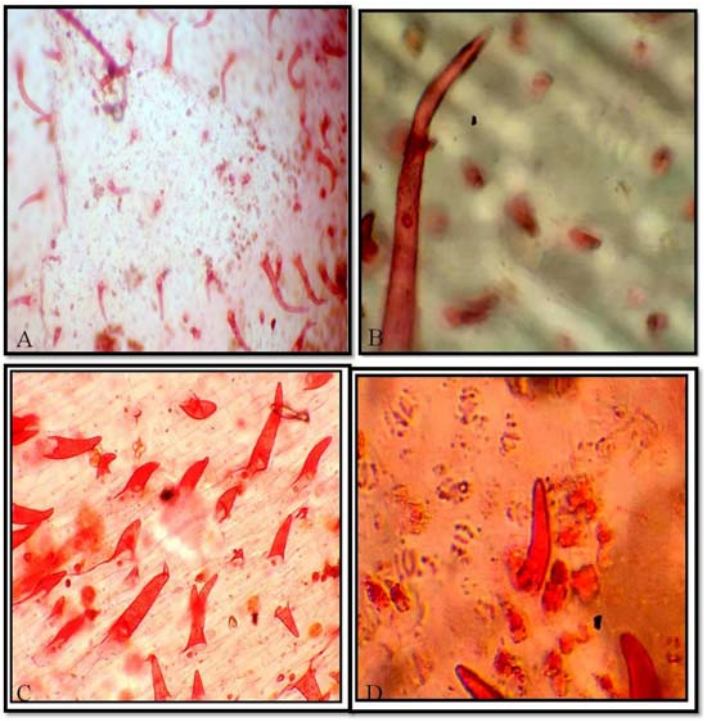

Figure 3. Trichomes of leaf samples *T. divaricata* and *H. patens* from control and polluted areas. Note clogged stomata in leaf sample from polluted area. (A) *T. divaricata* from control area; (B) *H. patens* from control area; (C) *T. divaricata* from polluted area reduced length and number; (D) *H. patens* from polluted area reduced length and number.

Table 2. Air pollution tolerance index of plant species growing in polluted site and control site.

SL No.	Species		Chl a	Chl b	RWC	TCH	pH	AA	APTI
1.	*T. divaricata*	C	0.55±0.02^a	0.35±0.01^a	70.2±4.32^b	0.91±0.05^a	5.4±1.02^b	20.9±2.21^b	20.2±2.10^b
		P	0.53±0.02^a	0.28±0.01^b	79.4±5.63^a	0.81±0.04^b	6.1±1.04^a	40.1±5.42^a	35.27±3.51^a
2.	*H. patens*	C	0.63±0.04^a	0.78±0.05^a	63.9±5.01^b	1.57±0.08^a	4.5±1.01^b	20.7±2.04^b	18.96±2.14^b
		P	0.40±0.03^b	0.43±0.03^b	72.1±4.85^a	0.84±0.06^b	5.1±1.12^a	50.1±6.21^a	36.42±3.84^a

Mean ± SD followed by same superscript are not statistically significant between the concentrations when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level. C = Control, P = Polluted, Chl a = Chlorophyll a; Chl b = Chlorophyll b; RWC = relative water content; TCH = Total chlorophyll content; AA = Ascorbic acid content; APTI = Air Pollution Tolerance Index.

Table 3. Ambient air quality monitoring at K. R. Circle.

SL No.	Month (2012)	SO_2 (ug/m^3)	NO_2 (ug/m^3)	SPM (ug/m3)	RSPM $_{(ug/m3)}$
1.	Feb	11.1	21.5	115	58
2.	Mar	11.1	22.5	138	67

Average at KSRTC building K. R. Circle, Mysore.

total chlorophyll reduced in the leaves of both the species growing at K. R. circle when compared to control. Chl b was found to be reduced in the leaves of *T. divaricatum* from 0.35 to 0.28 mg/g and in *H. patens* it reduced from 0.78 to 0.43 mg/g. Total chl reduced from 0.91 to 0.81 mg/g in *T. divaricatum* and from 1.5 to 0.84 mg/g in case of *H. patens*. pH in the leaf samples of K. R. Circle showed an increase over control plants from 5.4 to 6 in *T. divaricatum* and from 4.5 to 5 in case of *H. patens*. A two fold increase in the ascorbic acid content (40.1 and 50, respectively) was observed in the leaves of *T. divaricatum* and *H. patens* growing at K. R. Circle. The ambient air quality monitoring data at K. R. Circle was

obtained from KSPCB, Mysore (Table 3). The concentration of SO_2, NOx and SPM were within the permissible limits of CPCB. Both the plant species growing at K. R. Circle showed an increased APTI value of 35.27 and 36.42 respectively in *T. divaricatum* and *H. patens*. Based on the APTI values both the plant species were categorized as tolerant to air pollution.

Discussion

Leaf surface characteristics are sensitive to the air pollution. Response of leaf character to air pollution will indicate the adverse effects of air pollution, which thus can be used as bioindicator. *T. divaricata* and *H. patens* in the present investigation showed a reduction in leaf size compared to populations growing in control area. Similar reduction in leaf area was also observed in leaves of *Newbouldia laevis* (Kayode and Otoide, 2007) and *Albizia lebbek* under the stress of air pollution (Seyyednejad et al., 2009). Reduction of leaf area and petiole length under pollution stress was reported by Tiwari et al. (2006). Reduction in leaf size is an adaptation by the plants exposed to air pollution to minimize the entry of poisonous gases into the leaves (Zarinkamar et al., 2013).

In the present investigation clogging was observed in most of the stomata in the leaf samples of polluted area. Kulshreshtha et al. (1994b) had reported significant decrease in size of epidermal cells, stomata and trichome per unit area in auto exhaust polluted population of *Calotropis procera* and *Nerium indicum* collected from busiest roadside and also Kulshreshtha and Rai (2005) revealed that, as compared to the leaves from control, the frequency of epidermal cells and stomata had increased considerably in plants of polluted area and also Saadabi (2011) also reported that, in polluted sites leaves become smaller with reduced length and width and stomatal index per leaves area. In this study, stomata were found clogged in *T. divaricata* and *H. patens*. Kulshreshtha et al. (1994a) revealed that the diesel exhaust particles are very small and they are readily deposited on both surfaces of leaves and remained adhered to it for long time as they are unable to remove by wind, rain and physical washing. Kulshreshtha and Rai (2005) also reported stomata clogged in *Nyctanthes*, *Quisqualis* and *Terminalia*. Decrease in trichome number and length in polluted population has been observed in *Lantana* (Kamala et al., 1994) and *Calatropis* (Kulshreshtha et al., 1994b). On the contrary increase in trichome number has been observed by Sharma and Tyree (1973). According to Sharma (1977) trichomes help trap the particulate matter falling directly on the leaf surface which otherwise block the stomata pores and adversely affect the process of gaseous exchange. Thus increased number of trichomes seems to be another adaptation of the stress of air pollution providing an outline defense.

Reduction in the concentration of chlorophyll content in leaves of polluted area was observed in both the plant species. Similar changes in concentration of pigments were also observed in leaves of six tree species expose to air pollution due to vehicle emission (Joshi and Swami, 2009). Leaves from polluted area had significantly lower chlorophyll content than control (Tripati and Prajapathi, 2008; Stevvovic et al., 2010). In present study, Ascorbic acid concentration was increased in *T. divaricata* and *H. patens* ascorbic acid increased with the increase of dust deposition (Tripati and Prajapathi, 2008). Being a very important reducing agent, ascorbic acid also plays a vital role in cell wall synthesis, defense and cell division (Conklin, 2001). Increase in ascorbic concentration with respect to the control leaves also reported by Jyothi and Jaya (2010). The leaf pH values increased in the polluted area compared with that of control similarly increased in pH values in polluted site observed (Patel and Kousar, 2011).

In present study, increased in relative water content was observed in polluted site similarly increased in relative water content was observed (Patel and Kousar, 2011). Among two shrub species investigated for APTI *H. patens* showed more tolerance to air pollution than *T. divaricata.* Jyothi and Jaya (2010) observed that among the three shrubs species studied, *C. infortunatum* showed highest APTI values and found to be more

tolerant compared to other shrub species studies. Different plant species shows considerable variation in their susceptibility to air pollution. The plants with high and low APTI can serve as tolerant and sensitive species respectively. Also the sensitivity levels of plants to air pollutants differ from herbs, shrubs and trees with identical values, a tree may be sensitive but a shrub or a herb may be tolerant to given pollutant. Therefore, the indices for different plant types should be considered separately (Singh and Rao, 1983).

Conclusion

From the present study it may be concluded that *T. divaricata* and *H. patens* are categorized in to air pollution tolerant species according to their APTI values. Thus they can be considered as sinks of pollution. APTI determines the sensitivity or resistivity of the plant species to air pollutants. The results of such studies are therefore handy for future planning and for better understanding and management of air quality as well as in selection of suitable plant species with high APTI for plantation in polluted areas and they may become one of the strategies for abatement of cities of air pollution.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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