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Micro-morphological and biochemical response of *Muntingia calabura L*. and *Ixora coccinia L*. to air pollution

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The present study was carried out to assess the air pollution effects on micro morphological and biochemical parameters of *Muntingia calabura* and *Ixora coccinia*. In polluted area samples the number of stomata and unclogged stomata were found to be less than control, where as the number of clogged stomata are found to be higher in the polluted site when compared to control. The stomatal pore length and breadth were also found to be less in polluted area in both the plants when compared to control. The number of epidermal cells, trichome length values was found to be less than control in both the plants. While the trichome number was found to be less than control in *Muntingia calabura* and more then control in *Ixora coccinia*. The stomatal index in both the species is higher than the control. The amount of chl. a, chl. b, total chlorophyll content were found to be higher in control samples when compare to polluted samples. But relative water content, ascorbic acid, pH and air pollution tolerance index were found to be higher in polluted site samples when compare to control samples.

Key words: pollution, ascorbic acid, APTI

Air pollution is a major problem in modern society due to rapid urbanization and industrialization especially in metro-politan cities. Air pollution is defined as "Any atmospheric condition in which substances are present in concentrations high enough than their normal ambient levels to produce a measurable effect on man, animal, vegetation and also plants" (Seinfeld, 1986). Modernization and progress have led to air getting more and more polluted over the years. Increase in the population of industries, vehicles and urbanization are some of the major factors responsible for air pollution. The urban environment contains a complex mixture of air pollutants, the exact composition of which varies both over time and between individual towns and cities due to changes in patterns and sources of emissions. Cities in developing nations are being industrialized and increasing rapidly in size and diversity. It is accompanied by increasing emissions from vehicular traffic, industry, domestic heating, cooking and refuse burning which pose potential health risks for large scale of air pollution exposure. The rapid economic development combined with lack of emission controls makes megacities prone to more serious air pollution problems than similar cities in industrial nations.

Carbon monoxide (CO) is a colourless and odorless gas that is produced by the incomplete burning of carbon based fuels including petrol, diesel, and wood. It affects the plants, carbon monoxide gas which induced root initiation and caused stimulation of pre existing root primordia. It also caused epinasty and abscission of leaves, retarded elongation of stems, and anesthetized plants. Sulfur dioxide enters the leaves mainly through the stomata and the resultant injury is classified as either acute or chronic. Acute injury is caused by absorption of high concentrations of sulfur dioxide in a relatively short time. The symptoms appear as 2-sided lesions that usually occur between the veins and occasionally along the margins of the leaves. The colour of the necrotic area can vary from a light tan or near white to an orange-red or brown depending on the time of year, the plant species affected and weather conditions.

Particulate matter such as cement dust, magnesium-lime dust and carbon soot deposited on vegetation can inhibit the normal respiration and photosynthesis mechanisms within the leaf. Cement dust may cause chlorosis and death of leaf tissue by the combination of a thick crust and alkaline toxicity produced in wet weather. The dust coating also may affect the normal action of pesticides and other agricultural chemicals applied as sprays to foliage. In addition, accumulation of alkaline dusts in the soil can increase soil pH to levels adverse to crop growth. The interaction between plants and different types of pollutants influence physiological and ultra structural aspects (Heumann, 2002).

Environmental conditions may affect plant micro- morphology and the impact of environmental pollution on plants is well documented (Halloy, 1996; Lakshmi; 2010). Plant leaves usually perform basic functions such as capture of light energy and carbon molecules (Halloy, 1996; Rohit, 2010). These functions can best be fulfilled by flat, thin leaf surface full of chlorophyll (Cooper, 1993). Leaf micro-morphology has often been used as a sensitive indicator of environmental pollution and the effect of environmental pollution on the epidermal features of the plant leaves have been well documented (Bondad, 2006). Weyers and Travis (1981) have reported decrease in the size of stomatal openings, an increase in the frequency of epidermal cells and stomata in response to environmental pollution in some plant taxa.

The city of Mysore in Karnataka state, South India, is an important international tourist destination. In the recent past it has been designated as a heritage city. The total number of vehicles registered in the city has increased from 3, 06, 430 in 2004 to 4, 95, 513 in 2011 (RTO, Mysore). The emission from the vehicles will be high particularly at the traffic intersection that causes adverse effects not only on the health of the people who reside close to the roads but also to the vegetation growing nearby. The trees being stationary are continuously exposed to emission from the vehicles. Different plant species vary in their susceptibility to various pollutants. The present investigation was under taken to study the responses of the leaf characteristics in *Muntingia calabura* L. and *Ixora coccinia L.* growing near the traffic intersection with a view to investigate the feasibility of establishing it is a bio monitoring tool for environmental pollution.

Materials and Methods

Collection of plant materials

Leaves of *Muntingia calabura* and *Ixora coccinia* were collected from K.R. circle and Kaniyana hundi forest which served as polluted and control area respectively. K.R. circle located in the central part of the city of Mysore joins the main market area and bus terminal. It is one of the busiest intersections of the city with a very high traffic density. Kaniyana hundi forest located 16kms away from the city served as control area as it had negligible traffic.

Morphological studies:

Effect of air pollutants at morphological level such as foliar injury symptoms have been observed in the collected leaf samples and compared with control. The epidermal peel was obtained following the method of Ahmad (1974). The matured leaf samples were collected from polluted and control areas and cut into small bits. These cut pieces were taken in a test tube containing HNO_3 and incubated at 40°C. The bits were washed with water and stained in aqueous 2% safranin. The bits were again washed in water and mounted in glycerin jelly. The epidermal features like stomata and trichomes were measured using ocular micrometer under low power, high power and oil immersion magnification using a microscope.

Biochemical studies:

Fresh leaf samples were collected from the two tree species growing in polluted and control regions. Leaf samples were washed and stored in polythene bags. These fresh leaves were used for the estimation of chl a, chl b, total chlorophyll, ascorbic acid, relative water content and pH.

Chlorophyll estimation:

The chlorophyll content was estimated by following the method of Arnon, (1949). One gram of fresh leaves was ground with 20 ml of 80% acetone and centrifuged at 3000rpm for 15 min. The supernatant was saved for estimation; absorbance was measured at 645 and 663 for chlorophyll a, b and total chlorophyll. The amount of chl. a, b, total chlorophyll present in the sample was calculated by using the formula prescribed by Arnon.

Total ascorbic acid:

The total ascorbic acid was determined following the method of Behrens and Madere (1963). One gram of fresh leaves was ground with 20 ml of 80% acetone centrifuged at 3000rpm for 15 min. The supernatant was saved for estimation; 0.2ml of supernatant was taken and 1ml of 5% TCA (trichloroacetic acid) was added to this mixture and incubated on a water bath for 15 minutes to get the precipitate, 7ml of 80% concentrated sulphuric acid was added and the absorbance was taken at 540nm.

Relative water content:

Relative water content of the samples was determined following the method of Singh and Rao (1968) using the formula

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

Leaves extract pH:

pH of aqueous leaf extract was determined by digital pH meter.

Air pollution tolerance index (APTI):

The air pollution tolerance index of plants were determined following the method of Singh and Rao (1983) using the formula $\text{APTI} = \frac{A(T+P)+R}{10}$

A=Ascorbic acid concentration

T= Total chlorophyll mg/g fresh weight

P= pH of leaf extract

R= Relative water content.

Results and Discussion

The plants growing near K.R. circle are continuously exposed to different pollutants, which are released into the environment as a consequence of vehicular emission. The present study showed a decrease in the average area of leaf in both plants from polluted area compared to control area. The colour of the leaves of both the plants growing near K.R. circle was observed to be dark due to deposition of dust/smoke. Decrease in number of leaves of some plants by environment polluting gases emitted by vehicles has also been reported by Lalman and Singh (1990) and Kamalkar (1992).

Micro-morphological studies:

The quantitative and qualitative details of various parameters studied in polluted and control site has been depicted in table -1.The number, length, breadth and area of the stomata, trichome length, trichome number, epidermal cell number has been observed to be affected by the vehicular exhaust. The total number of stomata in *M. calabura* and *I. coccinia* growing in polluted area had decreased 20.7 and 28.4 compared to control 31.9 and 34.2 respectively. Similar results have reported by Bhiravamurthy and Kumar (1983) and Palaniswamy *et al*. (1995).

The length and breadth of stomatal pore and epidermal cell number was decreased in polluted areas of *M. calabura* and *I. coccinia* compared to control plants. In polluted areas the stomatal pore length and breadth decreased from 4.2 and 5.3µm to 2.6 and 4.1µm respectively, compared to control values of 7.7 and 8.2µm, 4.1 and 5.0µm respectively. The length and breadth of stomata and number of epidermal cells in two plant species decreased in polluted plants when compared to control areas plants, which may be helpful in preventing the entry of the pollutants into the leaves, which can otherwise cause injury and death of the tissues of leaves. (Rangarjan *et al*., 1995).The increase or decrease of epidermal cells might be to accommodate the decrease or increase of the stomata. Palaniswamy *et al*. (1995) have reported decrease in number of epidermal cells per unit area due to vehicular pollution.

The stomata were slightly raised from rest of the cells, were often filled with dust particles and at some place were also clogged. The clogging stomata were increased in both the species in polluted area 7.86 and13.4 compared to control 2.1 and 2.8 (Fig.1 B and D). The fine particles clog the stomata, affecting the gaseous exchange process and in turn affecting photosynthesis, water retention, respiration, and overall growth of plants. As the roadside plants covered with dust also suffered from water loss, water deficiency and any change in the original morphological structure make those plants more sensitive to water loss (Saneoka and Ogata 1987).

Stomatal index increased in polluted samples of *M. calabura* (89.9) while there was no change in *I. coccinia*. Vehicular pollution is known to affect the stomatal index and it has been reported in some plants (Palaniswamy et.al., 1995), while in some other plants vehicular pollution has been reported to increase its value (Salgare and swain, 1991). The Trichome number showed an increase in polluted area, while their length decreased in the polluted leaves of *I. coccinia* (18.2 and 6.6µm). In contrast *M. calabura* showed a twofold decrease in trichome number (6.0µm) and length (8.0µm) in polluted areas compared to control (12.1µm and14.16µm).

Decrease in trichome number and length in polluted population has been observed in Lantana (Kamala *et al*., 1994) and Calatropis (Kulshreshtha *et al*., 1994). 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.

Biochemical studies:

Air pollution tolerance index (APTI) plays a significant role to determine resistant and susceptibility of plant species. Air pollution tolerance index was calculated for two plant species *M. Calabura* and *I. coccinia* growing in heavy traffic area of Mysore city and control site (Table-2). The variation of the APTI can be attributed to the variation in any of the four physiological factors (total chlorophyll, ascorbic acid, pH and relative water content) which governs the computation of the index.

Chlorophyll is the principle photo-receptor in photosynthesis; its measurement is an important tool to evaluate the effect of air pollutants on plants. As it plays an important role in plant metabolism any reduction in chlorophyll content corresponds directly to plant growth (Joshi and Swami, 2009). The present investigation showed a reduction in chlorophyll a, chlorophyll b, and total chlorophyll in leaf samples of polluted area compared to control area in the two plant species. There was no variation observed in the chlorophyll a content in the leaves of *M. calabura* growing in K.R. Circle compared to leaves of control area; while it showed only a slight reduction in the leaves of *I. coccinia* (0.65 to 0.61mg/g). Chlorophyll b content and total chlorophyll reduced in the leaves of both the species growing at K.R. circle when compared to control. Chl b content in the leaves of *M. calabura* reduced from 0.73 to 0.53mg/g and in *I .coccinia* it reduced from 0.9 to 0.95 mg/g .Total chl reduced from 1.49 to 1.49 mg/g in *M. calabura* and from 1.51 to 1.2mg/g in case of *I. coccinia.*

pH is a biochemical parameter that acts as an indicator for sensitivity of air pollution (Scholz and Rick 1977). P^H in the leaf samples of K.R.circle showed an increase over control plants from 6.4 to 7.2 in *M. calabura* and from 6.4 to 7.1 in case of *I. coccinia.* Relative water content is an appropriate measure of plant water status in terms of physiological consequences of cellular water deficit or relative water content of a leaf is the water content present in it which help to maintain its physiological balance under the stress condition caused by pollution especially when the prevailing transpiration rate are high (Tiwari and Rai 2001).

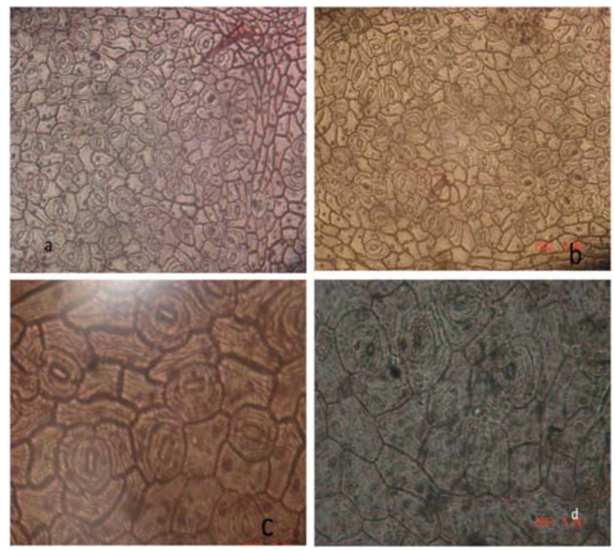

Fig.1. a and b: stomata in control and polluted areas of *Ixora coccinia*, c and d stomata in control and polluted areas of *Muntingia calabura*

Table-1 Micro-morphological characteristics of *Muntangia calabura* and *Ixora coccinia* from polluted and control site.

Plants	Stomata number	Clear stomata	Clogged Stomata	Stomatal pore length	Stomatal pore breadth	No. of epidermal cells	Trichome length	Trichome number	Stomatal index
M. calabura (control)	31.96± 1.7a	30.3± 0.96a	2.13± 0.83b	7.74± 0.425a	4.186± 0.18a	12.76± 0.41a	14.16± 0.20a	12.1± 3.75a	71.5± 1.17b
M.calabura (polluted)	20.76± 0.65b	12.43± 0.15b	7.86± 0.11a	4.20± 0.11b	2.67± 0.22b	2.86± 0.25b	8.32± 0.10b	6.25± 1.23b	89.9± 3.44b
I. coccinia (control)	34.26± 0.47a	30.5± 0.60a	2.8± 0.65b	8.2± 0.26a	5.06± 0.15a	12.86± 0.41a	11.3± 2.3a	12.4± 0.18b	87.1± 0.37a
I.coccinia (polluted)	28.4± 1.37b	12.06± 0.25b	13.46± 1.13a	5.37± 0.25b	4.166± 0.25b	3.43± 0.450b	6.6± 1.2b	18.2± 0.4a	87.7± 1.11a

Mean±SE followed by the same supercsript are not statistically significant between control and polluted plants, when subjected to Tukey's mean range test ($p<0.05$)

Table 2: Effect of air pollution on chlorophyll, ascorbic acid, RWC, pH and APTI of *Muntangia calabura* and *Ixora coccinia* from polluted and unpolluted site

	Chlorophyll a	Chlorophyll b	Total chlorophyll	Ascorbic acid	RWC	pH	APTI
Mutangia calabura (control)	0.752±0.02[a]	0.739±0.02[a]	1.49±0.045[a]	30.6±0.24[b]	46.83±0.75[b]	6.43±0.035[b]	28.82±0.35[b]
Mutangia calabura (polluted)	0.681±0.01[b]	0.537±0.001[b]	1.12±0.032[b]	40.1±0.64[a]	49.46±0.52[a]	7.26±0.054[a]	38.30±0.54[a]
Ixora coccinia (control)	0.650±0.01[a]	0.952±0.003[a]	1.51±0.051[a]	30.5±0.49[b]	49.26±0.49[b]	6.4±0.035[b]	29.05±0.45[b]
Ixora coccinia (polluted)	0.617±0.01[b]	0.900±0.034[b]	1.27±0.021[b]	40.9±0.58[a]	51.1±0.78[a]	7.16±0.04[a]	39.34±0.38[a]

Mean±SE followed by the same supercsript are not statistically significant between control and polluted plants, when subjected to Tukeys mean range test ($p<0.05$)

Relative water contents of the two plant species increased over control plants from (46.6 to 49.4) in *M. calabura* and from (49.2 to 51.1) in *I. coccinia.* Ascorbic acid, a natural antioxidant has been shown to play an important role in pollution tolerance, it activates many physiological mechanisms. Increased level of ascorbic acid may be due to the defense mechanism of plant to cope with stress condition since it retards leaf senescence (Garg and Kapoor, 1972). In the present study the ascorbic acid concentration was increased in polluted plants of *M. calabura* (40.1) and *I. cooccinia* (40.9) when compared to control plants (30.6 and 30.5). Thus, it acts as a biological indicator of air pollution. Air pollution tolerance index (APTI) values the plant was conveniently grouped as follows based on the reports of Lakshmi *et al*., (2009). APTI: response 30 to 100: tolerant; 29 to 17: intermediate; 16 to 1: sensitive; < 1: very sensitive. In the present study both the plant species growing at K.R. circle showed an increased APTI value of 38.30 and 39.34 respectively in *M. calabura* and *I. coccinia*. Based on the APTI values both the plant species were categorized as tolerant to air pollution.

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