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Physiological Responses of Some Tree Species to Air Pollution Stress around Bhadravathi Town, India

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ABSTRACT

Monitoring the effects of air pollutants on vegetation is very important to assess their possible damage to natural vegetation and crop plants. In this sense, ambient air quality monitoring was carried out during 2006-2008 at five sampling sites of Bhadravathi town. The concentration of suspended particulate matter was higher (41.02-236.56 μg/m^3) than the concentration of nitrogen oxides (4.15-19.69 μg/m^3) and sulfur dioxide (1.90-13.23 μg/m^3). Four tree species (*Polyalthia longifoia*, *Mangifera indica*, *Pongamia pinnata* and *Acacia auriculiformis*) were selected to determine the effect of air pollution; the tested trees showed variation in biochemical parameters between sampling sites. The reduction in the range of ascorbic acid (1.80-4.99 mg/g of tissue), pH (5.2-6.5), relative water content (49-79%) and total chlorophyll content (1.39-2.77 mg/g of tissue) in tested trees at sampling sites versus trees at control site was significant. The variation among the biochemical parameters in the leaves of tested trees was directly attributed to the air pollution at Bhadravathi town.

Keywords: air quality, APTI, ascorbic acid, chlorophyll

INTRODUCTION

The ambient air environment of an urban area may be contaminated with several pollutants and the plants growing there would be exposed not only to one but many air pollutants under different conditions. Air pollution and particulate matters emitted as smoke from a thermal power plant cause environmental stress to the nearby vegetation and normally inhibit the normal growth of plants (Saquib 2008). It is possible to estimate the overall effect of a large number of pollutants as total pollution by measuring changes in the plants (Agarwal 1985; Tiwari *et al*. 1993; Chauhan 2010). Pandey and Rao (1977) stated that air pollution-induced changes in vegetation are recognizable. They include morphological injury and many physiological and biochemical perturbations along with subtle changes in pH of the cell sap. Plants serve as indicators of air pollution due to the possible synergistic action of pollutants. Hence, plants are considered to be efficient bio-indicators that evidence changes in the environment and are thus valuable records of contamination, and very convenient for environmental monitoring.

The effects of pollutants, when in combination, are quite apparently of paramount importance rather than the individual pollutant. Study of the effects of pollutant mixtures on a single plant type and vegetation has now become a major area of research (Chauhan and Joshi 2008; Prajapati and Tripathi 2008; Tripathi *et al*. 2009; Chauhan 2010). The regional impact of air pollution on different local plant species is a major ecological issue. The climatic conditions, the physico-chemical properties of air pollutants and their residence time in the atmosphere impact surrounding plants and animals.

As such, researchers who concentrated mostly around large point sources of pollutants have started to focus on air pollution effects on vegetation (Agarwal 2000; Oliva and Mingorance 2006; Tripathi and Gautam 2007; Agbaire 2009; Jyothi and Jaya 2010). Plants are considered to be indicators and as well as mitigators of pollution (Mukherjee 1993). Certain organisms, by virtue of their adaptability to a prevailing environment, continue to exist or otherwise, if sensitive, become eliminated. Literature pertinent to the response of trees to specific pollutants will provide important information for monitoring specific pollutants and in their mitigation.

Thus, the need for monitoring the responses of vegetation to air pollution has increased more than ever, especially in urban and industrial areas. This may allow for the direct interpretation of the effect that air pollution exerts on the environment (Posthumus 1980).

The present study has been undertaken to assess the effect of ambient air pollution on physiological attributes of tree species selected near residential, commercial and industrial areas of the town to provide information of tolerant and sensitive tree species growing in these areas.

MATERIALS AND METHODS

Description of study area

Bhadravathi is an industrial town situated 13° 49′ 46″ N and 75° 42′ 22″ E in the Shimoga District of Karnataka state, India. It is situated at about 255 km from the state capital, Bangalore. The study area is notorious for its emissions from two large-scale industries (Visweswaraya Iron and Steel Plant and Mysore Paper Mills Ltd.) in addition to numerous small-scale industries of the town. The air quality of Bhadravathi town was compared with that of the control area (Kuvempu University campus).

Determination of ambient air quality

Ambient air quality monitoring was carried out by following standard methods of the National Ambient Air Quality Monitoring (NAAQM 2006). Air sampling was carried out using APM-410 (to trap SPM) and APM-411 (for gaseous pollutants) high volume air samplers. The sampling frequency was 24 h, twice a week at uni-

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form intervals and for a period of two consecutive years (July 2006 to June 2008).

Specification of high volume air sampler used for air monitoring

The flow rate of the instrument ranged from 0.9 to 1.2 m^3/min and the recommended Whatman filter paper No. 41 was used. Sampling time was normally 8 and 24 h (max). Power requirement for the instrument was 220 V, single phase, 50 Hz, and had an A.C. built-in voltage stabilizer with an automatic shut off.

Determination of suspended particulate matter

The light table surface was cleaned with a methanol soaked wiper and allowed to dry. The filter was handled with aseptic hands to prevent contamination. Before placing each filter on the filter chamber of the instrument it was examined for pinholes on a light table.

Suspended particulate matter (SPM) was measured by weight/volume and mass/quantity. In each case it was determined by weighing the filter paper before and after sampling with proper equilibrium each time. The monthly mean of SPM for each of the sampling stations was obtained separately using the values of all samples of each respective month.

SPM in ambient air was calculated (according to IS 5182 (Part 4) 2004):

$$SPM = \frac{(Wr\text{-}Wi) \times 10^6}{V} \ \mu g/m^3$$

where SPM = suspended particulate matter in $\mu g/m^3$, Wr = final weight of the filter in g, Wi = initial weight of the filter in g, V = volume of air sampled in m^3, 10^6 = conversion of g to µg.

Determination of sulphur dioxide (modified West and Gaeke method)

The ambient air was bubbled through an aqueous solution of potassium tetrachloromercurate (TCM). The SO_2 in air forms a dichlorosulphitomercurate complex, which resists oxidation by the oxygen in air. This complex was stable to strong oxidants such as ozone and nitrogen oxides and therefore, the absorber solution was stored for a couple of hours prior to analysis. This complex was made to react with pararosanilline and formaldehyde to form the intensely colored pararosaniline methylsulphonic acid. The absorbance of the solution was measured with a spectrophotometer (Systronics 367). The concentration of SO_2 in the sample is expressed in $\mu g/m^3$ and is calculated as follows:

$$C\ (SO_2\ \mu g/m^3) = \frac{(A\text{-}A_0) \times 1000\ (B)\ V_s}{V_a \times V_t}$$

where A = sample absorbance, A_0 = reagent blank absorbance, B = calibration factor, µg/absorbance, V_a = volume of air sampled in litres, V_s = final volume of sampling solution, V_t = aliquot taken for analysis and 1000 = conversion factor from litres to cubic meters.

Determination of nitrogen oxides

The gas was collected in the absorber of the air sampler and the mixture was analyzed through the sodium arsenite method (Jacob and Hochheiser 1958).

Ambient NO_x was collected by bubbling air through a solution of sodium hydroxide and sodium arsenite. The concentration of nitrite ion (NO^-_2) produced during sampling was determined colorimetrically by reacting the nitrite ion with phosphoric acid, sulfanilamide and *N*-(1-naphthyl)-ethylenediamine di-hydrochloride (NEDA) and measuring the absorbance of highly colored azo-dye at 540 nm using a spectrophotometer (Systronics 367). The concentration of the NO_x in the sample is expressed in $\mu g/m^3$ and was calculated as follows:

$$C\ (NO_x\ \mu g/m^3) = \frac{(S\ NO_2) \times V_s}{V_a \times 0.82 \times V_t}$$

where S NO_2 = NO_2 concentration in analyzed sample, 0.82 = sampling efficiency, V_a= volume of air sampled in litres, V_s = final volume of sampling solution and V_t = aliquot taken for analysis.

Selection of the sampling plants

In total 4 trees were selected. At each of the five sampling sites, including the control site, only few plants (5 species) were selected as sample plants for the study. From each tree the leaves were collected during air quality monitoring and care was taken that the sample plants were of almost the same age with more or less the same girth and height for a given species. 4-5 full grown leaves of the twigs at a suitable height (i.e., twigs should be well exposed to the atmosphere and free from the influence of dust blown at the ground level by any vehicles) above the ground level and at the outer periphery of the canopy were collected for the analysis. Sampling was conducted three times a year (April, August and December).

Estimation of ascorbic acid

Ascorbic acid (AA) or vitamin C is an antiscorbutic. AA content was determined by the method of Aberg (1958) and Sadasivam and Manickam (1997) by using the leaf extract of all tested trees.

1. Principle

AA reduces 2,6-dichlorophenol indophenol dye to a colorless leuco-base. The AA gets oxidized to dehydroascorbic acid. Even though the dye is a blue colored compound, the end point is the appearance of pink color. The dye is pink colored in acid medium. Oxalic acid is used as the titrating medium.

2. Materials

Oxalic acid 4%.

Dye solution: Weigh 42 mg sodium bicarbonate in to a small volume of distilled water. Dissolve 552 mg 2,6-dichloro phenol indophenol in it and make up to 200 mL with distilled water.

Stack standard solution: dissolve 100 mg AA in 100 mL of 4% oxalic acid solution in a standard flask (1 mg/mL).

Working standard: Dilute 10 mL of the stock solution to 100 mL with 4% oxalic acid solution. The concentration of working standard is 100 µg/mL.

3. Procedure

5 mL of the working standard solution was pipetted into a 100-mL conical flask and 10 mL of 4% oxalic acid was added and titrated against the dye (V_1 mL). The end point is the appearance of pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of AA. After extracting the sample (0.5-5 g depending on the sample) in 4% oxalic acid the volume was made up to a known volume (100 mL) and centrifuged. 5 mL of this supernatant was pipetted out and 10 mL of 4% oxalic acid was added and titrated against the dye (V_2 mL). The amount of AA mg/100 g sample was calculated by:

$$\text{Ascorbic acid} = \frac{0.5\ \text{mg}}{V_1\ \text{mL}} \times \frac{V_2}{5\ \text{mL}} \times \frac{100\ \text{mL}}{\text{wt. of sample}} \times 100$$

Estimation of chlorophyll

The chlorophylls (Chl) are essential components for photosynthesis that are found in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as ether or acetone. There are at least five types of Chl in plants. Chl *a* and *b* occur in higher plants, ferns and mosses. Chls *c*, *d* and *e* are only found in algae and certain bacteria.

1 g of gently washed, fresh leaves were cut and well mixed representative sample of leaf tissue in to a clean mortar and pestle and grained to a fine pulp after the addition of 20 mL of 80% acetone. After centrifuge (5000 rpm for 5 min) the supernatant was transferred to a 100-ml volumetric flask, and then the remaining residue was repeatedly grinded until the residue become colorless. Finally the mortar and pestle were thoroughly washed with 80% acetone and the clear washings were collected in the volumetric flask. The volume was then made up to 100 ml with 80% acetone. Chl contents were determined by measuring the absorbance of the centrifugate at 645, 663 and 750 nm with a spectrophotometer (Systronics-367). Calculations were made as suggested by Arnon (1949) and Sadashivam and Manickam (1997) using the following formulae.

$$\text{Chl } a \text{ (mg/g of tissue)} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chl } b \text{ (mg/g of tissue)} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total Chl (mg/g of tissue)} = 20.2\ (A_{645}) - 8.02\ (A_{663}) \times \frac{V}{1000 \times W}$$

where A = absorbance at wavelengths at 663 and 645 nm, respectively minus the absorbance at 750 nm, V = final volume of Chl extract in 80% acetone and W = fresh weight of plant leaves extracted.

Estimation of pH

The pH represents the acidity or alkalinity of the aqueous solution. About 5 g of thoroughly washed, fresh leaves were homogenized and made into paste using a mortar and pestle. The volume was gently mixed and made up to 25 ml using double distilled water and the pH of the extract was measured using Systronics 361 pH digital meter.

Estimation of relative water content

The leaves of all selected plants were gently washed in before measuring fresh weight (F) and they were dipped in double distilled water for 24 h to obtain a turgid weight (T). The dry weight (D) was determined after keeping the leaves in a hot air oven for 24 h at 80°C. Relative water content (RWC) was measured using the formula suggested by Sivakumaran and Hall (1978):

$$(\text{RWC}) = \frac{\text{F-D}}{\text{T-D}} \times 100$$

where F = fresh weight of the leaves, T = turgid weight of the leaves and D = dry weight of the leaves.

Air Pollution Tolerance Index

The Air Pollution Tolerance Index (APTI) was determined using the Rao and Dubey (1990) and Ghosh and Mukherjee (2003) methods. These authors derived the formula using four parameters: AA, pH, RWC and total Chl content to evaluate the tolerance of plants to air pollution. The formula used for calculating APTI is as follows:

$$\text{APTI} = \frac{\text{RA (T+P)}}{100}$$

where A = AA, T = total Chl, P = pH and R = RWC.

Even though the APTI of a plant species determined by this method may not be comprehensive, either for tolerance or for sensitivity, the index can be considered as a better indicator for comparative purposes among different populations or between different sites for a given population.

RESULTS AND DISCUSSION

Status of ambient air quality of study area

From the two years of continuous monitoring it is evident that all the pollutants are within the standards prescribed by CPCB but the concentration of SPM was higher than SO_2 and NO_x ranged from 20.08 ± 11.91 μg/m^3 at the control site during 2007-08 to 236.56 ± 83.22 μg/m^3 during 2006-07 at site 4. On the other hand, the concentration of SO_2 ranged from 0.033 ± 0.06 μg/m^3 at the control site to 13.62 ± 6.09 μg/m^3 during 2006-07 at site 4. NO_x ranged from 0.28 ± 0.31 μg/m^3 at the control site to 19.69 ± 7.88 μg/m^3 during 2006-07 at site 4. According to the pollution level the pollution load in the polluted sites could be ranked as: site 4 > site 3 > site 5 > site 1 > site 2.

Physiological response of tree species to air pollution

All the parameters of the tested plant species at all the sampling sites showed significant variation from site to site and as well as within sites (**Tables 2-7**). The maximum percentage reduction of AA followed the order: *P. pinnata* (46.15%), *M. indica* (44.90%), *P. longifolia* (29.96%) and *A. auriculiformis* (26.47%) from the control site to site 4. The AA concentration in the leaf extracts of all the tested plants at the 5 sites decreased in comparison to the control site. A reduction in AA content in the leaves of the tested plant species at the 5 sites was observed in all plant species. Tinku and Ambarish (2003) in Burdwan found low AA content in *Ficus benghalensis* resulting in it being rated the least pollution-tolerant among 13 tested plant species. All values of air pollutants were expressed as the mean of 96 trials. On the other hand, the biochemical parameters tested were expressed as the mean of 6 trials. Furthermore, the data obtained pertaining to the air pollutants and biochemical parameters of tested plants were subjected to one-way multifactorial analysis of variance (ANOVA) in order to determine which means were significantly different from others (**Tables 1-7**).

According to Keller and Schwager (1977) AA is a strong reductant which fulfils several functions in photosynthesis. AA may also influence the detoxification of polluted plants, e.g. by reducing SO_2. Further, they found that the AA content of forest tree species was distinctly decreased by shading, particularly in light-demanding species. Continued SO_2 fumigation depressed AA content long before visible symptoms of injury appeared. AA thus deserves more attention in physiological air pollution research.

The effects of fumigation of low levels of SO_2 (78.6, 131 and 262 μg m^{-3}) on AA were evaluated by Varshney and Varshney (1984) in three crop plant species, namely *Brassica nigra*, *Phaseolus radiatus* and *Zea mays*. Among the three crops, *Z. mays* was relatively SO_2-resistant, possessing a comparatively high amount of AA. The AA content of *B. nigra* and *P. radiatus*, which are relatively SO_2-sensitive species, decreased markedly within one week following SO_2 fumigation. However, fumigation of *Z. mays* with SO_2 was accompanied by a significant reduction in AA only after 6 weeks of fumigation with 131 and 262 μg m^{-3} of SO_2. The results of that investigation indicated a positive correlation between AA content and SO_2 sensitivity of plants.

The present investigation revealed considerable variation in the levels of AA in all the species at all sites. The reduction in AA may be attributed to its consumption for scavenging cytotoxic-free radicals generated during a chain reaction after the absorption of pollutants into foliage (Dwivedi and Tripathi 2007).

pH varied among all the plants at all the sampling sites of the study area (**Table 3**). Interestingly, the pH fluctuated considerably compared to the control. Only *M. indica* showed an increase in pH at sites 4 and 5 when compared to the control site while the remaining tree species showed a

Table 1 Annual concentration of air pollutants showing significant differences among the five sampling sites of Bhadravathi Town.

				SPM (µgm/m^3)				
Sites	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
2006-07	20.08 ± 11.91	62.02 ± 24.84	41.01 ± 17.28	207.16 ± 88.38	236.56 ± 83.22	120.17 ± 48.72	32.16	0.0001
2007-08	20.15 ± 16.14	63.12 ± 22.13	37.26 ± 13.44	202.60 ± 85.35	232.30 ± 81.63	131.26 ± 51.25	31.85	0.0001
SO_2 (µgm/m^3)								
2006-07	0.07 ± 0.09	2.97 ± 1.27	1.90 ± 1.19	8.91 ± 4.37	13.23 ± 4.75	4.22 ± 2.39	34.56	0.0001
2007-08	0.033 ± 0.06	3.45 ± 1.18	1.83 ± 0.91	10.57 ± 4.29	13.62 ± 6.09	4.05 ± 1.51	33.87	0.0001
NO_x (µgm/m^3)								
2006-07	0.35 ± 0.36	7.11 ± 4.17	4.15 ± 2.71	17.97 ± 9.40	19.69 ± 7.88	8.41 ± 4.78	36.21	0.0001
2007-08	0.28 ± 0.31	6.45 ± 3.49	4.09 ± 2.28	18.51 ± 8.18	19.15 ± 6.88	9.47 ± 5.62	26.01	0.0001

Table 2 Variation of ascorbic acid in mg/g of tissue (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
Polyalthia longifolia	2.57 ± 0.001	2.32 ± 0.02	2.50 ± 0.01	1.81 ± 0.21	1.80 ± 0.02	1.95 ± 0.02	105.17	0.0001*
Mangifera indica	5.30 ± 0.22	5.00 ± 0.40	5.20 ± 0.08	3.38 ± 0.07	2.92 ± 0.02	4.92 ± 0.02	112.24	0.0001*
Pongamia pinnata	3.51 ± 0.05	3.06 ± 0.02	3.36 ± 0.02	2.18 ± 0.04	1.89 ± 0.02	2.58 ± 0.16	1696.47	0.0001*
Acacia auriculiformis	4.99 ± 0.05	4.76 ± 0.01	4.78 ± 0.02	4.56 ± 0.05	4.36 ± 0.45	4.69 ± 0.14	4.72	0.006^{NS}

* Significant at $P < 0.05$; ^{NS} = non-significant at $P > 0.05$

Table 3 Variation of pH (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
Polyalthia longifolia	5.60 ± 0.16	5.57 ± 0.06	5.60 ± 0.08	5.30 ± 0.07	5.20 ± 0.07	5.50 ± 0.08	11.99	0.0001*
Mangifera indica	5.60 ± 0.21	5.50 ± 0.10	5.60 ± 0.09	5.70 ± 0.05	5.30 ± 0.08	5.40 ± 0.35	2.55	0.065^{NS}
Pongamia pinnata	5.90 ± 0.08	5.70 ± 0.21	5.80 ± 0.21	5.60 ± 0.05	5.30 ± 0.12	5.70 ± 0.02	8.70	0.0001*
Acacia auriculiformis	6.50 ± 0.35	6.40 ± 0.14	6.50 ± 0.08	6.30 ± 0.14	6.30 ± 0.17	6.40 ± 0.08	912	0.0001*

* Significant at $P < 0.05$; ^{NS} = non-significant at $P > 0.05$

Table 4 Variation of chlorophyll *a* in mg/g of tissue (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
Polyalthia longifolia	1.72 ± 0.02	1.69 ± 0.01	1.71 ± 0.02	1.55 ± 0.01	1.55 ± 0.01	1.69 ± 0.08	99.22	0.0001*
Mangifera indica	1.41 ± 0.02	1.40 ± 0.02	1.39 ± 0.01	1.37 ± 0.01	1.29 ± 0.02	1.39 ± 0.02	22.81	0.0001*
Pongamia pinnata	1.30 ± 0.06	1.24 ± 0.04	1.28 ± 0.01	1.02 ± 0.01	0.98 ± 0.07	1.18 ± 0.03	38.29	0.0001*
Acacia auriculiformis	1.86 ± 0.03	1.80 ± 0.03	1.86 ± 0.01	1.50 ± 0.03	1.49 ± 0.02	1.76 ± 0.03	129.45	0.0001*

* Significant at $P < 0.05$

Table 5 Variation of chlorophyll *b* in mg/g of tissue (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
Polyalthia longifolia	0.75 ± 0.01	0.70 ± 0.05	0.74 ± 0.01	0.68 ± 0.01	0.68 ± 0.01	0.70 ± 0.01	7.30	0.0010*
Mangifera indica	0.59 ± 0.06	0.44 ± 0.01	0.56 ± 0.008	0.41 ± 0.01	0.39 ± 0.06	0.42 ± 0.02	22.94	0.0001*
Pongamia pinnata	0.60 ± 0.02	0.49 ± 0.05	0.59 ± 0.03	0.48 ± 0.02	0.41 ± 0.01	0.49 ± 0.006	23.42	0.0001*
Acacia auriculiformis	0.91 ± 0.02	0.87 ± 0.008	0.89 ± 0.02	0.63 ± 0.03	0.67 ± 0.01	0.79 ± 0.01	134.02	0.0001*

* Significant at $P < 0.05$

Table 6 Variation of Total Chlorophyll in mg/g of tissue (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	**Control**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	***F* value**	***P* value**
Polyalthia longifolia	2.47 ± 0.01	2.39 ± 0.002	2.47 ± 0.02	2.24 ± 0.01	2.23 ± 0.003	2.39 ± 0.03	151.08	0.0001*
Mangifera indica	1.98 ± 0.01	1.83 ± 0.04	1.97 ± 0.01	1.78 ± 0.03	1.68 ± 0.001	1.82 ± 0.02	121.26	0.0001*
Pongamia pinnata	1.90 ± 0.10	1.68 ± 0.02	1.87 ± 0.07	1.51 ± 0.02	1.39 ± 0.03	1.70 ± 0.02	50.85	0.0001*
Acacia auriculiformis	2.77 ± 0.02	2.67 ± 0.01	2.72 ± 0.01	2.13 ± 0.02	2.21 ± 0.17	2.55 ± 0.04	52.12	0.0001*

* Significant at $P < 0.05$

decrease. The higher the pH, the higher the tolerance to pollution (**Table 3**). High pH may increase the efficiency of conversion from hexose sugar to AA, while low leaf extract pH showed good correlation with sensitivity to air pollution (Escobedo *et al.* 2008; Liu and Ding 2008). Choudhury and Rao (1977) related pollution tolerance of plants with their AA levels and concluded that the higher the level of AA, the greater the tolerance.

The Chl contents in the leaf extracts of the trees also showed a varied response from site to site (**Table 4**). The maximum reduction in Chl *a* was noticed in *A. auriculiformis* (46.28%) followed by *M. indica* (28.51%), *P. pinnata* (24.61%), and the minimum reduction was observed in *P. longifolia* (9.88%).

The maximum reduction in Chl *b* was noticed *P. pinnata* (32.78%) in followed by, *M. indica* (31.57%), *A. auriculiformis* (26.37%) and *P. longifolia* (9.33%) (**Table 5**). The maximum reduction of total Chl was noticed in *P. pinnata* (26.84%) followed by *A. auriculiformis* (20.21%), *M. indica* (15.15%) and *P. longifolia* (9.71%) (**Table 6**). Chl reduction was evidently observed more at sampling sites 3 and 4 indicating the direct effect of air pollution on vegetation. Chauhan (2010) carried out research to assess the effect of air pollution on biochemical parameters of *Ficus religiosa*, *Mangifera indica*, *Polyalthia longifolia* and *Delonix regia*. Reduction in Chl *a*, Chl *b*, total Chl content, AA, carotenoids, pH, RWC and APTI was recorded in the leaf samples of all selected trees collected from a polluted site at Dehradun City, India when compared with samples from a control area. There was maximum (43.36%) reduction of chl *a* content in the leaves of *F. religiosa* and minimum (26.57%) reduction in *M. indica* at the polluted site compared to the control site. Maximum (44.67%) reduction in AA was observed in the leaves of *D. regia* and minimum (22.93%) in the leaves of *P. longifolia*.

Dust may cause chlorosis and death of leaf tissue by a combination of the formation of a thick crust and alkaline toxicity produced in wet weather (Tariq *et al*. 2008). Senthilkumar and Paulsamy (2011) analyzed 24 tree species in the municipal town of Kothagiri, Tamil Nadu and found variation in leaf Chl content at the study area in response to air pollution.

Table 7 Variation of percentage Relative Water Content (mean ± SD) in tested plants at all the sampling sites of Bhadravathi Town.

Trees	Control	Site 1	Site 2	Site 3	Site 4	Site 5	*F* value	*P* value
Polyalthia longifolia	61.00 ± 2.16	60.00 ± 1.41	60.55 ± 0.91	58.00 ± 2.94	59.00 ± 1.63	59.00 ± 2.44	0.835	0.542 ^{NS}
Mangifera indica	58.00 ± 1.41	57.00 ± 2.16	58.00 ± 1.63	56.00 ± 4.32	49.00 ± 0.81	56.75 ± 0.50	9.67	0.0001*
Pongamia pinnata	65.00 ± 1.41	61.00 ± 2.16	63.00 ± 2.48	57.00 ± 2.16	60.25 ± 0.57	60.00 ± 1.63	8.50	0.0001*
Acacia auriculiformis	79.00 ± 2.16	78.00 ± 2.82	78.00 ± 2.16	75.00 ± 0.81	76.00 ± 1.41	77.00 ± 2.16	2.10	0.111^{NS}

* Significant at $P < 0.05$; ^{NS} = non-significant at $P > 0.05$

Arti and Pandey (2011) found maximum reduction (14.71%) of total Chl in maize (*Zea mays*) in response to cement dust pollution from Kalyanpur Cement Ltd., Banjari, Rohtas, Bihar.

Reduction in Chl content may be due to an increase in Chlorophyllase enzyme activities, which in turn affects the Chl concentration in plants (Mandal and Mukherji 2000). Chl is the principal photoreceptor in photosynthesis and its measurement is an important tool to evaluate the effects of air pollutants on plants as it plays an important role in plant metabolism and any reduction in Chl content corresponds directly to plant growth (Joshi and Swami 2009). SO_2 plays an important role in the reduction of Chl content (Rao and Dubey 1985; Mandloi and Dubey 1988). Although all the species showed significant variation in all the biochemical indicators, the extent to which plant species were affected varied from species to species and from site to site. Almost all the species showed maximum variation in biochemical indicators at site 4, where severe air pollution occurred. A considerable loss in total Chl in the leaves of plants exposed at site 4 (severe air pollution) supports the argument that the chloroplast is the primary site of attack by air pollutants such as SPM, SO_2 and NO_X. Air pollutants make their entrance into leaf tissues through the stomata and cause partial denaturation of the chloroplast and decrease pigment contents in the cells of polluted leaves (Tripathi and Mukesh 2007). Rao and Leblanc (1966) mentioned that a high level of gaseous SO_2 causes the destruction of Chl which might be due to the replacement of Mg^{++} by two hydrogen atoms and degradation of Chl molecules to phaeophytin.

Several researches found decreased levels of Chl content in several common plant species affected by air pollution in different parts of India. Dubey *et al*. (1982) noticed 27.64% reduction of Chl in *M. indica* at Ujjaini, Madhya Pradesh and 17.46-47.29% reduction among five sampled plants. At Haridwar, Joshi and Abhishek (2007) saw a 4.13% reduction in *M. indica*, but Rao *et al.* (1990) found a 90.33% reduction of Chl in *M. indica* leaves at Varanasi, UP.

M. indica and *P. pinnata* showed 80% each reduction in Chl at the Korba Power Plant, MP, India (Williams *et al*. 1995). Samal and Santra (2002) at Kalyani City, Bengal noticed a 5.66 and 12.08% reduction of total Chl content in *M. indica* and *P. longifolia*, respectively. Surprisingly, and in contrast, Subba Rao (2005) found a 35.21% increase of Chl in *A. auriculiformis* Visakapattanam, India.

The highest reduction in RWC of leaf extracts was noticed in *P. pinnata* (6.15%), *N. indicum* (5.63%), *A. auriculiformis* (3.79%) and *P. longifolia* (3.27%) (**Table 7**). Similar findings were also noticed by Joshi and Swamy (2007) at Haridwar in which the rank of four selected plants had tolerance as follows: *E. camaldulensis* > *A. lebbeck* > *C. salignus* > *P. juliflora*.

APTI

The APTI calculated for all the tested plants revealed their sensitivity to air pollution in the study area (**Table 8**). *A. auriculiformis* was more tolerant (higher APTI values) than the other plants in all 5 sites. *P. pinnata* was the most sensitive (lowest APTI values) among the tested plant species at all the sampling sites. Plants were ranked according to their tolerance as: *A. auriculiformis* >*M. indica* > *P. longifolia* > *P. pinnata.*

All the four tested trees showed the decline of APTI from Site-2 (where all the plant species have the highest tolerance indices) versus other sampling sites.

The changes in APTI values of a particular species from different sites may be due to the decreasing tolerance of the species because of prolonged or chronic exposure. Such a decline in the APTI of certain plant species from different sites of Bangalore city were reported by Madhusudhanan (2000) for eight species. Their study demonstrated that the APTI value of a species could vary among seasons. Out of 99 plants studied by Dwivedi and Tripathi (2007) at surrounding areas of coal-fired industries, *Ricinus communis* with APTI = 81.10 was the most resistant wild plant showing uniform distribution at all the polluted sites. On the other hand, *Lepidium sativum* with APTI = 5.27 was the most sensitive plant and was present only at the less polluted sites. Chauhan and Joshi (2008) stated that the concentrations of Chl *a*, *b*, total Chl, carotenoids, AA, relative moisture content, pH and APTI were always lower in leaves of plants of the same age at polluted sites than at the control site. Their findings were based on a study conducted at Dehradun, Uttarakhand to know the effect of air pollution on four selected trees; *Ficus religiosa*, *Mangifera indica*, *Polyalthia longifolia*, *Delonix regia*.

The leaf samples collected from 24 tree species in industrial areas of Visakhapatnam City were used to determine their APTI by calculating the AA, Chl, pH, and RWC and found a positive correlation between air pollution and these biochemical parameters (Srinvas *et al*. 2008). The APTI of 10 plant species around the Erhoike- Kokori oil exploration station of Delta State, Nigeria was determined by Agbaire (2009). From 10 species studied, *Psidium guajava* was most tolerant, followed by *Elaise guineensis* and *Musa paradisiaca*. The order was: *Psidium guajava* < *Elaise guineensis* < *Musa paradisiaca* < *Bambusa bambusa* < *Anacardium occidentale* < *Terminalia catappa* < *Manihot esculenta* < *Impereta cylindrical* < *Chromolaena odorata* < *Mangifera indica.* Combining several parameters (Chl, AA, pH and RWC) gave a more reliable result than the use of individual parameters. Chauhan (2010) found a reduction in APTI for all four selected trees (*Ficus religiosa*, *M. indica*, *P. longifolia*, *Delonix regia*) from polluted site of Dehradun City when compared with samples from a control area.

Seyyednjad *et al*. (2010) examined the APTI of four plant species around a petrochemical station in southwest Iran and compared it with an unpolluted area. Four physiological and biochemical parameters (AA, leaf RWC, leaf extract pH and total leaf Chl) were used to compute the APTI values. By combining a variety of these parameters, a more reliable result was obtained than if individual parameters were assessed.

APTI has been determined by pooling the attributes *viz*., total Chl, AA and moisture content of leaves and leaf extract pH for certain locally available tree species in and around a Kothagiri municipal town, Nilgiris, Tamil Nadu by Senthilkumar and Paulsamy (2011). Of the 24 species analyzed 6 tree species (*Alnus nepalensis*, *Callistemon lanceolata*, *Eucalyptus ficifolia*, *Ficus elastica*, *Michelia champaca* and *Toona ciliate*) recorded higher APTI (8.34-10.67) values. Further, these authors suggested that these tree species can be given priority for a plantation programme in and around industrial complexes, road sides and also new urbanized areas in Kotagiri so as to reduce the effect of air pollution and clean the environment. The APTI of some tree species for green belt development to mitigate traffic generated noise was evaluated in Varanasi (Tripathi *et al.* 2011). They determined that APTI ranged from 8.74 in *Nerium odorum* to 23.58 in *Polyalthia longifolia* among 35

plants.

Mondal *et al.* (2011) evaluated the APTI of 10 plant species collected from an urban area. High APTI values were recorded in *Psidium guajava* (31.75%), *Swietenia mahoganii* (28.08%), *Mangifera indica* (27.97%), *Polyanthia longifolia* (25.58%) and *Ficus benghalensis* (25.02%). The Anticipated Performance Index (API; based on APTI and some biological and socioeconomic parameters of plants) of these plant species was also calculated by considering their APTI values together with other socio-economic and biological parameters. Further, they concluded, according to API, that the most tolerant plant species for green belt development were *Ficus benghalensis* (87%), *Mangifera indica* (87%), *Swietenia mahoganii* (87%) *and Saraca indica* (81%).

Thawale *et al.* (2011) evaluated biochemical changes that occurred in four selected plant species, namely *Azadirachta indica*, *Mangifera indica*, *Delonix regia* and *Cassia fistula* of residential, commercial, and industrial areas of Nagpur City, India. Air pollution altered the foliar biochemical features of all the plants selected. The changes in APTI of plants were also estimated, revealing that these plants can be used as a biomarker of air pollution.

Possible secondary additive factors may not affect the morphology of tested plants, as the soil type and weather of the neighbouring areas are very much similar (Bhadravathi and Shankaraghatta).

CONCLUSION

Air pollutants in urban and industrial areas may get absorbed, accumulated or integrated in the plant body and, if toxic, may injure the plants in various ways (Ishii *et al*. 2007; Singh *et al*. 2008). The rate and total amount of pollutant taken up from the air can affect photosynthesis, respiration, leaf conductance and leaf longevity. All of these factors in trees adversely affect canopy carbon fixation and net accumulation of Chl. In sensitive species the level of injury is high resulting in less Chl and *vice versa* in tolerant species. Nrusimha *et al*. (2005) and Tripathi *et al*. (2009) recorded a similar observation as the sensitive species help to indicate air pollution while tolerant species helped to abate it. This is also supported by other studies on the physiological response under heavy industrial pollution stress (Chang *et al*. 2004; Cui *et al*. 2006; Dong *et al*. 2007).

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