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Full Length Research Paper

Analysis of Biochemical Parameters as Tolerance Index of Some Chosen Plant Species of Bhadravathi Town

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Abstract

Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases like carbon dioxide and oxygen and provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the air environment. Among the various strategies for controlling atmospheric pollution, absorption of gaseous pollutants by plants provides one of the natural ways of cleansing the atmosphere as they act as effective indicators of air pollution. Recent studies have explored the possibility of plants to remove and also act as sinks for air pollutants. The main focus of this work is to provide an assessment of the use of biochemical parameters of plants as indicators of air pollution so that these biochemical indicators can be used for air quality monitoring in urban areas. Also, an attempt has been made to identify suitable air pollution tolerant tree species based on air pollution tolerance index for air pollution control in Bhadravathi town.

Keywords: *Biochemical parameters, Atmospheric pollution, Industrialization and Bhadravathi.*

Introduction

The effect of air pollution on plants has long been known. It has also been reported that when exposed to air pollutants, most plants experience physiological changes before exhibiting visible damage to leaves (Dohmen, *et al.,* 1990). Studies have also shown the impact of air pollution on Ascorbic acid content (Hoque, *et al.,* 2007), chlorophyll content (Flowers *et a*l., 2007), leaf extract pH (Klumpp *et al* .,2000) and relative water content (Rao 1979). Plants sensitivity and tolerance to air pollutants varies with change in leaf extract pH, relative water content (RWC), ascorbic acid (AA) content and total chlorophyll content. There are many factors controlling tolerance in plants. For example, the importance of pH in modifying the toxicity of SO_2 has been shown. Plants with lower pH are more susceptible, while those with pH around 7 are more tolerant (Singh and Verma, 2007). Ascorbic acid content is another parameter that may be used to decide the tolerance of plant to air pollution. It plays a significant role in light reaction of photosynthesis (Singh and Verma, 2007), activates defense mechanism (Arora *et al.,* 2002) and under stress condition, can replace water from light reaction (Singh and Verma, 2007). Ascorbic acid is a natural antioxidant in plants that plays an important role in pollution tolerance (Joshi and Swami, 2007). It is also a factor in cell wall synthesis, defense and cell division. It is also a strong reducer and plays an important role in photosynthetic carbon fixation, with the reducing power directly proportional to its concentration. So, it has been given top priority and used as a multiplication factor in the formula (Escobedo *et al*., 2008; Pasqualini *et al*., 2001; Conklin, 2001).Chlorophyll itself is actually not a single molecule but a family of related molecules, designated as chlorophyll '*a'*, '*b'*, '*c'*, and'*d*'. Chlorophyll '*a'* is the molecule found in all plant cells and therefore its concentration is what is reported during chlorophyll analysis. Accessory pigments absorb energy that chlorophyll '*a'* does not absorb. Accessory pigments include chlorophyll '*b*', xanthophyll and carotenoid (Joshi and Chauhan, 2008). Depletion in chlorophyll immediately causes a decrease in productivity of plant which subsequently exhibits poor vigor. Photosynthetic efficiency was noted to be strongly dependent on leaf pH (Singh and Verma, 2007).

Water is a necessity for plant life. Shortage of water may cause severe stress to terrestrial plants (Singh and Verma, 2007). High water content within the plant body helps to maintain its physiological balance under stress conditions such as exposure to air pollution when the transpiration rates are usually high. However, the air pollution tolerance index (APTI) based on all the four parameters has been used for identifying tolerance levels of plant species (Yan-Ju and Ding, 2007; Singh *et al.,* 1991).

Materials and Methods

Study area

Bhadravathi is an industrial town and taluk in Shimoga District of Karnataka state, India. It is situated at a distance of about 255 Kilometers from the state capital Bangalore and at about 20 Kilometers from the district headquarters, Shimoga. The town is spread over an area of 67.0536 Square Kilometers (25.8895 Sq. mi) and has a population of 160,392 as per 2001 census. The town has two major industries i.e. Visvesvaraya Iron and Steel Plant and Mysore Paper Mills Ltd. Two national highways pass through the city: NH-206 and NH-13. Polluted sites were located around the factories, while the control site was located at Shankaraghatta, 19 Km away from polluted site, possessing dense forest and vegetation. The study was carried out from January to May 2013.

Sample collection

Samples were collected and brought to the laboratory in polythene bags and kept in an ice box to nullify the adverse effect of high light intensity and temperature. The leaves were plucked at a height of 1 to 2 meter from the ground level.

Table 1: Sampling sites taken.

Site no.	Area
Sampling Site I	New town
Sampling Site II	Near Sathya Sai school
Sampling Site III	Hutta colony
Sampling Site IV	Hosamane
Control site	Shankaraghatta

Table 2: Categories of tree species based on APTI

APTI value	Response
30-100	Tolerant
29-17	Intermediate
16-1	Sensitive
< 1	Very sensitive

Method

1) Leaf relative water content (RWC):

With the method described by Singh (1997), fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water over night, blotted dry and weighed to get turgid weight. The leaves were dried over night in an oven at 70^{o} C and reweighed to get dry weight.

RWC= (FW-DW/TW-DW) x100
FW = Fresh weight
DW = Dry weight
TW = Turgid weight.

2) Leaf extract pH

5g of the fresh leaves was homogenized in 10ml deionised water. This was filtered and the pH of the leaf extract determined after calibrating pH meter with buffer solution of pH 4 and 9.

3) Total chlorophyll content (TCh)

This was carried out according to the method described by Arnon, (1949). 3g of fresh leaves were blended and then extracted with 10ml of 80% acetone and left for 15 minutes for thorough extraction. The liquid portion was decanted into another test-tube and centrifuged at 2,500 rpm for 3 minutes. The supernatant was then collected and the absorbance taken at 645nm and 663nm using a spectrophotometer. Calculations were done using the below formulae.

Chlorophyll a= $(12.7_{Dx663}\text{-}2.69_{Dx645})$xV/1000 mg/g
Chlorophyll b= $(22.9_{Dx645}\text{-}24.68_{Dx663})$xV/1000 mg/g
TCh=Chlorophyll a + Chlorophyll b mg/ Dx

4) ***Ascorbic acid content*** (Bajaj and Kaur, 1981):

1g of leaf sample was taken in a test tube and 4 ml oxalic acid-EDTA (0.05M oxalic acid, 0.2 M EDTA) extracting solution was added. Then add 1 ml of orthophosphoric acid followed by 1ml of 5% sulphuric acid. To this mixture, 2ml of 5% m/v ammonium

molybdate and 3ml of water were added. The solution was allowed to react for 15 minutes at room temperature. After incubation period the absorbance was measured at 760 nm with UV-Visible spectrophotometer, VISI SCAN-167.
The concentration of ascorbic acid in samples was then calculated from a standard ascorbic acid curve. For this 0.1 ml to 0.6 ml aliquots of standard ascorbic acid solution was taken in a series of test tubes and chemicals were added as before. After incubation period, absorbance was measured at 760 nm and standard graph was prepared.

Air Pollution Tolerance Index (APTI) Determination

This was done following the method of Singh and Rao, 1983. The formula of APTI is given as
APTI= [A+ (T+P) R]/10
A = Ascorbic acid content (mg/g)
T = Total chlorophyll mg/g
P = pH of leaf extract
R = Relative water content of leaf (%)

Results and Discussion

Ascorbic acid

Average ascorbic acid content of selected nine plants for four sampling sites is represented in Table 3.Ascorbic acid is a natural detoxicant, which may prevent the damaging effect of air pollutants in plant tissues (Singh *et al.*, 1991) and high amount of this substance favours pollution tolerance in plants (Keller and Schwager, 1977; Lee *et al.,* 1984). A definite correlation between ascorbic acid content and resistance to pollution exists in plants (Varshney and Varshney, 1984). Resistant plants contain high amount of ascorbic acid, while sensitive plants possess a low level. The level of this acid declines on pollutant exposure (Keller and Schwager, 1977). Thus, plants maintaining high ascorbic acid level even under polluted conditions are considered to be tolerant to air pollutants. Its reducing power is directly proportional to its concentration. *Eugenia jambolana* (2.30 mg/g and 3.35 mg/g in sampling sites I and II) and *Psidium guajava* (2.27mg/g and 2.39 mg/g in sampling sites I and II) showed higher value of ascorbic acid for sampling site I and II. In sampling site III, the plant species which showed higher value of ascorbic acid were *Artocarpus heterophyllus* (2.03mg/g), *Eugenia jambolana*(2.15mg/g) and *Psidium guajava* (2.19mg/g). At sampling site IV, the plants were *Mangifera indica* (2.23mg/g), *Eugenia jambolana* (2.15mg/g)and *Psidium guajava* (2.19mg/g). The species which showed least value of ascorbic acid in sampling site I were *Ficus religiosa* (0.41mg/g), *Ficus racemosa* (0.32mg/g) and *Pongamia pinnata* (0.43mg/g). Similarly in sampling site II, *Ficus racemosa* (0.71mg/g) and *Polyalthia longifilia* (0.73mg/g) and in sampling site III, *Ficus racemosa* (0.49mg/g) and *Pongamia pinnata* (0.51mg/g) showed lower ascorbic acid values. At sampling site IV, *Pongamia pinnata* (0.26mg/g) and *Polyalthia longifilia* (0.29 mg/g) showed least values of ascorbic acid. The values of ascorbic acid ranged from 0.26 mg/g to 3.35 mg/g.

Table 3: Average ascorbic acid content (mg/g) of selected plants at four sampling sites.

Plants	Sampling site I	Sampling site II	Sampling site III	Sampling site IV
Mangifera indica	1.17	1.67	1.25	2.23
Azadirachta indica	1.36	1.31	1.21	0.70
Artocarpus heterophyllus	1.20	1.06	2.03	0.45
Ficus religiosa	0.41	0.99	0.65	0.77
Ficus racemosa	0.32	0.71	0.49	0.74
Pongamia pinnata	0.43	0.87	0.51	0.26
Polyalthia longifolia	1.19	0.73	0.62	0.29
Eugenia jambolana	2.30	3.35	1.39	2.15
Psidium guajava	2.27	2.39	2.29	2.19

Total chlorophyll

Average total chlorophyll content of selected nine plants for four sampling sites is represented in Table 4. The chlorophyll level in plants decreases under pollution stress (Speeding and Thomas, 1973). Bell and Mudd (1976) suggested that tolerance of plants to SO_2 might be linked with synthesis or degradation of chlorophyll. Thus, plants having high chlorophyll content under field conditions are generally tolerant to air pollutants. The TCh of *Polyalthia longifolia* and *Pongamia pinnata* are higher compared to other species in sampling site I, i.e. 2.81 mg/g and 1.91mg/g respectively. In sampling site II, P*olyalthia longifolia* showed higher value of TCh ie., 1.76 mg/g, followed by 1.19 mg/g. In sampling site III too , *Polyalthia longifolia* showed TCh content of 1.74mg/g followed by *Pongamia pinnata* (1.38mg/g). *Eugenia jambolana* (1.19 mg/g) and *Artocarpus heterophyllus* (1.19mg/g) showed highest value in

sampling site IV. *Psidium guajava* showed least value of TCh in sampling site I, II and III and *Polyalthia longifolia* at sampling site IV.

Among all the sampling sites, the highest chlorophyll was found in sampling site I i.e., 2.81 mg/g and 1.76 mg/g from *Polyalthia longifolia* and the minimum of 0.65 mg/g and 0.57 mg/g from *Psidium guajava* sampled at site I and III.

Table 4: Average total chlorophyll content (mg/g) of selected plants at four sampling sites.

Plants	Sampling site I	Sampling site II	Sampling site III	Sampling site IV
Mangifera indica	1.66	1.19	1.26	0.82
Azadirachta indica	0.70	0.55	0.90	0.64
Artocarpus heterophyllus	0.75	0.62	0.94	1.19
Ficus religiosa	0.91	0.66	1.02	1.06
Ficus racemosa	1.00	0.64	0.80	0.79
Pongamia pinnata	1.91	1.17	1.38	0.71
Polialthia longifilia	2.81	1.76	1.74	0.25
Eugenia jambolana	1.00	0.91	0.85	1.19
Psidium guajava	0.57	0.45	0.65	0.76

p^H

All the plant samples collected from polluted sites exhibited a shift in pH towards acidic range. Acidic pH was found in *Ficus religiosa* in sampling site I and sampling site II i.e., 4.6 and 5.1 respectively. In sampling site III, *Mangifera indica* showed lower pH i.e.5.5. *Eugenia jambolana* showed low pH of 5.03 in sampling site IV. The pH ranges from 4.6 to 7.6. (Table 5) The presence of SO_2 and NO_x in the ambient air causes a change in pH of the leaf sap towards acidic range. Upon diffusion of SO_2 through stomata, gaseous SO_2 dissolves in water to form sulphites, bisulphate and their ionic species with the generation of protons influencing the cellular pH. It is therefore concluded that the pH change towards acidic range observed in most species is due to entry of SO_2 into leaf mesophyll tissue.

Table 5: p^H of selected plants at four sampling sites

Plants	Sampling site I	Sampling site II	Sampling site III	Sampling site IV
Mangifera indica	5.52	5.72	5.56	5.65
Azadirachta indica	6.24	6.22	6.42	6.28
Artocarpus heterophyllus	5.37	5.67	5.72	5.42
Ficus religiosa	4.60	5.10	6.06	5.49
Ficus racemosa	7.60	7.68	7.15	5.1
Pongamia pinnata	6.53	6.22	6.56	6.35
Polyalthia longifilia	6.25	7.65	6.18	6.42
Eugenia jambolana	7.20	5.806667	5.75	5.03
Psidium guajava	5.64	5.66	5.60	5.9

Relative water content

Average relative water content of selected plants for four sampling sites is represented in Table 6. It has been reported that air pollutants increase cell permeability (Keller, 1986), which causes loss of water and dissolved nutrients, resulting in early senescence of leaves (Masuch *et al*., 1988). It is likely therefore, that those plants with high RWC under polluted conditions may be tolerant to pollutants. Among the 9 species, most of the plants showed maximum RWC in all the sites. However, *Azadirachta indica* (59.95%) and *Pongamia pinnata* (58.12%) showed lower RWC compared to others in sampling site II, and at sampling site III, *Azadirachta indica* (58.35%) was low. The average RWC ranged from 58.12% to 99.8%.

Table 6: Average relative water content (%) of selected plants at four sampling sites

Plants	Sampling site I	Sampling site II	Sampling site III	Sampling site IV
Mangifera indica	84.44	81.23	76.94	92.95
Azadirachta indica	87.08	59.95	58.35	79.15
Artocarpus heterophyllus	78.14	68.92	82.85	89.40
Ficus religiosa	87.32	84.39	70.93	93.13
Ficus racemosa	80.44	69.35	66.39	75.25
Pongamia pinnata	68.88	58.12	66.31	82.69
Polyalthia longifolia	85.33	76.44	99.8	94.83
Eugenia jambolana	82.27	77.96	81.94866	85.87
Psidium guajava	84.48	80.19	67.91	83.90

APTI calculation and discussion

Average APTI of selected nine plants for four sampling sites is represented in Table 7. It is evident from Tables 3, 4, 5 and 6 that no species has the maximum value for all the four parameters. Each parameter plays a distinct role in the determination of the susceptibility of plants. Thus, a combination of the four parameters represents the best index for the susceptibility of plants under field conditions.

The APTI values obtained for different plants were compared to find out the sensitivity or tolerance of these plants. It was reported that plants with relatively low index value are generally sensitive to air pollutants and plants with high index value were tolerant (Singh *et al*., 1991).

The APTI determination provides a reliable method for screening large number of plants with respect to their susceptibility to air pollutants. The method is simple and convenient to adopt under field conditions without adopting costly environmental monitoring gadgets. Among the nine tree species studied, *Polyalthia longifolia* was considered as relatively resistant to air pollution. The sensitive species can be used as bio-indicators and tolerant species can be used as a sink for air pollutants.

Among the 9 selected plant species , *Polyalthia longifolia (*75.20) and *Ficus racemosa (*69.27) were having the highest APTI in sampling site I followed by *Euginia jambolana > Azadirachta indica> Mangifera indica > Pongamia pinnata> Psidium guajava > Ficus religiosa> Artocarpus heterophyllus.* In sampling site II, *Polyalthia longifolia and Mangifera indica* ranked highest APTI value with 80.38 and 53.073 respectively, followed by *Ficus religiosa> Ficus racemosa> Psidium guajava > Eugenia jambolana> Artocarpus heterophyllus > Pongamia pinnata> Azadirachta indica.* Sampling site III had the highest APTI value in *Polyalthia longifolia (*79.38289), *Artocarpus heterophyllus (*55.60) followed by *Euginia jambolana> Ficus racemosa> Pongamia pinnata> Mangifera indica> Ficus religiosa> Azadirachta indica> Psidium guajava.* Sampling site IV had highest value of APTI with respect to *Polyalthia longifolia (*63.49785) and *Ficus religiosa (61.25657*) followed by *Mangifera indica> Artocarpus heterophyllus> Pongamia pinnata> Psidium guajava> Azadirachta indica> Eugenia jambolana> Ficus racemosa*.

The APTI values showed that all the selected plants were falling in the tolerant category as their APTI values ranged between 30 to 100 except *Azadirachta indica* at sampling site II (Table .2). *Polyalthia longifolia* with the highest APTI value at all the sampling sites, proves to be the most tolerant tree among the selected species and *Azadirachta indica* is the intermediate tolerant species.

Table 7: Average APTI of selected plants at four sampling sites.

Plants	Sampling site I	Sampling site II	Sampling site III	Sampling site IV
Mangifera indica	60.42	53.07	52.27	60.72
Azadirachta indica	61.81	25.89	44.04	54.85
Artocarpus heterophyllus	47.79	36.62	55.60	59.090
Ficus religiosa	48.30	51.49	48.57	61.25
Ficus racemosa	69.27	49.40	53.38	42.84
Pongamia pinnata	57.53	31.37	52.86	58.74
Polyalthia longifolia	75.20	80.38	79.38	63.49
Eugenia jambolana	67.27	41.47	54.22	53.65
Psidium guajava	52.78	49.29	42.45	56.08

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