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ASSESSMENT OF AIR POLLUTION TOLERANCE INDEX OF PLANTS GROWING ALONGSIDE NATIONAL HIGHWAY (21) OF HIMACHAL PRADESH IN INDIA

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ABSTRACT

The investigations were carried out during the year 2014-15 to study the air pollution tolerance of six plant species growing alongside a selected stretch (Bilaspur to Mandi) of National Highway 21 in Himachal Pradesh, India. The selected plant species were *Ficus carica, Morus alba, Toona ciliate, Melia azedarach*, *Ahotoda vasica* and *Murraya koengii*. Among all the plants *Toona ciliate* contained the highest leaf ascorbic acid (2.32 mg g^{-1}) and lowest value of 1.92 mg g^{-1} in *Melia azedarch*. The highest (83.09%) relative water content was recorded in post monsoon season while lowest value of 81.01% in the pre monsoon season. In the selected plant species the highest (3.04 mg g^{-1}) chlorophyll content was recorded in *Morus alba* and lowest (1.12mg g^{-1}) in *Ficus carica*. The highest (7.15) value of leaf extract pH was observed in *Morusalba*. The air pollution tolerance index of plant species followed the order *Toona ciliata* > *Melia azedarach* > *Adhotoda vasica* > *Ficus carica* > *Morus alba* and *Murraya koenigii* with the respective values of 10.72, 9.92, 9.88, 9.71, 9.47 and 9.29.The study suggested that in the study area *Toona ciliata* and *Adhotoda vasica* with the highest APTI act as indicator of pollution and can be planted to create green belt.

KEY WORDS
Ascorbic acid
Chlorophyll
Relative water content

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INTRODUCTION

With the advent of economic growth, the road development has become pre dominant, where in traffic and other commercial activities have increased significantly. Such activities has degenerated the living environment. Consequently the air is being continuously polluted through heavy traffic and also due to widening and maintenance of roads. Motor vehicles account for maximum air pollution as compared to any other source. They are responsible for virtually all of the carbon monoxide and lead in the air of cities, and a major portion of the NOx, VOCs (Volatile organic compounds), fine particles, and toxic chemicals (Chauhan, 2010; Narwaria and Kush, 2012).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, oxygen and also provide enormous area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the air environment (Escobedo *et al.*, 2008; Das, 2010). As air pollutant once released into the atmosphere cannot be traced or identified by any device chemically or mechanically, only plants can be useful in adsorbing and metabolizing these released pollutants from the atmosphere.Air Pollution Tolerance Index (APTI) is an unique index, which incorporates four biochemical parameters viz. total chlorophyll, pH of leaf extract, ascorbic acid and relative water content (RWC) (Shannigrahi *et al.*, 2003). These studies helps to provide an idea about the plant species tolerant and sensitive to air pollution (Rai *et al.,* 2014).

During the recent past the state of Himachal Pradesh has also achieved significant growth in terms of infrastructure especially roads. Being an important destination as tourist place the new National Highways are being constructed in the state. Therefore there is an urgent need to screen the plants already growing in the region according to their tolerance level to pollution. The present study was therefore conducted in the National Highway (21) in the stretch from Bilaspur to Mandi to work out the APTI of the plants growing alongside the roads based on four biochemical parameters for identifying the tolerance levels, so that suitable plant species can be selected to remediate air pollution in the study area.

MATERIALS AND METHODS

Area of study

The study was conducted on the National Highway 21 situated in Shivalik range having subtropical type of climate in lower Himalayas, which connects Chandigarh to Manali in Himachal Pradesh passing through many towns and villages, covering a distance of about 323kms. A stretch from Bilapsur to Mandi on the National Highway was selected for the study.The study area lies between

North latitude of 31°21′64" to 31°38′56" and East longitude of 76°56′77" to 76°46′46". The study area is on a fragile data and has high vehicular density since it leads to main tourist place Kullu – Manali of this country and passes through a major cement plant of the state of Himachal Pradesh.

Survey of the study area

In order to study the distribution of plants growing alongside the national highway a detailed survey was conducted from Bilaspur to Mandi. During the survey an account of plant species growing alongside the national highway was made. Based on the survey the commonly occurring species were identified for conducting study.

Treatment details

In order to identify the tolerance levels of plants growing alongside the National Highway six commonly occurring species were selected. The species selected were *Ficuscarica, Morusalba, Toonaciliata, Melia azedarach*, *Murraya koengnii* and *Ahotoda vasica*. In order to assess the impact of vehicular pollution on the plants growing alongside the national highway three horizontal distances namely; D1 (0-15m),D2 (15-30m) and D3 (30-45m) on both sides of the road were considered. To assess the seasonal impact of traffic pollution pre monsoon and post monsoon seasons were selected. The entire stretch of National Highway *i.e.* from Bilaspur to Mandi was divided into four segments of 15 km each and these were considered as replications. One replication was composed of four plants each of selected species , distance and side along the National Highway. In total there were 36 treatment combinations under RBD Factorial design. The data for the two sides (left and right) of the national highway was clubbed for statistical analysis.The results were statistically analyzed and interpreted by using Statistical Package Software version 21.

Sample collection

In order to maintain the uniformity,plants of same age and spread growing at iso-ecological conditions were selected for the study. To assess the APTI based on the four parameters namely leaf ascorbic acid, total chlorophyll, leaf extract pH and relative water content, fully matured leaves of selected species were collected in the morning hours at almost same diameter at breast height (DBH). The collected leaf samples were transported to laboratory for further analysis in ice box.

Sample analysis

The fresh leaf samples wereanalysed for total chlorophyll, ascorbic acid, leaf extract pH and relative water content using standard procedures as mentioned below:

Total chlorophyll

For the estimation of total chlorophyll content of the leaves, 10mg of the leaf sample was homogenized with 7ml dimethyl sulphoxide and was kept in oven at a temperature of 60-65°C for 30-35mins. The samples were filtered and volume was made to 25ml by dimethyl sulphoxide. The absorbance was measured at 663nm and 645nm in spectrophotometer and chlorophyll was estimated using the following equation given byHiscox and Istaelstam, 1979.

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{20.2\,A_{645} + 8.02\,A663}{\text{ax } 1000\text{x w}} \times V$$

Where

V = volume of extract made

a = length of light path in cell (usually 1cm)

w = weight of the sample taken

A_{645} is absorbance at 645nm

A_{663} is absorbance at 663nm

Ascorbic acid

To estimate leaf ascorbic acid content of selected plant species 10g of the sample was taken. The sample was homogenized in metaphosphoric acid (3%) and filtered. The volume of the filtrate was made to 100ml by metaphosphoric acid(3%). The aliquot measuring 10ml was taken and titrated against standardized dye to an end point of pink colour as per the standard procedure outlined by A.O.A.C (1980)**.**The ascorbic acid content was expressed in mg g^{-1}.

$$\text{Ascorbic acid (mg g}^{-1} = \frac{\text{Dye factor} \times \text{Titrer reading} \times \text{Volume made}}{\text{Weight of leaves taken} \times \text{Volume taken for estimation}}$$

Leaf extract pH

Leaf extract pH of the samplewas analyzed by the method suggested byBarrs and Weatherly (1962). Fresh leaf sample (10g) was homogenizedusing deionised water (50ml) and the supernatant obtainedafter centrifugation was collected for thedetermination of pH using a digital pH meter.

Relative water content

Relative water content of the samples was estimated using the method proposed by Singh (1977) and was computed by using following equation:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where

FW- Fresh weight, TW- Turgid weight, and DW- Dry weight of leaf samples

Air pollution tolerance index

The air pollution tolerance index (APTI) was estimated by considering four biochemical parameters namely ascorbic acid, total chlorophyll, leaf extract pHand relative water content and was computed by using the following equation given by Singh and Rao (1983).

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

Where, A- ascorbic acid (mg g^{-1} FW) , T- total chlorophyll (mg g^{-1} FW) ,P- leaf extract pH R- relative water content (%) of the leaves.

RESULTS AND DISCUSSION

Biochemical parameters

Leaf ascorbic acid

The different plant species growing alongside the National Highway (21) on the stretch from Bilaspur to Mandi found to exhibit significant variations in their leaf ascorbic acid content. Interestingly, each species growing at different distances from the road and during different seasons of the year also showed significant variations in the leaf ascorbic acid content

(Table 1). Among the selected six species the highest ascorbic acid content (2.60 mg g^{-1}) was found in *Toonaciliata*followed by *Ficuscarica*(2.31 mg g^{-1}), *Ahotoda vasica* (2.11 mg g^{-1}), *Morus alba* (2.10 mg g^{-1}), *Murayya koengii* (2.05 mg g^{-1}) and lowest (2.02 mg g^{-1}) in *Melia azederach*. The significant variation in the leaf ascorbic acid content in selected species may be ascribed to genetic variation. Whereas, the highest content in *Toona ciliate* may be because of its relatively higher tendency to tolerate pollution stress through the synthesis of ascorbic acid as compared to other plant species. The selected plants during post monsoon season exhibited significantly higher ascorbic acid content (2.29 mg g^{-1}) as compared to pre monsoon season (2.10 mg g^{-1}) in their leaves. The leaf ascorbic acid of selected plant species was found to decrease from the near roadside location (0-15m) towards the control site (30-45mm). The plants growing at a distance of 0-15m exhibited significantly higher ascorbic acid content (3.38 mg g^{-1}) followed by the plants growing at 15-30m and away from 30m (control) with the values of 1.71mg/g and 1.50 mg g^{-1}respectively.The trend of ascorbic acid content in the leaves of selected plant species may be ascribed to more synthesis in plants growing near roadside due to higher pollution stress. The results are in line with the findings of Yannawar and Bhosle (2013) who have also reported higher ascorbic acid content in the leaves of the plants growing near the roadside.

The species × season interaction was found to have a non significant influence on the ascorbic acid content of the leaves of plants. The interaction of species and distance resulted in a significant influence on the ascorbic acid content of the leaves. It is evident that the plants growing near the road found to exhibit higher ascorbic acid content in their leaves which decreased with the increase in the distance. The highest content of 4.41 mg $g^{-1}$was recorded in the leaves of *Toona ciliate* which was followed by *Ficuscarica* (3.72 mg g^{-1}) > *Morus alba* (3.26 mg g^{-1}) > *Adhotodavasica* (3.10 mg g^{-1}) > *Melia azerderach* (2.95 mg g^{-1}) > *Murrayakoenigii*(2.84 mg g^{-1}) at the same distance. Whereas, the lowest content of 1.44 mg $g^{-1}$was recorded in *Morusalba* growing away from 30m distance from the National Highway (control). The value was statistically at par with *Murrayakoengii, Melia azedarach ,Adhotodavasica*, *Ficuscarica*and*Toona ciliate*. The present trend of ascorbic acid distribution in leaves of plants growing at different distances from the National Highway (21) is in conformity with the findings of Jyothi and Jaya (2010). In a similar study Conklin (2001) has explained that the increased rate of production of reactive oxygen species during photo oxidation of SO_2 to SO_3 may be the reason for higher ascorbic acid content under such pollution. On the other hand Varshney and Varshney (1984) reported that higher ascorbic acid content of plants is its sign of tolerance against sulphur dioxide pollution. The season and distance interaction exerted a significant influence on the leaf ascorbic acid of selected plant species. The plants growing within a distance of 0-15m exhibited a higher amount (3.55 mg g^{-1}) of ascorbic acid in their leaves in the post monsoon which was followed by those growing at the same distance in the pre monsoon season. On the other hand the lowest content of 1.44 mg $g^{-1}$was recorded at the distance of more than 30m *i.e.* control during pre monsoon season. It is further evident from the data in Table 1(b) that the selected plants were found to contain higher ascorbic acid in their leaves during post monsoon season at all the distances as compared to pre monsoon season. In all the seasons the ascorbic acid content decreased with increasing distance. In the species × season × distance interaction the variations in the leaf ascorbic acid

Table 1(a): Season wise variation in ascorbic acid content (mg/g) of selected plant species growing at different distances on National Highway 21 in Himachal Pradesh

Distance	Pre monsoon				Post monsoon			
	Distance				Distance			
Species	D1	D2	D3	Mean	D1	D2	D3	Mean
F. carica	3.59	1.58	1.40	2.19	3.85	1.83	1.59	2.31
M. alba	3.06	1.52	1.38	1.99	3.46	1.65	1.49	2.10
A. vasica	3.02	1.63	1.42	2.02	3.19	1.75	1.66	2.11
M. azedarach	2.70	1.62	1.45	1.92	3.20	1.70	1.54	2.03
M. koenigii	2.74	1.74	1.50	1.99	2.94	1.83	1.55	2.05
T. ciliata	4.16	1.82	1.60	2.53	4.67	1.82	1.58	2.61
	3.21	1.65	1.44		3.55	1.76	1.56	

Table 1(b):

Species	Distance				Season	
	D1	D2	D3	Mean	Pre monsoon	Post monsoon
F. carica	3.72	1.70	1.49	2.31	2.19	2.42
M alba	3.26	1.59	1.44	2.10	1.99	2.20
A.vasica	3.10	1.69	1.53	2.11	2.01	2.20
M.azedarach	2.95	1.66	1.46	2.03	1.90	2.15
M.koenigii	2.84	1.78	1.53	2.05	1.99	2.11
T.ciliata	4.41	1.82	1.57	2.60	2.52	2.68
Mean (distance)	3.38	1.71	1.50	Mean (season)	2.10	2.29
Pre monsoon	3.21	1.65	1.44	2.10		
Post monsoon	3.55	1.76	1.56	2.29		

Species = 0.10;seasons = 0.06;Distance = 0.07; Species × distance = 0.17; Seasons× species× distance = NS; Season × distance = 0.10
Species × season = NS

Table 2(a): Season wise variation in total chlorophyll content (mg/g) of selected plant species growing at different distances on National Highway 21 in Himachal Pradesh

Species	Pre monsoon Distance				Post monsoon Distance			
	D1	D2	D3	Mean	D1	D2	D3	Mean
F. carica	0.48	1.06	1.83	1.12	0.79	1.30	2.04	1.25
M alba	2.36	3.09	3.66	3.04	2.22	3.17	3.92	3.07
A.vasica	0.92	1.64	2.71	1.76	1.16	1.83	2.14	1.73
M.azedarach	0.65	1.29	2.14	1.36	0.85	1.41	1.95	1.38
M.koenigii	1.61	2.63	3.66	2.63	1.90	3.06	3.83	2.78
T.ciliata	0.87	1.63	2.47	1.66	1.17	1.74	2.23	1.68
	1.15	1.89	2.74	1.93	1.35	2.09	2.68	1.98

Table 2(b):

Species	Distance				Seasons	
	D1	D2	D3	Mean	PreMonsoon	Post-Monsoon
F. carica	0.63	1.18	1.94	1.25	1.12	1.38
M alba	2.29	3.13	3.79	3.07	3.03	3.10
A.vasica	1.04	1.73	2.43	1.73	1.75	1.71
M.azedarach	0.75	1.35	2.04	1.38	1.36	1.40
M.koenigii	1.76	2.85	3.74	2.78	2.63	2.93
T.ciliata	1.02	1.68	2.35	1.68	1.66	1.71
Mean (distance)	1.25	1.99	2.71	Mean (seasons)	2.06	2.15
Pre monsoon	1.26	2.03	2.90	2.06		
Post monsoon	1.44	2.22	2.79	2.15		

$CD_{(0.05)}$

Species = 0.12	Species × season = 0.17	Seasons × species × distance = 0.30
season = 0.07	Species × Distance = 0.21	
Distance = 0.07	Season × distance = 0.12	

Table 3(a): Season wise variation in leaf extract pH of selected plant species growing at different distances on National Highway 21 in Himachal Pradesh

Species	Pre monsoon Distance				Post monsoon Distance			
	D1	D2	D3	Mean	D1	D2	D3	Mean
F. carica	4.7	6.1	6.6	5.80	5.0	5.9	7.2	6.03
M alba	5.2	5.9	6.6	5.90	5.1	5.9	6.9	5.93
A.vasica	5.1	6.2	6.5	5.93	5.2	6.3	6.5	6.00
M.azedarach	5.2	6.1	6.6	5.97	5.5	6.5	6.7	6.23
M.koenigii	4.8	6.1	6.5	5.80	4.9	6.2	6.6	5.90
T.ciliata	5.3	6.0	6.2	5.83	5.2	6.3	6.5	6.00
	5.05	6.07	6.50	5.87	5.13	6.18	6.73	6.02

Table 3(b):

Species	Distance				Season	
	D1	D2	D3	Mean	PreMonsoon	Post-Monsoon
F. carica	4.80	5.97	6.88	5.88	5.77	5.99
M alba	5.11	5.91	6.75	5.92	5.90	5.94
A.vasica	5.18	6.24	6.54	5.99	5.97	6.01
M.azedarach	5.30	6.29	6.64	6.08	5.94	6.22
M.koenigii	4.84	6.15	6.56	5.85	5.78	5.92
T.ciliata	5.26	6.14	6.35	5.92	5.85	5.98
Mean (distance)	5.08	6.12	6.62	Mean (seasons)	5.87	6.01
Pre monsoon	5.03	6.07	6.62	5.87		
Post monsoon	5.13	6.17	6.73	6.01		

$CD_{(0.05)}$

Species = 0.16	Species × season = NS	Seasons × species × distance = 0.30
season = 0.07	Species × Distance = 0.20	
Distance = 0.07	Season × distance = NS	

Table 4(a): Season wise variation in relative water content (%) of selected plant species growing at different distances on National Highway 21 in Himachal Pradesh

Species	Pre monsoon Distance				Post monsoon Distance			
	D1	D2	D3	Mean	D1	D2	D3	Mean
F. carica	87.11	80.86	76.99	81.65	85.99	80.54	80.72	82.04
M alba	78.61	75.22	70.7	74.84	82.28	78.16	76.98	76.99
A.vasica	84.6	82.45	80.21	82.42	88.68	84.38	80.39	83.45
M.azedarach	88.79	85.78	81.11	85.23	89.56	83.72	80.14	84.85
M.koenigii	75.31	72.83	70.51	72.88	83.72	80.15	75.17	76.28
T.ciliata	91.59	89.04	86.6	88.36	93.89	87.43	83.77	89.06
Mean	84.33	81.03	77.68	80.90	87.35	82.4	79.53	82.11

Table 4(b):

Species	Distance				Seasons	
	D1	D2	D3	Mean	Pre- monsoon	Post-monsoon
F. carica	86.55	80.70	78.86	82.04	81.65	82.42
M alba	80.44	76.69	73.84	77.00	74.84	79.14
A.vasica	86.63	83.42	80.30	83.45	82.42	84.48
M.azedarach	89.17	84.75	80.62	84.85	85.23	84.47
M.koenigii	79.51	76.49	72.84	76.28	72.88	79.68
T.ciliata	92.74	88.23	85.19	88.72	88.36	89.06
Mean (distance)	85.84	81.71	78.61	Mean (seasons)	81.02	83.09
Pre monsoon	84.33	81.03	77.68	81.01		
Post monsoon	87.35	82.40	79.53	83.09		

$CD_{(0.05)}$

Species = 0.08	Species × season = 0.11	Seasons × species × distance = NS
Season = 0.05	Species × distance = NS	
Distance = 0.06	Season × distance = NS	

Table 5(a): Season wise variation in APTI of selected plant species growing at different distances on National Highway 21 in Himachal Pradesh

Species	Pre monsoon Distance				Post monsoon Distance			
	D1	D2	D3	Mean	D1	D2	D3	Mean
F. carica	10.6	9.2	8.9	9.57	10.8	9.3	9.5	9.87
M alba	10.2	8.9	8.5	9.20	10.7	9.2	9.3	9.73
A.vasica	10.3	9.5	9.3	9.70	10.9	9.8	9.5	10.07
M.azedarach	10.5	9.8	9.3	9.83	11.0	9.6	9.3	9.97
M.koenigii	9.3	8.8	8.6	8.90	10.4	9.6	9.1	9.70
T.ciliata	11.7	10.3	10.1	10.70	12.4	10.2	9.7	10.77
Mean	10.42	9.42	9.12	9.65	11.03	9.62	9.4	10.02

istance Species	Distance				Season	
	D1	D2	D3	MEAN	Pre-monsoon	Post – monsoon
F. carica	10.68	9.25	9.20	9.71	9.55	9.88
M alba	10.45	9.07	8.90	9.47	9.19	9.76
A.vasica	10.59	9.65	9.39	9.88	9.71	10.05
M.azedarach	10.71	9.71	9.33	9.92	9.85	9.99
M.koenigii	9.82	9.19	8.86	9.29	8.88	9.70
T.ciliata	12.05	10.24	9.88	10.72	10.69	10.75
Mean (distance)	10.72	9.52	9.26	Mean (seasons)	9.64	10.02
Pre monsoon	10.41	9.42	9.10	9.64		
Post monsoon	11.03	9.61	9.42	10.02		

$CD_{(0.05)}$

Species = 0.17	Species × season = 0.25	Seasons × species × distance = 0.43
season = 0.10	Species × Distance× = 0.30	
Distance = 0.12	Season × distance = 0.17	

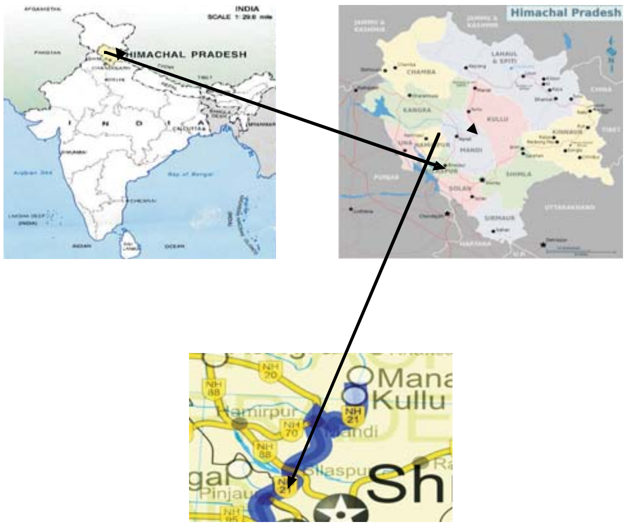

Figure: Map showing the study area

was found to be statistically non significant.

Chlorophyll content

The plants growing alongside the National Highway (21) showed a significant variation in the leaf chlorophyll content (Table 2) which was found to vary significantly with seasons of the year and changing distance from the road. The highest leaf chlorophyll content of 3.07 mg $g^{-1}$was found in the leaves of *Morus alba* followed by *Murrayakoenigii*(2.78 mg g^{-1}), *Adhotodavasica* (1.73 mg g^{-1}), *Toonaciliata*(2.35 mg g^{-1}), *Melia azedarach* (1.38 mg g^{-1}), and the lowest amount (1.38 mg g^{-1}) was found in *Melia azedarach*. This variation of chlorophyll content in the leaves of selected plants may be because of the genetic variations as well as due to vehicular pollution in the selected stretch of the National Highway. The results are in line with the findings of Ninave *et al.*, 2001. The seasons of the year also influenced the chlorophyll content of the leaves. In this part of the national highway the plants exhibited significantly higher chlorophyll content of 2.04 mg g^{-1} during post monsoon season where as the lowest (1.93 mg g^{-1}) was noticed during pre monsoon season. The season wise variation in chlorophyll content may be ascribed due to variation in environmental stresses and washing of pollutants. The results are in line with the findings of Jyothi and Jaya (2010). In contrast to ascorbic acid, the chlorophyll content was found to increase with increasing distance. The maximum amount (2.71 mg g^{-1}) of leaf chlorophyll was recorded at the control site (*i.e.* at a distance > 30m), the amount (1.99 mg g^{-1}) reduced at the distance of 15-30 and minimum content of 1.25 mg g^{-1} was recorded at 0-15m distance. The lower amount of chlorophyll at the roadside distance may be ascribed to higher traffic pollution alongside the road as compared to control site. The results are in conformity with the findings of Mir *et al*, (2008) who pointed out high level of automobile pollution the cause of decrease in chlorophyll content of plants near roadside.

The interaction between species and seasons exerted a significant influence on the total leaf chlorophyll content of the plant. Among all the species, *Morusalba*was found to have the highest chlorophyll content of 3.10 mg g^{-1} during the post monsoon season, which was at par with *Murrayakoengii*in the same season. The minimum content of 1.12 mg g^{-1} was noticed during pre monsoon in *Ficuscarica* (Table 2(b)). The species × distance interaction was found to be significant. Among the selected species *Morus alba* growing at a distance of more than 30m contained significantly highest chlorophyll content of 3.79mg/g which was statistically at par *Murrayakeonginii*at the same distance and followed by the plants growing at a distance of 15-30m while the lowest content of 0.63 mg g^{-1} was found in the leaves of *Ficuscarica*at the distance 0-15m from the road. The season and distance interaction also resulted in significant influence on leaf chlorophyll content. The highest amount of 2.90 mg g^{-1} was found at the control site (30-45mm) in pre monsoon season which was statistically at par with the content of plants growing away from 30m distance (control) in the post monsoon season. It was followed by leaf chlorophyll content of plants during post monsoon at the distance of 0-15m. The lowest content of 1.26 mg g^{-1} was found in the plants growing near national highway (0-15m) during pre monsoon season. It is evident from the data in Table 2(b) that the leaf chlorophyll content increased with increasing distance in both seasons. However the content was relatively higher in post monsoon as compared to pre monsoon season at all distances. The three way interaction between species × season × distance was found to be statistically significant with respect to chlorophyll content in the leaves of selected plant species. The chlorophyll content was found to be in the range of 0.48 – 3.92 mg g^{-1}. *Morus alba* was found to have maximum amount of 3.92mg/g of chlorophyll in the post monsoon season at the distance of more than 30m (control). However the highest content of *Morusalba* was found statistically at par with in the same season and distance. The lowest chlorophyll content of 0.48 mg g^{-1} was observed in *Ficuscarica*during the pre monsoon season near the national highway *i.e.* 0-15m.

Leafextract pH

The leaf extract pH of the plant species varied from 5.85 to 6.08. The leaf pH also varied significantly among species, seasons and distance from the national highway (Table 3). In the selected stretch , out of the six species *Melia azedarach*leaves found to contain the highest pH of 6.08 which was followed by *Adhotodavasica , Toonacilaita, Morus alba, Ficuscarica*and *Murrayakeonginii* the respective values of 5.99, 5.92, 5.91, 5.88 and 5.85. The present results are in line with Scholz and Reck (1977) who have reported that species vary in the leaf pH according to their sensitivity to acidic pollutant. The plants growing alongside the national highway exhibited decrease in their leaf extract pH from post monsoon (6.01) to pre monsoon season (5.87). The present trend may be ascribed due to more accumulation of acidic vehicular pollutants during pre monsoon as compared to post monsoon. The trend is in line with Aggrawal (1988). The distance from the national highway also exhibited significant variation with distance.

The highest value of 6.62 was observed at control site followed by the plants at 15-30m distance and lowest value of 5.08 in the plants near the roadside. Similar results were also observed by Singare and Talpade (2013) who also reported that the leaf extract pH tend to decrease with the increase in pollution with respect to control site.

The interaction between season and distance and between season and species was found to be statistically non-significant. The species × distance interaction exerted a significant influence on leaf extract pH of the plants. It is evident from the Table 3(b) that the pH decreased with the increasing distance in all the plant species. The highest value of 6.88 was recorded in *Ficuscarica*at the distance of more than 30m (control) which was statistically at par with *Morusalba*at the same distance. Whereas the lowest value of 4.80 was recorded in the leaves of *Ficuscarica*growing at a distance of 0-15m from the national highway. The interaction between species × season × distance for leaf extract pH was found to be statistically significant. Among the plant species *Ficuscarica* was found to exhibit the lowest value of 4.7 in the pre monsoon season at a distance of 0-15m from the roadside. The value was at par in the same species and distance during the post monsoon season .The highest value of 7.2 was again observed in same species during post monsoon season at the control site (beyond a distance of 30m) and it was statistically at par with *Morus alba* in the same season and distance. The plant species growing at control site induced higher leaf extract pH in their leaves during rainy season may be because of less pollution whereas those growing at traffic area contained minimum content during summer season this might be due to more stress conditions due to summer season and also because of high transportation activities at this stretch of the national highway. The results are in conformity with the findings of Jyothi and Jaya, 2010.

Leaf relative water content

The leaf relative water content (RWC) is the amount of moisture a plant holds and is an indicator hydration condition in the leaf matrix. The relative water content varied significantly among the selected plant species, seasons and at different distances from the roadside (Table 4). Among the plants *Toonaciliata*was found to have highest relative water content 88.72%, while the lowest value of 76.28% was observed in *Murrayakoenigii*. The variation in the relative water content among the plant species may be due to the difference in their genetic makeup. The highest value of relative water content in *Toonacilata*may be ascribed due to its higher resistant capacity to stress conditions. The plants of the National Highway contained the highest leaf water content of 83.09 % during post monsoon, whereas minimum content of 81.03% was noticed in pre monsoon season. This trend may be ascribed to higher relatively high soil moistureduring post monsoon season, which might have resulted in better uptake of water and consequently the higher water content in the leaves. Also the high amount of humidity in the post monsoon season results in less transpiration and therefore high relative water content. The results are in line with the findings of Jyothi and Jaya, 2010. Similar to ascorbic acid trend, significantly higher ascorbic acid content (85.84%) was noticed in the plants growing near the national highway *i.e.* at a distance of 0-15m. This was followed by the RWC of 81.71 % and 78.61% at 15-30m and distance more than 30m respectively. The present trend of higher RWC in the plants growing alongside the National Highway may be ascribed to relatively lower rate of transpiration at polluted sites which might have resulted in the leaves of the plants. The results are in line with Bora and Joshi (2014).

The species and seasons interaction had a significant variation in the leaf relative water content. Significantly, highest RWC of 89.06% was recorded in *Toonaciliata*during post monsoon season followed by *Adhotodavasica* , *Melia azedarach*, *Ficuscarica*, *Murrayakoenigii* and *Morus alba* with respective values of 84.48%, 84.47%, 82.42%, 79.68% and 79.14%. While the lowest value of RWC (72.88%) was recorded in *Murrayakoenigii*in pre monsoon season. the interaction between species × distance, season × distance and species × season × distance with respect to relative water content was found statistically non significant.

Air pollution tolerance index

The plant growing alongside the national highway (21) varied significantly in their tolerance levels which was found to vary significantly with the seasons of the year and with distances from the national highway (Table 5). Among the selected species the highest APTI of 10.72 was noticed for *Toonaciliata*which was followed by *Melia azedarach*(9.92), *Adhotodavasica*(9.88), *Ficuscarica*(9.71), *Morusalba*(9.47) while the lowest value of 9.29. The variation in the tolerance of the trees of a region to air pollution has also been reported by Lakshmi *et al*.(2008); Agbaire and Esiefarienrhe (2009). The higher value of APTI in *Toonaciliata*may be due to increased production of ascorbic acid and higher relative water content during pollution stress (Mohammed Kuddus *et al*., 2011). The seasons of the year also influenced the APTI values of the plants growing alongside the national highway. The plants were found to exhibit highest APTI values during post monsoon as compared to pre monsoon season with values of 10.02 and 9.64. The APTI for the plant species was found to decrease with the increasing distance from the roadside. The maximum APTI of 10.72 was observed at a distance of 0-15m which was followed by the plants growing at 15-30m whereas minimum (9.26) was noticed at control site. The highest APTI of the plants near the highway may be ascribed to the reason that the trees in order to adapt the stress due to auto exhaust pollution improve their tolerance capacity. The low APTI values of the plants away from 30m distance (control) may be due to less pollution at that site. The results are in line with the findings of Randhi and Reddy (2012).

The species and season interaction showed a significant variation in the air pollution tolerance index. The highest APTI of 10.75 was observed in *Toonaciliata* in the post monsoon season which was statistically at par with the same species during pre monsoon. The lowest APTI (9.19) was found in *Morusalba*in the pre monsoon season. The value of APTI also varied significantly with species × distance interaction. The highest value of 12.05 was observed in *Toonaciliata*growing at 0-15m distance which was followed by the same species growing at 15-10m. The lowest value of 8.86 was observed in *MurrayaKoenigii*growing away from 30m (control site). The interaction between distance and season was found to be statistically significant. During the post monsoon season the APTI values at different distances was higher and the value decreased in the pre monsoon season. The value of APTI decreased with increasing distance in both seasons. The highest APTI of 11.03 was found at the distance of 0-15m during the post monsoon followed by the APTI (10.41) at the same distance in the pre monsoon season. The lowest value of 9.10 was observed at the distance more than 30m (control) during pre monsoon. the interaction among species × season× distance was found statistically significant. The highest value (12.4) of APTI was recorded in *Toonaciliata*in post monsoon season near the national.

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