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Evaluation of Tolerance Level for Air Pollution on Road Side Grown Plant by Biochemical Analysis in Roorkee Region of Uttarakhand

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Abstract

The present investigation was carried out in School of Agricultural Studies, Quantum University, Roorkee. The experimental material used during the present investigation was procured from the Industrial area of Roorkee and controlled area of Roorkee in month of March-April 2022. Roorkee has industrial area and highly loaded by air pollution while the IIT, Roorkee is a covered area and noted as less polluted area. There were 7 plant species used in investigation viz., *Saraca indica*, *Psidium guajava*, *Syzygium cumini*, *Ficus religiosa*, *Citrus limon*, *Azadrachita indica* and *Mangifera indica*. For the present study young leaves of the selected plants were procured on the same day of experiment and used to study leaf area, air borne particulate matter, water content in leaf sample, chlorophyll content in leaf sample, pH of selected leaf samples and ascorbic acid in selected leaves. The results showed that air pollution tolerance index (APTI) of plants is a vital parameter because it assists the assessment of plant's tolerance to air pollution. In present study, high tolerance value of *Ficus religiosa* was observed and this plant can be planted near industrial areas and roadsides of Mandi Gobindgarh to mitigate air pollution. The results also showed that the plants *Ficus religiosa* and *Mangifera indica* are ranked as high tolerant plant species and are best to be planted around areas with poor air quality, since high tolerant species have ability to absorb air pollutants.

***Key words*:** Air pollution, Biochemical analysis, *Saraca indica, Psidium guajava, Syzygium cumini, Ficus religiosa, Citrus limon, Azadrachita indica, Mangifera indica*

In modern times pollution has become the biggest menace for the survival of the biological species. Earth was a beautiful landscape but man has ruthlessly exploited for his greed specially, in last century. With rapid industrialization and random urbanization environmental pollution has become a serious problem [1]. There has been substantive decline in environment quality due to increasing pollution, loss of vegetal cover and biological diversity, excessive concentration of harmful chemicals in the ambient atmosphere and in food chains, growing risks of environmental accidents and threats to life support systems.

According to World Health Organization, around two million people die prematurely from the effects of polluted air every single year. Air pollution is a huge problem and not just for people living in smog-choked cities; through such things as global warming and damage to ozone layer, it has the potential to affect us all. Air pollution results from mainly industries, thermal power plants, automobiles, domestic combustion etc. Vehicular pollution is one of the burning and serious environmental problems in big cities of India. As society develops it lead to more air pollution which is a global problem [2].

A number of cities in India are currently developing action plans to control and improve air quality especially the atmospheric gases and particulates by different ways. Different organizations and researchers are currently investigating the biomonitoring processes by which the trees can improve urban air quality by biofiltering the gases and particulates. To abate the impact of air pollutants, there is a need for perennial green envelope requiring large-scale afforestation and green belt development in and around urban areas. Green belt development envisages the multiplicity of objectives encompassing the micro level air pollutant abatement for enhancement of socio-economic value of the region [3]. The present research work was aimed to identify the plants having high pollution tolerance index, so that such type of plants can be recommended to be grown in the polluted area to improve the quality of air by reducing the air pollutants. Roorkee is one of the industrial areas of Uttarakhand and famous for the industries causing heavy amount of air pollution so the present

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work was conducted to study the effect of pollution on the road side grown plants and to identify the plants having high pollution tolerance index.

MATERIALS AND METHODS

The present investigation was carried out in School of Agricultural Studies, Quantum University, Roorkee. The experimental material used during the present investigation was procured from the Industrial area of Roorkee and controlled area of Roorkee in month of March-April 2022. Roorkee has industrial area and highly loaded by air pollution while the IIT, Roorkee is a covered area and noted as less polluted area. The leaf samples for present investigation of following plants *Syzygium cumini, Mangifera indica, Saraca indica, Psidium guajava, Ficus religiolsa, Azadrachita indica, Citrus limon* were collected from experimental area and controlled area.

For the present study young leaves of the selected plants were procured on the same day of experiment and used to study the following experiments.

1). *To determine leaf area*

Requirements: Fresh leaf samples, pencil, scale, graph paper

Procedure

a) Fresh leaves were taken and marked their margin on graph paper.
b) The number of full boxes, ¼ boxes, 1/8 boxes was counted on the graph paper.
c) The expected leaf area was calculated as:

$$\text{Area} = \text{Length} \times \text{breadth}\ (cm^2)$$

d) The actual leaf area was calculated as:

Actual leaf area = no. of full boxes + no. of half boxes × area 1/2+ no. of 1/4 boxes × 1/4 + no. of 1/8 boxes × 1/8= A cm^2

2). *Determination of air borne particulate matter*

Requirements: Dust full leaf samples, distilled water, hot air oven, weighing machine

Procedure

a) The polluted leaf samples were measured.
b) The leaves were washed using distilled water and dried in hot air oven for 5-10mins.
c) Then again measure and took the weight of leaf samples.
d) The difference in weight indicates the presence of air borne particulate matter on the surface of leaf.
e) The weight of air borne particulates was measured as:
f) Weight of air borne particulates = D-W.
g) Weight of dust full leaf (D), Weight of washed leaf (W)

3). *To determine water content in leaf sample*

Requirements: Fresh leaves, seven beakers, tap water, hot air oven, blotting paper, weighing machine.

Procedure

The fresh weight of leaves was measured as follow:

a) The leaves were washed thoroughly.
b) The leaves were dried on blotting paper.
c) The leaves were punched and measured the weight of leaves.

Turgor weight of the leaves were measured as follow:

d) The paunchered leaves were immersed in water for 24hours and measured to obtain their turgor weight.

Dry weight of leaves was measured as follow:

e) The leaves were finally dried in oven for 24hours at 70 °C and measured the weight of dried leaves to obtain dry weight.

The relative water content of the sample was calculated by:

$$RWC = \frac{(FW) - (DW)}{(TW) - (DW)}$$

Where;

Fresh weight (FW), Dry weight (DW), Turgor weight (TW).

4). *To determine chlorophyll content in leaf sample*

Requirements: Fresh leaves, 80% acetone, filter paper, pestle and mortar, beakers, funnels, and spectrophotometer.

Procedure

a) 1g of fresh leaves was crushed in 80% acetone
b) The crushed leaves were filtered to obtain the clear solution and made the final volume 80ml by 80% acetone.
c) The total chlorophyll content was measured as follow:

$$\text{Chlorophyll a} = (12.7(A663) - 2.69(A645)) \times V/(1000\times W)$$
$$\text{Chlorophyll b} = (22.9(A645) - 4.68(A663)) \times V/(1000\times W)$$
$$\text{Total chlorophyll content} = (20.2(A645) + 8.02(A663)) \times V / (1000\times W)$$

5). *To determine pH of selected leaf samples*

Requirements: pH meter, buffer tablets, distilled water and buffer samples of leaves.

Procedure

a) The 1g of fresh leaves was crushed in distilled water.
b) The crushed leaves were filtered and made the final volume 100ml.
c) The electrode was dipped in the sample.
d) The pH of sample was noted.
e) The same was repeated with other samples.

6). *To determine ascorbic acid in selected leaves*

Requirement: Fresh leaf samples, oxalic acid, ascorbic acid, 2,6-dichlorophenol indophenol, distilled water, beakers, burrette, flask etc.

Procedure

a) 4% oxalic acid was prepared by weighing 20g of oxalic acid and dissolved in 500ml of distilled water.
b) The dye solution was prepared by weighing 42mg of sodium bicarbonate and 52mg of 2, 6- dichlorophenol indophenol and dissolved in 200ml of distilled water.
c) Ascorbic acid stock standard was prepared by weighing 50mg in 50ml of 4% oxalic acid.
d) 5ml of ascorbic acid stock standard was diluted to 50ml with 4% oxalic acid to form ascorbic acid working standard.
e) 5g of fresh leaf samples was accurately weighed and ground in mortar with addition of 5ml of 4% oxalic acid. The mixture was further ground and strained through four layers of muslin cloth. The final volume of the extract was made up to 25ml with 4% oxalic acid in a standard flask. All the samples were similarly treated.
f) Determination of ascorbic acid content in leaf samples:

5ml of ascorbic acid working standard and 10ml of 4% oxalic acid were pipetted into 100ml conical flask. The contents in the flask were titrated against the dye solution (V1) until the appearance of a pale pink color that persisted for a few mins. 5ml of the test sample was similarly titrated against the dye solution (V2). Ascorbic acid content present in test samples were determined using the formula:

$$\text{Amount of ascorbic acid content (mg per gm)} = \frac{500 \times V2 \times 25 \times 100}{V1 \times 5 \times 5}$$

Where;
500 = ug of standard ascorbic acid taken for titration
V1 = Volume of dye consumed by 500ug of standard ascorbic acid
V2 = Volume of dye consumed by 5ml of test sample

Corresponds to total volume of the extract.

7). *Air pollution tolerance index (APTI)*

APTI is calculated by the formula proposed by Singh and Rao (1983).

APTI= A (T+P) + R/ 10

Where;
A= Ascorbic acid content; T = Total chlorophyll of leaf
R= Relative water content; P = pH of the leaf sample

RESULTS AND DISCUSSION

The effect of air pollution on morphological and biochemical characteristics of selected plant species was studied and the results showed under following heads:

Leaf area and air borne particulate matter

Seven different trees namely *Saraca indica, Psidium guajava, Syzygium cumini, Ficus religious, Azadrachita indica, Mangifera indica, Citrus limon* were randomly selected to study variation in leaf area of plants grown in experimental area and controlled areas. It was observed that samples from experimental area showed the reduction in leaf area as compared to the samples of controlled area. More reduction was observed in leaves of *Psidium guajava* 49.34% and less reduction was observed in *Citrus limon* 0.98% (Table 1). Whereas, the experimental area the dispersion of air borne particulate matter increases with reference to controlled area. The maximum increase was observed on leaf samples of *limon citrus* 270.96% and less was observed on leaf samples of *Ficus religiosa* 20.06% (Table 1). The increasing order of dust particles on canopy of leaves of selected plants as follow:

Ficus religiosa< Psidium guajava< Saraca indica< Azadrachita indica< Mangifera indica< Syzygium cumini< Citrus limon.

During the present studies, it was observed that the leaves of the plants collected from experimental area showed reduction in their leaf areas w.r.t leaves of controlled area. The maximum reduction was studied in *Psidium guajava* 66% while minimum reduction was studied in *Saraca indica* 1.5%. Suradkar and Kale [4] also reported that the leaves show variation in leaf area and color due to air pollution. Similarly, Mahajan *et al.* [5] reported that the pollutants deposited on the surface of leaves change their morphology. The reduction in leaf area is due to air pollution. Atmospheric particulates include fine and coarse particles consisting various heavy metals and trace elements. Their presence in a higher level and consequent transformation to biological organism induce a variety of health effects which currently considered as major problems in the highly urbanized regions of the world [6-7]. In particular, fossil fuel used in industry and automobiles as well as for domestic heating can become a significant source of airborne fine particulate matter. It is considered that traffic is one of the main sources of particulate pollution [8].

Table 1 Effect of air pollution on leaf area and air borne particulate matter on selected plant species

Names of plant species	Leaf area (c m^2) of leaf samples of experimental area	Leaf area (c m^2) of leaf samples-controlled area	% Reduction	Experiment area	Controlled area	% age increase
Saraca indica	31.5±15.17	38.63±8.6	19.36	0.290±0.026	0.131±0.02	121.37
Psidium guajava	14.25±1.35	28.13±9.42	49.34	0.208±11.4	0.109±0.035	90.82
Syzygium cumini	27.25±3.52	35.75±5.51	23.77	0.790±0.03	0.244±0.03	223.77
Ficus religiosa	25.12±2.50	25.15±7.34	4.09	0.389±0.03	0.324±0.02	20.06
Mangifera indica	18.75±5.90	23.50±5.38	20.21	0.27±0.25	0.10±0.03	170
Azadrachita indica	4.50±0.60	6.30±0.60	31.7	0.063±0.02	0.026±0.03	142.30
Limon citrus	37.25±6.1	37.63±.536	0.98	0.345±0.04	0.093±0.21	270.96

Relative water content and chlorophyll content

The water content of fresh leaf samples of selected plants was analyzed and percentage reduction of water content in leaves of experimental area was compared with controlled area. The maximum reduction 38.30% in water content was observed in *Psidium guajava* and minimum reduction 3.24% age reduction was observed in *Azadrachita indica* (Table 2). However, the most significant change in total chlorophyll content in selected leaf samples from experimental area and controlled areas was found in *Mangifera indica* i.e., large reduction from 3.29mg/g to 1.078 mg/g respectively. A decrease of about 67.23% of total chlorophyll content was observed in *Mangifera indica*. Least change was observed in *Saraca indica* from 4.59 mg/g to 4.22 mg/g where decrease about 8.06% was observed (Table 2). The reduction percentage of total chlorophyll content of leave samples of the selected plant species is as follow:

Mangifera indica> Azadrachita indica> Ficus religiosa> Syzygium cumini> citrus limon> Psidium guajava> Saraca indica.

Relative water content indicates the capacity of the cell membrane to maintain its permeability under polluted conditions [9]. Due to the air pollution, there is reduction in transpiration rate and damage to the leaf engine that pulls water up from the roots [10]. Consequently, the plants neither bring minerals nor cool the leaf. Reduction in relative water content in plants is due to impact of pollutants on transpiration rate in leaves [11]. During present investigation, it was observed that the relative water content reduces in the experimental area with reference to controlled area and maximum percentage reduction of water content in leaves of experimental area was observed in *Psidium guajava* 38.30% and minimum reduction 3.24% was observed in *Azadrachita indica.* Similarly, many workers reported reduction in water content in polluted area. Chlorophyll content of plants signifies its photosynthetic activity as well as the growth and development of biomass [11]. Suradhkar and Kale [4] studied that chlorophyll concentration of leaf is strongly affected by numerous external factors for example air, electrical current, light, pollutants etc. They had studied chlorophyll content in *Ficus religiosa* and *Hibiscus rosa-senesis.* The chlorophyll content was reduced in both plants in experimental area with controlled area. It indicates that chlorophyll reduced by the polluted air. Nayak *et al.* [12] studied that the decrease in chlorophyll content of leaves maybe due to the alkaline condition created by dissolution of chemical present in dust particles i.e., metals and polycyclic hydrocarbons in cell sap which block the stomatal spores for diffusion of air and thus put stress on plant metabolism resulting in chlorophyll degradation.

Table-2 Effect of air pollution on relative water content and chlorophyll content of fresh leaf sample on selected plant species

Name of plant species	Parameter Fresh wt. of Leaf [FW], Dry wt. of leaf [DW] Turgidity wt. of leaf [TW] $RWC = \frac{FW - DW}{TW - DW} \times 100$			Parameter Chlla= [12.7(A_{663})-2.69(A_{645})] X V/(1000/W) Chllb= [22.9(A_{645})-4.68(A_{663})] X V/(1000/W) Total Chll= [20.2(A_{645}) + (A_{663}) X V/(1000/W)		
	Controlled area	Experimental	Experimental	Experimental	Controlled	% age reduction
Saraca indica	78.54±2.4	4.22±0.4	4.22±0.4	4.22±0.4	4.64±0.75	9.05
Psidium guajava	51.07±1.9	2.48±0.5	2.48±0.5	2.48±0.5	2.78±0.46	10.79
Syzygium cumini	53.28±.1.9	4.178±0.08	4.178±0.08	4.178±0.08	4.9±0.32	14.73
Ficus religiosa	62.8±1.8	4.17±0.32	4.17±0.32	4.17±0.32	5.56±0.52	25.00
Mangifera indica	69.5±1.9	1.078±0.026	1.078±0.026	1.078±0.026	3.29±0.36	67.23
Azadrachita indica	51.41±3.06	3.09±0.42	3.09±0.42	3.09±0.42	4.98±0.18	37.95
Citrus limon	51.07±2.3	4.18±0.63	4.18±0.63	4.18±0.63	4.84±0.17	13.63

Table 3 Effect of air pollution on pH value and ascorbic acid of fresh leaf samples on selected plant species

Name of plant species	Parameter			
	Controlled area	Experimental area	Non-polluted area	Polluted area
Saraca indica	6.54±0.24	5.80±0.09	3.38±0.28	4.30±0.21
Psidium guajava	6.37±1.3	6.27±0.19	0.12±0.04	0.14±0.04
Syzygium cumini	5.60±0.33	4.70±0.11	6.75±3.2	7.34±0.03
Ficus religiosa	8.12±0.08	6.65±0.12	18.9±0.14	20.85±0.11
Mangifera indica	6.66±0.11	5.71±0.13	21.08±0.08	21.95±0.10
Azadrachita indica	7.75±0.11	6.07±0.15	12.51±0.35	15.67±0.21
Citrus limon	6.11±0.07	6.28±0.19	14.47±0.26	14.65±0.24

Table 4 Effect of air pollution on air pollution tolerance index (APTI) on selected plant species

Name of plants	Total chlorophyll		Leaf extract pH		Ascorbic acid		Relative water content		APTI	
	CA	EA	CA	EA	CA	EA	CA	EA	CA	EA
Saraca indica	4.64	4.22	6.54	5.80	3.38	4.30	78.54	67.01	11.64	11
Psidium guajava	2.78	2.48	6.37	6.27	0.12	0.14	51.07	31.51	5.2	3.3
Syzgium. cumini	4.9	4.18	5.60	4.70	6.75	7.34	53.28	51.04	12.41	11.62
Ficus religiosa	5.56	4.17	8.12	6.65	18.9	20.85	62.8	51.01	32.14	27.66
Mangifera india	3.29	1.08	6.66	5.71	21.08	21.95	69.5	49.66	27.93	19.87
Azadrachita indica	4.98	3.09	7.75	6.07	12.51	15.67	51.41	49.74	23.06	19.32
Limon citrus	4.84	4.18	6.11	6.28	14.47	14.65	51.07	52.15	20.95	20.54

Leaf extract, ascorbic acid content and air pollution tolerance index (APTI)

In present investigation, most plants showed alkaline pH. The maximum acidic value 4.70 pH was observed in *Syzygium cumini* whereas less acidic value or alkaline value 6.65 pH was observed in *Ficus religiosa* (Table 3) and ascorbic acid content in leaf samples of selected plant species were studied by using titration method. During present research, maximum increase of ascorbic acid content was observed in *Mangifera indica* 21.95mg/g and minimum increase of ascorbic acid content was observed in *Psidium guajava* 1.3 mg/g of experimental area with reference to controlled area (Table 3). However, maximum APTI was observed in *Ficus religiosa* 27.66 and 32.14 and minimum value was observed in *Psidium guajava* 3.3 and 5.2 in experimental area and controlled area respectively (Table 4).

pH of the leaf extract signifies the tolerant capacity of the leaf species. Plants with lower pH are more susceptible, while those with pH around 7 are more tolerant. Subramani and Devaanandan [9] and Mahajan *et al.* [5] studied that pH of more polluted site are more acidic than non- polluted site. This is due to the toxic gases, dust, smoke, SO_2, NO_2 and SPM which change the pH of cell sap deposited on leaf surface. Lohe *et al.* [11] studied that the change in leaf extract pH might influence the stomatal sensitivity due to air pollution. Some species exhibited a pH towards acidic side, which may be due to presence of SO_2 and NO_2 in the ambient air causing a change in pH of the leaf sap towards acidic site. Karda *et al.* [13] studied that ascorbic acid is water soluble and abundant component in plants. It is associated with chloroplasts and apparently plays a role in ameliorating the oxidative stress of photosynthesis.

Ascorbic acid also influences the resistance of plants to adverse environmental conditions including air pollution [14]. Tiwari and Tiwari [15] studied that the ascorbic acid being a strong reductant protects chloroplasts against SO_2 induced H_2O_2, O_2 and OH^- accumulation and thus protects the enzymes of the CO_2 fixation cycle and chlorophyll for inactivation together with leaf pH.

According to Singh and Rao [16] APTI is an index denotes capability of a plant to combat against air pollution. Plants which have high index value are tolerant to pollution and can be used to indicate levels of air pollution. Different plant species show considerable variation in their susceptibility to air pollution. The plants with high and low APTI can serve as tolerant and sensitive species respectively. In present study, *Ficus religiosa* and *Mangifera indica* with highest air pollution tolerance index was found to show more tolerant response to air pollution whereas *Azadrachita indica, Lemon citrus, Syzygium cumini* can be considered to show intermediate response and lastly *Saraca indica* and *Psidium guajava* can be considered show sensitive response.

CONCLUSION

The present study concluded that the air pollution tolerance index (APTI) of plants is a vital parameter because it assists the assessment of plant's tolerance to air pollution. In present study, high tolerance value of *Ficus religiosa* was observed and this plant can be planted near industrial areas and roadsides of Roorkee to mitigate air pollution. The plants *Ficus religiosa* and *Mangifera indica* are ranked as high tolerant plant species and are best to be planted around areas with poor air quality, since high tolerant species have ability to absorb air pollutants. The *Azadrachita indica*, Lemon citrus and *Syzygium cumini* are considered as intermediate tolerant plants. They can be planted near residential areas. While *Saraca indica* and *Psidium guajava* are grouped in sensitive plants and best to be planted near roadside so that they to be used as bio-indicators to air quality around roads of Roorkee.

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