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Assessment of Plants Sensitivity to Air Pollution using Physiological and Biochemical Parameters

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Abstract

Plants act as a main green belt enhancement component. Green plants create a surface that may absorb air pollutants and act as a sink for them, making greenbelts an efficient method of controlling air pollution. Thus attention is needed to promote and develop green belt zones in polluted areas. The current study is focused on evaluating the air pollution tolerance levels of *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa* already grown in polluted and unpolluted sites. By taking into account biochemical markers such as total chlorophyll level, ascorbic acid level, pH, water content, the Air pollution tolerance index (APTI) was calculated. APTI is an empirical relationship that assesses the degree to which different plant species can tolerate air pollution. Plants that possess APTI value less than or equal to 11 are known as sensitive, between 12 and 16 are moderately tolerant and greater than 17 are tolerant. The results obtained from the study showed that M. indica is more tolerant in nature. The rest of the plants used in the study are found to be intermittently tolerant. From this study, it can be concluded that planting *Mangifera indica, Psidium guajava*, *Annona squamosa*, *Ficus religosa* in urban areas can reduce air pollution. This study also concludes that use of the APTI evaluation for the identification of air pollution tolerant plants is a suitable method.

Introduction

Clean air is essential for the relationship between the environment and public health, but as pollution from many sources is released into the environment, the air quality is deteriorating, which has negative effects on both the climate and human health.[1-3] The main causes of air pollution emissions and poor air quality include growing urbanization,

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rising industrialization, and related anthropogenic activities. Fine dust particles are a major problem today due to their undesirable effects on plants and animals. By capturing particulates and absorbing gaseous pollutants, green belts are a natural way to reduce air pollution.[4]

Air pollutants and metals like mercury and lead can be filtered by plants. Conversely, certain air pollutants have the ability to negatively impact plant development and growth through their effects on the biochemical composition, photosynthetic rate, seed stomata, pedicle length, number of flowers on florescence, and germination. These alterations in biochemical traits are utilized to evaluate the plant's APTI.[5, 6]

More tolerant plant species serve as sinks for airborne pollutants and can be employed to create green belts, whilst more sensitive plant species serve as bioindicators.[7] The development of green belts surrounding urban and industrial areas will be aided by the addition of plants having capacity to withstand air pollution. The chlorophyll level, ascorbic acid, pH, and water content are helpful in calculating the tolerance level. This index has been used in studies on the construction of green belts, traffic noise reduction, and pollution mitigation near roadsides and next to businesses. It has been discovered that plants grown in conditions that appear to be contaminated have higher APTI than plants living in circumstances that appear to be less polluted. This is because various plants react to air pollution in different ways. The objective is to find out the air pollutant tolerance capacity using *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa* already grown in polluted and unpolluted site.

Materials and Methods
Leaf Sample Collection
Fresh leaves from the plants *Mangifera indica, Ficus religiosa*, *Psidium guajava*, and *Annona squamosa* were collected in sterile zip lock covers from polluted sites near Adyar and the control site(College Campus-Unpolluted site). How many trees?? how many leaves per tree?

Extract Preparation
In accordance with standard protocols, fresh leaves were used. The entire experiment was carried out with aqueous extract.

Biochemical Analysis
Evaluation of pH of leaves
1g of leaf sample was homogenized followed by centrifugation. Then supernatant was collected and using a pH meter, the pH was determined.[8]

Evaluation of Relative Water Content
Leaves weighing 5g were collected, washed, and weighed. After soaking the leaf samples in water for 24 hours, they were wiped dry and weighed to measure the turgid weight. After drying overnight at 70°C in a hot air oven, the leaves were reweighed. Singh's method for determining and computing RWC was employed.

$$RWC = ((FW-DW)/(TWDW)) \times 100$$

FW stands for fresh weight, DW for dry, and TW for turgid weight.

Estimation of Ascorbic Acid
Stock Ascorbic Acid Solution
100mg of ascorbic acid is weighed & dissolved in 100ml of distilled water in a standard flask.

Working Standard Ascorbic Acid
10ml of stock was pipetted out in to a measuring flask and made up to 100ml with distilled water in a standard flask.

Sample Preparation
5g of leaves crushed in mortar and pestle. Mix the sample with 50ml of 5% metaphosphoric acetic acid solution, transfer it into a beaker and add 50ml of phosphoric acid solution. Filter using whatmann filter paper

To the filtrate add few drops of bromine water. Then add few drops of thiourea solution to remove excess bromine water

85% H_2SO_4

DNPH
3g of 2,4 DNPH in 15ml of concentrated H2SO, then add 90ml of 74% ethanol.

Procedure
0.5, 1.0, 1.5, 2.0, 2.5 ml of working standard ascorbic acid was pipetted out into tubes labelled as standard from S1-S5. 0.1 ml of extract was pipetted out into

tubes labelled as T. With 5% TCA, the volume in each tube reached up to 3 ml. 3 mL of 5% TCA acts as a blank. To each tube, 1.0 ml of DTC reagent was added and thoroughly mixed. The tubes were kept in a water bath for 1 hour at 60° C. The tubes were cooled followed by the addition of 5 ml of 9 N sulphuric acid. The tubes were maintained at room temperature for 20 minutes. The intensity of the colour developed was recorded at 520nm. The amount of ascorbic acid was estimated from the standard graph.[9]

Estimation of Chlorophyll

Arnon's approach[10] was used to calculate the chlorophyll content. 1g of leaves were extracted in 20ml of acetone for 15 minutes. The liquid portion was transferred followed by centrifugation at 2,500rpm. In a normal flask, the contents were made up to 50 ml with 80% acetone. The optical density of chlorophyll a, b was recorded at 645nm and 663nm using a visible spectrophotometer, and the following formula was used to perform the calculations

Chlorophyll -a mg/g tissue =

$$\frac{12.7\,(Abs663) - 2.69\,(Abs645)\ x\ V}{1000} \ X\ W$$

Chlorophyll -b mg/g tissue =

$$\frac{22.9\,(Abs\ 645) - 4.68\,(Abs\ 663)\ x\ V}{1000} \ X\ W$$

Total chlorophyll: Chlorophyll a + Chlorophyll b

The air pollution tolerance indices for the selected plants were calculated using the following formula

APTI= (A (T+P) + R)/ 10 is the formula.

Ascorbic Acid, T=Total chlorophyll, P=pH of the leaf extract, R=Relative water content of leaf percent).

Estimation of Total Soluble Sugar

Anthrone procedure[11] was used to calculate the total soluble sugar content of leaf tissue. 20 ml of distilled water along with 1 g of leaf tissue was homogenised. The tubes were then hydrolyzed in a boiling conditon for three hours with 5 cc of 2.5 N hydrochloric acid. The tubes were allowed to cool to ambient temperature. After that, the sample was neutralized with sodium carbonate solids. The sample was diluted to 100 ml and centrifugation was performed. The supernatant was collected, and aliquots of one milliliter were utilized for estimate.

Working standards were prepared in separate tubes with various concentrations of glucose, and the volume of the standards and sample was reduced to 1ml using distilled water. The tubes were then filled with anthrone reagent (4 ml) and kept in a warm condition for 8 minutes followed by cooling at normal temperature. The reading was recorded at 630 nm using spectrophotometer. Total soluble sugar concentration in the sample was calculated using the standard graph.

Results and Discussion

Chlorophyll content in leaves of *Mangifera indica, Ficus religiosa*, *Psidium guajava*, *Annona squamosa* collected from unpolluted and polluted sites is shown in table 1.

Table 1: Chlorophyll content in leaves of Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa   obtained from unpolluted and polluted sites.

S. No	Botanical name	Chloro -phyll a in Unpo -lluted leaves	Chloro -phyll b in Unpo -lluted leaves	Chlorophyll Total (mg/g) in Unpo -lluted leaves	Chloro -phyll a leaves -lluted leaves	Chloro -phyll b leaves -lluted leaves	Chlorophyll Total (mg/g) in Polluted leaves
1	*Mangifera indica*	2.170	0.630	2.800	0.117	0.310	0.427
2	*Ficus religiosa*	0.920	0.254	1.174	0.582	0.184	0.766
3	*Psidium guajava*	1.398	0.533	1.931	0.316	0.132	0.448
4	*Annona squamosa*	0.928	0.261	1.189	0.069	0.057	0.126

From results obtained, it is well evident that chlorophyll level varied from 1.18 to 2.8 in unpolluted leaf samples and 0.12 to 0.42 in areas exposed to pollution. It is to be observed that if the pollutant level is high chlorophyll content will get decreased From the study carried out, *Mangifera indica,* showed the highest total chlorophyll level of 2.82 mg/g, *Psidium guajava* 1.9. mg/g while *Annona squamosa* and Ficus religosa showed the lowest chlorophyll content of 1.18 and 1.17 mg/g in leaves collected from unpolluted sample. The total chlorophyll content in polluted sample was found to be highest in Ficus religosa (0.76mg/g) followed by *Psidium guajava* (0.44 mg/g) and Mangifera indica (0.42 mg/g), very lowest in *Annona squamosa* (0.261 mg/g)

respectivelyThe levels of chlorophyll was decreased in samples obtained from polluted areas than in controlled sites. The average chlorophyll content of leaves has been frequently used to assess how air pollution affect the photosynthesis.[12] It is believed that chlorophyll serves as a measure of biomass output, photosynthetic activity, and growth. According to Kameswaran *et al.* (2019), Plants with a high chlorophyll content are often resistant to contaminants even after being exposed to traffic pollution.[13] This shows that the plant's tolerance to SO2 from vehicular exhaust may be causing lower chlorophyll production or deterioration in the already-present chlorophyll concentration.

Table 2: pH of the leaf extracts of *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa*

S.No	BOTANICAL NAME	pH (Un polluted leaves)	pH (Polluted leaves)
1	*Mangifera indica* (Mango)	6.2	5.9
2	*Ficus religiosa* (Peepal)	5.8	5.6
3	*Psidium guajava* (Guava)	5.5	5.3
4	*Annona squamosa* (Custard apple)	6.6	6.1

The pH of the plant species collected from unpolluted and polluted site is given in the table 2. The pH of the leaves collected from unpolluted site ranged from 6.6- 5.5 and in polluted samples it was found to between 6.1 to 5.3. The highest pH was observed in *A.squamosa* (6.6) followed by *M.indica* (6.2),

P.guajava (5.5), in unpolluted leaf samples. There was a moderate decrease in the pH of samples from polluted sites. The pH of A.squamosa from areas exposed to air pollutants was found to be 6.1 followed by followed by *M.indica* (5.9) , *F. religiosa* (5.6) and *P.guajava* (5.3),
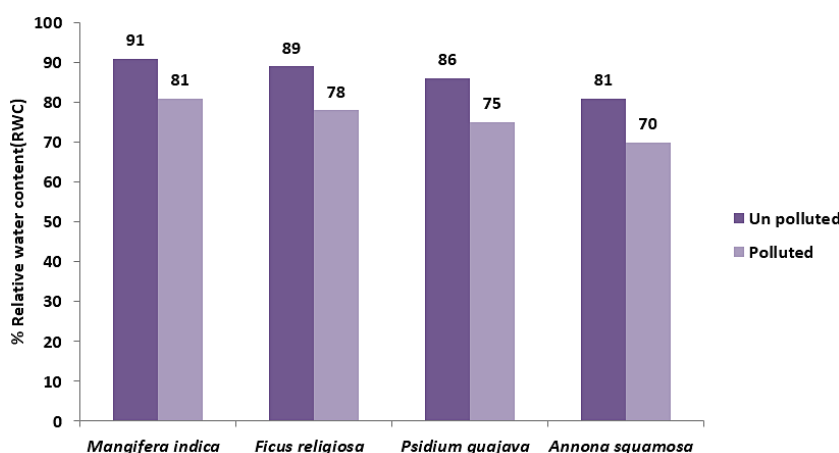

Fig. 1: RWC (%) of plant species

According to Escobedo (2008), increased pH favors the production of ascorbic acid from hexose, whereas decreasing pH is assumed to be related to susceptibility to air pollution[14] The pH of leaves decreases when they are exposed to acidic pollutants like SOX and NOX, and this pH decrease is more prominent in sensitive plants compared to tolerant ones,[15-16]

The RWC of the of *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa* collected from unpolluted and polluted sites is shown in Figure 1

M. indica showed the greatest RWC (91%) followed by *F. religiosa* by 89% and *P. gujava* by 86%, *A. squamosa* by 81% in leaves collected from unpolluted site. In this study it was surprising to note that there was nearly 10% decrease in water content of leaves collected from polluted site.

Under stressful circumstances, plants can maintain their physiological equilibrium thanks to the high relative water content (RWC) in their leaves. Hence, a high RWC number indicates that plants are highly resistant to stress.[17] Air pollution has been linked to reduced transpiration rates and damage to leaves that can pull water up from the roots, which prevents plants from bringing in minerals. The rate of transpiration in leaves is affected by the air pollutants with a decrease in relative water content that has been found in some plant species
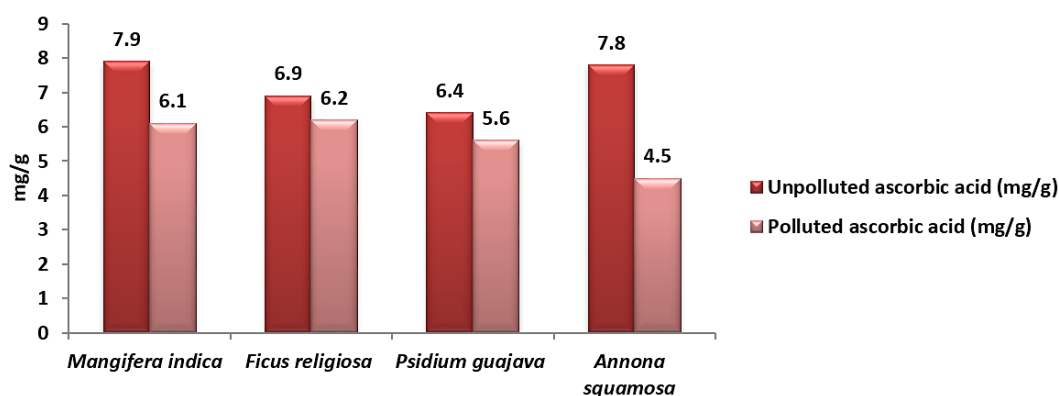
Fig. 2: Ascorbic acid content in *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa* collected from unpolluted and polluted sites.

Ascorbic acid of the leaves collected from unpolluted and polluted sites is shown in figure 2. From this study it was observed that there was slight change in the ascorbic levels of polluted and unpolluted leaves. The ascorbic acid level was found to be 7.9 mg/g in *M indica* and 7.8 mg/g in *A.squamosa* and the level was found to be 6.9mg/g in *F.religiosa* in unpolluted samples. A slight decrease was noted in polluted samples

Apart from synthesizing cell walls, ascorbic acid is essential for cell division, carbon fixation, and functions as a strong antioxidant to protect plants from the damaging effects of free radicals. Because of this, trees are better able to withstand air pollution. Studies reveal that ascorbic acid can lessen the quantity of ozone that passes through the cell wall and enters the plasma membrane.[18-19] Plant tolerance limits increase as ascorbic acid level increases. As a result, ascorbic acid is required for plants to endure the damage caused by air pollution to their tissues.[20-21] According to one study, when there is pollution, ascorbic acid levels in plants fall.[22] However ascorbic acid levels in plants increases if plants withstand pollution.[23-24]

Soluble sugar content in leaves of *Mangifera indica, Ficus religiosa, Psidium guajava, Annona squamosa* from unpolluted and polluted sites is depicted in figure 3. The sugar levels were found to be elevated in all the unpolluted leaf samples when compared to the polluted ones. The amount of Sugar is more in *M.indica* (24 mg/ g) and least in *F.religiosa* (21 mg/g) in Unpolluted leaves and in polluted leaves M.indica (20 mg/ g) and least in *A.squamosa* sp (17 mg/g).
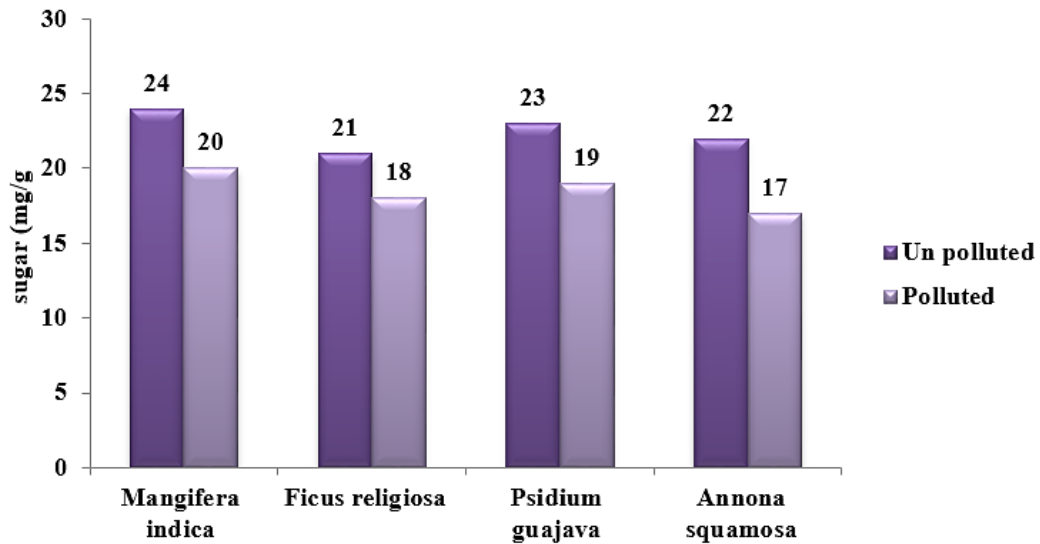
Fig. 3: Soluble sugar content in plant leaves collected from Polluted and unpolluted site

Starch is the end result of photosynthesis and is composed of glucose. In higher plants, sugar serves as both the primary result of photosynthetic energy production and the primary metabolic pathway for carbohydrates. It is believed that the amount of soluble sugar in a plant indicates its physiological activity and its susceptibility to air pollution. Polluted leaves may have less sugar because of decreased photosynthesis brought on by lower pH, less chlorophyll, and less CO_2 fixation. APTI showed variations among the various plants obtained from polluted and unpolluted areas. The APTI of plant species were represented in fig 4.
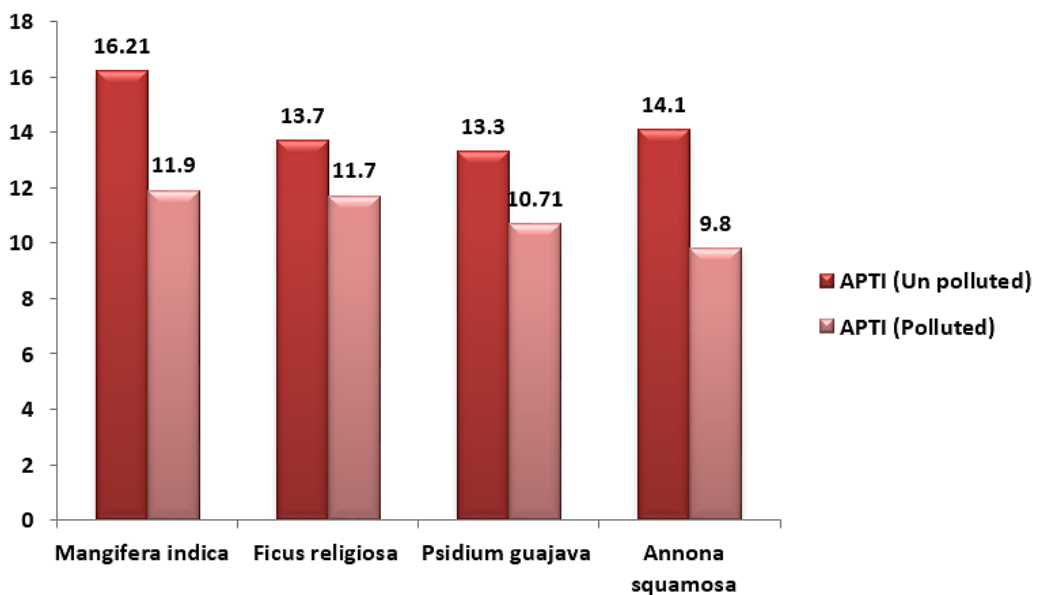

Fig. 4 : APTI of plant species

The APTI values were in the order M.indica (16.21) > A.squamosa (14.1) > F.religiosa (13.7) > P.guajava (13.3) in unpolluted leaves and in polluted leaves APTI values were 9.8 for A.squamosa, 10.7 for Psidium gujava, 11.7 and 11.9 for *F. religiosa* and *M.indica* respectively.

The APTI values provide a reliable way to assess the tolerance and vulnerability of any location's flora to automobile pollution. Plants with APTI values between 12 and 16 are categorised as intermediate, while those with APTI values between 11 and 17 are known to be tolerant.

Conclusion
Plants serve as living filters to reduce air pollution and are a major component in enhancing the green belt. From this study it can be concluded that planting plants like *Mangifera indica, Psidium guajava, Annona squamosa, Ficus religosa* in urban areas can reduce air pollution. Less than 11 for the APTI

value indicates sensitivity; values between 12 and 16 indicate moderate tolerance, values over 17 indicate high tolerance. It is shown that M. indica is extremely tolerant. Also, the other plants used in the study are found to be intermittently tolerant. Thus, attention is needed to promote and develop green belt zones in polluted areas using these type of plants.

Conflict of Interest
There is no conflict of interest

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