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Evaluation of Physiological and Biochemical Responses of Air Pollution in Selected Plant Species around Industrial Premises of Malappuram District, Kerala

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Air pollution impact on various physiological and biochemical parameters of fifteen plant species around industrial premises of Malappuram district, Kerala were investigated. The present study has been carried out with the aim of analyzing the air pollution tolerance mechanisms in the selected fifteen plant species collected from the nearby areas of these two textile and PVC industries and to screen the most tolerant plant species based on the air pollution tolerance index of selected plants and can provide a cost effective and eco-friendly technique to mitigate air pollution. The air pollution caused a lower value of leaf extract pH, total chlorophyll and carotenoid contents in selected plant species. The increased level of ROS in plants due to exposure to air pollution damages the biomolecules such as lipids and results in MDA formation. Moreover, antioxidants like proline, ascorbate, soluble sugar and phenolics work hand in hand to scavenge toxic ROS produced under air pollution in all plants and thus prevent the oxidation of vital components in the plant cells. However, the accumulation of antioxidants in selected plant species was varied in response to air pollution. The air pollution tolerance index (APTI) has been used for identifying tolerance levels of plant species and to rank plant species in their order of tolerance to air pollution. APTI of *T. grandis*, *H. brasiliensis*, *A. occidentale* and *P. pinnata* and the present study indicated that these four plant

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species are most suitable sink for air pollution, which can be utilized for green belt development in industrial area for reduction of the level of the air pollution. Moreover, lowest APTI was recorded in *G. floribunda*, *P. emblica* and *M. oleifera*, can be used for the biomonitoring of air pollution.

Keywords: Air pollution tolerance index; antioxidants; pollution.

1. INTRODUCTION

Air pollution is regarded as a major threat and causes serious environmental damages to all living beings on the earth. "Ambient air pollution in several large cities of India is among some of the highest in the world and more than three-quarters of the people in India are exposed to pollution levels higher than the limits recommended by the National Ambient Air Quality Standards in India and the World Health Organization" [1]. Air quality index is one of the important tools available for characterizing the quality of the ambient air at a particular location and it can be used as a measure to assess the pollutants concentrations in atmospheric air. The air quality index of Malappuram, Kerala is 95 and it follows moderate air pollution level. As the air quality index increases, it would be resulted in an increasingly large percentage of the population is experience increasingly severe adverse health effects. "A wide range of pollutants are emitted by the human activity on factories and industries, like carbon monoxide, nitrogen oxides, ammonia, particulate matter, lead, hydrocarbons, organic compounds and other chemicals" [2].

"Some of the damage caused by air pollution to plants results from the induction of oxidative processes that reduce peroxidic bonds and that consequently catalyse the production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$). These are very lethal and cause extensive damage to protein, DNA and lipids and thereby affect normal cellular functioning" [3]. There is no mechanical or chemical device, which can completely check the emission of pollutants at the source. Once the pollutants are released to the atmosphere, only the plants are the hope, which can mop up the pollutants by adsorbing and metabolizing them from the atmosphere. "Therefore, the plants, role in the air pollution abatement have been increasingly recognized in recent years. Plants act as a sink or even as living filters to minimize air pollutant by developing characteristic response and symptoms, hence to be examined for their biomonitoring potential" [4]. "Based on responses of plants towards a particular stress as well as gaseous stress, they can be categorized into 'sensitive' and 'tolerant'. Sensitive species are early indicators of pollution, and the tolerant species help in reducing the overall pollution load" [5, 6]. Moreover, the present investigation is based on identification of stress tolerant plant species towards air pollution from the study area. In present study emphasis is given on evaluation of tolerance level in fifteen selected plant species against industrial air pollution and calculation of air pollution tolerance index.

2. MATERIALS AND METHODS

Air pollution impact on various physiological and biochemical parameters of fifteen plant species were investigated. Preliminary screening was carried out inorder to find out the most common plant species growing vigorously around selected industrial premises of Malappuram district, Kerala.

2.1 Plant Material

Fifteen plant species namely *Hevea brasiliensis* (Willd.) Müll. Arg., *Anacardium occidentale* L., *Tectona grandis* L. 1f., *Artocarpus heterophyllus* Lam., *Terminalia catappa* L., *Phyllanthus emblica* L., *Getonia floribunda*, *Swietenia mahagoni* (L.) Jacq., *Pongamia pinnata* (L.) Pierre, *Cocos nucifera* L., *Piper nigrum* L., *Mangifera indica* L., *Gliricidia sepium* (Jacq.) Walp., *Moringa oleifera* Lam. *and Macaranga peltata* (Roxb.) Mull.Arg. were selected for the present study. Fresh leaf samples were collected from populated areas in selected industrial sites of Malappuram district, Kerala.

2.2 Experimental Design

Ten samples each from healthy and mature leaves of each selected plants were plucked by random selection in early morning hours (7.00-9.00 am) and brought in polythene bags to the laboratory. All the samples were analyzed freshly for various physiological and biochemical parameters.

2.3 Physiological Studies

Relative leaf water content of leaves was determined following the method described by Pathak et al. (2011). The total chlorophyll and carotenoid content in leaves were estimated following the method of Arnon [7].

2.4 Biochemical Parameters

Five grams fresh leaves of each sample were homogenized in 30ml de-ionised water using mortar and pestle. This was filtered through Whatmann No.1 filter paper and pH of the leaf extract was determined using the digital pH meter. The malondialdehyde content (MDA) estimation was done according to the method of Heath and Packer [8] and the MDA content was calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$. Proline content of experimental leaves of all fifteen plants was estimated after 60 days of treatments according to the method of Bates et al. [9] using L-proline as the standard. The total soluble sugar was estimated using the method proposed by Dubois et al. [10]. D-glucose was used as the standard. Moreover, total phenolic was estimated using Folin-Denis reagent according to the method of Folin and Denis [11] and total phenolic content in the plant tissue was calculated using tannic acid as standard. For the estimation of ascorbate content, the method of Chen and Wang [12] was adopted and ascorbate content was calculated from a standard curve prepared using different concentrations of ascorbate.

2.5 Air Pollution Tolerance Index (APTI)

Air pollution tolerance index (APTI) was determined following the method of Singh and Rao [13].

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

Where,

A = Ascorbic acid content (mg g^{-1} DW)
T = Total chlorophyll (mg g^{-1} DW)
P = $_p$H of leaf extract
R = Relative water content of leaf (%).

2.6 Statistical Analysis

The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9).

3. RESULTS

3.1 Physiological Studies

3.1.1 Relative leaf water content (RWC)

Variation in RWC of selected plant species at industrial site was depicted in Fig. 1. The RWC of plant species varied from 24.32% to 63.45%. RWC of *T. catappa* was found to be highest (63.45%), whereas, lower was of *M. Peltata* (24.32%). The trend of RWC was found as follows; *T. catappa* > *A. heterophyllus* > *P. nigrum* > *S. mahagoni* > *C. nucifera* > *H. brasiliensis* > *G. sepium* > *M. indica* > *M. oleifera* > *A. occidentale* > *T. grandis* > *P. emblica* > *G. floribunda* > *P. Pinnata* > *M. Peltata* (Fig. 1).

3.1.2 Photosynthetic pigments

In the case of total chlorophyll content, significant variation was observed in selected plants, i.e., it was ranged from 12.77 to 84.87 mg/g DW of leaf tissue. Maximum and minimum chlorophyll content was observed in *P. pinnata* and *G. floribunda*, respectively. Likewise, total carotenoid content varied significantly from 2.50 to 21.45 mg/g DW. Results revealed that maximum carotenoid content was found in *T. catappa* (21.45 mg/g DW) followed by *S. mahagoni* (18.54 mg/g DW) and *A. heterophyllus* (17.53 mg/g DW), whereas, the minimum carotenoid content was observed in *G. sepium* (2.5 mg/g DW) (Fig. 1).

3.2 Biochemical Studies

3.2.1 Leaf extract pH

The leaf extract pH of all plants was calculated and it was ranged from 3.2 to 6.4. Maximum leaf extract pH was observed in *G. Sepium* followed by *H. brasiliensis* (5.8), *A. heterophyllus* (5.7), *C. nucifera* (5.5) and least in *P. emblica* (3.2) (Fig 2).

3.2.2 Rate of lipid peroxidation

Rate of lipid peroxidation or the MDA content of all fifteen plants was calculated and in general it was ranged from 3.54 (µmol/g DW) to 16.4 (µmol/g DW). The rate of lipid peroxidation was maximum in *M. oleifera* (16.4 µmol/g DW) followed by *P. pinnata* (15.54 µmol/g DW) and minimum in *G. floribunda* and *C. nucifera* (3.54 µmol/g DW in both plants) (Fig. 2).

3.2.3 Proline content

The concentrations of total proline content markedly varied in all selected plants from 0.88 mg/g DW to 11.32 mg/g DW. Maximum proline content was recorded in *M. oleifera* (11.32 mg/g DW) and *M. peltata* (10.3 mg/g DW). The accumulation of proline content was least in *T. grandis* (0.95 mg/g DW) and *G. floribunda* (0.88 mg/g DW) (Fig. 2).

3.2.4 Ascorbate (AsA) content

The average ascorbate content was found to be ranged from 2.09 mg/g DW to 20.76 mg/g DW. The maximum ascorbate content was recorded in *T. grandis* (20.76 mg/g DW) followed by *A. occidentale* (17.87 mg/g DW) and *H. brasiliensis* (15.55 mg/g DW). However, the ascorbate content was least in *M. oleifera*, *M. indica*, *C. nucifera*, *P. emblica*, *G. floribunda*, *T. catappa*, *A. heterophyllus* (<5 mg/g DW) (Fig. 2).

3.2.5 Soluble sugar content

Total soluble sugar content in all plants was recorded and it was found to be ranged from 12.054 mg/g DW to 49.75 mg/g DW. The maximum soluble sugar content was recorded in *P. nigrum* (49.75 mg/g DW) followed by *C. nucifera* (48.65 mg/g DW) and *H. brasiliensis* (43.4 mg/g DW). The minimum soluble sugar content was observed in *P. pinnata* (12.054 mg/g DW) (Fig. 2).

3.2.6 Total phenolics content

Total phenolics content of all fifteen plants was calculated and in general it was ranged from 2.30 (mg/g DW) to 23.54 (mg/g DW). The results revealed that maximum phenolics content was found in *C. nucifera* (23.54 mg/g DW), whereas, the minimum phenolics content was observed in *A. Heterophyllus*, *M. oleifera*, *G. sepium*, *P. emblica* and *T. catappa* (<5 mg/g DW) (Fig. 2).

3.2.7 Air pollution tolerance index (APTI)

The present study revealed the variability among various plant species with respect to vulnerability to air pollution. APTI of selected plant species ranged from 7.80 to 100.52. Highest APTI was exhibited by *T. grandis* (100.52), *H. brasiliensis* (99.80), *A. occidentale* (92.47) and *P. pinnata* (90.92), whereas, lowest was found in *G. floribunda* (7.80), *P. emblica* (7.83) and *M. oleifera* (8.07). The APTI value of selected plants in the industrial site was found to be in the order of *T. grandis* > *H. brasiliensis* > *A. occidentale* > *P. pinnata* > *S. mahagoni* > *G. sepium* > *M. Peltata* > *P. nigrum* > *T. catappa* > *C. nucifera* > *M. indica* > *A. heterophyllus* > *M. oleifera* > *P. emblica G. floribunda* (Table 1).

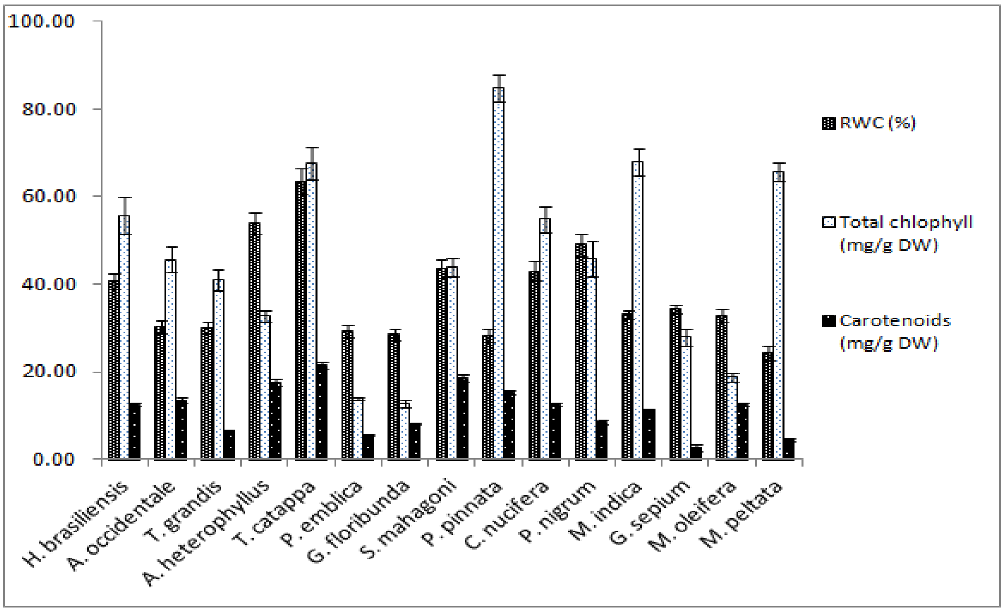

Fig. 1. RWC (%), total chlorophyll (mg/g DW) and carotenoid content (mg/g DW) in selected fifteen plants. The data is an average of recordings from three independent experiments each with three average of recordings from three independent experiments each with three replicates (*i.e.* n=9)

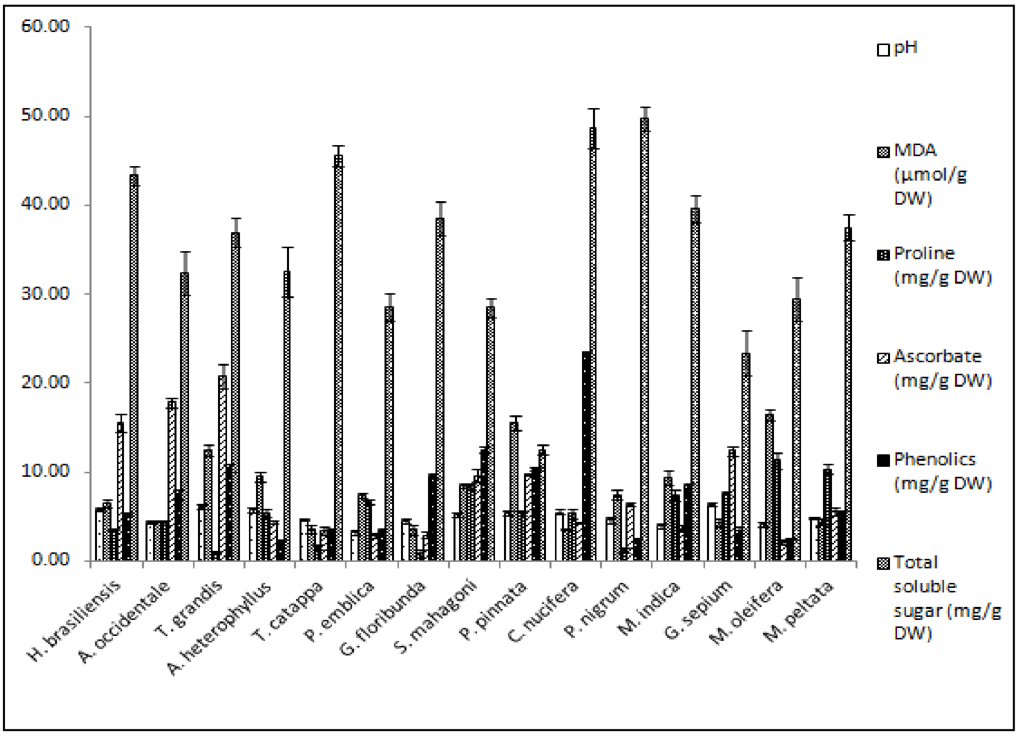

Fig. 2. pH, MDA (µmol/g DW), proline (mg/g DW), total ascorbate (mg/g DW), total phenolics (mg/g DW) and total soluble sugar content (mg/g DW) in selected fifteen plant species. The data is an average of recordings from three independent experiments each with three average of recordings from three independent experiments each with three replicates (*i.e.* n=9)

Table 1. Air pollution tolerance index (APTI) of selected fifteen plant species. The data is an average of recordings from three independent experiments each with three average of recordings from three independent experiments each with three replicates (*i.e.* n=9).

	Plant species	APTI
1	*Tectona grandis*	100.52±4.24
2	*Hevea brasiliensis*	99.80±3.62
3	*Anacardium occidentale*	92.47±5.76
4	*Pongamia pinnata*	90.92±4.88
5	*Swietenia mahagoni*	51.82±2.93
6	*Gliricidia sepium*	46.03±3.11
7	*Macaranga peltata*	41.40±2.98
8	*Piper nigrum*	37.20±1.85
9	*Terminalia catappa*	31.15±1.45
10	*Cocos nucifera*	30.51±2.13
11	*Mangifera indica*	29.64±2.17
12	*Artocarpus heterophyllus*	22.45±1.34
13	*Moringa oleifera*	8.07±1.05
14	*Phyllanthus emblica*	7.83±2.59
15	*Getonia floribunda*	7.80±3.54

4. DISCUSSION

Plants as successful candidates for biomonitors of air pollution are of incredible environmental significance since they are continuously exposed to the pollution due to their static nature. Higher concentrations of heavy metals in the polluted air may affect the physiological behaviour of plants and may block the electron transport chain. Similarly, the intake of phytotoxic gaseous pollutants may also affect the primary metabolic activities of plants like photosynthesis, respiration, transpiration, etc. [14, 15].

High water content within a plant body helps to maintain its physiological balance under stressful conditions, such as exposure to air pollution. The highest relative water content (RWC) was found in response to the high availability of soil water content and lower evaporation and transpiration rate. RWC of a leaf is the water present in response to its full turgidity. Under air polluted conditions, transpiration rates are frequently high, which leads to dryness. Therefore, the maintenance of RWC by the plant may determine its relative tolerance to pollution. “It has been reported that as the concentration of pollutant increases, cell permeability of plant leaves also increase, which results in loss of water and nutrient, and ultimately leads to early senescence” [16]. In this study, RWC in leaves of *T. catappa* was found to be highest and least in *M. Peltata* upon exposure to air pollution.

Chlorophyll is often measured in order to assess the impacts of environmental stress since the changes in the pigments are linked with visual symptoms of growth disorder and photosynthetic productivity. “One of the most common impacts of air pollution is the gradual disappearance of chlorophyll content which may be associated with a decrease in net photosynthesis rate” [17]. In the present study, *P. pinnata* showed high chlorophyll content than other plants which could be correlated to high photosynthetic productivity. In contrast, *G. floribunda* leaves exhibited lower chlorophyll content, revealing its sensitivity towards air pollution.

“Carotenoids are the structural components of the photosynthetic antenna and reaction center. They play a critical role in photosynthetic process and protect chlorophyll from photo-oxidative damage” [18]. In the present study the increased carotenoid content was observed in *T. catappa*, *S. mahagoni* and *A. heterophyllus* could be related to the protective role of carotenoids against potentially harmful photooxidative processes [19].

“The result showed pH of leaf extract of all plants was acidic which may be due to diffusion of gaseous air pollutants like NO_2, CO_2 and SO_2 in the cell sap and when plants are suffering from air pollutants as their cellular fluid would produce massive H^+ to react with SO_2, which enters through stomata and intercellular space from air so that H_2SO_4 is generated and then leaf extract pH reduces and the results were in line with the findings” of Kaur and Nagpal [20]. Photosynthetic process is reduced in plants when the leaf pH was low and low pH is more prone to air pollution in sensitive plant species, while those with pH around 7 are more tolerant [21].

The increased level of ROS in plants due to exposure to air pollution damages the biomolecules such as lipids and results in MDA formation as the breakdown product of polyunsaturated fatty acids of membranes and finally it results in reduction of membrane stability index. This is widely accepted as an important criterion for making the assessment of the severity of the oxidative damages [22]. The MDA content or membrane damage was highest in *M. oleifera* and it was related to the oxidative damage induced by excessive ROS production and membrane damage.

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen production during stressful conditions in plants, which altogether results in the reduced primary metabolic activities by altering membrane integrity. Plants synthesize a variety of osmolytes such as proline, ascorbate, phenolics and sucrose which are low molecular weight, highly soluble compounds which function as compatible solutes in plant cells and are nontoxic even when accumulated at high cellular concentrations [23]. Proline accumulation is one of the important non enzymatic antioxidant defence systems against ROS which are known to occur under air pollution in plants. Upon exposure to air pollution, significant increase in the accumulation of proline was recorded in all plants.

Antioxidant like ascorbate and glutathione work hand in hand with enzymatic antioxidants to scavenge toxic ROS produced under unfavourable environmental conditions and prevents the oxidation of vital components in the plant cells [24]. The ascorbate detoxifies tocopheroxyl radicals produced as a result of singlet oxygen induced lipid peroxidation in photosystem II. An elevated level of ascorbate *T. grandis*, *A. occidentale* and *H. brasiliensis* substantiates would help these plant species to minimize ROS production and greater tolerance towards industrial air pollution. However, the ascorbate content was least in *M. oleifera*, *M. indica*, *C. nucifera*, *P. emblica*, *G. floribunda*, *T. catappa*, *A. heterophyllus* and it was related to the oxidative damage induced by excessive ROS production in these plant species.

Soluble sugars have osmoprotectant and cryoprotectant roles and their presence is important for plasma membrane. They are important parts in plant structure and source of energy in all organisms. "The concentration of soluble sugars are indicative of the physiological activity of a plant and is determines the sensitivity of plants to air pollution" [25]. In this study, maximum sugar content was recorded in *P. nigrum*, *C. nucifera* and *H. brasiliensis* and the minimum soluble sugar content was observed in *P. pinnata*. Moreover, phenolics function as defence factors against various types of stresses caused by pollution or adverse environmental conditions. In the present study, an elevated level of phenolics content was recorded in *C. nucifera*, whereas, the minimum phenolics content was observed in *A. Heterophyllus*, *M. oleifera*, *G. sepium*, *P. emblica* and *T. catappa* could be related to its sensitivity towards air pollution.

"The impacts of air pollution on separate parameters such as ascorbate content, chlorophyll content, leaf extract pH and relative water content gave conflicting results for same species Hence, the air pollution tolerance index (APTI) based on all four parameters has been used for identifying tolerance levels of plant species and to rank plant species in their order of tolerance to air pollution viz., sensitive, intermediate and tolerant" [26, 6]. The selected fifteen plant species were found to be having significant variation in air pollution tolerance index and the APTI value of *T. grandis*, *H. brasiliensis*, *A. occidentale* and *P. pinnata* was maximum than other plants. Moreover, lowest APTI was found in *G. floribunda*, *P. emblica* and *M. oleifera*, can be used for the biomonitoring of air pollution. The APTI value of selected plants in the industrial site was found to be in the order of *S. mahagoni* > *G. sepium* > *M. Peltata* > *P. nigrum, T. catappa, C. nucifera, M. indica* and *A. heterophyllus* showed an intermediate level of tolerance towards air pollution. Thus the present study indicated that *T. grandis*, *H. brasiliensis*, *A. occidentale* and *P. pinnata* species were the most suitable sink for industrial air pollution, which can be utilized for green belt development in selected industrial area of Malappuram, Kerala for reduction of the level of the air pollution.

5. CONCLUSION

This study evaluated the air pollution impact on various physiological and biochemical parameters of fifteen plant species and to screen the most tolerant plant species. The air pollution caused a lower value of leaf extract pH, total chlorophyll and carotenoid contents in selected plant species. The increased level of ROS in plants due to resulted in high MDA content. The content of antioxidants like proline, ascorbate, soluble sugar and phenolics was highly enhanced in all selected plant species but it was varied in response to air pollution. The air pollution tolerance index (APTI) of *T. grandis*, *H. brasiliensis*, *A. occidentale* and *P. pinnata* was found to be maximum and the most suitable species to be used for green belt development in industrial area for reduction of the level of air pollution.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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