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IMPACTS OF AIR POLLUTION ON TREES GROWING BY THE ROADSIDE OF KARACHI WITH RESPECT TO THEIR TOLERANCE CHARACTERISTICS

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Abstract

An air pollution tolerance index (APTI) proposed by earlier workers is often employed by environmentalists or ecologists to investigate the relative susceptibility of plants to air pollution in mega cities. The APTI was estimated by determining ascorbic acid, total chlorophyll content of leaves, pH of leaf extract and relative water content (%). In the current investigation, this index was employed to evaluate the susceptibility/ tolerance of ten common tree species growing in a polluted site (Guru Mundar area) of Karachi city and relatively unpolluted habitat (Karachi University Campus). Ascorbic acid content, pH of leaf extract and relative water content were found elevated in polluted site over the unpolluted habitat, whereas, total chlorophyll content of leaves was invariably lesser at polluted site. In addition, to the above four parameters we also examined free amino acid content of leaves and the percentage of clogged and partially clogged stomata of the preferred species from the same areas. In general, ascorbic acid, leaf extract, pH and the relative water content of leaves were high in the polluted habitat compared to unpolluted sites, whereas, total chlorophyll content of leaves was lesser at polluted site for almost all the species examined. Tree species with a high APTI value were regarded as tolerant. Tolerant species included *Azadirachta indica*, *Ficus benghalensis*, *Ficus religiosa*, *Conocarpus erectus* and *Peltophorum pterocarpum* as indicated by greater magnitude of APTI under polluted regime. Three species including *Albizia lebbeck*, *Guaicum officinale* and *Polyalthia longifolia* were found sensitive to air pollution. *Delonix regia* did not differ with respect to APTI for polluted and unpolluted situations. The percentage of clogged and partially clogged stomata was in general higher in the polluted site compared to unpolluted habitat. Remarkably, the levels of free amino acid of all species were found accentuated in the polluted site compared to unpolluted habitat. Presumably, an improved APTI can be achieved if the levels of free amino acids and percentage of clogged and partially clogged stomata can be included in the computation of APTI. It is suggested that green belts in the city and its suburbs and developing industrial and residential areas can be planted with trees species having high APTI. This would not only beautify the city but such trees positively act to clean the air and serve as sinks for varied air pollutants leading to better human health.

Key words: Air pollutions, APTI, Ascorbic acid, Chlorophylls, Relative water content, Free amino acids, Clogged stomata.

Introduction

Air pollution of the metropolitan city of Karachi is rising to an alarming state predominantly due to vehicular exhaust, domestic use of fuel (cooking and power generators) and the gases released by a huge number of industries. In general, the atmospheric pollutants are being generated due to, economic growth, industrialization and urbanization. Over the last 35 years or so, population, roads, vehicular traffic, use of power generators in super-stores and homes, and number of industries have increased enormously in Karachi city resulting in enhanced particulate and gaseous pollution. Air pollution seriously damages plants, natural vegetation, health of human beings and other living organisms and materials such as buildings and stone-works. Atmospheric pollution is mainly due to gaseous pollutants including SOx, NOx, CO and O_3 and its precursors as well as VOCs (Olowoporoku *et al*., 2012; Longhurst & Brebbia, 2012; Tiwari & Kumar, 2020; Haakman *et al*., 2020). All these gases (or vapours) are harmful to humans, or creating discomfort and even can cause cancer (VOCs), the domesticated animals as well as to cultivated (ornamental) plants and the local flora. Besides, gaseous emissions particulate matter (e.g., PM_{10} and $PM_{2.5}$) is also a major source of air pollution (Mudakavi, 2010; Tiwari *et al*., 2019). It comprises of a complex mixture of dust, pollen, spores, soot, smoke and droplets (Colls & Tiwary, 2009; Longhurst & Brebbia, 2012). Some of the spores and pollen are highly allergenic (Afzal *et al*., 2004, Pourkhabbaz *et al*., 2010; Alam *et al*., 2012). The characteristics of air quality in urban areas has been changing substantially in the last two decades, while SO_2 level and black smoke concentration are declining, and are being replaced by higher concentration of vehicular exhaust (Brophy *et al*., 2007). Particulate and gaseous pollutants present in the atmosphere in combination or by themselves may cause severe disruption in the physiological system of plants (Kazmi *et al*., 2002; Siddiqui, 2009; Bytnerowicz *et al*., 2007; Kulshrestha & Saxena, 2016). Plants being an integral part of ecosystems and because of their particular structure, such as leaves and branches that are exposed to atmospheric pollutants, are generally targeted by air pollution. Their morphological features are particularly affected (Abbasi *et al*., 2020; Pourkhabbaz *et al*., 2010; Leghari & Zaidi, 2013). The various air pollutants, gaseous and particulate in combination also cause adverse synergistic effects. Because plants are sedentary, they are continuously exposed to air pollution, and the extent of injury or retardation in growth is directly proportional to pollution intensity and the duration of exposure. In this regard, trees suffer the most because of their perennial and arboreal habit which exposes them to air pollutants occurring at ground surface and upwards up to certain heights. Sensitivity to air pollution (and component gases singly) varies considerably among plant species and among growth forms. Thus, air pollution sensitive species of plants can be exploited as biological pointers of air pollution severity (Lakshmi *et al*., 2009; Zhang *et al*., 2020).

The control of air pollution is more challenging and difficult compared to other forms of pollution. Air pollution directly affects plants through leaves and stems and indirectly through the supporting soil (Steubing *et al*., 1989; Selmi *et al*., 2016). Acid rain formed in the atmosphere during rains affects the plants both through the shoot system of plants and by absorption from the soil. These municipal air pollutants are known to be threats to human health, the domesticated animals and as well as plants but also eventually lead to global environmental issues. The air-borne particulate pollutants not only include carbon particles and dust but also trace elements and heavy metals that constitute a significant proportion which needs to be constantly monitored (Kar *et al.,* 2010). The deposited dust and carbon particles on the leaves suppress the process of photosynthesis and cause stomatal closure and consequently plant productivity (Kazmi *et al*., 2002). With regards to monitoring and mitigation of air pollutants in a metropolitan city, workable policies should be made by the government to control and regulate the pollution saddle effectively. Plant responses to atmospheric pollution can provide relatively simple means to monitor the pollution intensity and also permit to take mitigation measures.

To ascertain the tolerance/sensitivity level of selected trees to air pollution some appropriate vital response variables are of special significance. Generally, the degree of tolerance levels of plants to air pollution shows variable behavior. The tolerance levels depends to a greater or lesser extent on soil type and its moisture, nutrient and pollutant regime, nearness from road traffic or other sources of heavy pollution, topography and local climatic conditions. Once the tolerance is evaluated the appropriate plants can be chosen to develop a strategy for planting such trees on the road sides and vacant spaces that will improve more greenery in the city and the quality of urban life (Colls & Tiwary, 2017).

Currently, the criterion for tolerance of plants to air pollution is the APTI (air pollution tolerance index) which is being employed in many countries (Thambavani & Kamala, 2010; Esfahani *et al*., 2013; Chibuisi *et al*., 2014; Vyankatesh *et al*., 2014; Lohe *et al*., 2015; Babu *et al*., 2013; Rai *et al*., 2013; Gholami *et al*., 2020; Chaurasia & Karan, 2016; Sahu *et al*., 2020). However, the extent of susceptibility of plants to air pollution varies considerably among the species. The air pollution tolerance capability of a plant species is an innate characteristic. Therefore, the determination of degree of tolerance/sensitivity is necessary to recognize plant species as biological indicator of air pollution whereby some toxic substances can be disposed through absorption by plants (Singh *et al*., 1991). Hence, pollution tolerant plants would have greater expectation to tolerate air-pollution or they would be able to survive if the air pollution level of an area is higher because of increased level of industrialization or greater traffic-load on the roads.

This study focuses on the evaluation of some common trees with regard to their susceptibility to air pollution from two different locations in Karachi, 1. Roadside trees exposed to vehicular exhaust air pollution as well as due to domestic consumption of fossil fuel at Guru Mandir area and 2. Karachi University campus which is relatively less exposed to atmospheric pollution owing to an open area and being far away from industries, lesser level of vehicular load and lesser number of households.

Material and Methods

The study sites: Karachi is a metropolitan city which has a population of nearly 22 million and there are over 7600 industrial units (including small industries). Karachi University campus is located at about 18 km from the city centre. The atmosphere of most part of the city is highly polluted due to heavy traffic, emissions released by the industries and burning of fossil fuel in the domestic and industrial sectors. The air of Karachi University Campus, being away from the city centre, is relatively less polluted. Within the campus, there is comparatively much lesser road traffic; there are no industries nearby and lesser number of residential houses. However, there are a lot of trees on the roadside and traffic islands; besides there are small groves and a large main nursery having a variety of trees in the campus. Semi-natural vegetation also prevails between departments and Institutes within the campus. Therefore, it was used as the control site. While Guru Mandir area, located close to the city centre where traffic load is extremely high, served as the polluted site.

Sample collection: For the study 10 plant species were selected that were present at both the study sites during March to June 2022. These species were: *Azadirachta indica* A. Juss., *Albizia lebbeck* (L.) Benth, *Conocarpus erectus* L., *Ficus religiosa* L., *Ficus benghalensis* L., *Delonix regia* (Boj. ex Hook.) Raf., *Guacum officinale* L., *Peltophorum pterocarpum*, *Polyalthia longifolia* (Sonn.) Thw. and *Terminalia catapa* L. Individual trees at each site were chosen in a random fashion. Likewise, the leaves from the trees were also collected by the same procedure with the condition that they were growing under similar environmental conditions (e.g., soil, water and light conditions). Four mature and undamaged leaves from each tree species were obtained from the two study sites. Old, yellowing or damaged leaves were avoided. The necessary parameters for the ATPT were ascertained in the laboratory as under. In addition, the extent of stomatal clogging by smoke (and dust) particles and the levels of free amino acid (that is usually elevated under stress conditions) were also determined.

Ascorbic acid content: Ascorbic acid (AA) content was determined following the method outlined by Bajaj & Kaur (1981). In a 4 ml of oxalic acid-EDTA solution, 1.0 g of leave tissue was extracted followed by the addition of 1 ml of orthophosphoric acid. To this 1 ml 5% tetraoxosulphate (VI) acid was added. Then, ammonium-molybdate (2.0 ml) was added followed by 3 ml of deionized distilled water. After 15 min the optical density was noted at 760 nm on a UV-Vis 1240 Shimadzu, Japan spectrophotometer. The amount of AA was measured using a standard curve and the concentration is reported in mg/g. Samples was replicated three times each.

Estimation of chlorophyll: Chlorophylls were extracted from leaves of the selected species from the two contrasting habitats and determination was performed by Arnon's method (1949). To start with this method, 1 g of leaf tissue was macerated in 10 ml of 100% acetone solution and the mixture was centrifuged at a speed of 568g for 10 minutes. Later by using a spectrophotometer (UV-Vis 1240 Shimadzu, Japan), OD was noted at 645 and 663 nm against 100% acetone as blank. Chlorophylls were estimated using Arnon's equation. Three replications were used for the leaf sample of each species from each habitat.

The pH of leaf extract: 5.0 gm of fresh leave extract was mixed in 10 ml deionized distilled water. This solution was filtered and the pH was measured on a pH meter (Jenway, England). Each sample of leaves had three replications.

Relative water content (RWC): Weatherley (1965) method was employed to quantify the relative water content. To obtain fresh weight the leaves were brought to laboratory, cleaned and weighed immediately (W1). The turgid leaf weight was determined in the laboratory by plunging the leaves in a water for 24 h. The excess water was removed and turgid weight measured (W2). Thereafter, the leaves were placed in an oven at 80ºC for overnight and weighted (W3). The RWC was estimated as: $[(W_1\text{-W3})/ (W_2\text{- W3})] \times 100$. Samples were replicated thrice.

Assessment of stomata clogging: To estimate the percentage of clogging of stomata with the particulate pollutants, leaf samples collected from both the habitats (sites), leaf samples were randomly collected and fixed in 15 % ethanol immediately after collection. In the laboratory impressions of lower epidermis were obtained using pearl transparent nail polish to determine the number of clogged, partially clogged and open stomata from five different fields of the microscopic view. This technique is found to be adequately effective for the examination of leaf epidermal surface.

Estimation of free amino acids (FAA): Ninhydrin assay was employed for the determination of FAA (Yemm & Cocking, 1955) with minor modifications. 5.0 ml of 80% ethanol was used for the extraction of 500 mg of leaf tissue (4 replicate each) and centrifuged at 568 g for 20 minutes followed by passing the sample through activated charcoal to avoid coloration if any. To estimate the free amino acids in the extract, a 0.1 ml aliquot was taken and FAA ascertained using 7.6 ml ninhydrin reagent. The mixture was thoroughly shaken and placed in a boiling water bath for 10 minutes. The OD was noted at 570 nm on a spectrophotometer (Shimadzu UV/36000, Japan) while 80% ethanol served as the blank. The standard curve was developed using glycine and the concentration of FAA was given as mg FAA/g fresh weight.

Estimation of APTI: The APTI of the plant species was determined by using the formula developed by Singh *et al*., (1991) reproduced below:

$$APTI = [A\ (T+P) + R]\ /10$$

Where,
A= Ascorbic acid content of leaf mg/g dry weight
T= Total chlorophyll content of leaf mg/g dry weight
P= P is the pH of leaf extract
R= Percent relative water content of leaf tissue

Table 1. Ascorbic acid content, total chlorophyll, pH of leaf extract and relative moisture content % of ten tree species.

Plant species	Ascorbic acid mg/g		Total chlorophyll (a,b) mg/g		pH of the leaf extract		Relative moisture content %	
	KU Campus	City Centre	KU Campus	City Centre	KU Campus	City Centre	KU Campus	City Centre
Azadirachta indica	7.0 ± 0.6	9.8 ± 0.7	5.9 ± 0.7	3.9 ± 0.8	6.2 ± 0.2	6.9 ± 0.5	72.6 ± 1.8	78.4 ± 1.3
Albizia lebbeck	5.4 ± 0.4	6.3 ± 0.4	4.8 ± 0.5	4.7 ± 0.6	6.0 ± 0.4	6.9 ± 0.4	70.5 ± 1.3	76.8 ± 2.5
Conocarpus erectus	5.7 ± 0.6	7.2 ± 0.6	5.8 ± 0.1	5.3 ± 0.4	6.3 ± 0.4	6.5 ± 0.6	73.7 ± 0.9	77.5 ± 1.5
Delonix regia	6.2 ± 0.5	6.5 ± 0.6	6.5 ± 0.3	4.7 ± 0.3	6.7 ± 0.6	7.1 ± 0.3	69.6 ± 2.5	74.5 ± 1.4
Ficus benghalensis	7.8 ± 0.8	9.4 ± 0.5	6.6 ± 0.8	4.7 ± 0.2	6.4 ± 0.3	6.9 ± 0.2	71.2 ± 2.2	78.4 ± 2.5
Ficus religiosa	5.8 ± 0.6	8.2 ± 0.6	5.9 ± 0.3	4.6 ± 0.4	6.3 ± 0.5	6.9 ± 0.2	69.0 ± 2.5	78.2 ± 2.4
Guaiacum officinale	6.1 ± 0.5	6.8 ± 0.4	5.8 ± 0.5	5.0 ± 0.1	6.6 ± 0.3	7.0 ± 0.1	72.5 ± 3.4	75.8 ± 2.5
Peltophorum pterocarpum	6.1 ± 0.2	6.1 ± 0.6	5.7 ± 0.4	4.9 ± 0.3	6.4 ± 0.1	7.3 ± 0.4	73.8 ± 1.1	79.7 ± 1.5
Polyalthia longifolia	4.2 ± 0.3	5.9 ± 0.2	6.8 ± 0.5	5.3 ± 0.3	6.7 ± 0.5	7.1 ± 0.2	75.1 ± 1.2	79.8 ± 2.1.
Terminalia catapa	5.2 ± 0.5	6.8 ± 0.6	6.2 ± 0.2	5.1 ± 0.4	6.4 ± 0.2	6.9 ± 0.3	73.5 ± 2.4	79.7 ± 2.3

Statistical analysis

The means and standard errors (SE) of each of the variable were computed. In addition, each variable was subjected to two factor (species=10 and habitat=2) analysis of variance. (ANOVA). The follow-up of ANOVA was performed using Fisher's LSD at 0.05 and Scheffe's multiple contrasts (Zar, 2014; Rosner, 2015).

Results and Discussion

The results of above mentioned biochemical parameters (ascorbic acid, leaf extract pH, relative water content and total chlorophyll content) are presented in Table 1 for both Karachi University Campus (relatively unpolluted due to least vehicular traffic) and the City centre (polluted mainly due to high traffic-load) localities (or habitats).

Ascorbic acid content: The concentration of ascorbic acid of tree leaves at K.U. Campus ranged between 4.2 mg g^{-1} (*Polyalthia longifolia*) and 7.8 mg g^{-1} (*Ficus benghalensis*), at unpolluted habitat it ranged between 5.9 2 mg g^{-1} (*Polyalthia longifolia*) and 9.8 mg g^{-1} (*Azadirachta indica*) (Table 1). Results of factorial ANOVA showed a significant differences in species (p<0.001) and also between unpolluted and polluted habitat (p<0.001). The interaction of species X habitats was also significant (p<0.01). Scheffes multiple contrast for ascorbic acid content in unpolluted and polluted locality depicted a significant (p<0.05) difference between the unpolluted and polluted habitats (sites). But the difference between leguminous and non-leguminous species was found non-significant.

The ascorbic acid plays a pivotal role in photosynthesis and photo-protection, in defence against ozone and other oxidative stresses because it acts as a major redox buffer; also possibly it is involved in cell expansion and cell division. It plays a role in hormone biosynthesis and in regenerating other antioxidants. It is known to activate some physiological and defense mechanism processes. Its reducing power varies with its concentration in plant (Raza *et al*., 1985). Owing to these vital functions it is considered as an important factor in plant health. High level of ascorbic acid confers tolerance of plant species towards pollution (Lee *et al*., 1984; Gallie, 2013). However, sometimes its levels tend to decline under constant pollution regime. Nonetheless, when a plant is capable of maintaining greater levels of ascorbic acid under persistent polluted conditions in that case because of its vital functions outlined above, it helps the plant to achieve pollution tolerance and, therefore, it is included as an important criterion variable in the computation of ATPI.

Total chlorophyll content: The total chlorophyll conecntration of leaves at K.U. campus (non-polluted) ranged between 4.8 (*Albizia lebbeck*) to 6.8 (*Polyaltia longifolia*) while in the polluted habitat (City centre) it ranged between 3.9 (*Azadirachta indica*) and 5.3 (*Conocarpus erectus* and *Polyalthia longifolia*) (Table 1). Scheffe's multiple contrast test showed lower (p<0.05) chlorophyll content for the polluted habitat compared to unpolluted site, while in *Albizia lebbeck* the difference in chlorophyll conecntartions were found non-significant. It is well known that total chlorophyll concentration decreases under importunate air pollution stress (Ahmed & Qadir, 1975; Kazmi *et al*., 2002; Giri *et al*., 2013). Photosynthetic pigments of five common tree species growing at about 1 Km away from a thermal power plant (air Polluted site) were substantially reduced and a sub unit of the rubisco enzyme was found degraded in *Azadirachta indica* at the polluted locality (Govindaraju *et al*., 2012). In general, stress condition is known to deplete chlorophyll content and brings about changes in the ratios of photosynthetic pigments (Darall & Jager, 1984). Maina & Wang (2015) found increase in chlorophyll a/b ratio with stress caused by depressed N-level in soil (N-stress). It is seen that plants growing under field conditions with unpolluted regime have usually high chlorophyll content (Singh *et al*., 1991). Total chlorophyll content therefore, considered as a pertinent parameter to include in the computation of APTI.

The pH of leaf extract: The pH values of leaf extract of all species at unpolluted habitat (K.U. Campus) site (range= 6.2 to 6.7) was significantly (p<0.001) lower than that at polluted site (range= 6.5 to 3) (Table 1). The pH values fluctuate significantly among the selected plant species (p<0.05) However, the interaction of Species X habitat was found non-significant. Scheffe's multiple contrast test disclosed a significant (p<0.05) difference in the leaf extract pH of unpolluted vs. the polluted habitat. It is demonstrated that in the accumulation or presence of acidic pollutants the leaf pH decreases and becomes acidic and with the increase in such pollutants it deciles further. It has been demonstrated that in air-pollution-tolerant plants leaf extract pH is higher than in non-tolerant plants (Singh *et al*., 1991; Klumpp *et al*., 2000). The greater levels of leaf extract pH in plant species under polluted regime may confer pollution tolerance under air pollution stress. This provides the basis of leaf extract pH as a factor for the determination of APTI.

Relative water content (RWC): The RWC % was in general high. The RWC% ranged between 70.5 % (*Albizia lebbeck*) to 78.1% (*Polyalthia longifolia*) at unpolluted habitat (KU Campus) and from 74.5% (*Delonix regia*) to 79.8 (*Polyalthia longifolia*) at the polluted habitat (city centre). Factorial ANOVA disclosed that differences among species were significant (p<0.05) while habitats were highly significant (p<0.001). Interaction of species X habitats was declared non-significant. RWC% was significantly greater (p at the most 0.05) in polluted compared to un-polluted site as shown by Scheffe's test (p<0.05) though Polyalthia *longifoilia* did not differ significantly in this respect at the two habitats. Singh *et al*., (1991) reported that RWC% was higher in deciduous trees compared to evergreen trees. The results of the current study seem to be in accordance with this observation to a considerable extent. It is demonstrated that RWC% is associated with protoplasmic permeability (Amoda *et al*., 2002; Vitali *et al*., 2016). Therefore, Singh *et al*., (1991) accepted it as an important feature to be included in the formulation of APTI. It is apparent from Table 1 that none of the plant species investigated had the greatest value for all the four selected parameters for APTI as each parameter is involved in sensitivity response to pollutants in accordance with its own peculiar intrinsic mechanism in the metabolism.

APTI (Air Pollution Tolerance Index): The APTI values for the ten selected species at unpolluted and polluted sites are given in (Table 2) which shows consistently greater values for polluted (city centre) habitat compared to unpolluted (KU Campus) with the exception of *Delonix regia. D. regia* generally seem to receive better environmental conditions as it was found growing slightly away from the direct gust of vehicular exhaust. The APTI magnitude ranged from 12.86 (*Albezia lebbek*) to 17.26 (*Ficus benghalensis*) at un-polluted site (KU Campus) while at polluted site, it ranged between 15.12 (*Delonix regia*) to 18.65 (*Ficus benghalensis*). For deciduous tree, *Albizia lebbeck* showed APTI of 16.63 at polluted site while *Terminalia catapa* yielded value of 16.13 in polluted habitat. In general, it seems that trees respond to polluted regimes by elevating their APTI. The sensitivity of plants, of course varies, in this respect. Among the species that were examined in the current study *Azadirachta indica* and *Ficus religiosa* seem to be sensitive to changing environmental conditions and respond well when exposed to air polluted conditions by changing their magnitude of APTI. When comparing deciduous trees (e.g., *Albizia lebbeck*, *Azadirachta indica*, *Terminalia catapa* and *Ficus benghalensis*) with the evergreen trees (*Ployaltia longifolia*, *Guaiacum officinales*, *Conocarpus erectus*, *Delonix regia*), usually the deciduous tree species have greater APTI relative to evergreen species. The deciduous characteristic is presumably one innate characteristic that enables the species to thrive under adverse (seasonal) condition. This could be in some fashion related to stress tolerance. Begum and Harikrishna (2010) found *Azadirachta indica* and *Ficus benghalensis* to have the highest value of APTI (at polluted site) among the 17 species tested. This result corresponds well with the finding of the current study. *Delonix regia* in this study seems to be sensitive to some degree. Likewise Singare & Dalpade (2013) among the four species tested for APTI found *D. regia* to be the least tolerant species.

Table 2. Air pollution tolerance index (APTI) of 10 selected tree species in Karachi under Unpolluted and air-polluted habitats.

Species	Unpolluted	Polluted
Azadirachta indica	15.69	18.42
Albizia lebbeck	12.86	16.63
Conocarpus erectus	14.26	16.83
Delonix regia	15.40	15.12
Ficus benghalensis	17.26	18.65
Ficus religiosa	13.97	17.25
Guaiacum officinale	14.81	15.74
Peltophorum pterocarpum	14.76	17.21
Polyalthia longifolia	13.48	15.30
Terminalia catapa	13.90	16.13

Clogging of stomata: The frequency of completely clogged (CC), partially clogged (PC) and open (O) stomatais shown in Table 3. The percentage of completely clogged (CC) stomata in unpolluted site ranged between 13.0% (*Guaicum officinale*) to 26% (*Ficus benghalensis*) whilst at polluted site they ranged between 25.2% (*Guaicum officinale*) to 41.4% (*Ficus beghalensis*). The partially clogged (PC) stomata in unpolluted habitat ranged between 27.9% (*Ficus relegiosa*) and 37.2 (*Peltophorum pterocarpum*) while at polluted site between 32.2 (*Guaicum officinale*) and 48.4% (*Azadirachta indica*). The result of factorial ANOVA yielded significant differences in species (p<0.001), habitat (p<0.001) and interaction of Spp. X habitat (p<0.001) for percentage of clogged stomata. For partially clogged stomata, significant difference (p<0.001) was depicted among species, habitats (p<0.05) and interaction of species X Habitats (p<0.001). Scheffe's test for difference between the two habitats (unpolluted vs. polluted) for CC stomata showed a significant difference (p<0.01), likewise for PC stomata also a significant difference was disclosed (p<0.05) though *Conocarpus erectus* did not differ appreciably in this respect at the two habitats. Atmospheric pollutants when prevailing in a locality even at low levels may adversely affect CO_2 absorption which interferes with the plant-water relations thereby controlling the stomatal aperture. Therefore, they are capable of deteriorating the water balance of the leaves (Robinson *et al*., 1998). Many atmospheric pollutants like SO_2 and O_3 are known to cause stomatal closure (McAinsh *et al*., 1996) while the former has the capability to damage the auxiliary cells (around stomata) in particular along with the damage to the epidermal cells. In addition, the stomatal openings may be clogged by particulate air pollutants such as dust and smoke particles (Ahmed & Qadir, 1975; Kazmi *et al*., 2002). Together the two different processes of stomatal closure mechanisms may cause significant impacts on the physiological processes such as water balance of plants and the photosynthetic rate.

Free amino acids (FAA): Free amino acid ranged between 2.95 (*Azadirachta indica*) and 5.51 (*Albizia lebbeck*) in the unpolluted habitat while the range was 4.51 (*Azadirachta indica*) and 8.49 (*Polyalthia longifolia*) under polluted regime. Consistently higher levels of FAA were detected in all selected species under polluted habitat compared to unpolluted site (p<0.001). The species factor was also found significant (p<0.001) and the interaction species X habitat (p<0.01). Scheffe's multiple contrast test for the unpolluted vs. polluted s disclosed a significant between habitat difference (p<0.001). Scheffe's multiple contrast performed to evaluate the difference between the two habitats (unpolluted and polluted) disclosed a significant difference (p<0.05).

In general, FAA content was found elevated in the plant leaves growing in polluted habitat compared to unpolluted site (Table 4). The amino acids are known to play varied roles in plants, for instance they act as osmolytes, cause detoxification of heavy metals, ion transport regulator and serve as source of energy.

Proline serves as a free radical scavenger that has potential to protect plants against oxidative stress damage. It has been also demonstrated that proline also has the capability to bind metals thereby avoiding the effect of metal toxicity. It also acts as an antioxidative defense molecule thereby playing a pivotal biological role as a response to air pollution stress (Liang *et al*., 2013). Proline also confers salinity and drought tolerance in some plants (Tabot & Adams, 2014). Hg and Cd concentration in shoots and roots of cajanus seedlings increase the concentration of FAA (Patnaik & Mohanty, 2013). Narwal *et al*., (1993) reported elevated concentration of FAA in reposnse to Cd in maize plants. Excessive cellular concentration of heavy metals reduces the consumption of amino acid and support protein hydrolysis which in turn affects the equilibrium concentration of cellular proteins (Tendon *et al*., 2004). Concentration of a number of amino acid including alanine, glutamic acid and arginine enhanced when subjected to stress condition in plants (Tendon *et al*., 2004). Plant metabolic pool is remarkably influenced by the levels of individual amino acids. Seceli *et al*., (2005) demonstrated that the FAA content enhanced in the foliage of two apple varieties subjected to drought stress. A buildup of higher levels of nitrogenous compounds is known to occur when plants are under stress conditions (Hasegawa *et al*., 2000; Meloni *et al*., 2001, 2003; Greenway & Munns, 1980).

Table 3. The frequency of completely clogged (CC), partially clogged (PC) and open (O) stomata of the ten selected species at two habitats, Unpolluted site (Karachi University Campus) and Polluted site (Guru Mandir area). Means followed by ± standard error of mean.

Species	Un polluted sites			Polluted sites		
	CC	PC	O	CC	PC	O
Azadirachta indica	24.2 ± 18	32.6 ± 2.9	43.2 ± 3.1	28.5 ± 3.6	48.4 ± 6.2	23.1 ± 4.8
Albizia lebbeck	22.6 ± 2.4	35.9 ± 3.4	41.5 ± 3.6	35.7 ± 4.1	43.1 ± 4.7	21.2 ± 4.1
Conocarpus erectus	18.5 ± 2.3	38.5 ± 3.6	43.0 ± 4.1	26.3 ± 2.8	38.9 ± 3.5	34.8 ± 3.2
Delonix regia	16.5 ± 2.0	28.5 ± 4.1	55.0 ± 3.7	30.5 ± 3.5	32.6 ± 3.0	36.9 ± 3.9
Ficus benghalensis	26.2 ± 3.0	36.4 ± 4.8	37.4 + 4.6	41.4 ± 3.9	35.3 ± 3.8	23.3 ± 3.3
Ficus religiosa	23.2 ± 2.6	27.9 ± 3.1	48.9 ± 3.8	36.9 ± 3.5	39.6 ± 3.6	23.5 ± 2.9
Guaiacum officinale	13.0 ± 1.8	28.6 ± 3.4	59.3 ± 4.1	25.2 ± 2.1	32.2 ± 2.9	42.6 ± 2.6
Peltophorum pterocarpum	24.4 ± 3.2	37.2 ± 4.1	38.4 ± 4.4	28.7 ± 2.5	35.0 ± 3.7	36.3 ± 3.5
Polyalthia longifolia	21.4 ± 4.1	32.6 ± 3.8	46.0 ± 4.2	30.6 ± 3.3	34.6 ± 2.9	31.8 ± 3.0
Terminalia catapa	15.7 ± 2.8	38.2 ± 3.9	46.1 ± 3.7	34.7 ± 4.1	41.6 ± 3.8	23.7 ± 2.8

Table 4. Free amino acid content mg g^{-1} in the leaves of 10 tree species in Karachi under Unpolluted and air-polluted habitats. Mean ± SE.

Species	Unpolluted	Polluted
Azadirachta indica	2.95 ± 0,18	4.51 ± 0,39
Albizia lebbeck	5.51 ± 0.27	8.28 ± 0.42
Conocarpus erectus	3..71 ± 0.22	5.76 ± 0.36
Delonix regia	4.02 ± 0.35	6.19 ± 0.39
Ficus benghalensis	3.27 ± 0.25	4..71 ± 0.40
Ficus religiosa	3.18 ± 0.38	6.80 ± 0.55
Guaiacum officinale	3.56 ± 0.45	5.82 ± 0.41
Peltophorum pterocarpum	3.62 ± 0.30	7.44 ± 0.63
Polyalthia longifolia	4.78 ± 0.39	8.49 ± 0.58
Terminalia catapa	3.92 ± 0.33	6.69 ± 0.50

An overview of the above results demonstrates that each of the investigated species for APTI responded differentially to air pollution as evident by varied values of APTI. Out of these 10 species 3 namely *Albizia lebbeck*, *Guaicaum officinale* and *Polyalthia longifolia* seem to be sensitive as indicated by their low APTI. On the other hand, tolerant species included *Azadirachta indica*, *Ficus beghalensis*, *Ficus religios*, *Conocarpus erectus* and *Peltophorum pterocarpum* as indicated by greater magnitude of APTI under polluted regime. Whereas three other species had intermediate response with respect to air pollution.

The result regarding air pollution tolerant tree species seem to correspond well with those of Begum & Harikrishna (2010) who conducted a study at Bangaluru, India. APTI is an extremely useful measure that can also be utilized for ranking species based on their air pollution tolerance capability that can be employed by landscape designers and developers to select tree species that can tolerate varying degree of anticipated air pollution for residential and developing areas in the outskirts of cities, and also for proposed industrial areas (Yan-Ju & Hui, 2008) taking cognizance with regard to current and expected pollution regime for residential and developing areas. The plant species susceptible to air pollution not only used as bio-indicators and but can be utilized for bio-monitoring purposes. The species with intermediate APTI can be planted in large university campuses (outside the city), large hospitals, industrial complexes, and city outskirts and in the suburbs where they can thrive well and at the same time play a significant role in improving the air quality index.

Green belts in the city can be developed using high APTI trees (plants) that not only beautify the city but positively act to clean the air and serving as sinks for many pollutants. Since no air cleaning devices have been developed for large scale cleaning it is imperative to resort to natural vegetation layer and planted trees (with high APTI) which can ameliorate the atmospheric pollution and improve the atmospheric conditions (Zhang *et al*., 2020). Many plants not only adsorb a variety of air pollutants but also metabolize them into nontoxic compounds (Shannigrahi *et al*., 2004; Agbaire & Esiefarienrhe, 2009). It is interesting to note that some other indices of air pollution tolerance have been adduced. Among these one noteworthy index is that developed by Banerjee *et al*., (2022) in which they employed twenty-one morphological and biochemical parameters in order to select plant species to combat air pollution in urban and industrial areas. Morphological characters are particularly useful as some like epithelial leaf layer is known to captures dust and soot particles in the waxy layer (above epithelium) forming flakes and crusts that capture particulate matter while other surface morphological characteristics are also important with regard to particulate matter removal. In particular, trichome length and stomatal frequency appeared to be important characteristics of plants with respect to pollution tolerance. Trichomes serve as protective tool by releasing certain secondary metabolites (Tian *et al*., 2017). PMs accumulate by the presence and density of epicuticular trichomes, e.g., Chen *et al*., (2017) found a significant correlation between trichome density and PM 2.5. Sahu *et al*., (2020) suggested EPI (expected performance index) which is more complex than APTI by including some other helpful characteristics with respect to their ability to be used in the plantation of greenbelts. EPI indicates the expected performance of plants and is obtained by integrating APTI scores with other phyto-socio-economic characters. For purpose of planting, plants with a high EPI score are recommended (>60%) for the following characteristics: (i) indigenous plants are preferred as they are more likely to thrive under local microclimatic conditions, soil, and human interactions, (ii) trees attaining a height of at least 10 m with generally thick leaves, and (iii) plants having rapid growth rate and (iv) leaf structure (Sahu *et al*., 2020). An assessment of the EPI of various indigenous plants is a promising step in selection of tree species for developing greenbelts therefore; the plants with higher APTI and EPI should be preferred. Another index, namely API (anticipated performance index) has been put forward by Noor *et al*., (2014) by synthesizing ATPI with some biochemical, physiological, economic, morphological and biological characteristics, which seems to be a promising index for selection of those species which are tolerant to air pollution and can be successfully exploited for the development of industrial and residential areas. Lastly, in the current study the FAA content was evaluated in plants from both polluted and unpolluted sites and it was demonstrated that the total FAA content was invariably found higher in the leaves of plants prevailing under air polluted habitat. In view of this fact it seems that free amino acid content also deserves a place in the computation of APTI. Furthermore, stomatal clogging is also found significantly associated with air pollution that cannot be ignored while estimating APTI.

Conclusions

The results of the current study deduced that ATPI considered being a promising tool to evaluate the sensitivity and tolerance of plants to air pollution and it can be employed for plantation in developing domestic and industrial sectors in accordance with the tolerance of particular plant species. However, this study clearly demonstrates that free amino acid contents of plants and the degree of stomatal clogging should also be included when computing ATPI. It is further suggested that the quality of air in Karachi can be enhanced while planting the native species that are tolerant as indicated by their APTI.

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