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Comparative leaf morphometrics of two urban tree species: an assessment to air pollution impacts

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Abstract

Leaf-size indices of two urban tree species namely: *Pterocarpus indicus* forma *echinatus* Willd. and *Sweitenia macrophylla* King growing in polluted and non-polluted areas of Cagayan de Oro City, Philippines were compared to verify if air pollution could directly affect leaf morphology. The streets with the heaviest influx of vehicles per day were considered as the polluted area while the non-polluted area was the peri-urban ecotourism village located approximately 2 kilometers away from the main highway. Fifty mature leaves from sampled trees were collected and measured to determine the variables such as length, width, area, petiole length including color, shape and deformities. Collected samples undergo tissue analysis to determine plant nutrient status. Results show that leaves of both species growing in the polluted area were significantly inferior in length, width, and area compared to those in the non-polluted site. However a variation in petiole length was observed with *P.indicus* forma *echinatus* being longer in polluted area than in non-polluted area. In contrast *S. macrophylla* has shorter petiole length in polluted area than non-polluted area. This implies a diverse effect of air pollution to leaf morphology. Statistical analysis for all variables evaluated revealed a significant difference at $p<0.005$ level. Moreover, both species in the polluted area exhibits pale green color while a dark green color in the non-polluted site. The shape of *P. indicus* forma *echinatus* in polluted area exhibited lobes and deformations while the most visible injury includes appearance of white streak lesions and scorched leaf apex for *S. macrophylla*.

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Introduction

Urban air pollution is a major environmental problem in most of the developing countries and in many heavily populated and industrialized areas in the world (Kambezidis *et al.*, 1996). In the last decades, there has been a sharp increase in air pollution. The increase in the number of automobiles in urban areas, industrialization along with expansion of cities, increase in energy demands and rapid economic development have been all the major factors influencing the level of air pollution (Tiwari *et al.,* 2008). Urban air pollution poses significant threat to all life forms. For instance, trees in the urban environment are subjected to a number of stresses than those in typical rural conditions. In the Philippines, the largest urban centers include Metro Manila, Metro Cebu, Davao and Cagayan de Oro where land, water and air pollution including space congestion have become very critical in the past years. In many urban areas of the world like these, motor vehicle traffic is a major source of air pollution contributing to about 57-75% of total emissions (WHO, 2006). Significantly, the role of urban trees and vegetation in reducing CO_2 has long been recognized. Trees can be significant sinks for gaseous pollutants since they provide large surface for pollutant uptake (Fowler, 2002).

Vegetation is considered to be an efficient sink for atmospheric pollutants, since leaves can absorb many kinds of toxic gases through their stomata (Hill, 1971) and are removed through dry deposition (Nowak *et al.*, 2006). However, these pollutants could have long term effects on plants by influencing CO_2 contents, light intensity, temperature and precipitation. Also, pollutants from auto emission can directly affect the plant by entering in to the leaf, destroying individual cells, and reducing the plant's ability to produce food (Saadullah *et al.*, 2013). Among the plant organs, leaves are the most sensitive part to be affected by various environmental conditions because most of the major physiological processes in plants occurred in the leaves. Several studies have been focused on the capacity of plants to ameliorate air quality however evaluating the leaf morphology as indicators of air quality has been overlooked thus, this study was conducted to determine if air pollution has direct impact to plants via leaf morphometrics.

Materials and methods

Locale of the Study

The study was conducted within Cagayan de Oro City. The streets with the heaviest volume of vehicles per day are considered the polluted area (PA).

Fig. 1. Map of the study site.

This includes Barangay Cogon and the streets of Velez, Neri, Capistrano, Corales, Pabayo, Abejuela, and Rizal. The site assumed to be a non-polluted area (NPA) was at Malasag ecotourism village, a peri-urban area located approximately 2 km away from the main highway and 6.28 km from the first study site (PA). Malasag ecotourism village was formerly a reforestation area turned into an ecotourism site which houses a botanical garden and wildlife sanctuary. The study sites lies between the geographic coordinates of 8°14'00" to 8°31'5"N and 124°27'00" to 124°49'00"E (Fig. 1). The climatic type falls under Type III of the Corona Climate Classification characterized by the absence ofvery pronounced maximum rain period with a short dry season lasting only from one to three months, either from December to February or from March to May (Bareja, 2011).

Leaf Sample Collection

Leaf samples of the six *Sweitenia macrophylla* and two *Pterocarpus indicus* forma *echinatus* trees found in both study sites were collected. Fifty (50) matured leaves were taken in every sampled tree. The leaf-size indices such as length, width, area including petiole length was determined. Variations in leaf color such as chlorosis, browning, yellowing, presence of spots and/or change in the leaf's normal pigment including the shape either normal or deformed were also assessed.

Plant Tissue Analysis

The collected leaf samples of both species were grounded and oven-dried at 80°C and undergo tissue analysis to determine the nutrient content and/or status at FAST Laboratories in Cagayan de Oro.

Statistical Analysis

For comparison of the leaf morphological variables such as leaf length, leaf width, leaf area and petiole length between different species in two site categories, an independent t-test was performed through SPSS to determine if the leaf samples in polluted area significantly vary from that of non-polluted area.

Results and discussion

Leaf Morphometrics

Table 1 shows the leaf morphological measurements *P. indicus* forma *echinatus* and *S. macrophylla* in both polluted and non-polluted area. Based on the results, the leaf morphological characteristics vary among species, between the same species and between study sites. For instance, *P. indicus* forma *echinatus* found in non-polluted area has the highest average in all the variables measured such as the leaf length, width and area compared to the same species found in polluted area. The same trend was also observed on *S. macrophylla*.

Leaf Length

For *P. indicus* forma *echinatus*, result showed that the leaf length between polluted and non-polluted area vary slightly with 3.9% difference between polluted and non-polluted area. However, for *S. macrophylla* the leaf length differs greatly between two sites, length of leaves in non-polluted area is 60.8% longer compared to polluted area. In fact, there is a great disparity between the polluted and non-polluted area for the six (6) sampled trees.

Table 1. Average leaf measurements of *P. indicus* forma *echinatus* and *S. macrophylla* in both polluted and non-polluted area.

Species	Tree No.	Average Length (cm)		Average Width (cm)		Average Area (cm²)	
		PA	NPA	PA	NPA	PA	NPA
P. indicus forma *echinatus*	1	11.2	11.13	5.18	7.24	38.5	54.74
P. indicus forma *echinatus*	2	9.23	10.21	5.43	7.25	34.26	54.15
S. macrophylla	1	11.34	14.06	4.40	4.30	34.71	41.29
S. macrophylla	2	7.11	15.75	2.87	5.07	13.44	58.76
S. macrophylla	3	12.88	18.33	4.30	5.26	41.31	63.84
S. macrophylla	4	10.75	17.30	4.11	5.49	27.50	67.05
S. macrophylla	5	8.03	17.48	3.21	5.83	19.11	74.40
S. macrophylla	6	12.06	17.03	4.09	5.96	35.52	73.28

Leaf Width

A disparity in the average width of *P. indicus* forma *echinatus* between the polluted and non-polluted area was also observed. In non-polluted area, the average width is wider by 36.8% compared to *P. indicus* forma*echinatus* in polluted area which was relatively narrower. For the *S. macrophylla*, the non-polluted area has 38.9% wider average width compare to *S. macrophylla* found in polluted area.

Table 2. Leaf color and shape of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Species	Tree No.	General Leaf Color		General Leaf Shape	
		PA	NPA	PA	NPA
P. indicus forma *echinatus*	1	Light Green	Green	Lobed	Typical
P. indicus forma *echinatus*	2	Light Green	Green	Lobed	Typical
S. macrophylla	1	Waxy green	Dark Green	Typical	Typical
S. macrophylla	2	Waxy green	Dark Green	Typical	Typical
S. macrophylla	3	Waxy green	Dark Green	Typical	Typical
S. macrophylla	4	Waxy green	Dark Green	Typical	Typical
S. macrophylla	5	Waxy green	Dark Green	Typical	Typical
S. macrophylla	6	Waxy green	Dark Green	Typical	Typical

Leaf Area

In the non-polluted sites, the average leaf area of *P. indicus* forma *echinatus* was larger by 46.7% compared to the same species found in polluted area. The same trend was observed with the *S.macrophylla* wherein the average leaf area in the polluted sites was 28.60cm while the non-polluted area was 63.10cm, this accounts a 120.6% average leaf area difference of *S. macrophylla* between the two study sites.

Table 3. NPK leaf nutrient contents of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Species	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	PA	NPA	PA	NPA	PA	NPA
P. indicus forma *echinatus*	3.60	2.50	0.13	0.18	2.51	0.77
S. macrophylla	1.46	1.76	0.10	0.10	1.15	1.14

In general, the results showed that the leaf length, width, and area vary greatly between the polluted and non-polluted area. Comparison of the results between leaf length measurement of *P. indicus* forma *echinatus* and *S. macrophylla* found in polluted area and non-polluted area indicated that the species length of leaves reflected the environment where it was planted.

Species in polluted area tend to have shorter leaves than those in non-polluted area. This is due to abiotic stresses dominantly caused by air pollution. According to Syyenejad *et al.* (2009), one way to increase tolerance in contrast with stress is by balancing water content of tissues by decreasing leaf area.

The interactions between plants and different types of pollutants were investigated by several authors such as Sikora and Chappelka (2004); Myers (2015); Ekpemerechi *et al.* (2014); Snejana (2004); Neverova *et al.* (2013); and Gostin (2009), all came up with the same findings that reduced leaf area is the most common observation.

The reaction of different species to the altered environmental conditions is strongly correlated with its structural and functional features. Under the action of pollutants, leaves develop different morphological changes. Plants usually adapt to high pollutant concentrations and unfavorable environmental conditions which is likely to result in different morphology (Gostin, 2009).

Table 4. Macronutrient requirement of plants.

Interpretation	Nitrogen (N)	Phosphorus (P)	Potassium (K)
Deficient	<2.2	<0.09	<0.40
Low	2.2 - 2.3	0.09 - 0.11	0.40 - 0.69
Optimum	2.4 - 2.8	0.12 - 0.16	0.70 - 1.09
High	2.7 - 2.8	0.17 - 0.29	1.10 - 2.00
Excess	> 2.8	> 0.30	> 2.30

Source: Agriculture and Food Development Authority.

A similar study of Saadullah *et al.* (2013) revealed that lower percentage of leaf length at polluted site as compared to non-polluted site might be due to effect of air pollution at that site which reduces the gases exchange for photosynthesis and productivity of leaf.

While plant growth generally depends on leaf size since it facilitates photosynthesis, pollutants entry via leaf stomata damages photosynthetic tissues resulting to a reduced leaf size. Moreover, according to Svetlana *et al.* (2010), different environmental conditions are always reflected to plants.

Table 5. Independent t-test Analysis of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Species	Average Leaf Length (cm)			Average Leaf Width (cm)			Average Leaf Area (cm^2)			Average Petiole Length (cm)		
	PA	NPA	p-value	PA	NPA	p-value	PA	NPA	p-value	PA	NPA	p-value
*P. indicus*forma *echinatus*	10.26	10.67	.239^{ns}	5.3	7.25	.000**	36.38	54.45	.000**	9.19	4.82	.001**
S. macrophylla	10.36	16.66	.000**	3.83	5.32	.000**	28.6	63.1	.000**	6.41	10.8	.000**

ns not significant

** highly significant.

Plant injury caused by air pollution is common near large cities. The type and concentration of pollutants, distance from the source, length of exposure, and meteorological conditions are among the factors that govern the extent of damage. Tiwari *et al.* (2008) stressed that automobile exhaust pollution was more damaging than the industrial pollution. For some pollutants, damage can occur at levels below Environmental Protection Agency (EPA) standards. Sikora and Chappelka (2004) mentioned about the damages of air pollution to plants included the following such as injured leaves develop light white streaks on either side of the mid-vein, on some species, chronic injury causes brown to reddish brown or black blotches, including burning at leaf apex (Fig. 2).

Petiole Length

Result in petiole length measurement of *P. indicus* forma *echinatus* revealed that the petiole in polluted area is longer by 90.6% than those in non-polluted area. However, in the case of *S. macrophylla*, the petiole length was longer in the non-polluted area than those found in polluted area with a difference of 68.5% between the two sites.

The results showed that species have different reactions towards certain areas. As mentioned by Ekpemerechi *et al.* (2014), the effects of pollution on the morphology of plants are quite diverse depending on the constituents of the pollutants as well as the plant itself. The *S. macrophylla* found in polluted area has shorter petiole length compared to non polluted area. Significant reduction in length, breadth, area of leaves and length of petiole which were collected from plants growing in polluted area has also been reported by Jahan and Iqbal (1992); Koochak and Syyednejad (2013).

Table 6. Independent t-test Analysis between *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Category site	Average leaf Length (p-value)	Average leaf Width (p-value)	Average leaf Area (p-value)	Average petiole Length (p-value)
Polluted Area (PA)	$.671^{ns}$	.000**	.000**	$.699^{ns}$
Non-Polluted Area (NPA)	.000 **	.000**	.002 **	.000**

ns not significant

** highly significant.

This trend of reduction has also been recorded in a species growing near a fertilizer plant with high concentrations of SO_2 and NO_2 (Anbazhagan *et al.*, 1989). Based on Cagayan de Oro City Clean Air Forum Report (2015), NO_2 is among the major pollutants present in the polluted area of the study site.

In the urban environment NO is oxidized to NO_2through reaction with ozone (O_3). In close to large sources of NO, such as busy road junctions and street canyons, the supply of O_3 rapidly exhausts and a large proportion of the NO left is unoxidized (Honour *et al.*, 2009). This leads to characteristically high levels of this pollutant in the area.

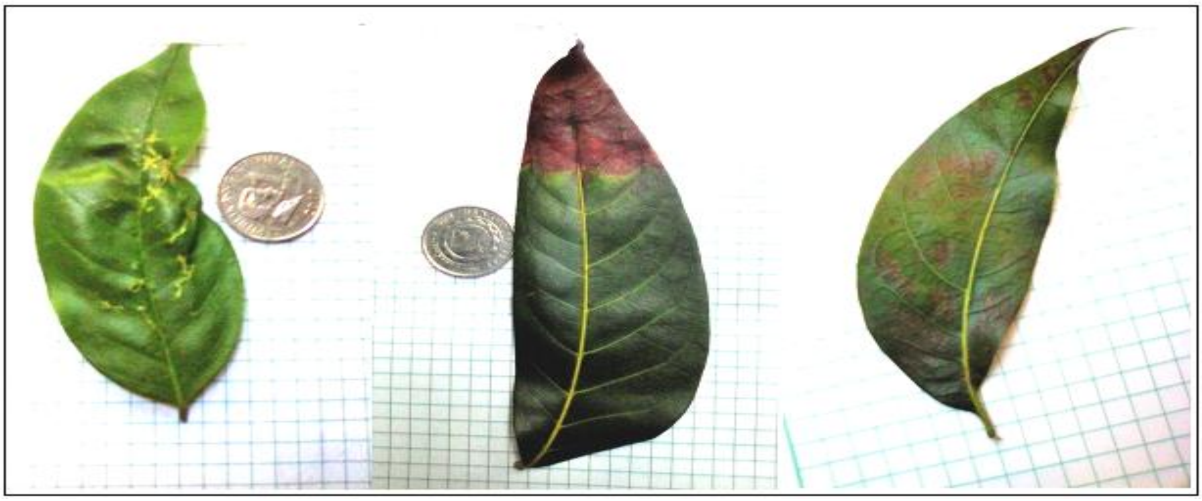

Fig.2. Leaf injuries of exhibited by the species under study in polluted site.

Leaf Color and Shape

Table 2 shows the leaf shape and color of the species under study in both polluted and non-polluted area. Species in polluted area showed significant difference compared to species found in the non-polluted area.

The color of leaves vary from light green in polluted area to generally green in non-polluted area for *P. indicus* forma *echinatus* while most of the leaves of *S. macrophylla* in polluted area are green and waxy while in non-polluted area the leaves are generally dark green.

Moreover, majority of the leaves of *P. indicus* forma *echinatus* were deformed and lobed-shape in polluted area while those found in non-polluted area exhibit typical leaf shape of ovate to elliptic with an acuminate apex. For both sites, the *S. macrophylla* has typical leaf shape, but those found in polluted areas are much smaller than those in the non-polluted sites.

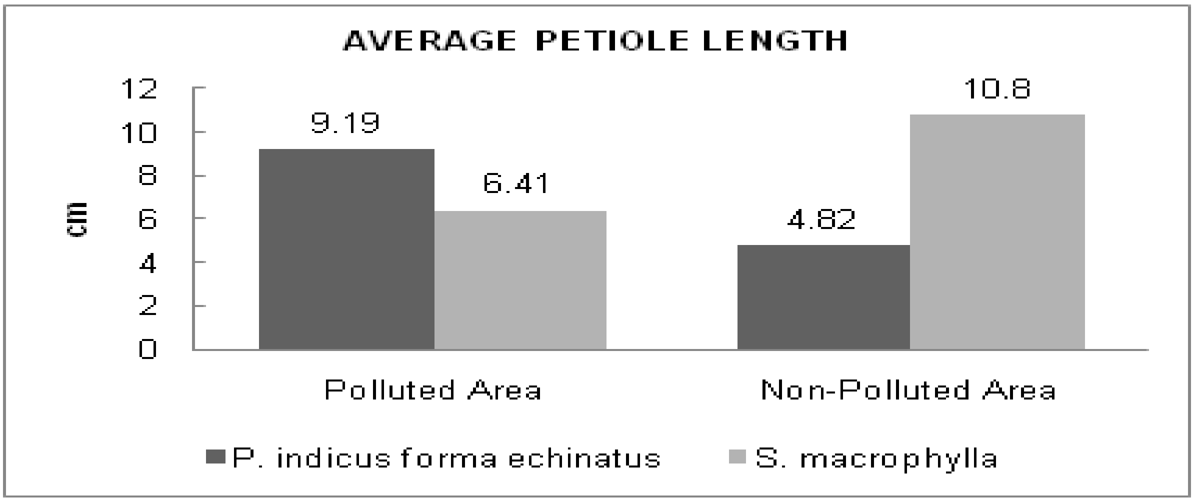

Fig. 3. Petiole length of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Leaf colors of a plant can be used to identify stress level due to its adaptation to environmental change.

Fig. 4. Deformed leaves of *P. indicus* forma *echinatus*in polluted site.

The surface of every leaf is characterized by a cuticle, it serves as the barrier between the interior of the leaf and the external environment. Since cuticle covers the surface of leaves, it serves as a sensitive and early indicator of exposure to air pollutants.

Fig. 5. Waxy and slighltly lobed leaf of *Sweitenia macrophylla* in polluted site.

Typically the symptoms of leaf exposure to air pollution includes fusion of wax crystals that eventually forms continuous layers of wax on top of the leaf which may appear more shiny (Berg, 1989).

This implies that *S. macrophylla* in polluted area are more exposed to air pollutants since it exhibited a more waxy appearance on its leaf surface compared to those found in non-polluted area. According to World Health Organization (2000), cuticle is recognized as an evidence of limitation for leaf uptake of SO_2 and other form of pollutants in polluted areas.

Fig.6. Leaf sample of *P. indicus* forma *echinatus* in non-polluted site.

While leaves of all the species under study are green, the species found in polluted area are relatively lighter. This implies that the chlorophyll pigment on leaves of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted area might be affected by air pollution. According to Giri *et al.* (2013), the photosynthetic pigments are the most likely to be damaged by air pollution and further explained that that under stress they may undergo several photochemical reactions such as oxidation, reduction, and reversible bleaching.

Plant Tissue Analysis

Table 3 shows the percentage nutrient leaf content of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted and non-polluted sites.

Results show that *P.indicus* forma *echinatus* has the highest nitrogen, phosphorus and potassium uptake in both polluted and non-polluted sites.

Based on the optimum NPK requirement of plants (Table 4), *P. indicus* forma *echinatus* found in polluted area exceeds the N requirement while it is high in non-polluted area. This observed values is because *P. indicus* forma *echinatus* is a leguminous species and that it could able to fix nitrogen efficiently compared to *S. macrophylla*. Phosphorus uptake of *P. indicus* forma *echinatus* is at optimum level in both polluted and non-polluted area while the potassium is high in polluted area and optimum in non-polluted area.

S. macrophylla is nitrogen deficient and low in phosphorus in both of the study sites although it is high in potassium. This is because *S. macrophylla* is not a leguminous species which are able of nitrogen-fixing.

Nitrogen is largely responsible for the growth of leaves, thus species deficient of N exhibits smaller leaves and a light-green or yellowing of foliage. On the other hand, when P is limiting, the most striking effects are a reduction in leaf expansion and leaf surface area, as well as the number of leaves (Agriculture and Food Development Authority, 2011). The plant tissue analysis indicated that the nutrient uptake of *P. indicus* forma *echinatus* and *S. macrophylla* vary depending on the sites. It can be observed that *P. indicus* forma *echinatus* is able to acquire more nutrients compared to *S. macrophylla* in both polluted and non-polluted area. This implies that *P. indicus* forma *echinatus* is more resistant to an air polluted environment compared to *S. macrophylla*.

Statistical Analysis

Table 5 shows the overall average leaf measurements and statistical analysis results of *P. indicus* forma*echinatus* and *S. macrophylla* in both polluted and non- polluted area. Based on the independent t-test results, all variables measured such as leaf length, width, area and petiole length of species under study are significantly different at $p<0.005$ level of significance with the exception of the leaf length of *P. indicus* forma *echinatus* with which the result is at 0.239. Nevertheless, all independent t-test results indicate that variables among two study sites vary significantly at $p<0.005$ from each other.

Fig. 7. Leaf sample of *S. macrophylla* in non-polluted site.

Table 6 shows the independent t-test analysis results between *P. indicus* forma *echinatus* and *S. macrophylla*. All variables vary significantly between the two species at $p<0.001$ level of significance except for the leaf and petiole length measurements. This implies that the leaf and petiole length of *P. indicus* forma *echinatus*is not statistically different to that of *S. macrophylla* in polluted area.

Conclusion

Based on the results, air pollution has profound effects on the leaf morphology of the species under study. The leaf length, width and area, including petiole length of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted area is significantly lower compared to the same species in non-polluted area. The leaf morphometrics of *P. indicus* forma *echinatus* and *S. macrophylla* in polluted area vary statistically at $p<0.005$ from those found in non-polluted area except the leaf petiole length of *P. indicus* forma *echinatus*. Evident damages of air pollution in species foliage include appearance of white streaks on the leaves of *P. indicus forma echinatus* and burned leaf apex of *S. macrophylla* both found polluted area. In terms of ecophysiology, air pollution seemingly affects the photosynthesis as indicated by lighter leaf color of species found in polluted area. In general, there is clear evidence that the variation on all the leaf-size indices investigated for *P. indicus forma echinatus* could be directly attributed to the effect of air pollution since tissue analyses revealed that *P. indicus* forma *echinatus* is not nutrient deficient. On the other hand, the leaf morphological variation including the coloration of *S. macrophylla* may not be wholly associated to the effects of air pollution because the nutrients deficiency as revealed via tissue analysis could not be totally disregarded.

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