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Research article

MORPHO-ANATOMICAL CHARACTERIZATION OF *TITHONIA DIVERSIFOLIA* (HEMSL.) GRAY GROWING ON SITES EXPOSED TO VEHICULAR EMISSIONS

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ABSTRACT: Vehicular emissions serve as the major driver of the deteriorating air quality in Baguio City, Philippines, and air pollutants from these emissions are known to affect both plants and animals including humans. This study aimed to investigate the effects of vehicular emissions on the morpho-anatomy of the aerial vegetative parts of *Tithonia deiversifolia* (Hemsl.) Gray, a plant usually observed along roadsides of Baguio City. Plants samples were taken from areas selected based on the intensity of vehicular emissions characterized by the volume of traffic. This study presents the effects of vehicular emissions on leaf length, internode length, trichome length, trichome density, thickness of the epidermis, and, for the first time, on number of vascular bundles.
Keywords: *Tithonia diversifolia*, vehicular emissions, plant morpho-anatomy, biomonitors, air pollution, roadside plants

INTRODUCTION

The worsening condition of air quality is a growing concern since exposure to air pollutants has been known to cause respiratory illnesses. Equally vulnerable, though, are the vegetations that are constantly exposed to air pollution, thus are commonly used as biomonitors for air quality [29]. Plants tend to absorb and accumulate heavy metals such as Pb, Zn, Cd, Cu, Fe, Ca and Mg [3, 6, 15,]. The responses of plants to these pollutants were commonly investigated using morphological and anatomical parameters, such as stomatal characteristics, trichome characteristics, cuticular features, epidermal features, leaf size and internode length [8, 9, 21, 25, 26, 29]. The state of the air quality in Baguio City, Philippines is affected by the its growing population. Based on the May 2010 data of the National Statistics Office (NSO), the population in the city dramatically increased from 183,000 in 1990 to 318,676 [2]. Consequent to the increasing population is the increase in vehicle use in the city, which contributes 65% of the pollutants present in the air [7]. It was also noted by Ward et al. [27] and Motto et al. [12] that these pollutants are found to be present as far as 100 m from the source of emissions. In Baguio City, *Tithonia diversifolia* (Hemsl.) Gray, locally known as marapait, thrives in areas with varying degree of traffic volume. This leads to differential exposure of *T. diversifolia* to vehicular emissions. *T. diversifolia* has been described as an effective agent for remediating heavy metals by accumulating Pb, Cu, and Cd in its roots and leaves [1, 15]. The effect of these heavy metals in the morphology and anatomy of the plant was not established, except for the pigment concentration in the leaves [15] which shows no significant differences between those plants exposed in heavy traffic and those exposed in light traffic. This study aimed to characterize the morpho-anatanomy of the vegetative aerial parts of *T. diversifolia* exposed to heavy urban traffic and compare it with the morpho-anatomy of those that are found in sites with light traffic volume.

METHODOLOGY

The study sites were selected based on the intensity of vehicular emissions, which is a function of the volume of traffic in these areas. The roadsides of UP Drive or UPD (16^{0}24’18.50” N, 120^{0}35’54.16” E) and Military Cut-off or MC (16^{0}24’05.99” N, 120^{0}35’53.30” E) were categorized as sites with high and medium exposure to vehicular emissions respectively, and South Drive or SD (16^{0}24’28.36” N, 120^{0}36’27.23” E) as the site with light exposure to vehicular emissions. The difference on vehicular volumes of these sites was found statistically significant. In a 24-hour cycle, UPD had an average vehicular volume of 48,063, while SD had an average vehicular volume of 3,458. Ten plants of *T. diversifolia*, all about one meter high, were sampled from each of the sites. Collected samples were washed with water and fixed using FAA.

Morphological analysis

The fifth to seventh expanded leaves from the shoot apex were taken for leaf length measurement, and the internode below each leaf was also measured. A total of 30 leaves and internodes per site were measured using a ruler.

Microscopy

The third to fifth expanded leaves from the tip of each plant were prepared for microscopic analysis. The characters observed using light microscope were trichome length, trichome density, thickness of the epidermis and number of vascular bundles. Trichomes were scraped from the leaf surfaces, and were measured using a calibrated ocular micrometer. Trichome density was calculated by counting the number of trichomes found in a 1 mm transect line across the leaf lamina. This was done three times using different spots in every leaf lamina. In each plant, the internode below the 7^{th} leaf was taken for stem anatomical observation. Samples were embedded in paraffin wax, sectioned, stained, and mounted. Counting of vascular bundles was done using 100x linear magnification. The thickness of the upper epidermis was observed in mounted leaf cross sections. Measurement was taken using a calibrated ocular micrometer.

Statistical Analyses

The data were analyzed using ANOVA followed by Tukey HSD. ANOVA was used to find if there are significant differences in the morpho-anatomical characteristics of *T. diversifolia* found in each site, while the Tukey HSD test was used to identify the degree of difference between significantly different sites.

RESULTS AND DISCUSSION

Leaf length

Results showed that shortest leaf length was observed among plants along UPD while the longest leaf was observed among plants along SD (Table 1). However, statistical analysis showed no significant difference on the length of the leaves collected from the three sites. This statistically not significant result contradicts the observations of Jahan and Iqbal [11] in which leaf breadth and area of leaves and length of petioles of *Ficus benghalensis, Guaiacum officinale* and *Eucalyptus* sp. are reduced due to exposure to vehicular emissions. On the other hand, the result in this study is parallel to what is seen in the study of Olivares [15] that leaves of *T. diversifolia* exposed to heavy urban traffic and were found to be lead-contaminated did not show visible symptoms of damage. Moreover, the ability of some other plants to remain normal and healthy in terms of general morphology even exposed to vehicular emissions has also been observed by Pal *et al.* [16] in *Asparagus racemosus*, *Azadirachta indica, Bougainvillea spectabilis, Cassia fistula, Ficus religiosa, Nerium indicum, Polyalthea longifolia,* and *Thevetia nerifolia.*

Internode length

Similar to the trend observed in leaf length, the internode length tends to increase as the level of exposure to vehicular emissions decreases. Plants found in UPD had the shortest internode length while plants found in SD were observed to exhibit the longest internode (Table 1). Based on statistical analysis, there is a significant difference between the internode length of plants in UPD and the plants in the other two sites. The reduction of internode length was observed in the studies using *Populus sp.* conducted by Woodbury et al. [25] and using *Commelina bengalensis* by Mishra [14]. In both studies, the reduction in internode length was attributed to air pollution. The same trend was also observed by Przedpełska & Wierzbicka [17] where *A. arenosa* growing in lead-zinc waste heap was found to have reduced growth. Moreover, it was reported by Matsushima and Brewer [13] that reduced internode length has been found as a response to elevated sulphur dioxide level. Rai and Kulshreshtha [18] stated that high tolerance to air pollution has metabolic costs and one of which is reduced growth. It is possible that the plant has to allocate photosynthate to leaves at the expense of other organs [28] such as the stem, thus reducing the internode length and the total linear growth of the plant while maintaining leaf production. In this study, it was observed that *T. diversifolia* in UPD that reaches the height of one (1) meter produced more leaves compared to those in MC and SD.

Trichome Length

Reduced adaxial trichome length has been observed in plants exposed to high vehicular emissions (Table 1). This result is parallel to the findings of Gupta and Ghause [10] using *Solanum melangona* exposed to coal-smoke pollutants, and to the results of Rao and Dubey [19] using tropical plants exposed to heavy metals present in polluted air. The result of Tukey test shows no significant difference between MC and UPD, while SD has significantly longer trichomes as compared to the other sites. Statistical tests also show that there is no significant difference in the trichome length in the abaxial side of the leaves. Aerial particulates are collected and trapped on the surfaces of the leaves, thus affecting the growth of trichomes on the adaxial side and causing no effect on the abaxial trichomes.

Trichome density

The average number of trichomes on the adaxial surface of the leaves in MC is significantly lower than those in SD. The difference in trichome density in UPD to either SD and MC is found to be statistically not significant. The high density of trichomes observed in populations exposed to high vehicular emissions is a common response in polluted environment, as proven by previous studies [22, 23, 24]. This will help in trapping the air particulates and prevent the upper leaf tissues from direct injury. Azmat et al. [5] also observed trichomes to release excess Pb to the environment thus protecting the plant against toxicity. In this study, UPD, the one exposed to heavy vehicular emissions had statistically the same trichome density as those populations found in SD which are exposed to light vehicular emissions. Other factors may contribute to higher trichome density of the populations in South Drive, such as defense against herbivory [30, 20]. Since the area was less disturbed and had thicker associated vegetations, herbivores are suspected to be present in the area, and these avoid surfaces with high level of trichomes [4].

Thickness of adaxial epidermis

Thinner epidermal layer was observed in sites with exposure to heavy vehicular emissions. The epidermal cells in the adaxial side of the leaves were found to be thicker in SD compared to UPD and MC (Table 1). In the abaxial epidermis, all sites show different mean thickness, however these were not statistically different from one another. The same effect of vehicular emissions was observed by Jahan and Iqbal [11] using *Eucalyptus sp.* and by Rai and Kulshreshta [18] using *Calotrois procera* and *Nerium indicum*.

Table 1. Morpho-anatomical characteristics of *T. diversifolia* found in areas with varying degree of vehicular emissions.

Parameters	UPD	MC	SD	ANOVA	Tukey test (0.05 α level)
Leaf length (cm)	28.46	20.014	29.468	0.823	ns
Internode length (cm)	7.043	2.9	5.61	0.000	UPD < [MC = SD]
Trichome length (μm)					
Adaxial	326.183	282.459	540.566	0.001	SD > [MC = UPD]
Abaxial	515.21	525.486	594.828	0.167	ns
Trichome density					
Adaxial	2.185	1.481	2.926	0.005	SD > MC
Abaxial	5.556	3.37	4.926	0.005	[UPD = SD] > MC
Leaf epidermal thickness (μm)					
Adaxial	10.463	9.259	12.87	0.001	SD > [MC = UPD]
Abaxial	11.944	11.3	14.63	0.068	ns
Number of vascular bundles	34.852	29.481	32.111	0.001	UPD > MC

ns = not significantly different between sites

Stem vascular bundles

The effect of pollution on the number of vascular bundles was used for the first time as a parameter in this kind of study, and results were significant. Populations of *T. diversifolia* exposed to heavy vehicular emissions had greater number of vascular bundles. The average number of vascular bundles in UPD was significantly higher compared to MC, while SD was not significantly different compared to either MC or UPD. The presence of greater number of vascular bundles in plants exposed to heavy vehicular emissions may have developed for better transport mechanisms, since contaminants such as Pb may physically block water uptake [5].

CONCLUSION

Plants used as biomonitors exhibit changes in their morphology and anatomy when exposed to varying conditions in their environment such as when there is air pollution. Vehicular emission is one of the major sources of air pollutants. These pollutants may induce plant responses leading to observable changes in their morphology and anatomy. Plant responses may vary depending on the amount of pollutants to which they are exposed.

In this study, *T. diversifolia* found in selected sites of varying level of exposure to vehicular emissions showed several changes on morpho-anatomy. A variation in leaf length was noticed, but this was not statistically significant. Internode length was shorter in populations exposed to high vehicular emissions. Reduced linear growth seemed to be a common response to presence of pollutants, and appeared to be an adaptation as they allocate more of their photosynthate to the leaves in expense of other organs such as the stem. Reduced trichome length was observed in populations exposed to high vehicular emissions. Greater trichome density was also observed in the same populations of *T. diversifolia*. Such an observation may be attributed to the fact that trichomes play a protective role by trapping the particulates that may induce direct injury to leaf surfaces. It is also noteworthy that the trichome density of *T. diversifolia* in sites with light vehicular emissions is not significantly different with those described above. This observation may be seen as response against herbivory. Adaxial leaf epidermis was found thinner in populations exposed to high vehicular emissions, similar to results of previous studies. This study presented the use of number of vascular bundles as parameter in describing the effect of vehicular emissions to plants. The number of vascular bundles was greater among plants exposed to high vehicular emissions. This is to facilitate more efficient transport mechanism as certain pollutants, such as lead may block the transport of water.

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