

Response of roadside tree leaves in a tropical city to automobile pollution

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Abstract

One of the sources of air pollutants in the surrounding environment is the automobile emissions. Automobiles produce gaseous and particulate matters which are toxic and inflict damage to roadside plants. Roadside trees are notable for the absorption, sequestering of contaminants and the effective interceptor of airborne pollution. In view of this, the present work was based on investigating the macro-morphological and micro-morphological changes that boost the tolerance and continued existence of four roadside trees, namely *Ficus platyphylla*, *Mangifera indica*, *Polyalthia longifolia* and *Terminalia cattapa* in the incidence of vehicle exhaust emissions in Kumasi Metropolis, Ghana. Three arterial roads representing three different traffic volumes of extreme, heavy and severe were considered as observational sites. Kwame Nkrumah University of Science and Technology Campus was selected as the control site. The macro-morphological characteristics of the four tree species showed reduced leaf area, whilst the micro-morphological results revealed that stomata size, number and index were reduced at the arterial roadsides in all the four tree species. There was increased epidermal cell number and length and trichome length at the polluted arterial roadsides when compared to the control. These variations can be considered as pointers of environmental stress and could be used as indicators of urban air pollution.

Keywords: bioindicator; leaf dimension; roadside tree species; stomatal dimensions; vehicular emissions

Introduction

Exhausts from vehicles are one of the sources of air pollution that generates approximately 70% of all harmful emissions (Balat and Balat, 2009). Vehicles emit pollutants such as CO₂, CO, oxides of nitrogen, SO₂, hydrocarbons, unburnt petrol and carbon particles (Wang and Xie, 2009; Bhandarkar, 2013). These gaseous and particulate pollutants arising from the vehicle exhaust are toxic and causes damage to roadside trees (Joshi and Swami, 2009). Roadside plants are noteworthy in the absorption, sequestering of pollutants and a proficient interceptor of airborne contaminants. Studies have recorded changes in plants caused by wide range of environmental pollutants, and the vast majority of these works allude to physiological modifications (Patra and Sharma, 2000). The morphological modifications that occurs in plants resulting from air pollution are changes in stomatal and epidermal cell size, lower recurrence, thickening of cell wall, epicuticular wax deposition alterations and chlorosis (Uka *et al.*, 2017). Several studies in this area have been conducted to

investigate morphological, structural, physiological and biochemical variations in trees (Pourkhabbaz *et al.*, 2010; Maheswari, 2012; Pandey *et al.*, 2015; Lohe *et al.*, 2015; Deepika and Haritash, 2016). Surface leaf characters, including stomata and epidermal cells, have been reported to be drastically altered in plant species growing along the roadside due to the stress of car exhaust pollution with high traffic intensity in urban areas (Rai and Mishra, 2013). A few authors consider foliar epidermis as a bioindicator of environmental quality (Alves *et al.*, 2008; Balasooriya *et al.*, 2009). Srivastava (1999) observed that general plant development was influenced with serious mutilations in foliar epidermal characters. It likewise brought up the significance of the cuticle and epidermal features in the determination of resistance/ sensitivity of each species to environmental pollutants.

Gostin and Ivanescu (2007) observed structural and micro-morphological traits of the stomata in *Salix alba* leaves due to air pollution. Kapoor *et al.* (2014) reported that reduction in size of epidermal cell and stomata, and increment in the quantity of epidermal cells, stomata and trichomes occur on exposure to SO₂ contamination. *Platanus orientalis* leaves subjected to automobile air pollution had reduced stomatal thickness and stomata widths than those leaves from the urban area (Pourkhabbaz *et al.*, 2010). It was also observed that vehicular air pollution demonstrated checked modification in epidermal attributes, with decreased number of stomata and epidermal cells per unit zone, while length and width of stomata and epidermal cells increase were observed thus its usage as biomarkers of auto pollution (Verma and Chandra, 2014). The use of tree leaves as accumulative biomonitors of air pollution is of great environmental significance (Bargagli, 1998). The leaves go about as air pollution receptors and biological absorbers or filters of pollutants (Free-Smith *et al.*, 2004), understanding responses that varies with different plants species to air pollution, would be better with utilising some of the selected tree species. As traffic and air pollution persists in the Kumasi Metropolis, attention is geared towards ameliorating the problems of environmental pollution. Morpho-anatomical adjustments are promising means to measure the air quality of the urban habitat (El-Khatib *et al.*, 2011). Scientists have likewise reported that plants developed along roadsides showed impressive harm because of vehicular air pollution (Iqbal and Shafiq, 1999; Shafiq and Iqbal, 2003; Shafiq and Iqbal, 2005). In this study, leaves from *Ficus platyphylla*, *Mangifera indica*, *Polyalthia longifolia* and *Terminalia catappa* growing in the arterial road sites of Kumasi Metropolis with distinctive gridlock were collected to determine the adverse effect of vehicular pollutants on their macro-morphological and micro-morphological traits of their leaf epidermal structure.

Materials and Methods

Study area

Kumasi is the second biggest city in Ghana. It is situated around 270 km north of the national capital, Accra, 397 km south of Tamale (Northern Regional capital) and 120 km South-east of Sunyani (Brong Ahafo Regional capital). Kumasi is situated between latitude 6.35° - 6.40° and longitude 1.30° - 1.35° and has a land area of 254 km². The minimum temperature in the area is around 21.5 °C with the maximum temperature of 33.7 °C. Kumasi- Accra, Kumasi- Mampong and Kumasi-Offinso roads were selected for sampling because these major roads experience extreme congestion using average vehicle speed as a parameter. These major arterial roads: Accra road I (Arterial road I); Offinso road (Arterial road II) and Mampong road (Arterial road III) representing three different traffic volumes, were considered as polluted areas, while Kwame Nkrumah University of Science and Technology Campus was selected as the control for the purposes of comparison (Table 1).

Tree species and collection of samples

Four tree species (*Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia*) were selected for the study. The basis of their selection is anchored on easy recognition, high distribution and abundance along key arterial roads in the Kumasi Metropolis. Triplicate samples of leaves of *Ficus platyphylla*,

Mangifera indica, *Polyalthia longifolia* and *Terminalia cattapa* were collected from each sampling site. Twenty (20) physiologically active fully expanded leaves, third from the tip of phyllotaxis position were collected from each of the four tree species (three individuals per species). The Total sample size of 240 leaves per sampling site was collected for the morphometry and micromorphometry analyses. Sampling intervals between each tree species replicate ranged from 2.0 to 4.8 km along each road. Leaf samples were collected between the months of August-November, 2015 before the onset of the harmattan season in December when trees shed their leaves.

Table 1. Description of sampling sites in Kumasi Metropolis

Sampling site (Arterial roads)	Length of road (km)	Average daily traffic per lane	Congestion category	Latitude (N)	Longitude (W)	Classification
Arterial road I	14.3	43, 403	Extreme	6°41'20.85''	1°36'50.79''	Extreme traffic congestion
Arterial road II	8.1	19, 998	Heavy	6°46'43.01''	1°35'43.30''	Heavy traffic congestion
Arterial road III	6.1	24, 269	Severe	6°43'52.65''	1°37'40.74''	Severe traffic congestion
Control	3.1			6°40'38.28"	1°34'24.24"	Control

Morphometry studies

The measurement of leaf length was from leaf tip along the midrib of the leaf lamina to the leaf base at the point of attachment of the lamina to the petiole. The leaf breadth was measured along the widest breadth across the lamina. The leaf area was determined using a graph paper. The margin of leaf samples was traced on graph sheets with 1 mm² square cells. Leaf area was determined by counting the number of squares enclosed in each tracing (Ogoke *et al.*, 2009).

Micromorphometry studies

The leaf samples were thoroughly washed with tap water to eliminate dust particles from their surfaces. The leaves were allowed to dry and leaf epidermal peel slides were made by the methods of lasting impressions (Rai and Mishra, 2013; Otuu *et al.*, 2015). On the sampled leaves 1 cm² portion of the leaf abaxial and adaxial surfaces were painted with a thick layer of clear nail polish and left to dry for 10 minutes. Second and third coatings were successively applied at 5 minutes intervals and then left to completely dry for 30 mins. Epidermal strips of the leaf samples were scrape up gently using forceps and placed in drops of glycerine on clean microscopic slides. They were covered with cover slips and viewed under the light microscope at both low and high-power magnification. Photomicrographs were taken with Leica DM750 Microscope at different magnifications. Measurements were performed on ten observations. The stomata length and breadth, epidermal cell length and length of trichomes were measured with the aid of the stage ocular micrometer. Stomatal and epidermal cell numbers (densities) were observed and counted per microscopic field view diameter of 1mm² on the epidermal strips of sampled leaves. Stomatal size (SS) was calculated using the equation of Franco (1939). Stomata size = Length × breadth × K, where K (Franco's constant = 0.78524). Stomatal index (SI), which is the number of stomata present in a unit area of leaf in percentage, was calculated according to Salisbury (1927) as follows: $S.I\% = SD/(SD+ECD) \times 100$

where SD is stomatal density; ECD is epidermal cell density.

The diurnal analysis of CO, NO₂, SO₂ and VOC was monitored in the sampling sites using Aeroqual Series 500 (S500) gas monitors (Aeroqual Limited, Auckland, New Zealand). The Aeroqual monitors were placed at 1.5 m elevation above the ground. The Aeroqual Monitors were programmed to record 5 min average concentrations of the monitored air quality parameters continuously for 8 hrs at three points in each sampling

site. The 5 min averages were summed up to hourly means. The ambient air quality in each site was monitored for 6 days in week for three months.

Data analysis

Multiple regression analysis was carried out using Statistica software version 7.0 for the evaluation of relationship between air quality parameters and Micro-morphological features. Furthermore, One-way analysis of variance (ANOVA) was conducted to test for differences in plant morphological and among the different roads; this analysis was conducted to reinforce the regression analysis.

Results

Air quality of the studied sites

The ambient CO, SO₂, NO₂ and VOC concentrations in the arterial roadsides and the control is as presented in Table 2.

Table 2. Ambient air quality of Kumasi Metropolis during the study period

Parameter/ Sampling sites	CO (ppm)			SO ₂ (ppm)			NO ₂ (ppm)			VOC (ppm)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Arterial road I	5.64	11.06	7.96 ± 1.62 ^b	0.14	0.16	0.15 ± 0.01 ^b	0.07	0.08	0.08 ± 0.00 ^a	0.08	0.20	0.12 ± 0.29 ^a
Arterial road II	6.49	7.02	6.81 ± 0.16 ^b	0.18	0.25	0.21 ± 0.02 ^b	0.06	0.10	0.08 ± 0.01 ^a	0.04	0.10	0.07 ± 0.02 ^a
Arterial road III	5.16	5.38	5.26 ± 0.06 ^b	0.20	0.23	0.21 ± 0.01 ^b	0.09	0.10	0.10 ± 0.00 ^a	0.08	0.09	0.08 ± 0.00 ^a
Control	0.38	1.19	0.85 ± 0.24 ^a	0.03	0.11	0.07 ± 0.02 ^a	0.05	0.07	0.06 ± 0.01 ^a	0.00	0.02	0.01 ± 0.00 ^a

Mean ± SE in the same column with different letters in superscript are significantly different (P < 0.05)

Source: Uka *et al.* (2019)

Morphological characteristics of the selected tree species in the study area

All four plants, *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia* exhibited a tree growth habit and were perennials. Except for *Terminalia catappa* which is semi deciduous the other tree species are evergreen. *Mangifera indica* and *Ficus platyphylla* had dense crown structure, whilst *Terminalia catappa* and *Polyalthia longifolia* had spreading and irregular crown structures respectively. The leaf surface textures of all species were leathery except for *Polyalthia longifolia* which had a smooth texture. Leaves of all four tree species were hardy in nature (Table 3).

Table 3. Morphology of the selected roadside trees at study sites in the Kumasi Metropolis

Tree species	Sampling site	DBH (cm)		Tree height (m)		Leaf texture	Leaf	Life cycle	
		Range	Mean	Range	Mean				
<i>Terminalia catappa</i>	Arterial road I	41.40 – 47.77	43.52 ± 2.12	17.05 – 21.55	19.38 ± 1.30	Leathery	Hardy	Perennial	
	Arterial road II	32.15 – 38.22	35.03 ± 1.76	14.31 – 16.75	15.67 ± 0.72	"	"	"	
	Arterial road III	38.22 – 76.43	53.08 ± 11.82	13.03 – 21.75	17.41 ± 2.52	"	"	"	
	Control	32.48 – 78.98	52.33 ± 13.85	12.95 – 23.35	18.68 ± 3.05	"	"	"	
	Total average		45.99 ± 4.25		17.79 ± 0.81				
<i>Mangifera indica</i>	Arterial road I	35.03 – 47.77	40.44 ± 3.80	10.25 – 14.45	12.88 ± 1.33	Leathery	Hardy	Perennial	
	Arterial road II	39.52 – 92.36	70.29 ± 15.86	11.25 – 16.15	13.65 ± 1.42	"	"	"	
	Arterial road III	41.40 – 92.35	70.06 ± 15.05	12.35 – 19.75	15.22 ± 2.29	"	"	"	
	Control	48.87 – 75.54	64.51 ± 8.04	10.15 – 15.35	12.73 ± 1.50	"	"	"	
	Total average		61.33 ± 7.09		13.62 ± 0.57				
Tree species	Sampling site	DBH		Tree height (m)		Canopy (crown) structure	Leaf texture	Leaf	Life cycle
		Range	Mean	Range	Mean				
<i>Ficus platyphylla</i>	Arterial road I	41.40-88.53	59.23 ± 14.76	14.05 – 23.50	17.87 ± 2.88	Dense	Leathery	Hardy	Perennial
	Arterial road II	22.29 – 42.99	31.95 ± 6.02	10.15 – 14.65	12.28 ± 1.30	"	"	"	"
	Arterial road III	63.69-108.28	88.11 ± 13.05	12.75 – 18.75	15.08 ± 1.86	"	"	"	"
	Control	37.09 – 54.61	44.79 ± 5.17	13.03 – 15.02	14.20 ± 0.60	"	"	"	"
	Total average		56.02 ± 12.06		14.86 ± 1.16				
<i>Polyalthia longifolia</i>	Arterial road I	31.85 – 57.32	42.46 ± 7.65	14.35 – 16.45	15.68 ± 0.67	Irregular	Smooth	Hardy	Perennial
	Arterial road II	35.03 – 45.22	41.08 ± 3.09	13.62 – 18.35	16.71 ± 1.54	"	"	"	"

	Arterial road III	32.17 – 35.03	33.40 ± 0.85	12.62 – 18.35	14.61 ± 1.87	''	''	''	''
	Control	32.80 – 34.08	33.44 ± 0.37	13.25 – 14.25	13.75 ± 0.29	''	''	''	''
	Total average		37.60 ± 2.43		15.19 ± 0.64				

Effect of vehicular air pollution on macro-morphological characteristics

Leaf area

The leaf area of all the tree species at the arterial road sites were lower and significantly different from those at the control except for *Ficus platyphylla* ($P < 0.05$) (Table 4). The highest leaf area was observed in leaves of the studied tree species growing in the Control, whilst the lowest leaf area was recorded at the arterial roads. In *Terminalia catappa* the mean leaf area was highest at the control site and the lowest mean value of 61.18 mm² was recorded at the Arterial road I. There was no significant difference in the leaf area among the arterial road sites. However, there was significant difference ($p = 0.02$), when compared to the Control. The maximum mean leaf area for *Mangifera indica* was 29.03 mm² at the control, while the minimum was 15.54 mm² at Arterial road I. Leaf area mean values of *Mangifera indica* was not significant at the arterial road sites, on the contrary, there was a significant difference in the leaf area mean values between the arterial road sites and the Control ($p = 0.01$). *Ficus platyphylla* had the highest leaf area of 107.13 mm² at the Control site, while the least leaf area of 86.84 mm² was recorded at Arterial road III. There was no statistical difference between the sites at $p = 0.56$. The maximum leaf area for *Polyalthia longifolia* was 22.29 mm² at the Control, while the minimum was 13.21 mm² at the Arterial road III. Leaf area mean values of *P. longifolia* was not significant at the arterial road sites, on the contrary, there was a significant difference in the leaf area mean values between the arterial road sites and the Control ($p = 0.01$).

Table 4. Effect of vehicular air pollution on leaf area (mm²) of four street tree species in the Kumasi Metropolis

Sampling site	Tree species			
	<i>Terminalia catappa</i>	<i>Mangifera indica</i>	<i>Ficus platyphylla</i>	<i>Polyalthia longifolia</i>
Control	120.21 ± 1.48 ^b	29.03 ± 2.11 ^b	107.13 ± 9.53 ^a	22.29 ± 1.28 ^{ab}
Arterial road I	61.18 ± 10.93 ^a	15.54 ± 0.29 ^a	91.08 ± 16.43 ^a	13.61 ± 0.58 ^a
Arterial road II	114.86 ± 8.32 ^b	19.62 ± 2.71 ^a	89.26 ± 8.11 ^a	17.60 ± 1.92 ^{ab}
Arterial road III	93.43 ± 16.48 ^{ab}	18.48 ± 1.49 ^a	86.84 ± 5.67 ^a	13.21 ± 1.59 ^a

Mean ± SE in the same column with different letters in superscript are significantly different ($P < 0.05$)

Effect of vehicular pollution on micro-morphological variations of the studied tree species in the Kumasi Metropolis

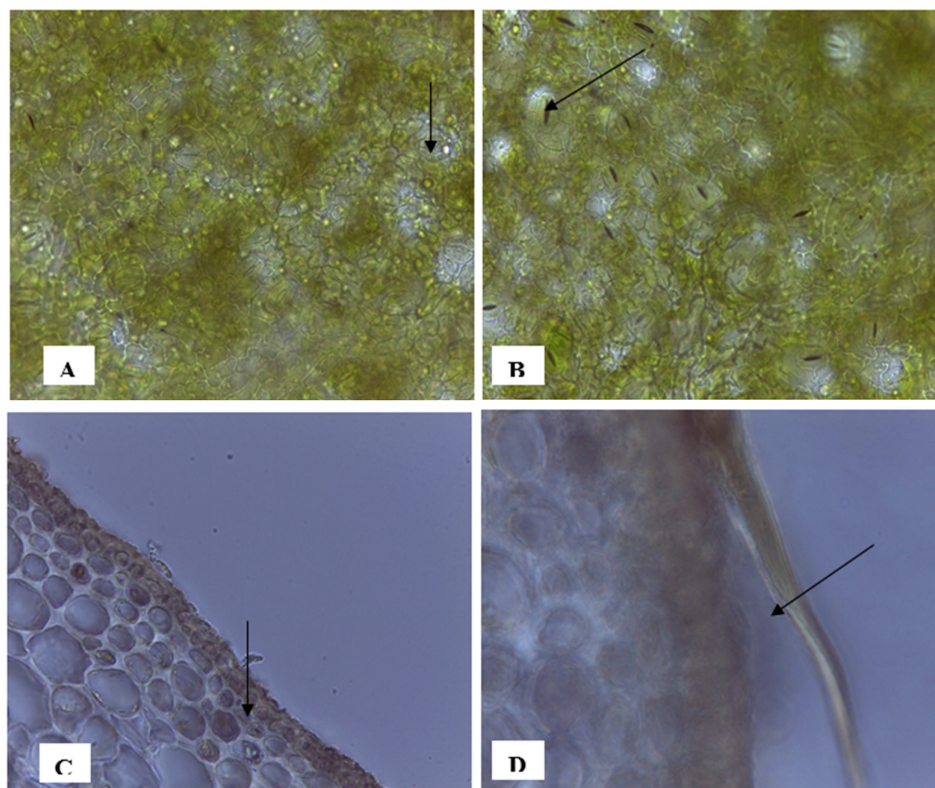
Terminalia catappa

In *Terminalia catappa* leaves, there was a general reduction in stomata size, stomata number and stomata index in all the arterial road sites when compared to the Control. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) stomata size, stomata number and stomata index were lower in the arterial road sites, when compared to the control (Table 5). On the other hand, there was a general increase in epidermal cell number, epidermal cell length and trichomes length in all the arterial road sites when compared to the Control (Table 7). There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 5). Stomata in the abaxial surface of leaves in the Control were observed to have little or no occlusion (Plate 1a) whilst most of the stomata in abaxial surface of leaves in the arterial road sites were occluded (Plate 1b). The trichomes in the abaxial surface of leaves in the Control were observed to be shorter (Plate 1c) whilst those on abaxial surface of leaves in the arterial road sites were longer (Plate 1d)

Table 5. Effect of vehicular pollution on micro-morphological variations of *Terminalia catappa* leaves in the Kumasi Metropolis

Micro morphological characters						
Sampling site	SS (μm)	SN (mm^2)	ECN (mm^2)	SI	ECL (μm)	TL (μm)
Control	361.48 \pm 96.2 ^a	556 \pm 134.4 ^a	666 \pm 223.11 ^a	45.5	31.47 \pm 8.7 ^a	64.1 \pm 33.4 ^a
Arterial road I	294.88 \pm 99.1 ^a	272 \pm 107.1 ^a	1480 \pm 292.5 ^a	15.5	41.1 \pm 7.7 ^a	98.8 \pm 80.9 ^a
Arterial road II	328.13 \pm 89.4 ^a	489 \pm 111.3 ^a	1306 \pm 310.7 ^a	27.2	36.1 \pm 9.3 ^a	65.4 \pm 29.8 ^a
Arterial road III	305.56 \pm 79.6 ^a	518 \pm 130.5 ^a	1337 \pm 303.7 ^a	27.9	38.3 \pm 8.7 ^a	82.6 \pm 38.9 ^a

Mean \pm SE in the same column with different letters in superscript are significantly different ($P < 0.05$), SN = Stomatal number; ECN = Epidermal cell number; SI = Stomatal Index; ECL = Epidermal cell length; TL = Trichome length

**Plate 1.** Photomicrographs of *Terminalia catappa* leaves

(A) *Terminalia catappa* leaf sample from control site showing normal stomata. (B) *Terminalia catappa* leaf sample from arterial road site showing occluded stomata. (C) *Terminalia catappa* leaf sample from the control showing short trichomes. (D) *Terminalia catappa* leaf sample from the arterial road site showing long trichomes. Magnification $\times 400$

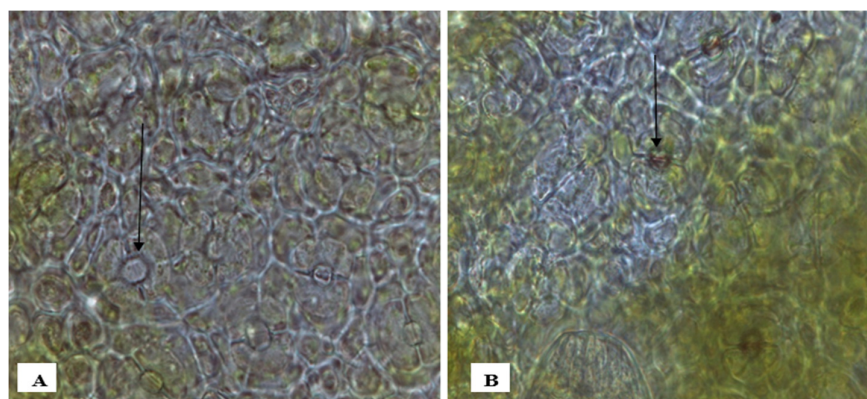
Mangifera indica

In general, stomata size, stomata number and stomata index in the leaves of *Mangifera indica* were lower in all the arterial road sites when compared to the Control site. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 6). As observed in *Terminalia catappa* epidermal cell number and epidermal cell length in all the arterial roads were higher when compared to the Control site. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 6). As in *Terminalia catappa* the stomata in the abaxial surface of the leaves of *Mangifera indica* in the Control site were observed to have little or no occlusion (Plate 2a) whilst most of the stomata in abaxial surface of the leaves of *Mangifera indica* in the arterial road sites were occluded (Plate 2b).

Table 6. Effect of vehicular pollution on micro-morphological variations of *Mangifera indica* leaves at the study sites in Kumasi Metropolis

Sampling sites	Micro morphological parameters				
	SS (μm)	SN (mm^2)	ECN (mm^2)	SI	ECL (μm)
Control	665.3 \pm 217.1 ^a	914 \pm 112.7 ^a	1379 \pm 308.9 ^a	39.9	17.9 \pm 3.1 ^a
Arterial road I	251.1 \pm 72.9 ^a	470 \pm 160.9 ^a	2419 \pm 479.7 ^a	16.3	29.1 \pm 5.3 ^a
Arterial road II	444.3 \pm 89.6 ^a	605 \pm 121.0 ^a	1408 \pm 156.6 ^a	30.1	22.2 \pm 4.4 ^a
Arterial road III	371.6 \pm 75.1 ^a	508 \pm 180 ^a	2147 \pm 353.2 ^a	19.1	24.8 \pm 4.1 ^a

Mean \pm SE in the same column with different letters in superscript is significantly different ($P < 0.05$), SN = Stomatal number; ECN = Epidermal cell number; SI = Stomatal Index; ECL = Epidermal cell length

**Plate 2.** Photomicrographs of *Mangifera indica* leaves

(A) *Mangifera indica* leaf sample from control showing normal stomata. (B) *Mangifera indica* leaf sample from arterial road site showing occluded stomata. Magnification $\times 400$

Ficus platyphylla

In *Ficus platyphylla* leaves, stomata size, stomata number and stomata index were lower in all the arterial road sites when compared with the Control. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 7). Epidermal cell number, epidermal cell length and trichome length increased in all the arterial road sites. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 7). Stomata in the abaxial surface of leaves in the Control were observed to have no occlusion (Plate 3a) whilst majority of the stomata in abaxial surface of leaves in the arterial road site were occluded (Plate 3b). The trichomes in the abaxial surface of the leaves in the Control were shorter (Plate 3c) than those on abaxial surface of leaves in the arterial road site (Plate 3d).

Table 7. Effect of vehicular pollution on micro-morphological variations of *Ficus platyphylla* leaves at the study sites in Kumasi Metropolis

Sampling sites	Micro morphological characters					
	SS (μm)	SN (mm^2)	ECN (mm^2)	SI	ECL (μm)	TL (μm)
Control	470 \pm 134.9 ^a	432 \pm 134.3 ^a	1374 \pm 8.5 ^a	23.9	34.20 \pm 11.2 ^a	32.8 \pm 9.7 ^a
Arterial road I	319.4 \pm 88.8 ^a	239 \pm 3.06 ^a	2045 \pm 235.6 ^a	10.5	42.50 \pm 14.4 ^a	42.4 \pm 22.2 ^a
Arterial road II	444.5 \pm 79.3 ^a	402 \pm 54.8 ^a	1868 \pm 243.1 ^a	17.7	35.80 \pm 6.5 ^a	33.6 \pm 15.8 ^a
Arterial road III	421.8 \pm 112.7 ^a	269 \pm 74.1 ^a	2044 \pm 311.8 ^a	11.6	35.70 \pm 7.6 ^a	37.7 \pm 15.0 ^a

Mean \pm SE in the same column with different letters in superscript are significantly different ($P < 0.05$), SN = Stomatal number; ECN = Epidermal cell number; SI = Stomatal Index; ECL = Epidermal cell length; TL = Trichome length

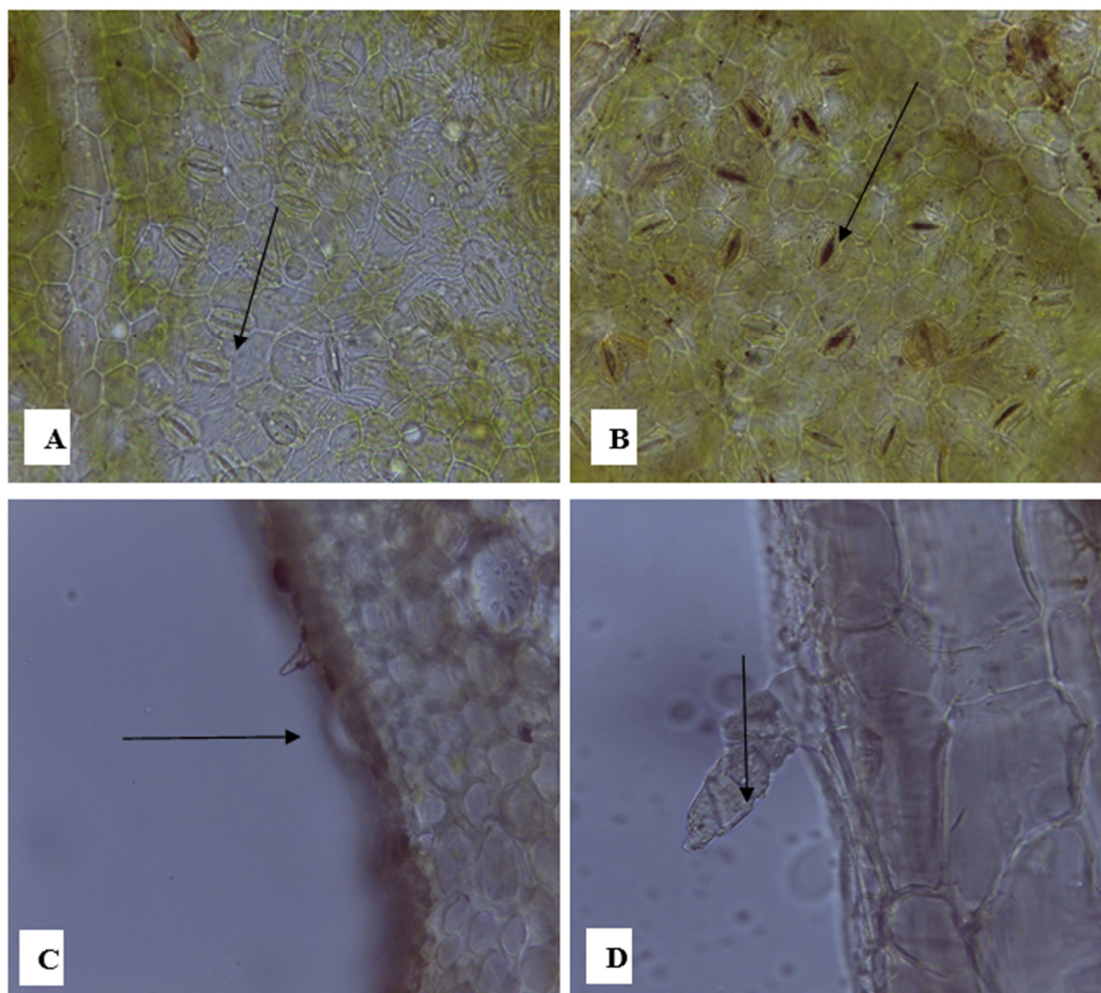


Plate 3. Photomicrographs of *Ficus platyphylla* leaves

(A) *Ficus platyphylla* leaf sample from control showing normal stomata. (B) *Ficus platyphylla* leaf sample from arterial road site showing occluded stomata. (C) *Ficus platyphylla* leaf sample from the control site showing short trichomes. (D) *Ficus platyphylla* leaf sample from the arterial road site showing long trichomes. Magnification $\times 400$

Polyalthia longifolia

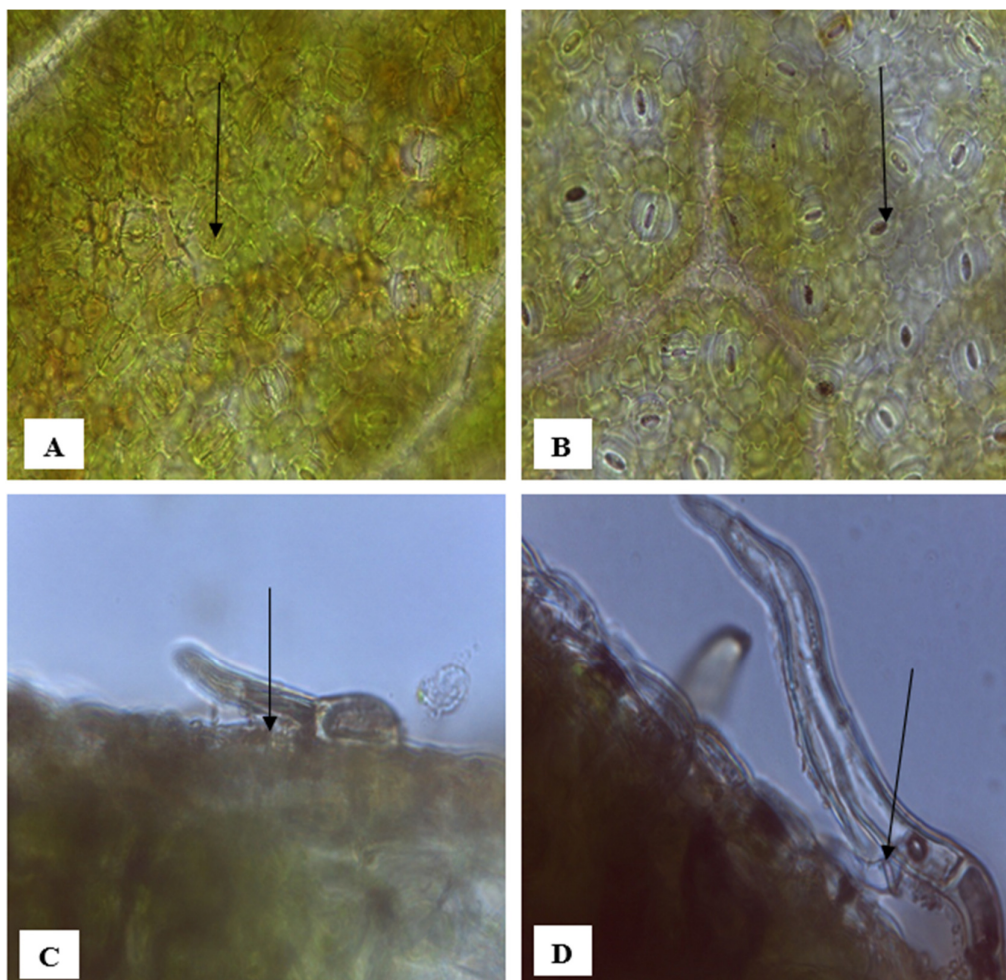
In the leaves of *P. longifolia*, stomata size, stomata number and stomata index were much lower in all the arterial road sites when compared to the Control. There was no significant difference among the arterial road sites and also when compared to the Control ($p > 0.05$) (Table 8). Epidermal cell number, epidermal cell length and trichome length increased in all the arterial road sites when compared to the control. There was no significant difference among the arterial road sites, however, there was statistical difference between Arterial road I and Control ($p = 0.035$) (Table 8). Stomata in the abaxial surface of the leaves of *P. Longifolia* in the Control were observed to have no occludation (Plate 4a) whilst most of the stomata in the abaxial surface of the leaves in the experimental sites were occluded (Plate 4b). The trichomes length in the abaxial surface of leaves in the Control were observed to be shorter (Plate 4c) than those on the abaxial surface in the arterial road sites (Plate 4d).

Table 8. Effect of vehicular pollution on micro-morphological variations of *Polyalthia longifolia* leaves at the study sites in Kumasi Metropolis

Sampling sites	Micro morphological characters					
	SS (μm)	SN (mm^2)	ECN (mm^2)	SI	ECL (μm)	TL (μm)
Control	713.5 \pm 71.3 ^a	307.00 \pm 5.4 ^a	1911.00 \pm 384.1 ^a	13.8	24.1 \pm 6.2 ^a	19.10 \pm 1.7 ^a
Arterial road I	520.6 \pm 120.5 ^a	287.0 \pm 1.2 ^a	2547.0 \pm 877.1 ^a	10.1	29.5 \pm 3.2 ^a	97.80 \pm 19.8 ^b
Arterial road II	599.7 \pm 117.9 ^a	306.0 \pm 32.1 ^a	2287.0 \pm 510.5 ^a	11.8	24.7 \pm 5.1 ^a	31.70 \pm 21.5 ^{ab}
Arterial road III	549.4 \pm 60.1 ^a	298.0 \pm 50.2 ^a	2458.0 \pm 555.6 ^a	10.8	28.0 \pm 5.8 ^a	35.4 \pm 13.5 ^{ab}

Mean \pm SE in the same column with different letters in superscript are significantly different ($P < 0.05$);

SN = Stomatal number; **ECN** = Epidermal cell number; **SI** = Stomatal Index; **ECL** = Epidermal cell length; **TL** = Trichome length

**Plate 4.** Photomicrographs of *Polyalthia longifolia* leaves

(A) *Polyalthia longifolia* leaf sample from control showing normal stomata. (B) *Polyalthia longifolia* leaf samples from arterial road site showing occluded stomata. (C) *Polyalthia longifolia* leaf sample from the control showing short trichomes. (D) *Polyalthia longifolia* leaf sample from the arterial road site showing long trichomes. Magnification $\times 400$

Relationship between ambient air quality and micro-morphological variations

The relationship between ambient air quality and micro-morphological variables of *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia* was investigated using the multiple regression analysis. The result is presented using the Pareto chart of t-values for the regression coefficients (Figures 1-4). CO, SO₂, NO₂ and VOC were used as the independent or predictive variables, while stomata number, stomata

size, epidermal cell number and trichome length were the response or dependent variables. In *Terminalia catappa*, CO was a significant predictive variable for epidermal cell number and stomata size, and SO₂ was a significant correlate for epidermal cell number (Figure 1). SO₂ was a significant predictive variable for epidermal cell number in *Mangifera indica* (Fig. 2b). In *Ficus platyphylla*, CO and NO₂ were significant predictors of epidermal cell number (Figures 3a and c). SO₂ was significant as a predictor of the epidermal cell number and stomata size in *Ficus platyphylla* (Figure 3b). It was also observed that none of the air pollutants acted as a significant predictive variable for stomata number, stomata size, epidermal cell number and trichome length in *Polyalthia longifolia* (Figure 4).

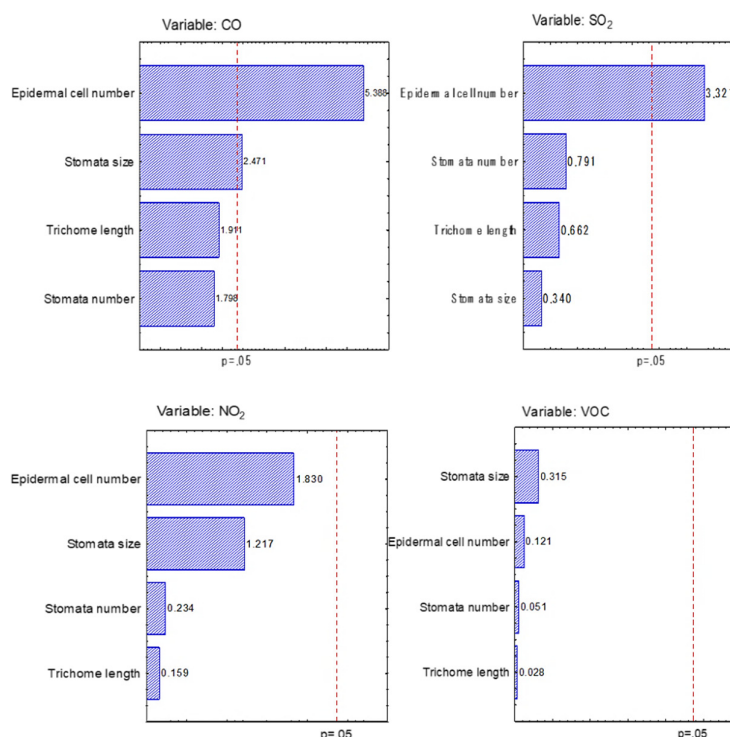


Figure 1. Pareto chart of t-values for coefficients from multiple regression for *Terminalia catappa*

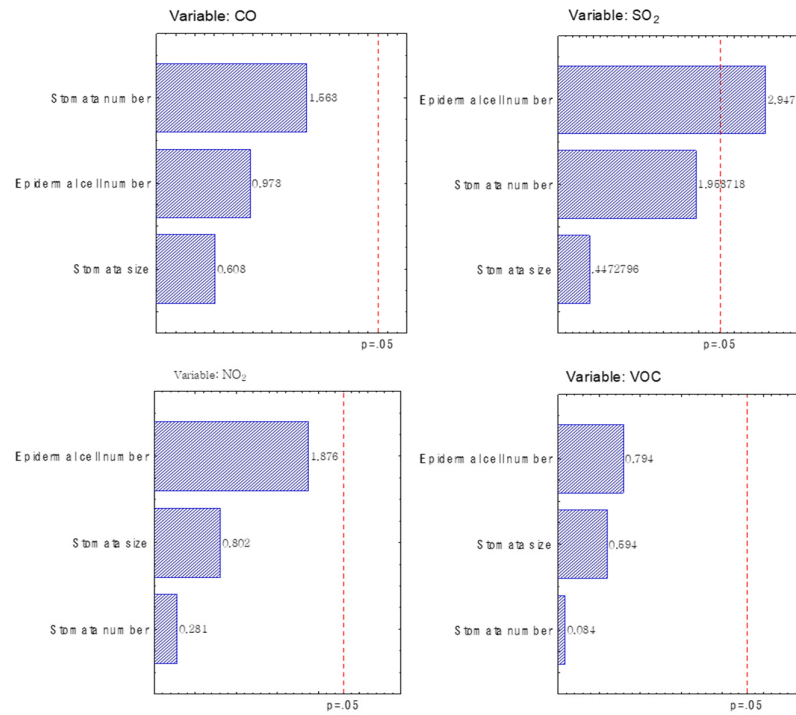


Figure 2. Pareto chart of t-values for coefficients from multiple regression for *Mangifera indica*

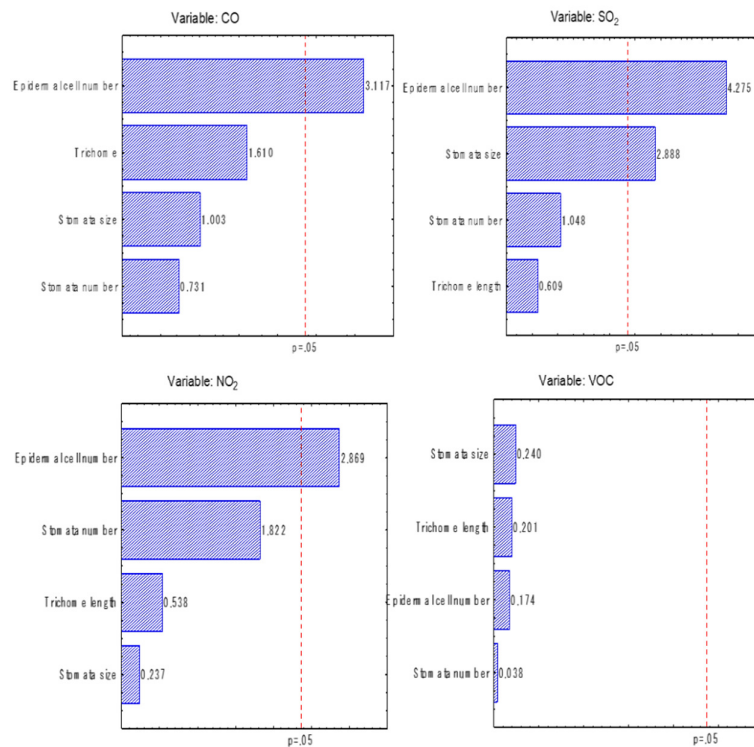


Figure 3. Pareto chart of t-values for coefficients from multiple regression for *Ficus platyphylla*

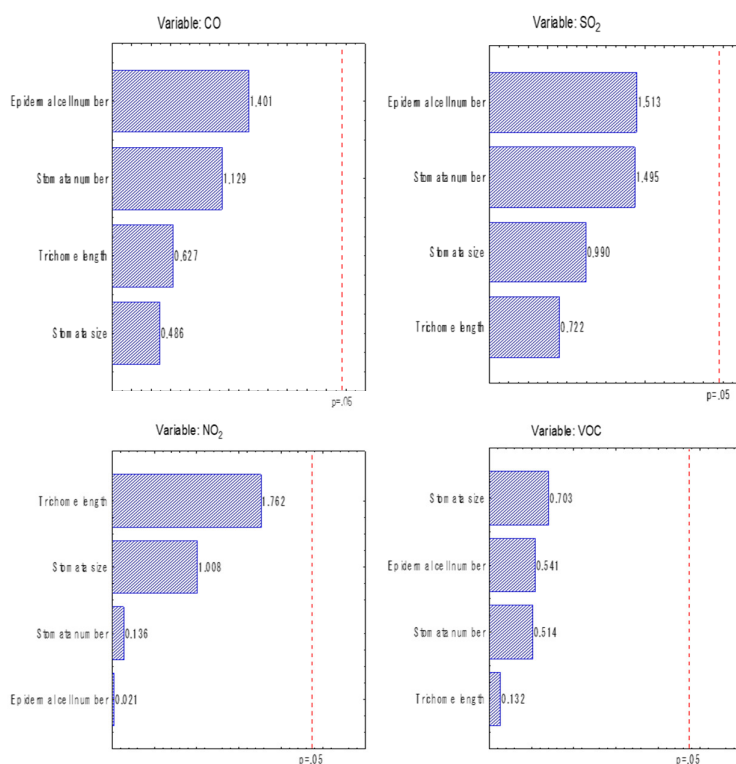


Figure 4. Pareto chart of t-values for coefficients from multiple regression for *Polyalthia longifolia*

Discussion

In this study, the leaf area in all the four tree species (*Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia*) studied were lower in the arterial roads compared to the Control site, suggesting that vehicular emissions probably affected this morphological feature. Similar findings were reported by Leghari and Zaidi (2013) and Shafiq *et al.* (2009) that the leaves taken from the polluted sites showed decline in leaf area. The reduced leaf area was not only an indication of retarded growth, but also was a morphological adjustment to highly polluted condition, on the grounds that with the smaller leaf size, the lesser will be the absorption of obnoxious gas (Zarinkamar *et al.*, 2013). The reduction in the leaf area may be due to more amount of damaging pollutants like SO₂ and NO_x emitted by the automobiles, which incidentally affects cell elongation mechanism and photosynthetic capacity of the leaves (Wagh *et al.*, 2006). It was striking to notice that in the leaves undergoing vehicular pollution visible morphological injuries was not seen. This result is consistent with the findings of Agrawal *et al.* (1991) and Salgare and Thorat (1995). Perhaps, hidden injuries or physiological disturbance could have resulted in reduction in the leaf proportions in all the four tree species (*Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia*) studied. It has been documented that plants experience physiological changes before displaying visible damage to leaves when exposed to air pollutants (Dohmen *et al.*, 1990). In this study, *Mangifera indica* and *Polyalthia longifolia* showed significant decrease in the leaf area among the sites, hence it appears that these tree plants are susceptible to these pollutants. However, *Terminalia catappa*, *Ficus platyphylla* tends to be more resilient to the pollutants for the fact these species were not significantly different from each other. This is in conformity of Honour *et al.* (2009) that plants response to air pollutants differs between species.

Stomata are the gateway for exchange of gases, but it can be overburdened by air pollutants resulting in variations in the micro-morphological characteristics of the leaf. Stomata size, stomata number and stomata

index were observed to be lower in the arterial roads, whilst epidermal cell number, epidermal cell length and trichome length were higher when compared to the control, an indication that automobile air pollutants most likely altered the micro-morphological parameters of the studied tree species. It was also observed that SO₂ pollution could possibly lead to stomata size reduction and increased epidermal cell number in *Ficus platyphylla*. NO₂ pollution could lead to increased epidermal cell number in *Ficus platyphylla*. CO, SO₂, NO₂ and VOC did not relate significantly to the stomata size, stomata number, epidermal cell number and trichome of *Polyalthia longifolia*. This suggests that the air quality parameters had minimal impact on the *Polyalthia longifolia*. The decrease in the stomata size in the leaves of the studied tree species suggests that stomata size allows a reduced amount of gas exchange between the environment and the trees, thus shielding the trees from intake of toxic gases. The decrease in the stomata size could be an avoidance mechanism against inhibitory effect of a pollutant on physiological activities such as photosynthesis (Pathak and Pancholli, 2014). These changes can be used as indicators of environmental stress (Pawar, 2016).

The reduction of leaf area in the arterial road sites brings about growth retardation and reduced surface area. Therefore, reduced surface area of the leaves of *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia* possibly have capacity for a smaller number of stomata in this manner toxic pollutants entering the leaves are reduced. The decrease in stomata number arising from vehicular air pollutants is an indication that the quantity of gaseous pollutants entering the leaves via the stomata is reduced and the plant becomes tolerant to the pollution. Larcher (2003) reported that a slight modification for general gas exchange control and pollutant entry through the stomata singularly is initiated through reduction of pollutant uptake by plants by merely decreasing their stomata number.

Stomata index is the ratio of stomatal number and number of both epidermal and stomatal cell. In this case, the dividend is the stomata number, while the divisor is summation of epidermal cell number and stomata number. When compared with the control, it indicates variations in number of stomata and in number of epidermal cells. It has been opined that reduction in stomatal index could be considered as a favourable adaptation to air pollution, as it might help in reducing the absorption of gaseous pollutants (Chauhan *et al.*, 2004).

There was increased epidermal cell number in all the four studied tree species at the arterial road sites. The increase in epidermal cells could be a favourable adaptation in plants found in arterial road sites for pollutant detoxification. The epidermis is the major site air pollutants are first confronted upon by free radical scavengers (Pawar, 2016). Epidermal cells increase ensures greater quantity of antioxidants, thereby enhancing the detoxification of pollutants (Shah *et al.*, 2000). There was increase in epidermal cell length at arterial road sites when compared with the control. In view of the fact that, differentiation of stomata mother cells involves the division of epidermal cells, reduction in stomata number is followed by epidermal cell size increase (Sant'anna-Santos *et al.*, 2008). Trichomes length at the arterial road sites when compared with the control was found highest. Consequently, the increase in trichomes for *Terminalia catappa*, *Ficus platyphylla* and *Polyalthia longifolia* appears to be an added adaptation to air pollution stress. Trichomes helps to trap particulate matter falling directly on the leaf surface which incidentally block the stomata pore and adversely affect gaseous exchange processes (Sharma, 1977).

In this study, clogging of stomata pores resulting from the effect of vehicular pollution were noticed in all the four tree species. This could be due to high concentrations of particulate matter arising from vehicular emissions that is subsequently deposited on the stomatal pores. According to Das and Pattanayak (1978), aerosols larger than the pore of stomata are largely accumulated on the pore opening and thus interfere with exchange of gas, photosynthesis and a reduction in plant growth. Verma and Chandra (2014) had similar observations regarding the clogging of stomata on the leaves of *Sida cordifolia* and *Catharanthus roseus* receiving the burden of auto pollution. Relatedly, stomata clogging in the leaves of *Citrus medica* attributable to diesel exhaust were observed by Kaur (2004).

It appears that they were tree species differences based on the response of the trees to vehicular pollution. The tree specific differences in its air pollution absorption agrees with Zhang *et al.* (2013) assertion that plants

as living entity varies individually in their adaptations to the environment and abilities to absorb pollutants. The difference in plants species ability to lessen air pollution is attributed to the change in the leaf surface features namely cuticle, epidermis, stomata and trichomes (Neinhus and Barthlott, 1998). It was also interesting to note that the air pollutants were the most predictive variable for the stomata size and epidermal cell number variations in the studied tree species. This is consistent with the report that air pollutants such as SO₂ make entrance into leaf tissues through the stomata (Tripathi and Mukesh, 2007). More so, increased epidermal cells ensures greater quantity of antioxidants, thereby enhancing the detoxification of pollutants (Shah *et al.*, 2000), hence epidermis is the major site air pollutants are first confronted upon by free radical scavengers (Pawar, 2016).

SO₂ had more effect on the micromorphological variables in the tree species except *Polyalthia longifolia* than the other pollutants. This is in keeping with Hill (1971) and Bennet and Hill (1973-1975) report that plants have more preference for SO₂ and less preference for NO₂ to be absorbed and metabolized in the plant tissue. They outlined the plants preference for air pollutants in the following order HF > SO₂ > Cl > NO₂ > O₃ > PAN > NO > CO. The high of SO₂ uptake by plants is due to its high solubility and rapid hydration in the aqueous phase in the plant (Pfan *et al.*, 1987; De Kok, 1990).

CO and NO are ineffectively taken up by plants. It has been reported that CO and NO which are insoluble are ineffectively taken up by plants (Williams, 1990). The major sink for CO is the soil organisms and in the case of NO, when slowly taken up is converted to other forms that may be taken up readily (Baby and Goel, 1995). None of the predictive variables related significantly to the prediction of VOCs in all the plants, this could be due to emission of VOC 's by plants (Niederbacher *et al.*, 2015).

Conclusions

In this study, the tree species response to automobile air pollution was by adjusting its leaf macro- and micro-morphological characteristics and such there was reduction in leaf area and stomata size, stomata number and stomata index as well as an increased epidermal cell number, length, and trichome length at the arterial road sites. In this study, stomata clogging with occluded stomata pores resulting from the effect of automobile pollution were noticed in all the four tree species. These alterations can be considered as indicators of environmental stress for initial revelation of urban air pollution

Authors' Contributions

Conceptualization: UNU and EJD; UNU; Investigation: UNU; Supervision: EJD; Writing - original draft: UNU; Writing - review and editing: EJD. All authors read and approved the final manuscript. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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