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Original Research Paper

Study of the Activity of Air Pollution on the Leaves of *Urena lobata* Growing along Busy Roads

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The leaves of *Urena lobata* growing along the sides of busy roads of Oluku, Agbor and Sapele in Benin City were studied vis-a-vis those growing in forest areas of Ebvomodu, Eyaen and Ebvoneka villages in the outskirt of Benin City for the activity of air pollution. Collections made from along busy roads constituted the polluted populations while those from the villages constituted the non-polluted populations. The polluted populations were collected within the distances of 0-0.5m from the tarred portions while those for the non-polluted populations were collected at distances of 50-60km away from the busy roads. The lengths and widths of the leaves were measured with the aid of a metre rule to determine the average leaf area (LA) while the upper and lower epidermis were peeled and mounted temporarily on slides for microscopic observations and line drawings. The average leaf areas (cm^2) of 61.64 ± 0.68, 12.18 ± 0.48 and 8.21 ± 0.55 were calculated for Oluku, Agbor and Sepele Roads respectively, while, 64.99 ± 0.18, 77.33 ± 0.28 and 67.81 ± 0.41 were calculated for Ebvomodu, Ebvoneka and Eyaen villages respectively. Consequently, the leaves from the polluted road sides were smaller than those from the non-polluted villages. The epidermal cell walls of leaves of the polluted and non-polluted populations of this species were sinuous in both leaf surfaces and comprised of diacytic stomata. Stomatal pores were plugged in both the upper and lower leaf surfaces of the polluted populations. The lengths and widths of stomatal pores for the non-polluted populations were 1.39 ± 0.10, 1.35 ± 0.46 and 1.31 ± 0.17 and 0.40 ± 0.33, 0.44 ± 0.92 and 0.46 ± 0.36 for the upper leaf surface, respectively while, at the lower leaf surface, the lengths and widths of pores of stomata were 1.59 ± 0.67, 1.51 ± 0.54 and 1.48 ± 0.14 and 0.42 ± 0.18, 0.43 ± 0.11 and 0.49± 0.86 respectively. Microscopic observations of the leaves from the polluted microhabitats revealed that the stomatal pores were plugged at both the upper and lower surfaces. This was suspected to be as a result of the cumulative effects of the air pollutants that dominated busy roads where the plant grows. This was, however, not the case with the leaves from non-polluted microhabitats in which the stomatal pores were not plugged. It was opined that this specie could serve as a pointer to the negative activity of pollution common in busy roads.

Keywords: Busy roads, Leaves, Air pollution, *Urena lobata*, Plugged stomata, Lengths and widths, Non-polluted, Polluted, Populations, Leaf epidermis, Microhabitats.

INTRODUCTION

Urena lobata Linn is an erect, bushy perennial shrub up to 2m high that reproduces from seeds. The stem is woody, round and fibrous, often scabrous and covered with white stellate hairs. The leaves are alternate, variable in shape, usually 3- to 5- lobed. The lobes are often shallow, but sometimes deep, about 2-8cm long and 8cm wide, irregularly dentate at the margins and with stalks up to 10cm long. The leaf is prominently veined, the main vein always bears a gland at the base. The lower leaf surface has soft whitish or stellate hairs. The inflorescence is a solitary, axillary cyme. The flowers are pink, about 2.5cm in diameter and sessile or with short stalks. The fruits are 5- seeded, globose capsules about 9mm in diameter, warty with rigid hooked bristles. It is found near settlements (Akobundu and Agyakwa, 1998). Leaves are often pruned to the effect of air pollution than any other parts of the plant. This could probably be due to their position in the habit of the plant. They are exposed to the atmosphere where they are liable to the influence of polluted and non-polluted air. It has been established that individual organisms within a population vary in their ability to withstand the stress of

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environmental changes. This, according to Garner (2002) is based on their genetic constitution (genotype), stage of growth at time of exposure, and microhabitat in which they are growing.

Plants are exposed in their environment to numerous stress factors that may act either simultaneously or consecutively with different intensities and frequencies. Plants, according to Bonneau et. al. (2007) experience stress from several environmental influences. Often, the leaves and the flowers were the first to show symptoms of air pollution, which according to Marianne and Wayne (2007) include unusual discolorations, spotting, twisting or turning of leaves and abortion of flowers followed by poor growth. Previous study by San (2006) had now revealed that increased carbon dioxide does not accelerate plant growth. It is now established that doubling the volume of atmospheric CO_2 is capable of causing leaf stomata to close by 20-40% in diverse plant species, thus reducing CO_2 intake.

The main sources of air pollution are the industries, agriculture, traffic, energy generators. During combustion and other production processes, substances that can pollute air are emitted. Reports from LENNTECH (2005) revealed that, traffic is responsible for one-third of the greenhouse gas emissions. Emissions caused by traffic are mainly those of carbon dioxides, VOC (Volatile Organic Compounds) and small dust particles.

In the recent times, Kayode and Otoide (2006, 2007a and b) have investigated the effects of gaseous pollutants in form of soot on the leaves of *Chromolaena odorata, Newbouldia laevis* and *Amaranthus spinosus* growing along roadsides. They reported reduction in the sizes of leaves and damages of leaf cuticles and epidermis on these plants (Otoide and Kayode, 2013).

More recently, Otoide (2014) reported damages such as eroded epicuticular wax, epidermal cell alterations, plugged stomatal apertures, blurred epidermal surfaces, polluted cells, ruptured stomatal ledges and eroded cell walls observed on the leaf epidermis of *Polyalthia longifolia, Digitaria gayana and Trianthema portulacastrum* growing very close to exhaust-pipes of power generators.

MATERIALS AND METHODS

Collection of plant samples

Matured leaves of *Urena lobata* were collected from along busy roads: Benin-Lagos, Benin-Agbor, and Benin-Sapele within the distance of 0-0.5m from the tarred portions, at the outskirts of Benin City, Edo State. Another collection of leaves of same specie was made in the forest areas of Ebvomodu, Ebvoneka and Eyaen villages which were 50-60km away from those busy roads in Edo State. The collections from along busy road sides and the ones from the villages constituted the polluted and the non-polluted samples of *Urena lobata,* accordingly.

Leaf Dimension

Ten (10) leaves per species from the polluted and non-polluted areas were randomly selected, their sizes were measured with the aid of plastic ruler and the data were recorded. The leaf Area (LA) of each leaf was then determined according to Otoide and Kayode (2013) as:

LA: L x W x 0.75

Where, L = Length of Leaf
W = Width of Leaf
0.75 = Constant

Preparation of Slides

The epidermal peels of each leaf sample were obtained using the methods of Metcalfe and Chalk (1988) and Olowokudejo (1990). The leaves were placed, with the outer surface facing downward, on a flat surface and flooded with 8% sodium hypochlorite solution (NaOCl). An area of about 1cm square was removed from a central / standard position, always midway between the base and the apex of the leaves. The peels were mounted temporarily on slides. 10 slides (each of adaxial and abaxial surfaces) were prepared per population.

Measurement of pores, guard cells and collection of data

The slides were examined under the light microscope using x40 objective. Data were collected from 10 microscopic fields, selected at random from each slide. The lengths and widths of stomatal pores and guard cells were measured using ocular micrometer. Data were collected from 50 stomata per leaf surface. This was done in 10 replications. The data obtained were subjected to relevant statistics using mean, standard deviation and ANOVA. Significant differences were determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

The epidermal features of leaves of *Urena lobata* from polluted and non-polluted microhabitats have been shown in Tables 1 and 2 respectively. The average leaf areas (cm^2) of 61.64 ± 0.68, 12.18 ± 0.48 and 8.21 ± 0.55 were calculated for Oluku, Agbor and Sepele Roads respectively, while, 64.99 ± 0.18, 77.33 ± 0.28 and 67.81 ± 0.41 were calculated for Ebvomodu, Ebvoneka and Eyaen villages respectively.

The epidermal cell walls of leaves of the polluted and non-polluted populations of this species were sinuous in both leaf surfaces and comprised of diacytic stomata (Tables 1 and 2). The Stomata Index (%) for the polluted populations were 25.00, 16.66 and 22.22 for the upper epidermis while they were 20.00, 43.58 and 25.00 for the lower epidermis respectively. On the other hand, the Stomata Index of the upper leaf surface of the non-polluted populations were 26.92, 25.00 and 21.00 while at the lower leaf surface the Stomata Index were 31.02, 37.12 and 42.69 respectively.

Stomatal pores were plugged in both the upper and lower leaf surfaces of the polluted populations. The lengths and widths of stomatal pores for the non-polluted populations were 1.39 ± 0.10, 1.35 ± 0.46 and 1.31 ± 0.17 and 0.40 ± 0.33, 0.44 ± 0.92 and 0.46 ± 0.36 for the upper leaf surface, respectively while, at the lower leaf surface, the lengths and widths of pores of stomata were 1.59 ± 0.67, 1.51 ± 0.54 and 1.48 ± 0.14 and 0.42 ± 0.18, 0.43 ± 0.11 and 0.49 ± 0.86 respectively.

The lengths and widths of guard cells for the polluted populations of this specie were 2.07 ± 0.46, 2.30 ± 0.84 and 2.16 ± 0.48 and 1.47 ± 0.16, 1.43 ± 0.52 and 1.45 ± 0.79 for the upper epidermis respectively, while in the lower epidermis, they were 2.01 ± 0.68, 1.76 ± 0.91 and 2.09 ± 0.13 and 1.51 ± 0.29, 1.47 ± 0.12 and 1.48 ± 0.51 respectively.

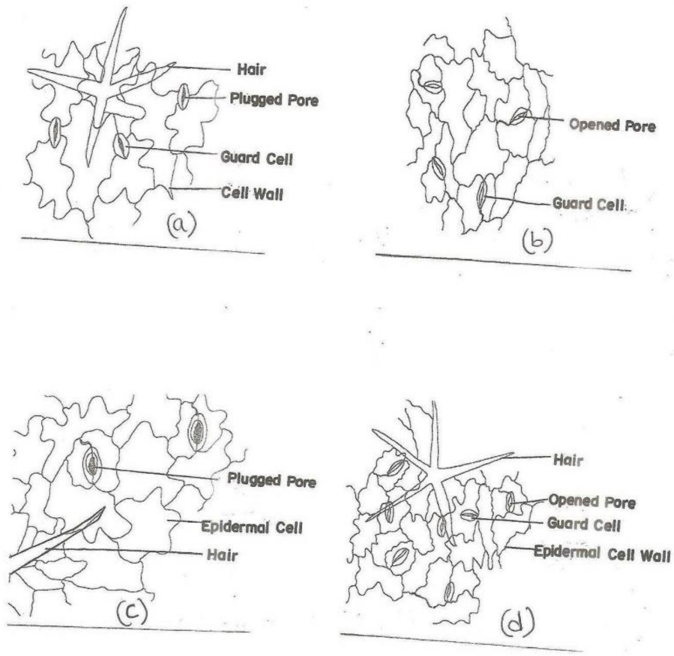

Fig.1: (a) The abaxial epidermis of *Urena lobata* from Oluku road.x200;
(b) The abaxial epidermis of *U. lobata* from Ebvomodu village.x200;
(c) The adaxial epidermis of *U. lobata* from Oluku road.x200 and
(d) The adaxial epidermis of *U. lobata* from Ebvomodu village. X200.

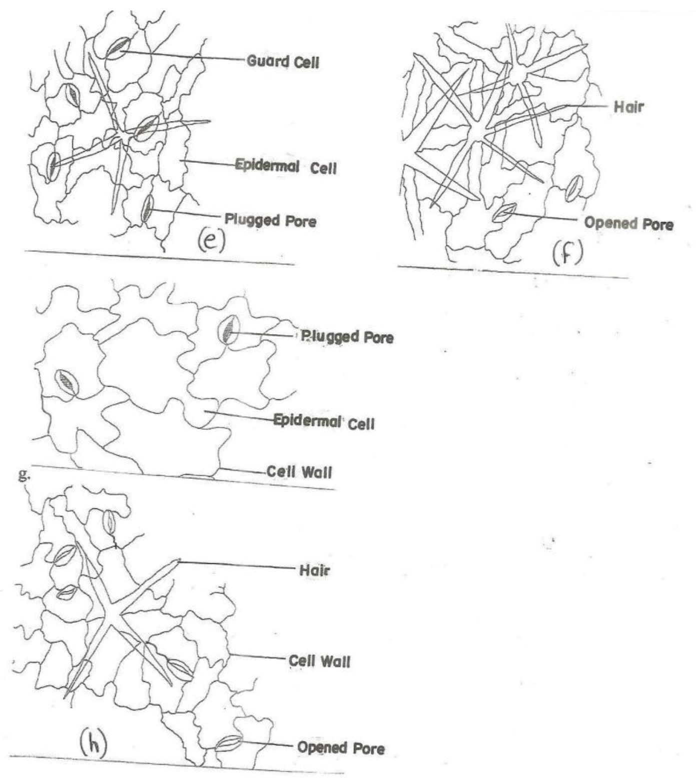

Fig.1: (e) The abaxial epidermis of Urena lobata from Agbor road.x200;
(f) The abaxial epidermis of U. lobata from Ebvoneka village.x400;
(g) The adaxial epidermis of U. lobata from Agbor road.x400;
(h) The adaxial epidermis of U. lobata from Ebvoneka village.x200.

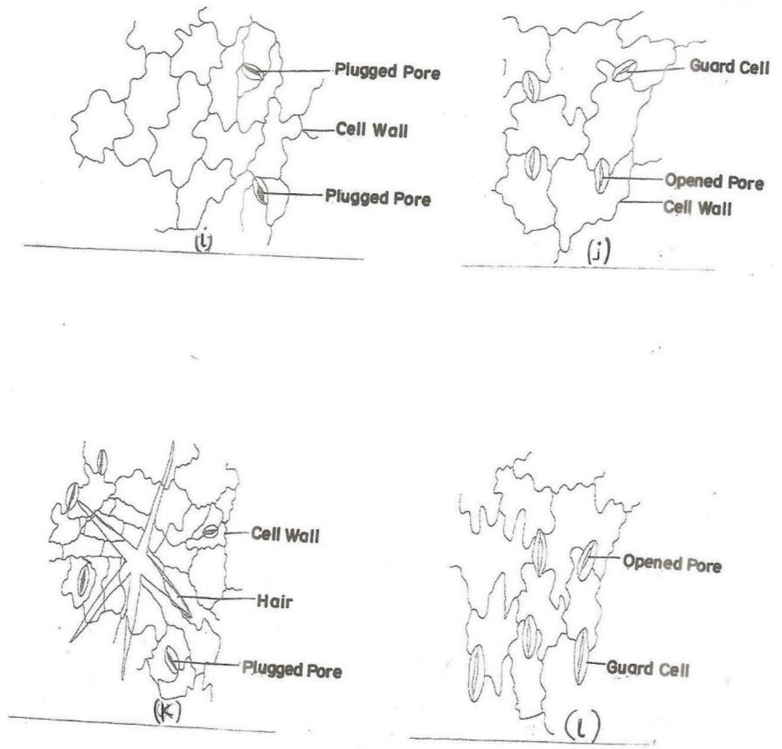

Fig.1:(i) The abaxial epidermis of *Urena lobata* from Sapele road.x200;
(j) The abaxial epidermis of *U.lobata* from Eyaen village.x200;
(k) The adaxial epidermis of *U.lobata* from Sapele road.x200 and
(l) The adaxial epidermis of *U.lobata* from Eyaen village.x400.

Table 1. Leaf epidermal characteristics of *Urena lobata* from polluted micro-habitats

Descriptions surface		**Polluted micro-habitats**		
		Oluku road	**Agbor road**	**Sapele road**
Nature of epidermal cell wall	U	Sinuous	Sinuous	Sinuous
	L	Sinuous	Sinuous	Sinuous
Type of stomata	U	Diacytic	Diacytic	Diacytic
	L	Diacytic	Diacytic	Diacytic
Stomata index (I) (%)	U	25.00	16.66	22.22
	L	20.00	43.58	25.00
Mean length of stomatal pore (µm)	U	Plugged	Plugged	Plugged
	L	Plugged	Plugged	Plugged
Mean width of stomatal pore (µm)	U	Plugged	Plugged	Plugged
	L	Plugged	Plugged	Plugged
Mean length of guard cells (µm)	U	2.07± 0.46	2.30± 0.84	2.16± 0.48
	L	2.01± 0.68	1.76± 0.91	2.09± 0.13
Mean width of guard cells (µm)	U	1.47± 0.16	1.43± 0.52	1.45± 0.79
	L	1.51± 0.29	1.47± 0.12	1.48± 0.51

U = Upper epidermis.
L = Lower epidermis.

Table 2. Leaf epidermal characteristics of *Urena lobata* from non-polluted micro-habitats

Descriptions surface		Non-polluted micro-habitats		
		Ebvomodu	Ebvoneka	Eyaen
Nature of epidermal cell wall	U	Sinuous	Sinuous	Sinuous
	L	Sinuous	Sinuous	Sinuous
Type of stomata	U	Diacytic	Diacytic	Diacytic
	L	Diacytic	Diacytic	Diacyti
Stomata index (I)(%)	U	26.92	25.00	21.00
	L	31.02	37.12	42.69
Mean length of stomatal pore (µm)	U	1.39± 0.10	1.35± 0.46	1.31± 0.17
	L	1.59± 0.67	1.51± 0.54	1.48± 0.14
Mean width of stomatal pore (µm)	U	0.40± 0.33	0.44± 0.92	0.46± 0.36
	L	0.42± 0.18	0.43± 0.11	0.49± 0.86
Mean length of guard cells (µm)	U	2.22± 0.14	2.25± 0.23	2.19± 0.97
	L	2.12± 0.12	2.08± 0.70	2.35± 0.81
Mean width of guard cells (µm)	U	1.26± 0.15	1.23± 0.93	1.28± 0.19
	L	1.57± 0.12	1.46± 0.29	1.76± 0.24

U = Upper epidermis.
L = Lower epidermis.

On the other hand the lengths and widths of guard cells in the upper epidermis of the non-polluted populations were 2.22 ± 0.14, 2.25 ± 0.23 and 2.19 ± 0.97 and 1.26 ± 0.15, 1.23 ± 0.93 and 1.28 ± 0.19 respectively, while in the lower epidermis, the lengths and widths were 2.12 ± 0.12, 2.08 ± 0.70 and 2.35 ± 0.81 and 1.57 ± 0.12, 1.46 ± 0.29 and 1.76 ± 0.24 respectively.

Polluted populations of this species from the sides of busy roads were observed to be different from the populations from the non-polluted villages due to aberrations (which ranged from mild to severe) discovered on the surfaces of the leaves. At the points of leaf collections it was observed that on the upper and lower leaf surfaces of this specie were deposits of dust particulates, films of soot and other particles resulting from exhausts, sand and the wear and tear of automobile tyres which were continuously deposited on the leaves.

The possibility of these extraneous materials undergoing chemical reactions with the leaves to form complex acids or solutions which may be injurious to the leaves cannot be underrated. No wonder there were patches of burnt epidermis and eroded cuticular wax on the leaf blade. These conditions of the leaves were not observed in the non-polluted populations as the leaves were free of dusts, soot and other extraneous materials. This could be as a result of their locations (micro-habitats in villages far away from busy roads and other sources of environmental air pollution).

Comparatively, the leaf area (LA) of the leaves from polluted microhabitats were smaller than those of the non-polluted microhabitats. This, no doubt, could be due to the adverse environmental conditions created for the species by air pollution along busy roadsides where it grows. It is genuine to suspect that the air pollutants that filled the busy roads where the samples were collected might have had negative influence on the photosynthetic activities of the leaves, thereby, making them not to develop like their counterparts in the non-polluted microhabitats.

These views seem to corroborate the previous reports of Socha (2002), Sylvia *et. al.,* (2005) and Otoide and Kayode (2008) who reported damages of leaves of some botanicals due to air pollutants such as Surphur dioxide, Nitrogen Oxides, Ozone and Peroxyacetylnitrates (PANSs) which enter the pores in leaves. Microscopic observations of the leaves from the polluted microhabitats revealed that the stomatal pores were plugged at both the upper and lower surfaces. This might be as a result of the cumulative effects of the air pollutants that dominated busy roads where the plant grows.

This was, however, not the case with the leaves from non-polluted microhabitats in which the stomatal pores were not plugged. It is worthwhile to assert that a leaf surface that is full of plugged stomata would experience difficulties in thriving in any environment since, exchange of gases and other metabolic activities would be prevented in the leaves. These assertions are in line with the reports of Edwardo (2002) that dust on leaves blocks stomata and lowers their conductance to CO_2 and interfered with photosystem II (water-plastoquinone oxidoreductase). He further reported that exposure of leaves to pollutants such as SO_2 and NOx had been found to cause stomata closure and curtailed photosynthesis. Also, the assertions tend to confirm the previous observations made by the author and his collaborator, such as: Kayode and Otoide (2006), Kayode and Otoide (2007a&b),

Otoide and Kayode (2008), Otoide and Kayode (2013) and Otoide (2014) on *Chromolaena odorata, Newbouldia laevis, Elaeis guineensis, Amaranthus spinosus, Euphorbia heterophylla,Chromolaena odorata, Commelina diffusa, Kyllinga pumila, Polyalthia longifolia, Digitaria gayana and Trianthema portulacastrum.*

Conclusively, results obtained from this study showed that the leaves of *Urena lobata* from along busy roads suffered some damages due to the cumulative effects of air pollutants that characterised busy roads where this specie grows. This is a pointer to the facts that busy roads are usually full of damaging air pollutants from automobiles plying them. Additionally, the possibilities of the pollutants to contaminate the raw and processed food items that are commonly displayed along the sides of busy roads by food vendors in Nigeria cannot be undermined.

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