



EFFECT OF AUTOMOBILE EXHAUST ON SOME LEAF MICROMORPHOLOGICAL CHARACTERISTICS OF SOME MEMBERS OF *Verbanaceae*, *Annonaceae* AND *Euphorbiaceae* families

¹Haruna, H., ¹Aliko, A.A., ¹Ahmad, F.A., ²Abubakar, A.W.

¹Department of Plant Biology, Bayero University, P.M.B. 3011, Kano - Nigeria.

²Department of Biological Sciences, Federal University, Dutse - Nigeria.

*correspondence author: hajarah556@yahoo.com

ABSTRACT

The study was carried using the Botanic Garden of the Kano State Zoological Garden as the control site in comparison with roadside in order to determine the effect of exhaust from vehicles on the number of stomata, epidermal cells, stomatal length, stomatal breadth, pore length and pore breadth. Results obtained showed an increase in the number of stomata and epidermal cells per unit area in leaf samples collected from polluted sites than those from unpolluted site. However, the length and breadth of stomata, pore length and pore breadth in the polluted sites was lower than that of the control site. Furthermore, there was no significant difference based on the type of stomata in the species studied at the two different sites. An increase in number of stomata, number of epidermal cells and stomatal density was found in plants growing on the road side both on the abaxial and adaxial surfaces, a decrease was found on the stomatal length, stomatal breadth, pore length, pore breadth and stomatal index of the plants growing on the road side when compared with those in the control site. These changes in stomata and epidermal cells per unit area could be used as an indicator of environmental stress which could be recommended in high traffic density areas for the early detection of urban air pollution.

Key words: Automobile exhaust, Micromorphology, Leaf, *Verbanaceae*, *Annonaceae*, *Euphorbiaceae*.

INTRODUCTION

Today air in most large cities is severally polluted and this pollution has a tremendous impact on the health and population. Industrialization, the growth in number of vehicles in an area and the burning of biofuels have led to the rapid deterioration of indoor and outdoor air quality, clean air has so far been treated as an unlimited and free natural resources only now as the health cost of polluted air are mounting, people are beginning to realize that clean air is valuable, the health impact of pollution is considerable premature death due to respiration and cardiovascular diseases and illness due to chronic respiratory diseases like asthma and bronchitis have increases.

In urban areas the transport sector causes the most pollution, producing 74% of the CO₂ and all the lead emitted. The number of vehicles in Kano has been increasing leading to a concurrent increase in pollution and plants being static i.e. immobile are therefore more exposed to dangers of road side due to intense vehicular traffic emission and their leaves are in constant exchange of gases with the atmosphere through transpiration by an organ called stomata (Kabata *et al.*, 2010).

Stomata are very minute openings found in the epidermal layer in the green aerial part of the plant, particularly the leaves. The roots of non-green part of the plants are free from them. Each stoma is surrounded by two semilunar cells, known as the

guard cells, the term stoma is often applied to the stomatal opening plus the guard cells.

The leaf stomata are the principal means of gas exchange in vascular plants. Stomata are small pores which may occur on the upper side of the leaf (adaxial) or lower side (abaxial) which open or close under the control of a pair of banana – shaped cells called guard cells, when open stomata allow CO₂ to enter the leaf for synthesis of glucose, and also allow for H₂O and free O₂ to escape. In addition to opening and closing of the stomata (stomatal behavior) plants may exert control over their gas exchange rates by varying stomata per unit area (stomata density) the more CO₂ can be taken up and the more water released.

Stomatal density refers to the number of stomata within a given part on the epidermis of the leaf, the stomatal density, index and distribution may vary due to factors such as temperature, humidity and light intensity in the natural environment of plant (Kimball, 2010) Atmospheric CO₂ is also known to affect the stomatal density of leaves. This relationship is inverse meaning that as CO₂ increases, the number of stomata decreases and vice versa. This occurs because as CO₂ concentration in the air surrounding the leaf increases a lesser volume of air is necessary to satisfy the need for gas exchange the reverse happens in low CO₂ condition as a greater volume of air is necessary to sustain the plant. Stomatal index is defined as the ratio of stomata to all epidermal cell

and it is influenced by environmental changes such as

Atmospheric pollution is becoming a worldwide concern, particularly in regions with intense motor vehicle traffic Isidori *et al.*, (2003) and researches have revealed that plants growing on the road side present changes regarding to color, length and even in their micromorphology, therefore it will be of great importance to determine the changes that occur on some micromorphological characteristics of plants exposed to automobile exhaust In this research project is aimed at knowing the effect of pollution on the number of stomata, epidermal cells, stomatal length, stomatal breadth, pore length, pore breadth, stomatal index and stomatal density of plants grown on selected roadsides in Kano.

MATERIALS AND METHOD

Sample collection

The most attention was given for the collection of plants samples which were closer to the traffic pathway getting direct contact to automobile - exhaust. Fresh collections were made from two sites which were recognized to be busy and the sites are Sharada Kwanar Freedom which has high number of vehicles passing by and the second site which is court road close to Aminu Kano Teaching Hospital, all located in Kano state and both two sites were recognized for the presence of automobile exhaust pollution. For comparison, collections were done also from a relatively clean area which is Kano state Botanical garden located inside the Kano State Zoological garden, which was considered as control. The plant samples collected were leaves from *Lantana camara*, *Euphorbia tithymaloides* and *Polyalthia longifolia*. The plants were selected based on their availability on both location i.e. the control site and the road sites

Method of Studying the Leaves

The leaves were first washed in water to remove dust particles and then fixed in formalin acetic acid (FAA) for 24 hours as described by Salgare and Iyer (1991). Epidermal peels were obtained by direct peel method or by scraping off with a safety razor blade as described by Gill and Karatela (1982). Epidermal peels were stained with 2-3 drops of safranin and mounted in aqueous glycerol and used immediately. Observations were made from ten good slides of each plant sample per site in counting of stomata, epidermal cells, and measurements of stomatal length, breadth, pore length and breadth. Camera lucida were used at $\times 400$ magnification. Measurements of stomatal length, stomatal pore size were made using a calibrated eyepiece graticule (micrometers) and recorded. Stomatal densities were measured following the formula described by Mustapha (1984).

$$S/\pi r^2 \text{ where}$$

S= Number of stomata counted

$$\pi r^2 = \text{Area field of view}$$

Where $r=0.65$

$$\pi=3.142$$

Stomatal index was calculated according to the work at Ferris and Taylor, (1994) using the formula

light and CO₂ concentration (Wood and Kelly, 1995).

$$S.I = \frac{\text{Number of stomata}}{\text{Number of epidermal cell} + \text{number of stomata}} \times \frac{100}{1}$$

Where

S=Number of stomata

E=Number of epidermal cells

Data Analysis

The data obtained was analyzed using t-test in order to compare between the two treatments and see whether automobile exhaust have effect on some micromorphological characteristics using Ms-Excel.

RESULTS AND DISCUSSION

The result obtained from the adaxial and abaxial surfaces of the plants leaves studied. It was observed that number of stomata on the adaxial surface in control plants was found to be less than 50% of the number of stomata recorded in those exposed to automobile exhaust. Similar trend was also observed with regards to the number of epidermal cells. The measurement of stomatal length, stomatal breadth and pore length (μm) of plants exposed to automobile exhaust was found to be 1.55, 1.64 and 2.08 in *E. tithymaloides*, *P. longifolia* and *L. camara* respectively and this is statistically similar in those plants raised in the control site. Similar trend was also observed on the abaxial leaf surface (Table 1).

The stomatal index (%) and stomatal density on adaxial surface of plants exposed to automobile exhaust were also found to be significantly greater than what was observed in the control plants on the adaxial surface. However, stomatal index on the abaxial leaf surface was found to be significantly not affected by the automobile exhaust (Table 2).

The increase in number of stomata, epidermal cells, stomatal index and stomatal density in the polluted site was due to vehicular emission. This response was due to the fact that more stomata are needed by the plants to release poisonous substance that might get into the plants' system through the openings. Similar observations were made in *Azadirachta indica* and *Dalbergia sissoo* (Sharma and Roy, 1995); *Azadirachta indica*, *Polyalthia longifolia* and *Cassia siamea* (Aggarwal, 2000); *Nyanthese arbortristis*, *Quisqualis indica* and *Terminalia arjuna* (Rai and Kulshreshtha, 2006). Other studies which are in line with these studies include Lakshmi (2010), Salgare (1990) and Salgare (1989). However, similar reports on the decrease in number of stomata and epidermal cells recorded on the leaves of plants treated as control were also reported by Marie and Gomez (2008) and Prakash *et al.* (2008), and which according to them could be regarded as an adaptive feature developed by plants in order to prevent the entry of toxic substance from the atmosphere in to plants through the stomata.

Reduction in the size of stomata resulted from inhibited cell elongation, leaf area and consequently the increase in cell frequency, as suggested by Rai and Kulshreshtha (2006). Similar result was also observed in studies of the foliar epidermal traits from other works (Trivedi and Singh 1990; Aggarwal 2000; Kaur 2004; Dineva 2004; Rai and Kulshreshtha, 2006; Privanka *et al.*, 2013).

A decrease in stomatal index occurs just as in the work of Verna *et al.* (2006). An increase in stomatal density occur just as in the work of (Rai and Kulshrestha 2006; Priyanka and Mishra, 2013).

In the study, paracytic type of stomata was observed in all the plants and surfaces. This is an indication that the automobile exhaust did not affect the type of stomata as seen in plates i - xii

Table 1: Mean Stomata measurement on adaxial (upper) surface of plant leaves studied

Species		<i>Euphorbia tithymaloides</i>	<i>Polyalthia longifolia</i>	<i>Lantana camara</i>
NO.ST	Control	16	4	14
	Polluted	29	10	27
NO.E.C	Control	30	14	40
	Polluted	56	48	70
S.L(μ m)	Control	1.64	1.53	2.5
	Polluted	1.55	1.64	2.08
S.B (μ m)	Control	1.38	1.64	1.66
	Polluted	1.12	1.61	1.60
P.L(μ m)	Control	1.25	0.47	1.85
	Polluted	1.21	0.43	1.51
P.B (μ m)	Control	0.34	0.34	0.34
	Polluted	0.35	0.37	0.37
S.I %	Control	34.36	16.90	25.66
	Polluted	45.49	21.12	28.31
S.D	Control	12.00	2.72	10.45
	Polluted	22.00	6.00	20.00

Table 2: Mean stomata measurement on abaxial (lower) surface of plant leaves studied

Species		<i>Euphorbia tithymaloides</i>	<i>Polyalthia longifolia</i>	<i>Lantana camara</i>
NO.ST	Control	20	18	18
	Polluted	34	26	31
NO.E.C	Control	37	77	52
	Polluted	61	128	89
S.L(μ m)	Control	1.82	1.77	2.60
	Polluted	1.60	1.77	2.10
S.B (μ m)	Control	1.50	1.72	1.60
	Polluted	1.20	1.70	1.57
P.L(μ m)	Control	1.40	1.12	1.90
	Polluted	1.23	1.28	1.30
P.B (μ m)	Control	0.44	0.47	0.44
	Polluted	0.35	0.43	0.36
S.I %	Control	34.5	18.51	26.2
	Polluted	33.8	17.1	26.23
S.D	Control	14.54	13.4	14.01
	Polluted	26.00	19.81	23.67

KEYS: **NO.ST**=Number of stomata, **NO.E.C**=Number of epidermal cells, **S.L**=Stomatal length, **S.B**=Stomatal breadth, **P.L**=Pore length of stomata, **P.B**=Pore breadth of stomata, **S.I**=Stomatal index, **S.D**=Stomatal density

Adaxial surfaces of plant leaves studied

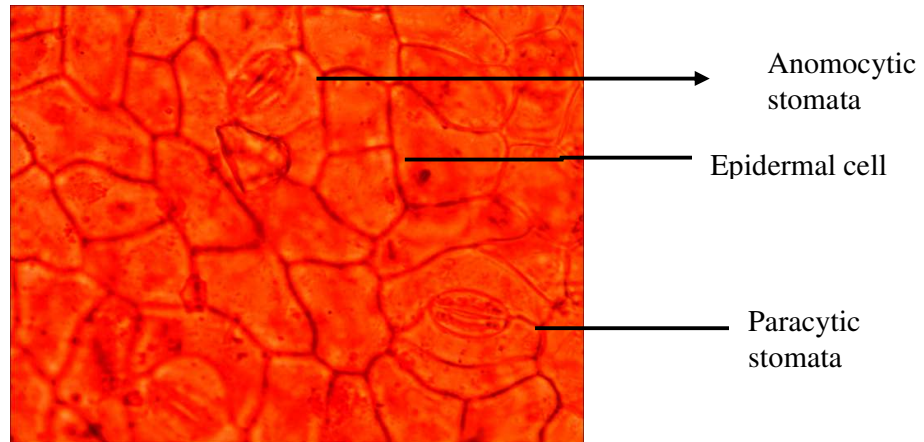


Plate i: A photomicrograph of polluted *Euphorbia tithymaloides* showing anomocytic and paracytic type of stomata with epidermal cell $\times 400\text{mg}$

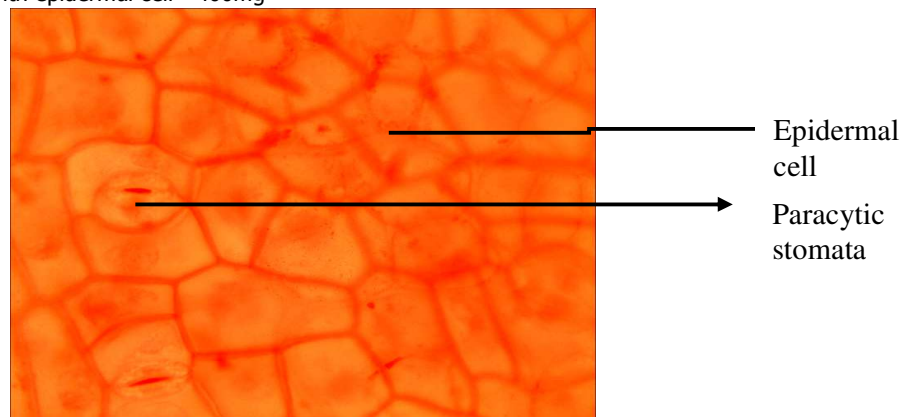


Plate ii: A photomicrograph of control *Euphorbia tithymaloides* showing paracytic type of stomata and epidermal cell $\times 400\text{mg}$

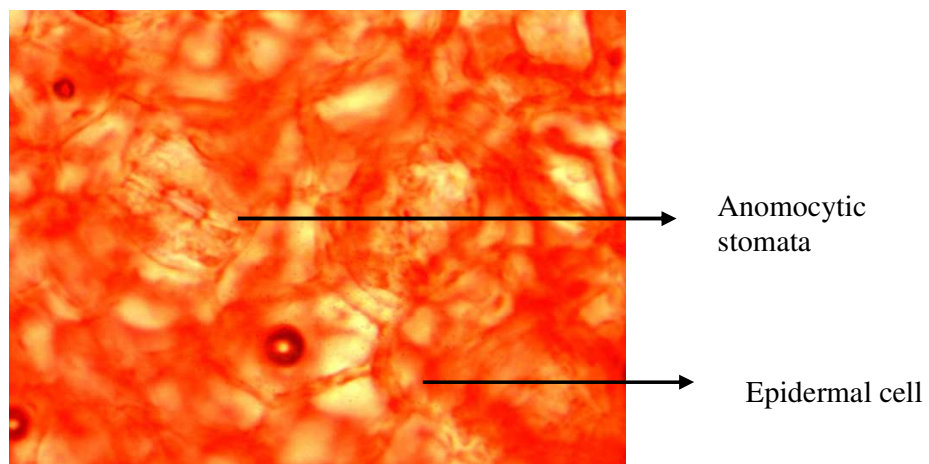


Plate iii: A photomicrograph of control *polyalthia longifolia* showing anomocytic type of stomata, guardcell and epidermal cell $\times 400\text{mg}$

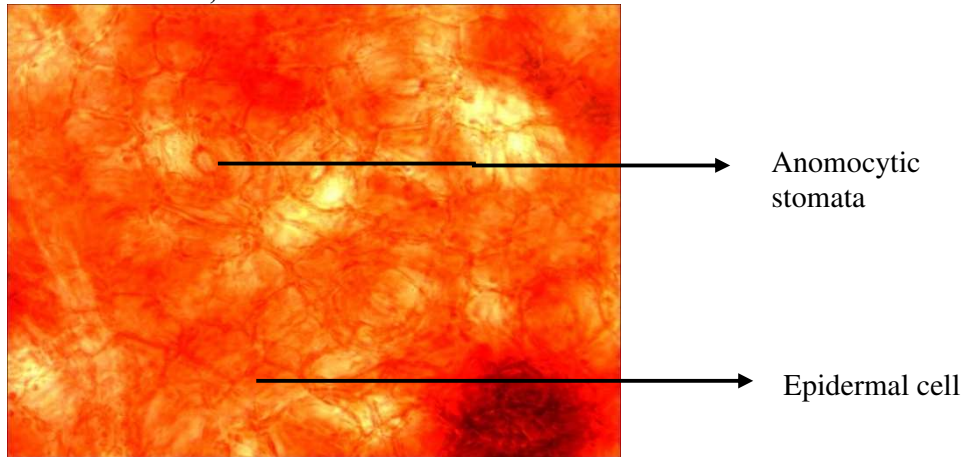


Plate iv: A photomicrograph of polluted *polyalthia longifolia* showing anomocytic type of stomata, guard cell and epidermal cell $\times 400\text{mg}$

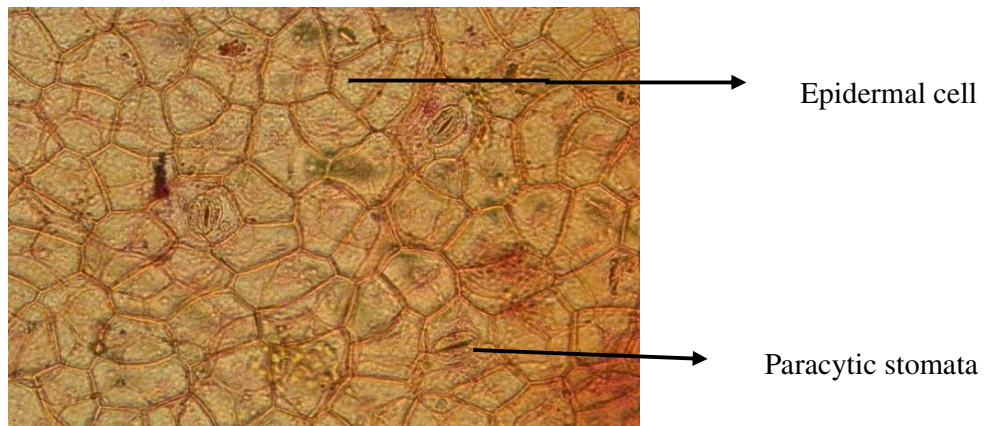


Plate v: A photomicrograph of Polluted *Lantana camara* showing paracytic type of stomata and epidermal cell $\times 400\text{mg}$



Plate vi: A photomicrograph of control *Lantana camara* showing paracytic type of stomata and epidermal cell $\times 400\text{mg}$

Abaxial surface of plant leaves studied

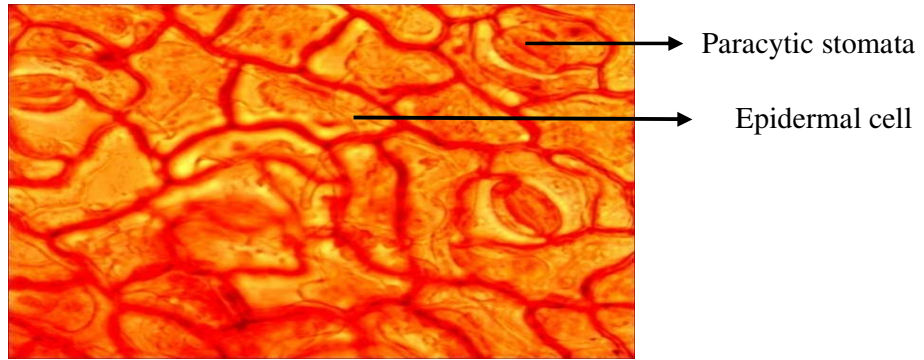


Plate vii. A photomicrograph of polluted *Euphorbia tithymaloides* showing stomatal guard cells, paracytic type of stomata and epidermal cells at $\times 400$ mg

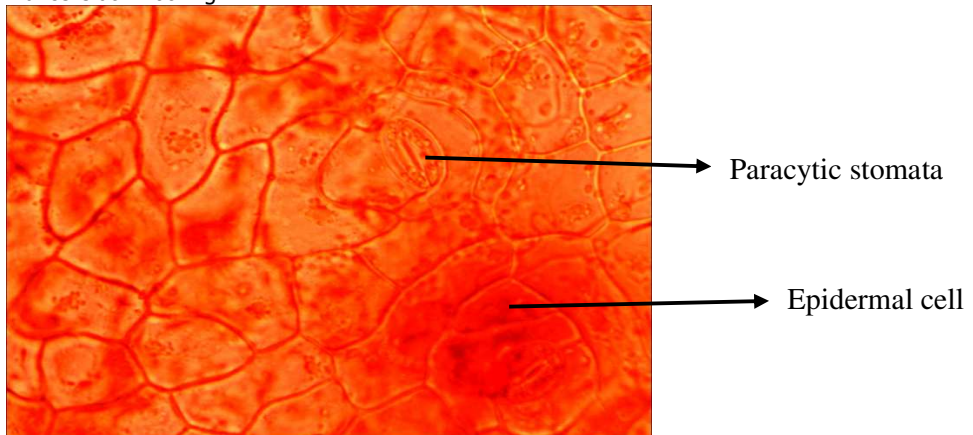


Plate viii. A photomicrograph of control *Euphorbia tithymaloides* showing stomatal guard cells, paracytic type of stomata and epidermal cells at $\times 400$ mg

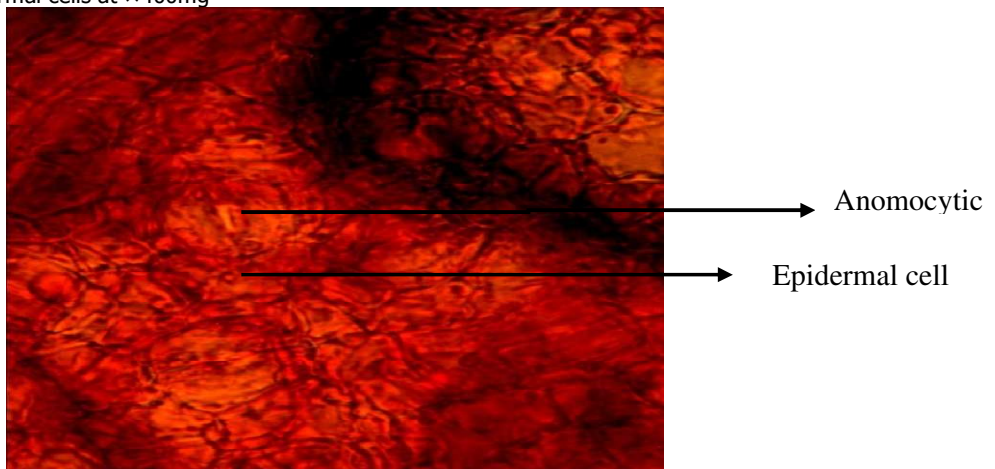
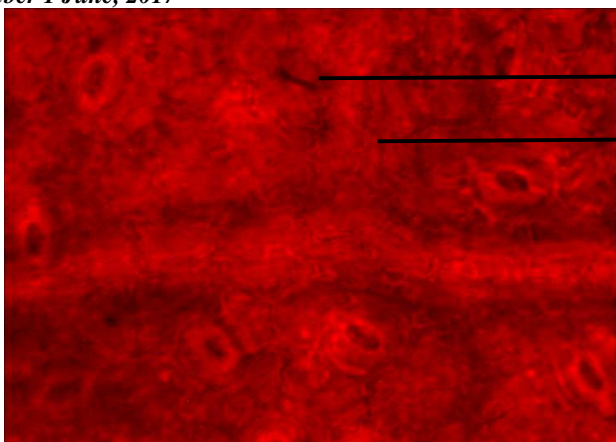
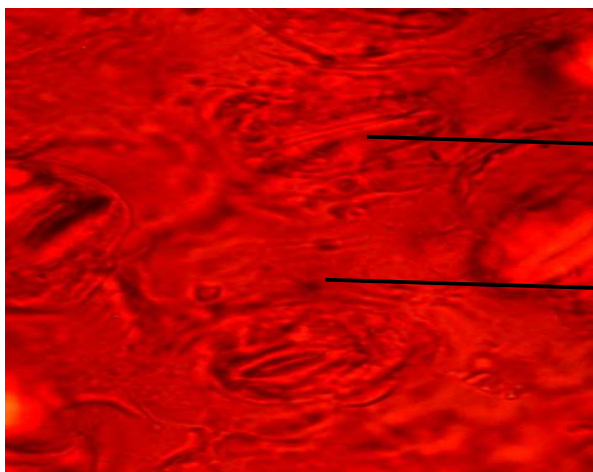


Plate ix. A photomicrograph of control *Polyalthia longifolia* showing anomocytic type of stomata and epidermal cells $\times 400$ mg



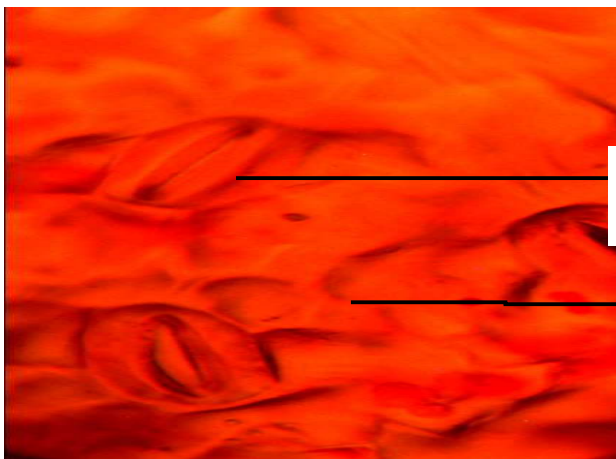
Anomocytic stomata
Epidermal cell

Plata x. A photomicrograph of polluted *Polyalthia longifolia* showing anomocytic type of stomata,guard cell and epidermal cells ×400 mg



Paracytic stomata
Epidermal cell

Plate xi: A photomicrograph of polluted *lantana camara* showing paracytic type of stomata, guardcell and epidermal cell mg×400



Paracytic stomata
Epidermal cell

Plate xii. A photomicrograph of control *lantana camara* showing paracytic type of stomata and epidermal cell ×400mg

CONCLUSION

BASED ON THE result obtained it is apparent that automobile exhaust has effect on the leaf micromorphology because of an increase in number of stomata, number of epidermal cells, stomatal index and stomatal densities was observed in plants

exposed to automobile exhaust both on the abaxial and adaxial surfaces when compared with what was recorded in the plant treated as control. These changes could be considered and exploited towards establishing presence of significant level of air pollution in an environment.

REFERENCES

- Aggarwal, P. (2000). The effect of auto-exhaust pollution on leaf surface of *Cassia siamea* (L.), a road side tree. *Acta Ecologica* **22**:101-106.
- Dineva, S. (2004). Comparative studies of the leaf morphology and structure of white ash *Fraxinus Americana* L. and London plane tree *Platanus acerifolia* willd. growing in polluted area. *Dendrobiobiology* **52**:3-8
- Gill, L.S. and Karatela, Y.Y. (1982). Epidermal structures and stomatal ontogeny in some Nigerian Cucurbitaceae. *Wildenowia*. **12**:303-310
- Isidori, M., Ferrara, M., Lavorgna, M., Nardelli, A. and Parrella, A. (2003). In situ monitoring of urban air in southern Italy with the tradescantia micronucleus bioassay and semipermeable Membrane devices (SPMD's). *Chemosphere* **52**:121-126
- Kabata – pendias, A. and Pendias, H. (2010). *Trace elements in soil and plants*. Boea Raton, Florida CRC Press.
- Kaur, S. (2004). Stomatal responses of lemon (*Citrus medica*) to exhaust emissions from vehicles using different types of fuel. *Pollution Research* **23** (3): 451-454.
- Kimball, John W. Gas Exchange in Plant *Kimball's Biology* p. **22**. Jan, 2010
- Kulshreshtha, K., Srivastava, K., Ahmed, K.J. (2005). Effect of automobile exhaust pollution on leaf surface structures of *Calotropis procera* L. and *Nerium indicum* L. *Feddes repertorium*, **105**: 185- 189.
- Kulshreshtha, K., Yunus, M., Dwivedi, A.K. and Ahmad, K.J. (1980). Effect of air pollution on the epidermal traits of *Jasminum sambac* Ait. *New Botanist* **7**: 193-197.
- Lakshmi, B.O. (2010). *Environmental pollution – Biological Risks Oxidative Stress and Natural Antioxidant Principles*. Paper presented in the sixteenth NCA National Symposium on applications of biotechnology in the environment management and medicine 19-21 November, Davangere, India.
- Marie-Duldulao, C.G. and Gomez-Romeo, A. (2008). Effect of vehicular emission on morphological characteristics of young and mature leaves of sunflower and Napier grass. *Benguet State University, Research Journal*. **16** :142151.
- Mustapha, O.T. (1984). *Biosystematics studies of *Pancratium hirtum* A.Chev. (Amaryllidaceae) in Nigeria*. An M.sc Thesis submitted to the University of Ilorin.
- Prakash, G., Joshi, C., Chauhan, A. (2008). Performance of locally grown rice plants (*Oryza sativa*) exposed to air pollutants in a rapidly growing industrial area of Haridwar. *Life Science Journal*. **5**(3): 57-61.
- Priyanka, R. and Mishra, R.M. (2013) Effect of Urban air pollution on epidermal traits of road side tree species, *Pongamia pinnata* (L) Merr, *Journal of Environmental science, Toxicology And Food Technology*. **2**:04-07.
- Rai, A. and Kulshreshtha, K. (2006). Effect of particulates generated from automobile emission on some common plants. *Journal of food, Agriculture and Environment* **4**(1): 253-259.
- Ramanathan, M. and Kanabiran, B. (1989). Effects of soil dust pollution on foliar epidermis of *Calotropis gigantea* (L.) R.Br. and *Ipomea carnea* Jac. (Abstract). *Journal of Indian Botanical society*. **67**: 100.
- Salgare, S.A. and V.B. Thorat, (1989). Effect of auto-exhaust pollution at Andheri (West) on the micromorphology of some trees- II (Monsoon collection). Proc. 8th National Conf. Soc. Toxi. and Workshop Reproductive Toxi. Deptt. Zool. College of Sci. Sukhadia Univ., Udaipur. Ab. No. 91.
- Salgare, S.A. and Rawal, M. (1990). Effect of auto-exhaust pollution at Andheri (West), Bombay on anatomy of some cultivated plants (monsoon season). *Journal of Ecobiology*, **2**(4): 273-280.
- Salgare, S.A. and Iyer, M.P. (1991). Effect of auto-exhaust pollution at Byculla on the Micro-morphology of Some weeds. *New agriculturist*, **1**(2): 123-128.
- Salisbury, E.J. (2006). On the causes and ecological significance of stomatal frequency with special reference to woodland flora. *Phill. Trans, R. Soc. B.*, **216**: 1-65.
- Sharma, M. and Roy, A.N. (1995). Effect of automobile exhaust on the leaf epidermal features of *Azadirachta indica* and *Dalbergia sissoo*. *Int. Journal of Mendel* **12** (1-4): 18-19.
- Sunstar .com. ph/ Static/ bag/2009 /02/10/news.
- Trivedi, M.L. and Singh, R.S. (1990). Effect of air pollution on epidermal structures of *Croton bonplandianum* Baill. *New Botanist* **17** (3-4): 225-229.
- Verna, R.B., Mahmooduzzafar, T.O. and Siddiqi, M.I. (2006). Foliar Response of *Ipomea pes-trigidis* L. to coal smoke pollution, *Turkish Journal of Botany*. **30**(5):413-417
- Wood and Kelly (1995). Isolation and physiological characterization of *Thiobacillus thysaris* **152**:160-166.