Effects of vehicle smoke on anatomical features of Soybeans [*Glycine max*] plant in Katsina State, Nigeria

Fatima Abdullahi Hari, Kalimullah Saghirand, * Nasir Hassan Wagini.

Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria

IJASR 2021
VOLUME 4
ISSUE 4 JULY – AUGUST **ISSN: 2581-7876**

Abstract: A study on the effect of vehicle smoke on anatomy of Soybeans [*Glycine max*] was conducted in the Biological Garden and Postgraduate laboratory of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK), and Nigeria. An effort was made in the study to screen out the anatomical features of *G. max* growing in unpolluted environment as compared to the ones growing in polluted environment. Four experiments were set, (three as test experiments and one as control) using a poly house containing five pots each. Polluted and unpolluted leaves and stems of the *G. max* were cut into sections in the laboratory and identified under microscope for the anatomical studies. All the parameters were analysed using one way analysis of variance (ANOVA) and means between the treatments were compared using Tukey's-b test at the significance level of $p \leq 0.05$. Results of the study revealed that the polluted leaves and stems were showing less epidermal cells, palisade and spongy parenchyma cells, xylem and phloem, vascular bundles, stomata and trichome size compared to the unpolluted ones. Findings of this study showed that*G. Maxhad* developed anatomical changes under polluted environment that further led to injury of the plant which could either be acute or chronic depending on the exposure time. Therefore, it is suggested that *G. max* farmers should avoid planting it along busy roads.

Keywords: Anatomy, vehicle smoke, *Glycine max*, Pollution

INTRODUCTION

Air pollution may be defined as any atmospheric condition in which substances are present at concentrations high enough above their normal ambient levels to produce a measurable effect on man, animals, vegetation, or materials (Grossi and Brimblecombe, 2007).Air pollution is arguably the most disturbing and dangerous type of pollution not just to plant but all life in ecosystem (Gostin, 2009).

The sources of pollution are either natural sources which include those that are not directly under human control, and those which are caused by human activities that pollute the environment (Sanderfoot and Holloway, 2017). Air pollution is a major problem arising mainly from industrial emissions and vehicular exhaust, the exposure of these pollutants affects the plants productivity (Giri*et al*., 2013). The release of vehicle exhaust into the air is one of the major sources of air pollution especially in the urban centres where many people owned vehicles leading to an increase in the release of gases which causes air pollution (Onokala, 2008).

Even though air pollution is usually a greater problem in cities, pollutants contaminate air everywhere, these substances include various gases and tiny particles that can harm human health and damage the environment.The reaction of different species to the altered environmental conditions is strongly correlated with their structural and functional features(West *et al*., 2012).

Studies concerning the anatomy of the vegetative organs under conditions of pollution were carried out by some researchers (Alves*et al*., 2008; Ahmad *et al*., 2005; Silva *et al*., 2005,Verma*et al*., 2006).However, in developing country like Nigeria little research has been carried out on measuring the effects of air pollution levels on agriculture (Ekiyor*et al.,* 2019).Farmers conduct their usual arable farming business in smoke polluted environment in Nigeria with no regard the impact of air pollution on their crops due to lack of information and data to support the allegations (Lugadiru, 2017).The best way of understanding and gaining knowledge on air pollution in Nigeria is continuous monitoring (using very sensitive plants) for proper conclusive recommendations.

MATERIALS AND METHODS

Study area

Katsina State is located in the north-western zone of Nigeria with 34 Local Government Areas. Katsina town is the state capital and is the largest settlement in the state. Katsina State is bounded byJigawa and Kano state to the east, Zamfara State to the west, to the south by Kaduna State and bordered by the Republic of Niger to the north. Apart from farming during the raining season, dry season farming is done along urban streams and along numerous dams built by the state and federal governments (Abaje*et al.*,2015).

Soil preparation and planting of *Glycine max*

To analyze the effects of exhaust pollutants on the growth and development of *G. max* plants, the soil was obtained from the Biological garden of Umaru Musa Yar'adua University, Katsina (UMYUK). The dry soil was sieved through a 2 mm sieve to remove gravels and other materials. The soil was mixed with organic manure at the ratio 1:3 to fill the experimental pots of 19 cm diameter and 11 cm in height, water was added to the soil to make suitable moisture for seed germination. At the bottom of the pots holes were made and a filter paper was placed to prevent leaching of soil.The *Glycine max* seeds were planted in twenty five pots, there were five pots for each replicate and five seeds were sown in each pot.(Zafar*et al.*, 2016).

Source and Application of Smoke

Four frames were prepared and covered with white polythene to absorb light forphotosynthesis and also help to maintain temperature, holes were dug on the ground in which five pots of each replicates were placed and covered with the frames to avoid destruction by rainfall or wind. The first poly house was used for control (not exposed to smoke), second poly house was exposed to one (1) minute exhaust smoke, the third poly house was exposed to two (2) minutes exhaust smoke and the fourth poly house was exposed to three (3) minutes exhaust smoke to observe the effect of smoke pollution on the *Glycine max*plants.

The smoke was applied using old (Jincheng) motorcycle emission;it ran on petrol, whereas lubrication oil was also provided for better performance. The smoke passed into the polyhouse using a 10 feet rubber pipe in which one-end of the rubber pipe was firmly fixed to the motorcycle exhaust (muffler) outlet while the other free end of the rubber pipe passed through a preferable water system (cooling system) horizontally, there by having it outlet into the polyhouse. The exhaust (smoke) passes into the polyhouse usually by igniting the motorcycle while rotating the throttle in a fixed manner for the specified durations of one (1), two (2) and three (3) minutes respectively; Finally, when the smoke exhaustion limit have been reached, the throttle was released and the motorcycle was switched off immediately. This method was repeatedafter every3 days. The plants were well taken care of with intensive watering, weeding, pest control and other necessary measures.

Anatomical Examinationof *Glycine max*Leaves

Sample collection: Leaves for the anatomical studies were collected randomly from each replicate between 9am to 10am and transferred into formalin acetic acid alcohol (FAA) instantly to be fixed and preserved.

Fine sections of the leaves were taken transversely through the midrib using dissecting blade, the section was placed on a glass slide stained with safranin and glycerin covered with a cover slip, the sample was mounted under a light microscope for observation. The observed features include Thickness of cuticle, Number of epidermis, Length and width of parenchyma cells, Thickness of xylem and phloem (Jahan and Iqbal, 1992).

Stomata: The preserved leaf specimen were cut into pieces and placed in scanning electron microscope to observe the stomata.

Determination of size and frequency of stomata: The stomata sizes were determined by measuring guard cell length and width of stomata on each treatment. Measurements were made using an image tools software. The frequency of the stomata in each treatment was determined as a number of occurrences per microscopic field of view at 750× magnification of scanning electron microscope (Allahnouri*et al.,* 2018).

Anatomical Examination of *Glycine max* Stem

The transverse section of each replicate were gently cut into small pieces using a dissecting blade Akomolafe*et al.*, (2019) and placed inside a petri dish containing water and few drops of safranin and left for 3 minutes. The thinnest section of the material was taken with the help of a delicate brush and placed on a glass slide, a drop of glycerine was added over the section and covered with a cover slip, the sample was placed under a light microscope for observation. The observed features include thickness of epidermis, thickness of cortex, thickness of vascular bundles, thickness of phloem, thickness of xylem, Diameter of pith, Number of vascular bundles.

Trichomes size and frequency: The preserved leaves and stems of each treatment were gently cut into small pieces and mounted on a glass slides, the samples were stained with safranin and placed under microscope for observation. The size of the trichomes (length) was measured using an Image tool softwareand the frequency of the trichomes was determined as a number of occurrences per microscopic field of view at 100x magnification of light compound microscope. The numbers of trichome cells were counted visually.

Statistical Analysis

All the analysis were carried out using SPSS version 20.0 and charts were plotted in Microsoft excel 2010. The data obtainedwere subjected to one-way analysis of variance (ANOVA) and means between treatments were compared using Tukey's-b at the significance level of $p \leq 0.05$ confidence level.

RESULTS AND DISCUSSIONS

Vehicle Smoke on Stomata and Trichomes of *Glycine max*

The modification of the frequency and sizes of stomata and trichomes as a response to the environmental stress is an important manner of controlling the absorption of pollutants by plants. Analysis of variance shows a significant difference ($p \leq 0.05$) in the upper and lower stomata length and width, control with the highest followed by one and two minutes exposure.Table 1shows a significant difference in the leaf and stem trichome length ($p \leq 0.05$). Control with the longest trichome had more cells than the other treatment exposed to exhaust emission.

Table 1: Vehicle smoke on stomata and trichome of *Glycine max*

Parameters		Mean±S.E		
	Control	1 min exposure	2 min exposure	3 min exposure
USL (µm)	25.70±0.46[a]	20.45±0.41[b]	19.00±0.56[b]	0.00±0.00
USW (µm)	14.17±0.27[a]	11.68±0.48[b]	9.76±0.46[c]	0.00±0.00
LSL (µm)	25.70±0.46[a]	20.45±0.41[b]	19.00±0.56[b]	0.00±0.00
LSW (µm)	14.17±0.27[a]	11.68±0.48[b]	9.76±0.46[c]	0.00±0.00
LTL (µm)	385.17±39.48[a]	271.26±34.49[b]	191.56±23.89[b]	0.00±0.00
STL (µm)	484.57±38.89[a]	368.72±33.00[b]	295.00±27.17[b]	0.00±0.00
NLTC	5.20 ±0.44[a]	3.80±0.30[b]	2.30±1.23[b]	0.00±0.00
NSTC	7.00±0.50[a]	4.30±0.60[b]	2.60±0.83[c]	0.00±0.00

Means followed by the same letters in the same column are not significant at $p \leq 0.05$
Key:

Min= minutes**; USL:** Upper Stomata Length **USW:** Upper Stomata Width **LSL:** Lower Stomata Length **LSW:** Lower Stomata Width **LTL:** Leaf Trichome Length **STL:** Stem Trichome Length **NLTC:** Number of Leaf Trichome Cells **NSTC:** Number of Stem Trichome Cells.

Stomata and trichome were found to be reduced in plants exposed to exhaust gas pollution compared with those exposed to clean air (Plates I and II). This could be due to a direct effect of one or more of the pollutant gases on the functioning of the plants.

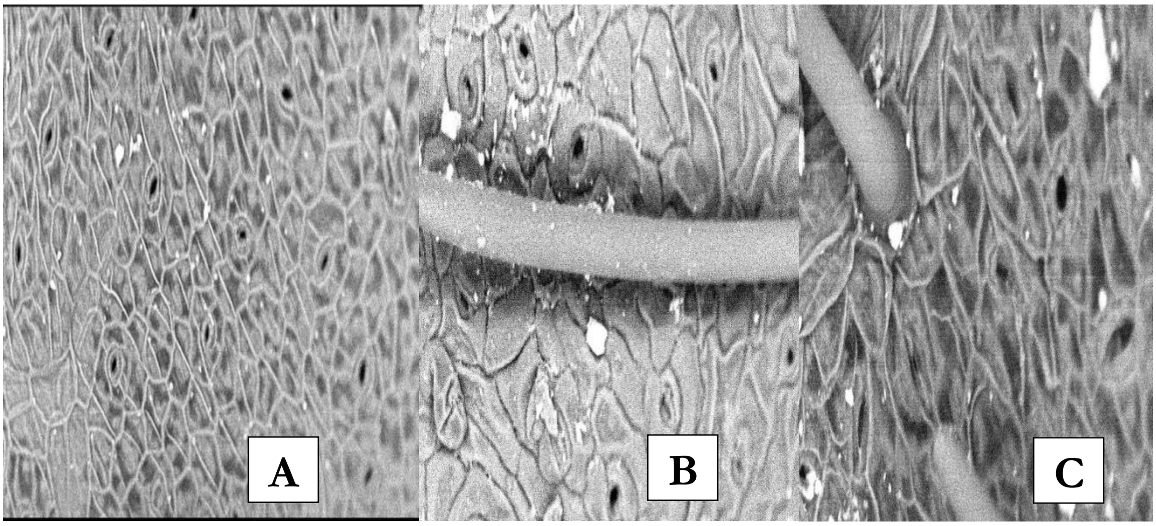

Plate I: *Glycine max* stomata of: A. control B. one minute vehicle smoke C. two minutes vehicle smoke.

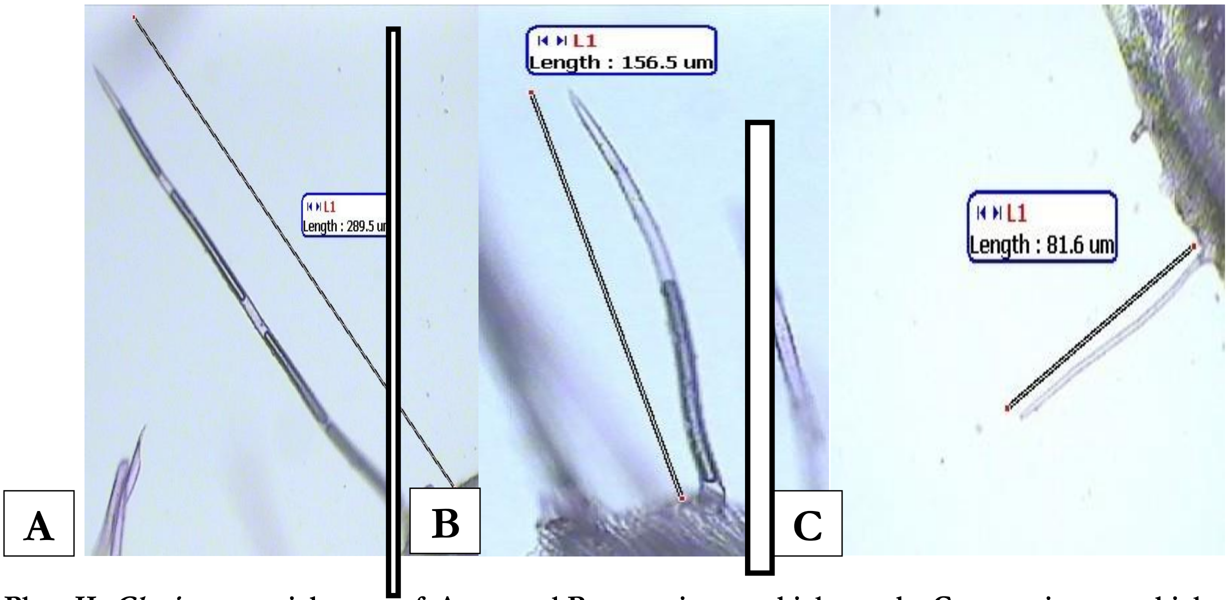

Plate II: *Glycine max*trichomes of: A. control B. one minute vehicle smoke C. two minutes vehicle smoke

This finding reflects to that of Verma*et al.* (2006) that report a significant decrease of stomatal density and stomatal index in *Ipomeapestigridis* grown undervarious degrees of environmental stresses (coal-smoke pollutants). Stomatal size was decreased significantly in (*Lotus corniculatus* L., *Trifoliummontanum* L., *T. pratense* L. and *T. repens* L.) analyzed by(Gostin, 2009) when exposed to air pollution, while stomatal index was increased to about a maximum of 20% in *Trifoliumrepens*leaves.

Vehicular pollution affect the stomatal index of both the plant species exposed to pollution as compared to control whereas length of trichomes decreased in *C. siamea* in the polluted area but *P. lanceolata* trichome length has increased (Kumar *et al.*, 2018).

A Study on the epidermal traits of single polluted and nine healthy leaves of *Psidiumguajava* was conducted by (Yunus and Ahmad, 1980) Leaves of guava collected from the cement factory, showed higher stomata and trichome frequency and smaller epidermal cells and trichome size compared to the leaves collected from the unpolluted areas. The length and width of guard and epidermal cells reduced in *Pongamiapinnata* due to exhaust pollution (Rai and Mishra, 2013)

Studies of macro- and micro-morphological features of plants growing on the road side of Pondicherry shows a decrease in the stomata size and the trichome morphology appears to be severely affected by air (vehicular) pollutants (Ramasammy, 2009).

Leaf anatomical studies of *Glycine max*

The Results in Table 2 show the effect of leaf anatomical features in control, one, and two minutes exhaust emission in *Glycine max*. In comparison with control, findings show a significant difference between the treatments. Leaves from polluted area showed a decrease in number of upper and lower epidermal cells, thickness of cuticle, length and width of parenchyma cells, thickness of xylem and phloem.

Table 2: Vehicle smoke on leaf anatomical parameters of *Glycine max*

Parameters (µm)	Control	Mean±S.E One minute	Two minutes	Three minutes
TOUC	14.10±2.18[a]	9.87 ± 0.42[b]	7.53 ± 0.67[b]	0.00±0.00
TOLC	12.54±0.16[a]	9.41 ± 1.30[b]	9.41± 1.30[b]	0.00±0.00
NOUE	52.44±2.56[a]	46.39± 2.56[b]	42.22±0.32[b]	0.00±0.00
NOLE	47.22±0.32[a]	40.74 ± 3.09[b]	38.38 ± 0.78[b]	0.00±0.00
L&WOPP	85.25 ± 8.60[a]	77.32±4.20[b]	72.25±4.14[b]	0.00±0.00
L&WOSP	70.32± 2.10[a]	63.26±1.51[b]	61.27±0.78[b]	0.00±0.00
TOPLM	1.15 ± 0.05[a]	0.57 ± 0.03 [b]	0.49 ± 0.1[b]	0.00±0.00
TOXLM	0.73 ± 0.52[a]	0.63 ± 0.02[b]	0.61±0.04[b]	0.00±0.00

Means followed by the same letters in the same column are not significant at $p \leq 0.05$
Key:

TOUC: Thickness of upper cuticle. **TOLC:** Thickness of lower cuticle **NOUE:** Number of upper epidermis. **NOLE:** Number of lower epidermis. **L&WOPP:** Length and width of palisade parenchyma. **L&WOSP:** Length and width of spongy parenchyma. **TOPLM:** Thickness of phloem. **TOXLM:** Thickness of xylem.

Plants growing in extreme environmental conditions specially, besides the roadside have anatomically and morphologically changes in their leaves due to influence of air pollution than plants growing in environmentally friendly conditions (Gielwanowaska*et al.,* 2005). The findings on leaf anatomical traits of these species growing at polluted sites have been compared with the findings of control site. Results revealed reductions in length and width of palisade and spongy parenchyma, thickness of cuticle, Number of epidermis and Thickness of xylem and phloem of polluted leaves as compared to control.

Changes in shape and structure of the cells have been widely reported. The cells of spongy parenchyma become flattened due to continuous exposure to pollutants. Significant reduction in spongy parenchyma on leaves of *F. bengalensis*of a polluted area was reported by (Jahan and Iqbal1992). Similarly, (Iqbal, 1985) has shown significant reduction in palisade and spongy parenchyma in leaves of white clover of a polluted population.Histochemical study by (Rhimi*et al.,*2016) reported that several structural changes such as decrease in the size of epidermal cells, tightening of palisade parenchyma cells and decrease in epidermal cells in both upper and lower epidermis was observed in Morpho-anatomical and physiological changes in grapevine leaves exposed to atmospheric pollution.Significant anatomical changes was observed in leaves of Mango, Mahogany and Koroi growing in polluted site as compared to non-polluted ones (Mitu*et al.,* 2019).

Leaf anatomy of Tansy also showed reduction in palisade parenchyma and upper and lower epidermis in polluted area as compared to leaves collected from non-polluted area, Significant reduction on leaves of Tansy plants from Ada Huja are adapted to the continuous effect of different pollutants (heavy metals, building materials, oxides of nitrogen and sulphur, etc.) which are released into the environment (Stevovice*et al.,* 2010).

Stem anatomical studies of *Glycine max*

Table 3 reveals findings in*Glycine max,* which are growing under the polluted atmosphere and unpolluted area. The polluted soybean shows highly significant (p≤0.05) growth loss. Thickness of the epidermis, cortex, phloem, xylem and vascular bundle shows significant difference as compared to control, on the other hand diameter of the pith area slightly increase in the polluted samples.

Table 3: Vehicle smoke on stem anatomical studies of *Glycine max*in polluted and unpolluted site

Parameters(μm)		Mean ± S.E		
	Control	One minute	Two minutes	Three minutes
TOEPDS	0.78±0.11[a]	0.67±0.13[b]	0.65±0.07[b]	0.00±0.00
TOCTX	0.46±0.99[a]	0.26±0.05[b]	0.22±0.06[b]	0.00±0.00
TOPLM	0.57±0.05[a]	0.31±0.07[b]	0.29±0.12[b]	0.00±0.00
TOXLM	0.79±0.05[a]	0.65±0.26[a]	0.60±0.22[b]	0.00±0.00
NOVB	7.00±0.50[a]	4.30±0.60[b]	2.60±0.83[b]	0.00±0.00
TOVB	5.05±0.20[a]	3.41±0.59[b]	3.28±0.14[b]	0.00±0.00
DOP	60.2±11.61[a]	76.5±10.47[b]	78±7.25[b]	0.00±0.00

Means followed by the same letters in the same column are not significant at p≤0.05
Key:

TOEPDS: Thickness of Epidermis **TOCTX**: Thickness of Cortex **TOPLM**: Thickness of phloem **TOXLM**: Thickness of xylem **TOVB**: Thickness of Vascular Bundle **DOP**: Diameter of Pith **NOVB**: Number of Vascular Bundle

An overall reduction is apparent in the thickness of epidermis, cortex, phloem, xylem and vascular bundle of the plant exposed to smoke exhaustion as compared to control; there is a reduction in the diameter of the pith cells in control as compared to the polluted samples Plate III.

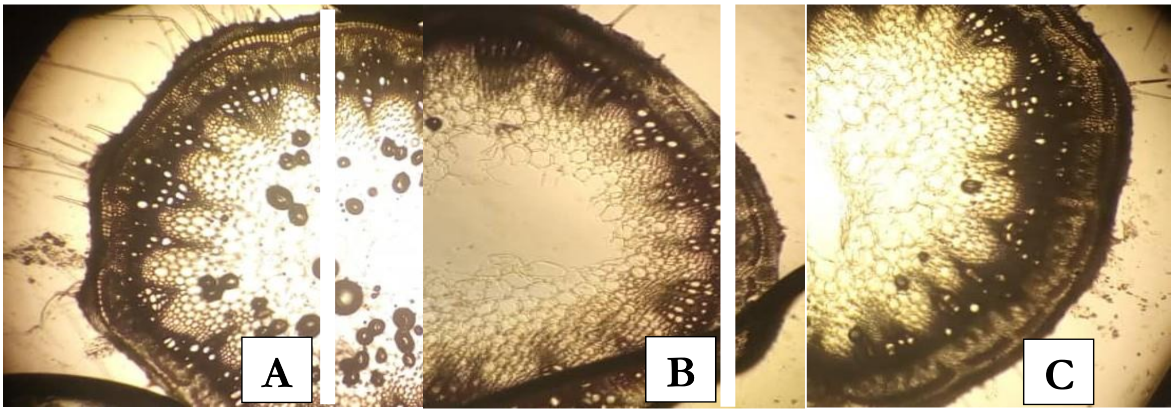

Plate III: Stem anatomy of *Glycine max*:A. control B. one minute vehicle smoke C. two minutes vehicle smoke

Study of two plant species *U.lobata* and *H.suaveolens* was carried out by (Akomolafe*et el.* 2019) *U. lobata*growing on the road side shows a reduction in the thickness of the epidermis and vascular bundle as compared to the one growing far from the roadside, on the other hand *H.suaveolens* growing on the road side shows an increase on the wall thickness and thickness of the vascular bundle which tends to exhibited higher protective covering of its internal structures on the road side. Pollutants emitted from the industry and automobile exhaust exercised a decisive influence on plant anatomy (Dipu, 2014).

To the best of the researcher's knowledge, in spite of the various related studies carried out in this research area, yet, more gaps are still left unfilled to further on, as there are limited report documented on the subjectarea of this work. Thus, recognizing the great importance of this study and it contribution to knowledge field and humanity in general, gave the required justification for this study and hence the need to carried out theresearch.

CONCLUSION

The anatomical findings of the study shows that the leavesof*G.max*incontrol has well-developed Stomata,trichomes, xylem, phloem, palisade parenchyma, spongy parenchyma and epidermal cells compared to polluted leaves. However, the Stem anatomical studies of the soybeans plants growing on the polluted sites also show significant reduction as compared to the control ones.It is concluded that *G. max* is affected by exhaust emission and long term exposure could be more toxic than short term exposure. Overall, the result of this study reveals that *Glycine max*growing in the polluted environment are adversely affected due to higher concentrations of automobile pollutants as compared to the lower pollutant.

REFERENCES

1. Abaje, I.B., Ogoh, A.O., Amos, B.B and Abasiya, M. (2015). Climate change, flood disaster assessment and human security in Katsina State, Nigeria, *Journal of Ecology*, **4**(4):47-56.
2. Ahmad, S. H., Z. Reshi, J. Ahmad and M. Z. Iqbal (2005).Morpho-anatomical responses of *Trigonellafoenumgraecum* Linn.to induced cadmium and lead stress. *Journal of Plant Biology.***48 (1):**64-84.
3. AkomolafeG.F. David O.A. and Nkemdy A.J. (2019). Comparative anatomical studies of responses of some roadside plants to highway automobile exhausts.*J.researchinforestry, wildlife& environ.***11 (3):** 246-255.
4. Allahnouri M., Ghashemi A.F., Phazhouhan, I. (2018). Traffic effects on leaf macro and micro morphological traits. *Journal of Folia Oecologica.***45** (2): 92-101.
5. Alves, E. S., M. BaêssoMoura and M. Domingos (2008). Structural analysis of *Tillandsiausneoides*L. Exposed to air pollutants in São Paulo City–Brazil, Water Air and Soil Pollution. **189:** 61-68.
6. C.M Grossi, P.Brimblecombe (2007). Effects of long term changes in air pollution and climate on the decay and blackening of European stone buildings.*Geo. Soc. Pub.***271** (1): 117-130.
7. Dipu S. (2014). Effect of Air Pollution on the Anatomy Some Tropical Plants.*Applied Ecology and Environmental Sciences.***2 (1):** 32-36.
8. Ekiyor T.H., Horsfall I.T., Kalagbor A.I., Egbara-dedu B.A. (2019). Evaluating the effects of air pollution on the Agricultural yield of selected crops in Eleme, Nigeria.*Journal of Applied Science and Environmental Management.***23** (10): 1771-1778.
9. Giełwanowska I., Szczuka E., Bednara J., Gorecki R. (2005). Anatomical features and ultrastructure of *Deschampsiaantarctica* (Poaceae) leaves from different growing habitats. *Annals of Botany.***96:**1109-1119.
10. Giri, S., Shrivastava, D., Deshmukh K. and Dubey, P.S. (2013).Effect of Air Pollution on Chlorophyll Content of Leaves.**1** (2):93-98.
11. Iqbal M.Z. (1985). Cuticular and anatomical studies of white clover leaves from clean and air polluted areas. *Pollut. Res.* **4:** 59-61.
12. Irina N.Gostin.(2009). Air pollution effects on the leaf structure of some fabaceae species.*Not.Bot.Hort.Agroboot.* **37**(2): 57-63.
13. Jahan S. and Iqbal M. Z. (1992). Morphological and anatomical studies on leaves of different plants affected by motor vehicles exhaust.*J. Islamic Acad. Sci.* **5:** 21-23.
14. Kumar L., N. K. Hemanth Kumar and Jagannath S. (2018).Assessment of air pollution impactonmicromorphological and biochemical properties of *Pentaslanceolata*Forssk.And *Cassia siamea*Lam. *Tropical Plant Research.***5 (2):** 141–151.
15. Mitu K.J., Islam M.A., Biswas P., Marzia S., Ali M.A. (2019)Effects of different environmentalpollutants on the anatomical features of roadside plants. *Progressive Agriculture.***30 (4):** 344-351.

16. Onokala, P.C. (2008). Contribution of road transport to environmental degradation in Nigeria Urban Centers: A Critical Analysis, 2008 Maiden Edition of LAMATA Annual Conference on Public Transport in Lagos held at Sheraton Hotel and Towers, Ikeja Lagos from 6th – 8th May 2008.
17. Rai, P., Mishra, R.M. (2013). Effect of urban air pollution on epidermal traits of road side tree species, *Pongamiapinnata (L.)Merr. J Environ SciToxicol Food Technol.* **2 (6):** 2319–2402.
18. Ramasamy, V. (2009).Effect of vehicular pollutants on road side plant of puducherry.*Atmos.Environ.*1-70.
19. Rhimi N., Ben Ahmed C., Elloumi N., Athar H.R., Noreen S., Ashraf M., Ben Abdallah F., Ben Nasri-Ayachi M. (2016). Morpho-anatomical and physiological changes in grapevine leaves exposed to atmospheric pollution. *AEAER.***14 (5):** 77-89.
20. Sanderfoot O.V. and Holloway T., (2017). Air pollution impacts on avian species via inhalation exposure and associated outcomes. *Environ. Res. Lett.* **12:** 1-16.
21. Silva, L.C., A. A. Azevedo, Silva and M. A. Oliva (2005).Effects of simulated acid rain on the growth and anatomy of five Brazilian tree species.*Australian Journal of Botany*. **53:**789-796.
22. Stevovice S., Mikovilovic V.S, Calic-Dragosavac D. (2010).Environmental impact on morphological and anatomical structure of Tansy.*African Journal of Biotechnology*, **9:**2413-2421.
23. Verma, R. B., Mahmooduzzafar, T. O. Siddiqi and Iqbal M. (2006).Foliar Response of *Ipomeapes-tigridis* L. to Coal-Smoke Pollution, *Turkish Journal of Botany*.**30(5):** 413-417.
24. West C.P., Tan A.D., Shanafelt T.D. (2012). Association of resident fatigue and distress with occupational blood and body fluid exposures and motor vehicle incidents.*Mayo clin proc.* **87:** 38-44.
25. Yunus, M., and Ahmad, K.J., (1980). Effect of air pollution on *Psidiumguajava*L .Indian J. Air pollution control.**3:** 62 – 66.
26. Zafar, N., Athar, M., Iqbal M.Z., Shafiq M., (2016).Effect of diesel generator exhausts pollutants on growth of *Vincarosea* and *Ruelliatuberosa*.**20** (4): 1191-1197.

