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Distribution of stomata on Lycopersicon esculentum leaves for plants growing in air pollution related to vehicle fumes along Waiyaki Highway Nairobi County Kenya

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Abstract

Air pollution is a worldwide environmental negative phenomenon affecting global climate, humans, animals and plants. Vehicle fumes as a leading major source among smoke producing air pollutants, was examined in this study. Nairobi, a mega-city with increasingly high levels of air pollution was studied with special emphasis on one of its roads, i.e. Waiyaki Highway. This road was investigated for scientific understanding of effects of vehicle fumes on plants stomata behavior and distribution. Hotspots of air pollution averaging PM_{10} 42.6µgm/M^3 along this Highway were identified as locations for monitoring of effects of air pollution on plants. A control (at NARL) had similar environmental conditions but no significant air pollution (measured air pollution at environment of control at NARL, had PM_{10} 0.6µgm/M^3). The control experiment was protected from air pollution related to vehicle fumes by lengthy distance away from the road plus vegetation thriving in between. Sites selected for treated experiments were: ICEA buildings, ABC Place, and Kangemi market. These hotspots were also evidenced by some existing trees/or plants which were moribund and all trees at the sites had darken tree trunks and leaves due to air pollution. *Lycopersicon esculentum* Mill. (1691), tomato plant that highly susceptible to air pollution was exposed to air pollution at selected sites. The experiments looked at distribution of stomata account after 3 months. Collected data was analysed using Poisson distribution in Genestat software. Means were separated by use of Least Significant Difference (LSD) or Tukey in Genestat software. The stomata were located under leaf mainly with plants in treated experiments exposed to air pollution. Significantly (at $F_{2,6}$=3.37, P<0.05) more of open stomata on the treated plants were found on lower epidermis. In the experiment, plants germinated developed normally but differences were experienced in distribution of stomata analysis. The unfavorable atmospheric condition due to air pollution related to vehicle fumes, physiologically plants react by leaf stomata pores distribution. Physiologically plants distribute stomata pores to where there is no obstruction as a way of adoption to air pollution and this case; it happened on treated experiment plants having more stomata and open stomata on lower epidermis upper epidermis.

Keywords Air pollution, Vehicle fumes, Leaf distribution of stomata, *Lycopersicon esculentum* Waiyaki Highway

Justification of the study

Plants are planted along roads for different purposes which could include, beautification, offering resilient to air pollution, sequestration of carbon dioxide. These functions are well accepted but plants leaves are dark in colour due to soot. These PM, which cause dark colour, are particularly trapped by hairy plants like of *Lycopersicon esculentum*. Since this PM obstructs stomata pores, how does a plant physiology allocate the pores in respond? This study will then help in understanding of effects that air pollution related to vehicles fumes have on plants.

Objective

Study the distribution and state of stomata on leaves of *Lycopersicon esculentum* in air polluted by vehicle fumes.

Hypothesis

The hypothesis that exhaust fumes from moving vehicles do contribute to leaf stomata distribution and behavior of *Lycopersicon esculentum* plants

MATERIALS AND METHODS

Reconnaissance *and Selection of Study Sites*

Reconnaissance was conducted by walking along Waiyaki Highway carriage way from where it starts from Nairobi City. While walking, tree leaves with darken colour and tree trunk of dark color were noted and site

noted and marked. Dark color was the main characteristic for choosing points to put up experiments with added feature of vegetation, which had signs of dying, or already dead plant materials. The intensity of dark colouration was used in choosing the experimental sites for observation. Four main sites were identified with quite evident air pollution. This as reported in Script of Lugadiru, (2016)

A site, immediately after the junction from Waiyaki Highway to Chiromo Campus of the University of Nairobi with coordinates, latitude -1.26903^0 S and longitudes- 36.8055^0 E was the first site to be selected (at ICEA building). The second site was at ABC Place with coordinates, latitude -1.25958^0 S and longitudes - 36.7757^0 E. The third experimental site was at Kangemi market along Waiyaki Highway with coordinates, latitude - 1.26431^0 S and longitude 36.7493^0 E. The control site was identified within the National Agricultural Research Laboratories (NARL) of the Kenya Agricultural Livestock Research Organization (KALRO) with coordinates, Latitude - 1.25821^0 S and longitudes - 36.7727^0 E.

The chosen sites were characterized by hyper air pollution produced by engines of the vehicles while climbing the lane (going uphill) and strategically positioned at a point with frequent vehicles and traffic is constantly streaming past or snarled up in slow-moving jams i.e. vehicles stationary and in idling state. However, the control experiment was set at a site free from air pollution as shown in figure 1

All treatment sites, that is, at ICEA building, ABC place and Kangemi market had one control site, at NARL. The control experiment was at given site by management of NARL which was appropriate site because the distance was 257 meters from Waiyaki Highway with insignificant deposits of lead from air pollution related to vehicle fumes. (The distance appropriateness confirmed by calculations based on a study made in Karachi, Pakistan (Shamsu and Mirza 2000) with results that at zero distance (at Finance and trade Centre position) from road had 30.0ppm lead deposits in soil and another site at Quad's tomb 157metres from road had 6.47ppm so with such graduations in decrease of lead deposit then at 257 meters could have very insignificant influence of air pollution.

Reconnaissance of study Sites

Reconnaissance was done by walking along Waiyaki Highway carriage way from Nairobi. While walking, tree leaves with darken color and tree trunk of dark color were noted. Dark color was main characteristic for choosing points to place experiment with added feature of vegetation which had signs of dying, or already dead. Four main sites were identified with quite evident air pollution. Area after junction from Waiyaki Highway to Chiromo University coordinates latitude -1.26903^{0}S longitudes 36.8055^0 E. Second, area at ABC place coordinates latitude -1.25958^{0}S and longitudes 36.7757^0 E. Control was within NARLO i.e. National Agricultural Research Laboratories of KALRO (Kenya Agricultural Livestock Research Organization) coordinates latitude 1.25821^{0}S and longitudes 36.7727^0 E). Fourth, was at Kangemi market along Waiyaki Highway. Figure 1 show all points used and marked with a black arrow.

In figure 1, study area on Waiyaki highway is indicated by red strip in Nairobi county map and in expanded map, arrows in blue show sites that were used to place experiments.

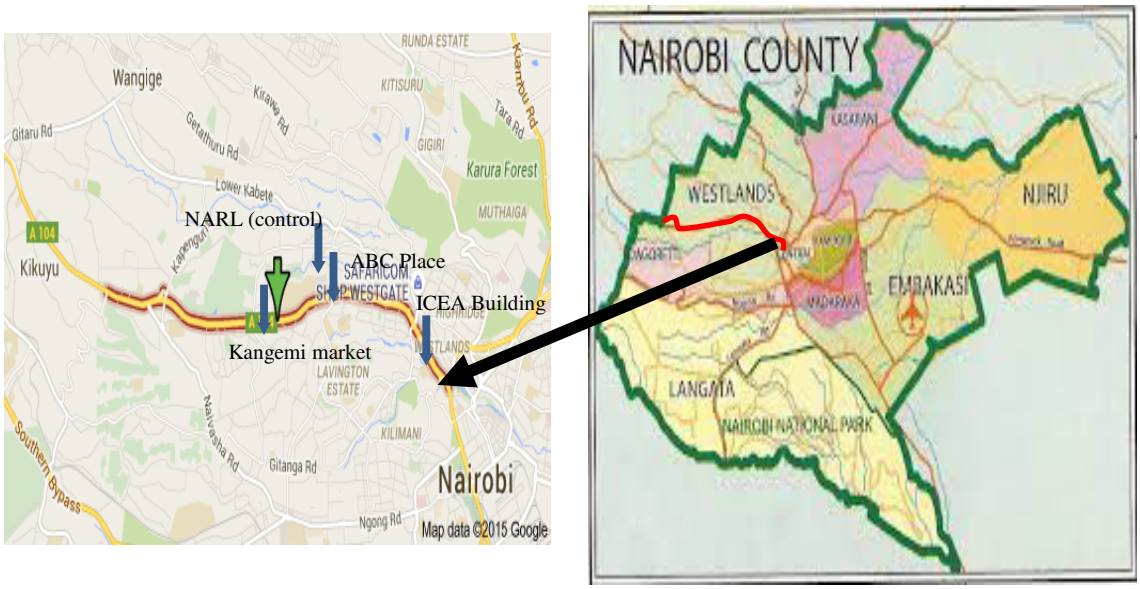

Figure 1 . Map of Nairobi County showing Waiyaki Highway and Experiments sites

Chosen sites were characterized by hype air pollution produced by vehicles going uphill and strategically

situated at a point with frequent vehicle where traffic is constantly streaming past or snarled up in slow-moving jams i.e. vehicles stationary and in idling state.
All experimental sites i.e. at ICEA building, ABC place and Kangemi Market had one control site.
Siting of control at NARL Kabete had added advantage because of former studies made on site by a soil survey scientist. There was a study at Pakistan (Shamsu and Mirza 2000) on deposits of lead as a result of air pollution from supper petrol vehicle fumes. These results gave a paradigm of using lead deposits as another way of ascertaining air pollution free zones as one move away from polluted roads. Chosen site to place control experiment was decided on inside compound of NARL away from Waiyaki Highway and confirmed by calculation shown elsewhere in this script. This gave extend at which control experiment would be place where there were minimum air pollution related to vehicle exhaust fumes.

Climate of the study site

Temperature of Nairobi avarages 23.4 ^{o}C at day time, rainfall of 1,024.2mm per year, which has a bimodal characteristics with two picks in April and October. Nairobi is relatively not windy but sometimes having westelies breeze. Between month of June and September, tempretures range from 10^{0} C and 15^{0} C as lowest and less windy.

Description of Study Area

Area used for study is along Waiyaki Highway road. The Highway stretches from Museum round about to Uthiru Flyover Bridge about twelve kilometres in length. The Highway is a dual carriage way with a steady uphill climb. The road has general gradient as being less than 10%. Trees planted on both sides in a non-professional manner, a project referred to as city beautification by Nairobi County.
Waiyaki Highway has linear air pollution from vehicle fumes. At time of doing this project, there were a lot of vehicle fumes because southern by pass had not been opened. Opening of southern bypass removed heavy transit trucks and Lorries travelling to and from Mombasa port and serving Uganda, Rwanda, Burundi, Southern Sudan and Republic of Congo. Along the Highway there were sites which had evidence of hype air pollution. Some of such points were selected for siting air monitoring structures. Sites selected are referred to in this study as ICEA buildings, ABC place, Kangemi market and KALRO Kabete. KALRO was used as control because it was a way from the road (257m).

Particulates Matter (PM10) measurements at various sites of the study

Particulates Matter (PM_{10}) at various sites of the study was determined by instruments acquired from Ministry of Labor, Occupation Health Services section. Figure 2 is Photograph of the instrument of measuring particulate matter in air to indicate the level of air pollution.Particulate matter instrument has a diaphragm which was placed at sites and sucked in air from environment at a rate of one cubic meter per second for twelve hours. This action was done three times, one at beginning of the study another when experiment was half way done and lastly at the end of experiment. The diaphragm has a sieve which traps particles in air which passes through. Initial weight of sieve was taken (by balance that measures in µgm) before and after twelve hours. Since the amount of air passing through the diaphragm was known, calculation of µgm per cubic meter was performed. Table 1 shows how calculation was done after measurement of particulate matter (PM_{10}). This was as done by Lugadiru, (2016) paper.

Figure 1 MEASURING OF PM ALONG WAIYAKI HIGHWAY

Data collection to determine effects of air pollution on Stomata number on the leaf

Stomata account was made to determine how physiological natures of plants exposed to air pollution are behaving. Leaves to be used for study on stomata were washed with clean water both upper and lower sides. The leaves were carefully selected from all tomato varieties at each and every experiment. Leaves were eventually painted with clear nail paint. After the paint drying, clear seal tape was laid on top of the portion of the leaf with clear nail paint. The clear seal tape was then reaped off. The seal tape carries itself with the nail paint. The nail pant will come out with leaf surface impression where stomata and other features are seen. The account was made by counting stomata impressions made on clear nail paint, reaped off using a clear seal tape. The clear seal tape was place in well market envelopes according to tomato variety and site. The clear seal tape then place in an electronic microscope with scale graduation showing micro-area (on a graticule a transparent glass with readings or graduations). The area was standardized to be 0.0016mm^2. Any stomata falling within this area were counted. This was repeated for upper leaf surface and lower leaf for each variety in the experiments. In each case open and closed were counted separately. This method was described by Pourkhabbaz *et al*., (2010). The results was recorded in excel for further analysis.

State of affairs of air pollution in the study sites

In table 2 shows state of air pollution at the chosen study sites at beginning, during and at end of experimentation period. Analysis show that there were no significant with each experiment at F2,9= 1.00, P<0.05 and that there is strong significant difference ($F_{2,9}$ = 0.001 P<0.05). When LSD at (p=0.05) of 33.55 µgm^3, was applied it was shown that, main difference was mainly between any one of treated and control experiments. Within experiments no differences between any two was equal or more than calculated LSD of 33.55 µgm^3

Table 1 Show particulate matter levels at different stages of the experiments

S/No.	Experimental site	Mean level of pollution before start of experiment (µgm^3)	Mean level of pollution during experimentation (µgm^3)	Mean level of pollution at the end of experimentation (µgm^3)	p-value
1	ICEA building	42.5±0.03^{ab}	42.2±0.03^{ab}	43.3±0.03 ^{ab}	1.00
2	ABC Place	42.5±0.03 ^{ab}	42.4±0.03 ^{ab}	41.4±0.03 ^{ab}	1.00
3	Kangemi market	43.6±0.03 ^{ab}	42.9±0.03 ^{ab}	41.5±0.03 ^{ab}	1.00
4	NARL	0.56±0.03^{a}	0.61±0.03^{a}	0.55±0.03 ^{a}	1.00
	P value	0.001	0.001	0.001	

In Table 2 are levels of air pollution at different times of the experiment. showing average pollution level between within experiments, a numerical value followed by letter ‘a’ in a superscript version within rows show they are same. While letter ‘b’ after numerical value in the column, i.e. between three tomato hybrids not statistically significantly different at p = 0.05

Analysis of stomata on different varieties of *Lycopersicon esculentum* plant leaves

Number of stomata (per area of 0.0016mm^{2}on leave),distribution of both opened and closed in upper epidermis of treated experiments and control experiment are different statistically at $F_{2,6}$=10.57, P<0.05 when Poisson distribution is used to analyze. Hence air pollution from moving vehicles has effect on functions of *Lycopersicon esculentum* Mill. leaves when upper epidermis is exposed to air pollution within a period of three months.

When Poisson distribution is used to analyse average number (per area of 0.0016mm^{2}on leave) stomata situation of lower epidermis in reference to either closed and open in treated and control experiments, the difference was highly significant ($F_{2,6}$=3.37, P<0.05) is significant. Hence air pollution from moving vehicles has effect on functions of *Lycopersicon esculentum* Mill. leaves when lower epidermis is exposed to air pollution within a period of three months.

Table 2 Shows stomata number situation in upper epidermis for treated and control experiments

Average No. Stomata per 0.0016mm2 at upper epidermis

	Control experiment	Treated experiment	Deviance	P/value
Open	3	1	±2	<.001
Closed	3	4	±2	<.001

There is strong significant within distribution of stomata (open or close) on upper epidermis

Table 3 Shows stomata number situation in lower epidermis for treated and control experiments

Average No. Stomata per 0.0016mm2 at lower epidermis

	Control experiment	Treated experiments	Deviance	P/value
Open	4	8	±1	0.001
Closed	8	9	±1	0.001

There is strong significant within distribution of stomata (open or close) on lower epidermis see figure 3

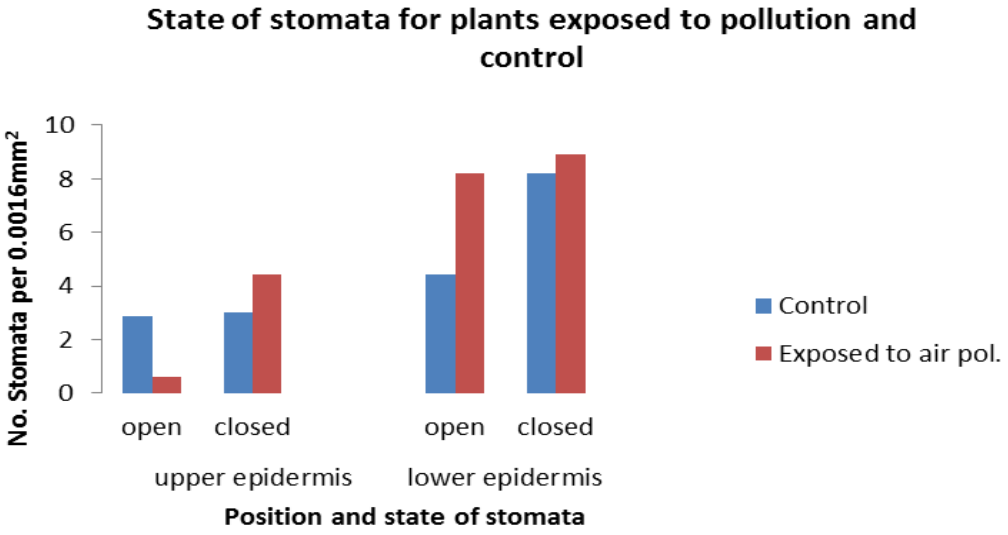

Figure 3 Number of stomata in either lower or upper of tomato leaves and if open or closed

Discussion on Plant Physiology

Plant physiology affects plant productivity. Stomata number, state and the position on a leaf, determines how much a plant will produce in terms of biomass production. Stomata are more under leaf than upper epidermis in most plants. Plants in air polluted tend to have more stomata underneath leaf than control and fifth of the stomata open, while on the upper epidermis are fewer and all are closed as shown in figure 4. This is a natural resistance mechanism as stated by Barnes *et al*. (1999) who reports there natural and manmade plant resistance to air pollution. The control had more stomata on the upper epidermis 50% open unlike plants exposed to air pollution. Plants in treated experiment had more stomata underneath the leave open compared to control experiment.

Tissue development of plants is a result of functionalities in biophysical-chemical reactions, which produces biomass. If a leaf has most stomata pores closed during sunny periods then less photosynthesis and less plants tissue developments. There is more vehicle related air pollution along Waiyaki Highway during day than at night, which then implies plants leaf functions of producing food is seriously reduced.

Difference in stomata distribution (either on upper or lower epidermis) and different behavior of either closure or open between treated and control experiments was statistically significant. Deviance of stomata on *Lycopersicon esculentum* leaves study shows effects of air pollution along Waiyaki Highway. According stomata behaviour, the hypothesis that exhaust fumes from moving vehicles do contribute to leaf stomata distribution of *Lycopersicon esculentum* plants is accepted. Hence air pollution from moving vehicles have effect on functions of *Lycopersicon esculentum* Mill., plant, causing stress and stomata deviance, at various sites for plants in treated

experiment within a period of three months. The facts that plants are stress, more flowers were produced.

Conclusions

Plants are sensitive to air pollution related to vehicle fumes whereby the response is by distribution of stomata pore to have more underneath leaf epidermis and have more open stomata in lower epidermis. The upper epidermis is left for were PM settle and do less physiological activities.

Recommendations

It is highly recommended that, more plants species should be studied to clearly ascertain, and conclude that plants do have peculiar behaviour and also distribute of stomata when exposed to air pollution related to vehicle fumes along Waiyaki Highway of Nairobi County in Kenya.

ock of farmers to the other while comparing with control experiment. The test also indicated non-germinated seeds if they differed from farmer to farmer or block to block and control.

Table 2 Number of seed of *Melia volkensii* germinated and not-germinated as used for $\chi 2$ test

Blocks of farmers	No. germinated		No. not germinated		Total
	Occurrence	expected	Occurrence	expected	
Kabati	16	11.2	84	88.8	100
Chuluni	1	3.36	29	26.64	30
Kyanguthi	10	12.32	100	97.68	110
Control	1	1.12	9		10
Total	28		222		250

Value of $\chi 2$ test at 3 degree of freedom at $p<0.05$ is 7.824 which more than calculated $\chi 2$ which is 7.43 showing there is no significant difference in the seeds germinated/ not germinated, between farmers/control and blocks of farmers

Acknowledgement

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