# EFFECTS OF AIR POLLUTION ON TOMATO PLANTS (*LYCOPERSICON ESCULENTUM*) GROWN ALONG WAIYAKI HIGHWAY IN NAIROBI COUNTY, KENYA

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**A Thesis submitted in partial fulfillment of the Requirements for the Award of the Degree of Master of Science in Environmental Management, in the Department of Environmental Science and Technology, School of Environment and Natural Resources Management, South Eastern Kenya University**

**2017**

## DECLARATION

I understand that plagiarism is an offence and I therefore declare that, this thesis is my own original work and it has never been submitted to any other institution of higher learning for award of a degree in any field of study and all materials cited in this write up, which are not mine have been duly acknowledged.

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# DEDICATION

This thesis is dedicated to Jehovah God (Isaiah 57:15), my son Horace Opote Kikumba, my father Erastus Lugadiru Kilinga and my family.

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## ABSTRACT

Vehicle exhaust fumes emission, as one of 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, Waiyaki Highway. Objectives were to find if there is air pollution, air pollution effects growth and stress of plants. This road was investigated to gain scientific understanding of the effects of vehicle fumes on plants. Hotspots (ICEA building, ABC Place, and Kangemi Market), had unique characteristics, which included moribund trees or plants, dark tree trunks and leaves due to air pollution. *Lycopersicon esculentum* Mill., (1768), tomato plants that is highly susceptible to air pollution was exposed to the air at selected sites along the highway. Four experiments were set, (three as test experiments and one, control) using a raised platform containing twelve plants in polythene bags and irrigated frequently. For the study analysis, data was collection on pollution level, seed germination rate, height growth, leaf length, flower count, biomass, stress and stomata account for three months. The data was analysed using Analysis of Variance (ANOVA), Kruskal Wallis and Poisson distribution in Genestat software. Means were separated by use of Least Significant Difference (LSD) $\alpha = 0.05$. The Waiyaki Highway had consistence air pollution, for whole period of this study (averaging $PM_{10}$ 42.6µgm/m$^3$ for test sites). The control experiment at NARL had similar environmental conditions but no significant air pollution ($PM_{10}$ 0.6µgm/m$^3$). The control experiment was protected from air pollution related to vehicle fumes by long distance away from the road plus canopies of trees thriving in between to shield experimental plants. Seed germination rate results, showed that, there were no significant differences at ($P < 0.05$). Growth pattern was determined using height, which proved significantly different at $P < 0.05$. Leaf length of tomato plants was not significantly different where $P < 0.05$. Results of flower count showed significantly difference ($P < 0.05$). Stress was significantly different where $P < 0.05$. Plants biomass was not significantly different between experiments ($P < 0.05$). Open stomata in upper epidermis was significantly different. Air pollution affects plants as evidenced by significance noticed on height growth, number of flowers, plant stress and distribution and status of stomata. Leaves elongation is independent of air pollution. Plants in air pollution had different height to control, more flowers and were stressed more due air pollution. Plants in air polluted environment had high stomata density underneath leaf than control and no open stomata on the upper epidermis. Stomata number, position and, if open or closed on leaves of plants is a quick way of determining air pollution. Three months was not enough for air pollution to affect biomass production. It is important for precise threshold levels of air pollution effects to plants be determined. Comprehensive study on effects of air pollution on seed germination, height growth, leaf growth, biomass and plants physiological behaviour in heavily air polluted environment be instituted on long term basis.

## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| AQI | Air pollution Quality Index |
| BRT | Bus Rapid Transport |
| EIA | Environment Impact Assessment |
| EPA | Environment Protection Agencies - US |
| GDP | Gross Domestic Product |
| GPS | Geographical Positioning System |
| KALRO | Kenya Agricultural Livestock Research Organization |
| KEFRI | Kenya Forestry Research Institute |
| LSD | Least Significant Difference |
| NARL | National Agricultural Research Laboratories |
| NCBD | Nairobi Central Business District |
| NEMA | National Environment Management Authority - Kenya |
| PM | Particulate matter |
| SEKU | South Eastern Kenya University |
| UNEP | United Nation Environment Program |
| USA | United States of America |
| WHO | World Health Organization |

# CHAPTER ONE: INTRODUCTION

## 1.0 Background Information

Air pollution is caused by anthropological activities especially from vehicle exhaust fumes (Odhiambo *et al.*, 2010 and Kinney *et al.*, 2012). Vehicle fumes originate from burning of hydrocarbons in motor vehicle engines, giving rise to carbon dioxide ($CO_2$), carbon monoxide (CO), sulfur dioxide ($SO_2$), nitrogen compounds ($NO_x$), and ethylene ($C_2H_4$), as well as a variety of other minor hydrocarbons in varying proportions (Zeiger, 2006). These gases are released from motor vehicle engines and are known to cause air pollution in an environment in which they occur (UNEP, 2010). Over 90% of air pollution in the world is attributed to vehicle exhaust fumes (UNEP, 2010).

Particulate matters (PM), which are solid in nature and measured in micrometers (µm), are released from motor vehicle engines and significantly contribute to air pollution (Wargo *et al.*, 2006). The PM from vehicle engines are un-burnt particles, which are part and parcel of composition of vehicle exhaust fumes (Kinney *et al.*, 2012). Gases produced from motor vehicle engines as fumes together with particulate matters are known as pollutants because they cause harm to environment, especially to living organisms such as plants (Kinney *et al.*, 2012). A study by Pourkhabbaz *et al.* (2010) found that leaves of plants, in an air-polluted environment were heavily covered with dust from particulate matter having a diameter between 2.5µm and 10µm. This cover, comprising of dust particles, reduces photosynthetic activities of leaves because chlorophyll that absorbs light energy is obstructed (Vliet and Kinney, 2007).

Plants are very susceptible to air pollution because they are exposed constantly to air and its environment (Chen-Xiao *et al.*, 2003). Plants with hairy cover are most affected because they trap a lot of particulate matters (Plate 1). Air pollution reduces plant productivity because leaves, which are primary food manufacturers are greatly affected (Pourkhabbaz *et al.*, 2010). Air pollution emanating from vehicle fumes is a common problem in many developing urban centers such as Nairobi (UNEP, 2010).

UNEP (2010) indicated that, air pollution in Nairobi is at a high level due to nature and conditions of vehicles used within and around the city. The level of air pollution is above recommended level of air pollution of 50µgm /$m^3$ by World Health Organization (WHO, 2005). Kinney *et al.* (2012) in their study confirmed that Nairobi has high levels of air pollution due to particulate matters. Air pollution is experienced mostly at certain times of the year depending on prevailing climatic conditions such as rain and calm weather conditions, which makes it spatial and temporal (Dioniso *et al.*, 2010). Hence, air pollution due to motor vehicle exhaust fumes has hot spots along Waiyaki Highway. Therefore, the study sought to investigate effects of air pollution from motor vehicle exhaust fumes on plants growing along Waiyaki Highway from seed germination to flowering stage.

## 1.1 Statement of the Problem

Gaseous and particulate matters from engines of moving motor vehicles continue to pollute environment along roads. Expensive new building for offices and residential purposes are mushrooming along Waiyaki Highway with no regard to air pollution. Business along Waiyaki Highway continues, with no facts to show there are hazards from air pollution related to vehicle fumes. Farmers conduct their usual arable farming business in air polluted environment along Waiyaki Highway with no regard to impact of air pollution on their crops due to lack of information and data to support the allegations.

Studies on air pollution have been previously undertaken along many roads around Nairobi County but not along Waiyaki Highway. Most of the previous studies on air pollution along roads in Nairobi had other goals other than effects of air pollution on plant growth. For example, Kinney *et al*. (2012) studied air pollution with respect to distance from roads. Points close to the road showed more air pollution (being twenty fold) as compared to points away from the road. Another study by Odhiambo *et al*. (2010) was generally concerned with occurrence of air pollution in the city. Their studies revealed that air pollution in Nairobi City was strongly correlated with commuting of people to and from work and that air pollution happened in particular areas of roads especially where traffic was constantly streaming past or snarled up in slow-moving jams. According to Odhiambo *et al*. (2010), the effect of air pollution was not persistent throughout the day but would reduce during off-peak period when traffic was low. Odhiambo *et al.* (2010) also noted that, not every day of the year had same level of air pollution. Studies conducted by Odhiambo *et al*. (2010) focused on the effects of air pollution on humans and not plants. According to Odhiambo *et al.* (2010) air pollution is therefore spatial and temporal in Nairobi City. These findings can be applied to Waiyaki

Highway, which is within Nairobi County where vehicle rush-hours and off picks do exist. It is against this backdrop that a comprehensive study was required to understand underlying science of the effects of air pollution on plants along Waiyaki Highway, so that consolidated efforts of management of the air pollution menace can be put into place.

Plants are affected by air pollution in two ways. One is by particulate matter (PM) in air pollution, which after landing on a surface, be it on plant leaf or stem, forms a cover (Omafra, 2014). Apart from forming a cover over leaf or stem, the particulate matter hinders photosynthesis and evapotranspiration (Pourkhabbaz *et al.,* 2010). Some particulates especially small size would penetrate into plant tissue systems and either cause blockage or alter internal chemical composition (Pradeep, 2008**)**. Gaseous part of air pollution, which can directly enter into a plant leaf through stomata during gaseous exchange. Once inside plant tissues, they change internal plant chemical composition (Pradeep, 2008**)**. The internal composition affects plant metabolism, which affect plant growth. Therefore a plant would be stressed, growth retarded and dirty.

## 1.3 Objectives

### 1.3.1 General Objective

Main objective of this study was to evaluate the effect of air pollution related to vehicle fumes on tomato plants grown along Waiyaki Highway in Nairobi County.

### 1.3.2 Specific Objectives

1. To determine levels of air pollution caused by exhaust fumes from moving vehicles in selected study sites, along Waiyaki Highway.
2. To evaluate effect of pollution by exhaust fumes from moving vehicles on seed germination, height growth, leaf development, flower formation and biomass of *Lycopersicon esculentum* plants at selected sites along Waiyaki Highway.
3. To determine contribution of pollution by exhaust fumes from moving vehicles on stress as shown by plant stress and stomata of *L. esculentum* plants grown at selected sites along Waiyaki highway.

## 1.4 Research Hypotheses

**$H_o$** There is no significant difference in levels of air pollution between the selected study sites along Waiyaki highway and control at NARL.

**$H_o$** There is no significant difference in effects of exhaust fumes from moving vehicles on seed germination, height growth, leaf development, flower formation, and biomass of *L. esculentum* plants in selected study sites along Waiyaki highway and control at NARL.

**$H_o$** There is no significant difference in effects of exhaust fumes from moving vehicles in plant stress and stomata between plants in selected study sites along Waiyaki highway and control at NARL.

## 1.5 The Conceptual Framework

In Nairobi City, along Waiyaki Highway, there are plants, which have darkened due to vehicle fumes (gaseous and particulate matter emanating from engines of moving motor vehicles). Darker coloration shown in Appendix 2, may be an indicator that plant leaves are not biologically functioning appropriately. Figure 1.1 illustrates conceptual framework. The conceptual framework, illustrates variables on plants of *L. esculentum* that have possibilities of being affected by air pollution related to exhaust fumes from moving vehicles along Waiyaki Highway for a period of three month, which is the time experimental plants will be monitored to assess the effects of air pollution emanating from vehicle fumes along Waiyaki Highway.

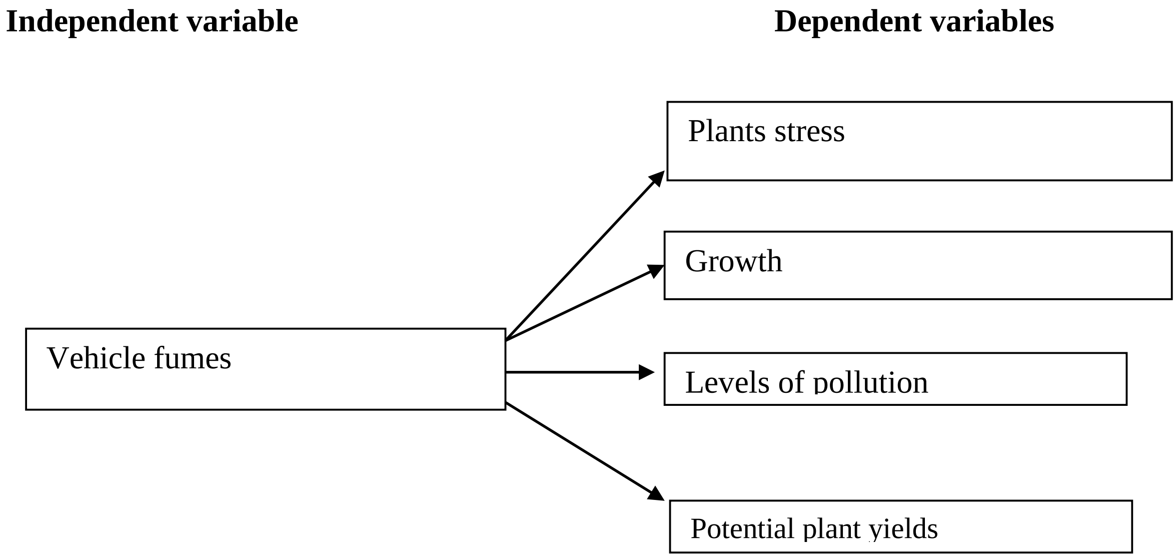


Figure 1 1 Conceptual framework of influence of pollution on plants leaf length

## 1.6 Justification of the Study

It has been estimated that every year, one billion people are exposed to air pollution resulting in one million premature deaths and one million pre-natal deaths in the world (UNEP, 2010). These exposures and the resulting deaths continue to take place due to lack of information. Studies are urgently required to generate a database of credible data and information on risks involved in incidences of air pollution and henceforth awareness and visible indicators explained to laymen, especially in developing countries for public good. A good approach to studying the effects of air pollution on human beings can be by, investigating effects of air pollution on plants, which can act as a biological barometer as described by Sowidis *et al*. (1995). Some places in Nairobi, air pollution have been estimated to exist at 500µgm /$m^3$, which is a level far above the recommended 50µgm /$m^3$ by WHO (Odhiambo *et al*., 2010). Studies are therefore required in order to understand and curb it before it reaches dangerous levels like in China, where in some areas; pollution is reading 700µgm /$m^3$, which are well above the international safe levels of 50µgm/$m^3$. Because Nairobi is a complex cosmopolitan city in a developing world, where air pollution is on increase due to uncontrolled human activities, it is important to evaluate pollution effects on environment in general and biosphere components such as plants.

Vehicle fumes constitute varying levels of air pollution at different times of the day at one spot along roads, which can be implied on Waiyaki Highway. Air pollution does not uniformly occur every day throughout the year, which implies that plants along Waiyaki Highway experience effects of air pollution emanating from vehicle fumes differently at every stage of their life. This, then, calls for comprehensive studies, so that interplay of factors contributing to this air pollution can be understood.

It is also reported by Odhiambo *et al.* (2010) that most of the air pollution in Nairobi is due to vehicle fumes, and it is very evident usually at a high level when there is vehicle congestion or heavy traffic where motor vehicles constantly stream past or snarled up in slow-moving jams (Appendix 5). The air pollution affects Nairobi commuters because it reaches pick levels in morning and evening when people are either going to work or going home. High picks of air pollution are most felt at some hot spots due to vehicle congestion or slow moving traffic. Air pollution at such points occurs because engines are running or on idling, pumping exhaust fume into a calm environment (Odhiambo *et al.*, 2010). Due to this exposure of air pollution, thousands of commuters every day unknowingly face premature deaths from pulmonary and cardiac arrest diseases plus cancer problems. Both Odhiambo *et al.* (2010) and Kinney *et al.* (2012) agree that no one has made an attempt to scientifically illustrate air pollution related to vehicle fumes and effects it has on organisms. This thesis looked into effects of air pollutions in Nairobi on plants as a biological indicator of its hazardous effects to organisms (Sowidis *et al*,. 1995) and, which is thought to be sufficient to cause harm to people in Nairobi. Air pollution is temporal and spatial in Nairobi, which requires more studies as shown by Gatari *et al.* (2005) so as to map hot spots and offer resilience to effects it may cause. In Nairobi Kenya, climatically talking, seasons change in a year and are times when air pollution is not quickly diffused thereby causing more harm than other times. This study does not address the spatial and temporal state of air pollution in Nairobi city. It is also hoped that long-term studies are made in Nairobi city to understand air pollution's trends. There is a strong belief by the writer that, there are threats in future of high levels of air pollution due to a high rate of population and infrastructure. Measurements are required in Nairobi to evaluate if this alarming situation is deteriorating (Gatari *et al.*, 2005). The best way of understanding and gaining knowledge on air pollution in Nairobi is continuous monitoring (using very sensitive plants) for proper conclusive recommendations as suggested by both Galcano *et al.* (2001) and Gatari *et al.* (2013).

## 1.7 Significant of the Study

The study on the effects of air pollution on tomato plants grown along Waiyaki Highway will be used to generate information which can be used by landscape designers, arable farmers along the highway, Nairobi City road planners and general public using Waiyaki Highway. The study will also bring to limelight the effects caused by air pollution on growing and developing plants. Those planning infrastructure will benefit, because results from this study will influence decision of positioning highways on appropriate distance from where people live or where they do business. Awareness created could influence Government policy makers to reduce air pollution at source, for example vehicles could be fitted with gadgets that reduce pollution from exhaust pipes.

# CHAPTER TWO: LITERATURE REVIEW

## 2.0.Introduction

Chapter two, deals with review of general air pollution in the World, Africa, Eastern Africa, Kenya and Nairobi. It shows how air pollution has affected people, plants and other organisms in their respective environments. In the chapter, a detail of air pollution sources and composition is explained. Particulate matter and its composition are elaborated. Air pollution on plants is explained.

## 2.1 Air Pollution in the World

Environment Protection Agency (EPA) US has set Air pollution quality Index (AQI) so as to set standards (EPA, 2014) as follows – good AQI is from 0μgm$^{-3}$ to 50μgm$^{-3}$ and poses no risk. From 51μgm$^{-3}$ to 100μgm$^{-3}$ is considered as moderate and could only affect few people sensitive to ozone air pollution, while unhealthy AQI starts from 101μgm$^{-3}$ to 150μgm$^{-3}$ which has high risk to older adults and children and those sensitive to ozone pollution. AQI of between 151μgm$^{-3}$ and 200μgm$^{-3}$ poses risk to everyone and begin to show adverse health concerns plus members of the sensitive groups may experience more serious effects. Very unhealthy AQI is 201μgm$^{-3}$ to 300μgm$^{-3}$ and everyone may experience serious health effects. The entire population is more likely to be affected at AQI of 300μgm$^{-3}$ and above.

It is estimated by United Nations Environment Program (UNEP, 2010) that more than 1 billion people worldwide are exposed to outdoor air pollution annually. It is also reported that urban air pollution causes up to 1 million premature deaths and 1 million pre-natal deaths each year in the whole world (UNEP, 2010). In developed countries, 2% of Gross Domestic Product (GDP) is spent on issues related to urban air pollution while in developing countries 5% (UNEP, 2010). A Recent report by WHO (2016), indicated that urban air quality of 98% of cities in low and middle-income countries (high income 56%) do not meet levels of air pollution set by World Health Organization (WHO) and populations in those cities are mostly affected.

Air pollution from Vehicle exhaust fumes and other sources contribute to forming brown cloud over cities with heavy pollution (WHO 2016). In general, environmental, air pollution causes acid rain which acidifies lakes, soils, buildings and monuments made of limestone or marble as reported by Rogers (2015). The air pollution does not only contribute to human diseases, it affects plants either physically or chemically. In plants the pollution effects develops over time (Kurczynska *et al.,* 1998). The chemicals affect mainly leaves and in severe state the whole plant dies (Myers, 2015). The same report by Myers (2015) reports that, plant's growth is slowed down by air pollution. Plants affected by air pollution are weakened and prone to pests and diseases as contained in Myers (2015) report.

In a study conducted by Chen-Xiao *et al.* (2003) it was found that plants were affected adversely by air pollution more than people. This leads to a conclusion that, "what affects human being would adversely affect plants due to continuous exposure". Vehicle fumes contain particulate matter, which is solid in nature and gaseous components. The physical composition of particulate matter is mainly soot of varying sizes, which are technically grouped in two, either of a diameter of 2.5μm or of 10μm (Ensor *et al.,* 2013). In animals, particulate matter of 2.5μm is very lethal, because it penetrates into the blood stream causing cardiac arrest disease (Ensor *et al.,* 2013). Influences on plant leaves by particulate matter differ from gaseous influence as demonstrated by Omafra, (2014). Adverse negative influence by gaseous air pollution to plants is at the secondary level where gaseous compounds react with other elements in the atmosphere to produce other chemical compounds (Omafra, 2014). Particulate matters in vehicle fumes affect plants in raw form, that is, they are deposited on their surfaces (Omafra, 2014). Some plants have a higher susceptibility to PM in air pollution than others due to either smoothness or roughness of surfaces, as reported by Gardener (2011).

Air pollution is a most studied aspect of urban pollution and its influence on genetical, physiological, biochemical composition, growth and yield patterns of plants, in both developed and developing countries (Singh *et al.,* 2003). In a long time, due to chemical build up in the plant, adverse effects are evident (Kurczynska *et al.,* 1998). In another study by Williams *et al.* (1996) showed that a reduction in chlorophyll, ascorbic acid, carotenoid and protein in plants is associated with air pollution leading to plant stress, which agrees with that of Polle and Schützendübel, (2003) who found out that, plant organs are stressed due air pollution. Stress makes plants in air polluted sites develop more flowers (Winchester 2015) and leaf senescence take place (Blande, 2014).

A study was done in Mashhad in Iran by Pourkhabbaz *et al.* (2010) found out that leaf size and stomata density were lower at air pollution sites compared to those in non-air polluted sites after prolonged exposure. Pourkhabbaz *et al.* (2010) found that, at air pollution site, leaf surfaces were heavily loaded with dust particles, after a period of time exposure. Accumulation of dust did not trigger stomata to occlude that is, never was sealed off but continued to function normally (Pourkhabbaz *et al.,* 2010). Pourkhabbaz *et al.* (2010) report further indicate that cuticle were thinner as compared to plants in non-polluted, indicating physiological effects of air pollution on leaves of plants. Pourkhabbaz *et al.* (2010) study had a limitation because, only one tree species was involved as *Platanus orientalis L*., which had been growing for some years in air pollution environment. It is not clear from the report at what stage air pollution effect started to be felt by plant of *Platanus orientalis L*. Research conducted in Russia in heavily industrialized sites by Lyanguzova (2011) had results, that general plants leaves tend to have significant resistance to air pollution, which corresponds to findings by Schembari *et al.* (2014) study in Barcelona and Cuza and Carol (2009) in Romania.

Most of the urban pollutants of the world are in developed countries and are slowly emerging in developing countries (UNEP, 2010). Initially, it has been assumed that underdeveloped countries had low levels of air pollution (WHO, 2005). However given increased use of fossil fuel exacerbated by the adoption of advanced technology in production, air pollution is a reality in both developed and developing nations (WHO, 2005). In addition, low–income solutions to daily transport (in developing countries), due to ever increasing number of commuters, have resulted in rapid increase in air pollutants (WHO, 2005). These air pollutants are from vehicles used to commute workers (WHO, 2005). This is compounded by a fact that majority of low-income countries import used vehicles, which emit more pollutants than new ones (WHO, 2005).

According to Pradeep, (2008), air pollutants include; sulphur dioxide ($SO_2$), nitrogen dioxide ($NO_2$), nitrogen compounds ($NO_x$), fluorides, chlorine ($Cl_2$), hydrogen chloride (HCl), ammonia ($NH_3$), organic gases (such as ethylene), minor gaseous pollutants, hydrogen sulphide ($H_2S$), carbon monoxide (CO), carbon dioxide ($CO_2$), bromine ($Br_2$), iodine ($I_2$), mercury vapor (Hg), cement-kiln dust as lime and gypsum; soot or particulates matter, magnesium oxide, boron, chlorides of sodium; potassium and calcium; sodium sulphate pesticides, insecticides and herbicides; and photo-oxidants, PAN (Peroxyacetylnitrate-$CH_3CO.O_2.NO_2$), Ozone ($O_3$). These pollutants are dispensed into the air and become air-pollution affecting plant and other organisms. Secondary pollution is formed from acid vapors, organic matter vapors, detergent vapors, industrial effluent deposition, oil deposition, waste heat deposition, soil acidification, pesticides, herbicides, sewage, ash pollution, chemical fertilizers and radioactive pollutants. These compounds change after they are dispersed in the environment to become secondary pollutants. Elstner and Osswald, (1994) reports, harmful gases in air pollution are readily absorbed as a part of the photosynthetic process, so a plant responds by stomata closure to reduce the effect. Same studies by Elstner and Osswald, (1994) noted that there are various gases in air pollution that can affect plants but most notorious of them all is excessive sulphur dioxide. After going through stomata and into leaf, Sulphur dioxide will combine with other inorganic element to produce lethal compounds like sulphur acid.

Rapid urbanization has resulted in increasing urban air pollution in major cities, especially in developing countries (UNEP 2010). Over 90% of air pollution in developing countries is attributed to vehicle emissions, which are composed of a high number of older vehicles coupled with poor vehicle maintenance, inadequate infrastructure and low fuel quality (UNEP, 2010). There is no modern transport means in these countries such as Bus Rapid Transport (BRT) or light commuter’s rail systems to decongest motorists on city roads and reduce air pollution per capita.

In the world, certain plant species are known to resist air pollutions. These include: *Aucuba japonica, Aquilegia vulgaris, Berberis, Bergenia cordifolia,* Cotoneaster, Elaeagnus, *Fatsia japonica,* Forsythia, *Garrya elliptica, Geranium endressii, Helleborus niger, Helleborus orientalis,* Hemerocallis, Hosta, *Ilex aquifolium, Iris Lamium, Ligustrum spp., Olea riaxhaastii,* Philadelphus, Pulmonaria, Rudbeckia, Symphytum, Viburnum, *Weigela florida, Acacia auriculiformis, Albizzia lebbek, Azadirachta indica,* Bougainvillea cultivars, *Bauhinia purpurea, Butea monosperma, Senna siamea, Senna surattensis, Dalbergia sissoo, Ficus infectoria, Diospyros embryopteris, Lagerstroemia duperreana, Melia azedarach, Millingtonia hortensis, Murraya paniculata, Nerium oleander, Nyctanthes arbortristis, Parkinsonia* *aculeate, Polyalthia longifolia, Putranjivar oxburghii, Terminalia arjuna, Terminalia muelleri, Thevetia neriifolia.* The plant species mentioned above are found in various countries, such as India, United Kingdom and German (Sharma and Roy, 1999; Barnes *et al.* 1999). Some of the species have been introduced in Kenya, for example, Neem (*Azadirachta indica*), which is well adapted to sulfur pollution conditions and can uptake sulfur hence reducing its environmental effect (Sharma and Roy, 1999).

Plants, which have shown to be most susceptible to air pollution, due to its hairy nature, include Tomato (*Lycopersicon esculentum*), *Citrullus lanatus* variety, or common name watermelon, squash (*Cucurbita argyrosperma*), potato (*Solanum tuberosum), Phaseolus vulgaris* or string beans, snap beans, pinto beans, *Nicotiana tabacum* (tobacco plant), soybeans, water cantaloupe (*Citrullus lanatu* ssp), muskmelon or summer melons *(Citrullus lanatu* ssp*), Medicago sativa* common name alfalfa, beet roots plant (*Beta vulgaris*), sunflower (*Helianthus annuus*), carrots (*Daucu scarota*), sweet corn (*Zea mays* var. *saccharata*), gourds (*Lagenaria siceraria*), green peas (*Pisum sativum*), turnips (*Brassica rapa*) (Holmes and Schultheis, 2001).

### 2.2 Air Pollution in Africa

In the African Region urban air pollution data remains very sparse. However, available data revealed particulate matter (PM) levels above the median of the world (WHO, 2016). In Africa, currently, WHO is monitoring urban air pollution in Algeria, Angola, Benin, Botswana, Burkino Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Namibia, Niger, Nigeria, Rwanda, Sao Tome & Principe, Senegal, Seychelles, Sierra Leone, South Africa, South Sudan, Swaziland, Togo, Uganda, United Republic of Tanzania, Zambia, and Zimbabwe. Measurements are taken as $PM_{2.5}$ and $PM_{10}$ and mainly in the cities with considerable varying results (WHO, 2014).

Air pollution in Africa is on increase because of a high rate of urbanization, the pollution accelerating at between 4% and 8% per year in many cities (WHO, 2005). Low-income, which results in daily commuting from slums dwellings to the place of work triggers increased use of vehicles owned by individual or “Matatus”(Kenya), as paid transport. These vehicles are bought as second hand with a high cost of maintenance because of old age. Maintenance is high and normally postponed by owners. Postponement reduces combustion capability hence more of un-burnt hydrocarbons are emitted as vehicle fumes. Due to many such vehicles operating on city roads, more air pollution emission is bound to increase (UNEP, 2010). Therefore, underlining air pollution is mainly poverty in developing countries; since poor people in African countries are not able to buy new vehicles hence opt for imported second-hand vehicles, which are poorly maintained (UNEP, 2010). The same UNEP report (2010) observed that poverty also contributes to use of cheap fuels, which does not combust to 100 per cent. These studies have not linked increasing air pollution and influence to plants or human’s life or other organisms, especially in developing countries (Sowidis *et al.*, 1995). In Africa, air pollution is not uniform but differs from region to region. Gatari (2005) reported that Nairobi experience more air pollution environment than other African cities such as Dar es Salaam, Gaborone, and Khartoum. Plants growing along roads, especially in Towns and Cities in Africa are black due to elevated air pollution emitted from exhaust fumes from vehicles and it is on increase (Gatari, 2005).

In countries of Angola, Lesotho, Botswana, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe, the air polluting is on increase because, the locals are looking for survival mechanisms alternatives, after exhausting natural resources and the actions are not environmental secure but pollute. Among environmental issues adopted in southern Africa which increase pollution are, air pollution and deforestation. The environmental harm, affects not only the population’s health, but also the plant species that are there. In these regions there is acid rain as results of air pollution with adverse effects on plants (EPA, 2014).

### 2.3 Air Pollution in eastern Africa

In East African, environmental air pollution does exist at a level comparable to other regions in developed countries, although restricted to big cities and along roads with heavy traffic loads. Air pollution related to vehicle fumes in Dar-es-salaam and Nairobi is at a level comparable to London or Vienna as reported by Bennet *et al.* (2005). Levels of air pollution in

Dar es Salaam are similar to data from other African and European countries. Air pollution distorts ecological systems in East Africa like other regions in Africa and in the world and should not be left out when dealing with its effects and corrections (Bennet *et al,* 2005). Gatari *et al.* (2013) showed mean concentrations of air pollution in East Africa were lower than those found in Asia and United States of America, but higher than those of some European cities. A study by Vliet and Kinney (2007) showed that air pollution was concentrated along roadways in urban settings, and it is approximately 20-fold higher than background sites (or sites away from roads) in South of Sahara particularly eastern Africa. Since eastern Africa is developing fast, attention paid to air pollution needs to be integrated into infrastructural plans to avoid or minimize effects. City planners, in East Africa, need scientific facts to help in positioning infrastructure away from roads to minimize negative effects of air-pollution to people.

The similarities of air pollution in East Africa and other places in the world need to be studied to curb effects on people and plants (Bennet *et al.*, 2005). Actual air-pollution effects on living organisms like plants have been studied elsewhere in the world, but not in East Africa. There are many studies in East Africa that can be done to graduate air-pollution influence on organisms and structures. Recommendations and data from such studies can be used to do tree configuration and sidewalk along roads as more towns are built in developing countries as in eastern Africa. Some trees, if well sited, capture air pollution reducing effects on other organisms, structures and even people in walk paths. In eastern Africa no specific studies on the effect of air pollution on plants are available.

## 2.4 Air Pollution in Kenya

Air pollution in Kenya has government limits in both areas of controlled environment (where pollution is not desired to be) and industrial areas. In air controlled environment, ambient concentrations of $PM_{10}$ of a 24-hour average of 180µg$m^{-3}$ and 24-hour annual average of 60µg$m^{-3}$ should not be exceeded. In industrial areas of Kenya and places with pollution, ambient concentrations of total suspended solids may not exceed a 24-hour average of 300µg$m^{-3}$ and the 24-hour an annual average of 100µg$m^{-3}$ (NEMA, 2005)

It is reported that 14,000 Kenyan die every year due to air pollution (Omulo, 2016). Maina *et al.* (2006) demonstrated that air pollution common in any developed countries was there in Kenya particularly along roads of Mombasa and Nairobi. Kinney *et al.* (2012) and (Odhiambo *et al.,* 2010) confirmed the existence of air pollution along Nairobi roads. Maina *et al.* (2006) also noted that air pollution is significant and worthwhile to be considered when investigating and planning climate change and sustainable development programs in Kenya. Air pollution is also found around factories in rural areas and this has affected people as reported by Omanga *et al.* (2014). The effects of air pollutions should be investigated and findings disseminated, so that people are aware of their effects and support any efforts to curb their menace (Maina *et al.*, 2006).

Air pollution contribute a lot to acid rain which occurred in Kenya in year 2000 and 2001 and damaged agricultural soil, fish resources and vegetation (Galcano *et al.,* 2001). The acid rain which occurred in Kenya had been due to air pollution taking place far away. Galcano *et al.* (2001), reports that, air pollution causes smog which scatters light hence reducing visibility. The air pollution causes offensive odours in addition of soiling buildings monuments in Kenya (Galcano *et al.*, 2001).

### 2.4.1 Air pollution in Nairobi

Air in Nairobi has been considered poisonous and causing which, may serious ailments like Lung and heart diseases as well as cancer and it is ten times above the recommended level by WHO (Elisaveta 2013). Air pollution in Nairobi generally is above-accepted level (WHO, 2006) especially on its streets (Kinney *et al.*, 2012). Daytime concentrations of $PM_{2.5}$ were seen to be very high at sites located adjacent to roadways (Kinney *et al.*, 2012), the report says it is ranging from 50.7 to 128.7 $\mu g/m^3$ across Nairobi Central Business District (NCBD) sites and commuter route. Comparison of levels of air pollution of Nairobi and another standard in the world started in 1996 by WHO. This followed studies, which had been undertaken by Ngugi (1983) and Karue (1991) who had noted a deterioration of air quality in Nairobi. Similarly, Kinney *et al.* (2012) observed that presence of air pollution (away from the source) is not as significant as compared to that close to the source. Therefore air pollution in Nairobi is significant. Nairobi being green city in the sun, its plants close to roads constantly are exposed to air polluting agents from vehicle exhaust fumes of moving vehicles, and have to endure more, like others in the world (Chen-Xiao *et al.*, 2003).

It is also reported by Odhiambo *et al.*, (2010) that most of the air pollution in Nairobi is due to vehicle fumes, and it is very evident usually at a high level when there are vehicles congestion or heavy traffic where motor vehicles constantly stream past or snarled up in slow-moving jams (Appendix 5). The air pollution affects Nairobi commuters because it reaches peak levels in morning and evening when people are either going to work or going home. High peaks of air pollution are most felt at some hot spots due to vehicle congestion or slow moving traffic. Air pollution at such points occurs because engines are running or on idling, pumping exhaust fume into a calm environment (Odhiambo *et al.*, 2010). Both Odhiambo *et al.* (2010) and Kinney *et al.* (2012) agree that no one has made an attempt to scientifically illustrate air pollution related to vehicle fumes and effects it has on organisms. This thesis looked into effects of air pollutions in Nairobi on plants as a biological indicator of its hazardous effects to organisms (Sowidis *et al.*, 1995) and, which is thought to be sufficient to cause harm to people in Nairobi. Air pollution is temporal and spatial in Nairobi, which requires more studies as shown by Gatari *et al.*, (2005) so as to map hot spots and offer resilience to effects it may cause. In Nairobi Kenya, climatically talking, seasons change in a year and are times when air pollution is not quickly diffused thereby causing more harm than other times. This study does not address the spatial and temporal state of air pollution in Nairobi city. It is also hoped that long-term studies are made in Nairobi city to understand air pollution's trends. For any infrastructure developments in Nairobi, Proper Environment Impact Assessment (EIA) is done before undertakings. In those already in existence EIA audit be taken. The best way of for audit on effects of air pollution in Nairobi is continuous monitoring (using very sensitive plants) for proper conclusive recommendations as suggested by both Galcano *et al.* (2001) and Gatari *et al.* (2013).

## 2.5 Composition of Vehicle Fumes

Vehicles engines need oxygen for combustion, which is drawn in through air filters. The air sucked in, by engines is wholesome. Such air is composed of by volume of oxygen ($O_2$), which is 20.947%, nitrogen ($N_2$), which is 78.084%, carbon dioxide ($CO_2$), which is 0.0350%, water vapor ($H_2O$) or atmospheric humidity and other gases that make up 0.934% of total air.

The gases (especially oxygen) are taken into engine combustion chambers as a vital component of combustion. Hydrocarbons, which are derived from diesel (with additives of sulfur) or hydrocarbons derived from petrol (with lead elements as additives), are pumped into engines combustion chamber, through turbo mechanism in the case of diesel engine's or through carburetor in the case of petrol. Vaporized hydrocarbons (fuel) are mixed and pumped by carburetor (modern petrol engines us turbo mechanism) into combustion chambers referred to as cylinders. In case of diesel engines, air is pumped into combustion chamber or cylinder, by turbo mechanism. Once gaseous solutions are compressed by pistons in the cylinders, (in case of petrol engine) are ignited by spark plugs, made to produce electrical sparks, the mixture ignites and explodes forcing piston down, an action which producing power stroke. This power stroke is responsible for vehicle movements.

In the case of diesel engines, gases are compressed and explode when fuel is injected in the mixtures. This is a power stroke stage, which is responsible for vehicle movements. Another stroke movement to piston (in both petrol and diesel engines) is initiated by the crankshaft, which is post power stroke, for purpose of expelling exploded gaseous out from the combustion chamber into the exhaust pipe, which is known as vehicle exhaust fumes. After the expulsion of exploded vehicle exhaust fumes, a piston moves down while sucking in a fresh mixture to repeat the cycle.

Chemical reactions in the chamber are mainly oxidation processes. Pure nitrogen is oxidized with oxygen in presence of high temperature to produce nitrogen oxide, nitrogen compounds and a substantial portion of nitrogen is not oxidized and remains as inert nitrogen. Hydrocarbon compounds are oxidized by oxygen to produce carbon dioxide, carbon mono oxide, and water. Un-burnt hydrocarbon compounds (HC) become soot of various sizes known as particulates matter ($PM_{10}$ and $PM_{2.5}$).

Additives to fuel produce other compounds. In diesel, sulphur is added to enhance engine performance. After the combustion, sulfur is oxidized to produces sulphur dioxide and particulates matter or soot. In petrol, lead is added to produce super petrol. After combustion lead produces lead oxide and un-burnt lead as soot or particulates matter. The addition of lead in Kenyan petrol is discouraged due to the effects it has on people, as one of air pollution (Omulo, 2016).

The chemical composition of vehicle fumes is, therefore; nitrogen compounds, nitrogen oxide, pure nitrogen, carbon dioxide, carbon mono oxide, water, un-burnt hydrocarbon compounds, particulates matter, sulphur dioxide and lead monoxide. Vehicle fumes are two varieties of products, one part in gaseous form, and other in solid form. All these byproducts (as vehicle exhaust fumes) affect plant growth in one way or another as reported by Wagh *et al.* (2004).

## 2.6 Particulate Matter in Air

Particulate matter is composed of fine organic solid matter of un-burnt lead, sulfur particles, un-burnt carbon, which altogether forms soot. This particulate matter is normally classified into two categories according to sizes. Very fine matters are $PM_{2.5}$, while bigger particles are $PM_{10}$. These matters are measured in micrograms per cubic meter of air ($\mu gm^{-3}$). The organic particulates are ejected from engines through the exhaust pipe. Sulfur particles are from diesel engines, deliberately added to diesel fuel to act as the catalyst to enhance performance. The sulphur is released as sulphur dioxide through the exhaust pipe. Lead is added to petrol hydrocarbons to enhance combustion. After combustion lead is oxidized to form a lead oxide which is released through the exhaust pipe. Since the lead compounds are heavy in nature they precipitate very close from a source. This is why along roads there is high air pollution (Kinney *et al.*, 2012). The lead compounds pollute soil along roads. The lead compounds as particulate matter land on plants along roads and darken plants with bad consequences (Guggenheim *et al.*, 1980). In this study particulate matter has been used as a measure of pollution which, includes gaseous (not measured) and solids in the air pollution. Air pollution affects photosynthesis, respiration, transpiration and allows the penetration of phytotoxic gaseous pollutants into plants.

## 2.7 Effects of Air Pollution on Plants

Plants are affected by air pollution in two ways (Braun and Fiuckiger, 1987). One is by particulate matter (PM) in air pollution, which after landing on a surface, be it on plant leaf or stem, forms a cover (Thompson *et al.*, 1984). Second are gases which can directly enter into a plant leaf through stomata during the gaseous exchange. Gaseous pollution combines with other elements in air and form complex compounds, then enter into a leaf as secondary air pollution with profound lethal consequences (Taylor *et al.*, 1986).

Oxidized PM in the air are black hence darken surfaces of a plant exposed to air pollution. This dark color disfigures the plant and loses its natural beauty for authentically values. PM forms covers which obstruct stomata. The stomata are affected by the cover in various ways. Stomata regulate evapotranspiration, which is a key function in plant metabolism (Fluckiger *et al.,* 1979). During evapotranspiration, as a drop of water escapes from stomata, it pulls another drop down a plant through capillary action (Thompson *et al.,* 1984). Evaporation of water from stomata causes water pull from the ground. This water pulls, turn into a chain reaction from leaf to stem to roots as water uptake. Obstruction caused by particulate matter from air pollution stops such important plant reactions (Gheorghe and Ion, 2011).

During photosynthesis process, water is mixed with carbon dioxide in presence of sunlight and green chlorophyll ($6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + O_2\uparrow$) to produce carbohydrates to build plant cellulose which translates into plants development and growth. When stomata are blocked by PM, then normal diffusion of carbon dioxide in an exchange with oxygen from a plant is stopped (Beasley, 1942). An important function in plants metabolism comes to halt, due to deposits on leaf of PM (Thompson *et al.,* 1984).

Plants use phloem and xylem vessels to transport and translocate water and manufactured carbohydrates in the whole plant. These vessels can be blocked by particulate matter, which enters in plant system and does not dissolve in water (Gheorghe and Ion, 2011). These actions interfere with plant metabolisms, hence reduced plant development.

### 2.7.1 Lead

Lead which is added to petrol to form super petrol, and during combustion lead oxidized to form lead oxide which is a heavy oxide and will pollute soils then up-taken by plants as one of heavy metal. Lead oxide is a particulate matter not gaseous. The lead oxide has little effect to plants as compared with the effects it has on people (Guggenheim *et al.*, 1980).

### 2.7.2 Effects of Sulphur dioxide on Plants

Very small particulate matters ($PM_{2.5}$) which may contain sulphur dioxide are capable of entering plant stomata, hence into plants tissues. Once inside the tissues where water is present, chemical reactions take place. Water combines with oxides to form acids (in case of non-metallic) or alkaline (in case of metallic). In case of sulphur oxide, it will form sulphuric acid ($H_2O + SO_3 = H_2SO_4$). The sulphuric acid is toxic to plant cells. The acidity condition distorts osmotic pressure thereby altering water movements (Omafra, 2014). This results in plant cells necrosis. Other gaseous compounds air pollution form complex lethal compounds outside with sulpher, which then enter into a plant through stomata causing damage to plant tissues (Pradeep, 2008)

At secondary level, sulfur dioxide forms acid rain which, injure plant leaves. The leaves lose their green color in scattered manner and form blotchy white spots (Omafra, 2014). Some leaves can develop red, brown or black spots. In severe condition leaves, start to senescence (Ricks *et al.*, 1975). Plant productivity is greatly reduced (Gheorghe and *Ion, 2011*).

The effects of $SO_2$ to plants are mostly felt in high humidity, windy conditions, in the early morning, and places with excess of sulphur in the soil. In $SO_2$ polluted sites, plants show initial reduction of photosynthesis and decreased respiration. The sulphur gas causes stomata closure and thus induces general water stress in plants. $SO_2$ interferes with amino acid metabolism and reduces the synthesis of proteins and enzymes (Gheorghe and *Ion, 2011*).

### 2.7.3 Nitrogen Dioxide

Nitrogen Dioxide ($NO_2$) affects leaves and young plants. This is a results of nitric acids formed on or inside a leaf after combination of $NO_2$ and water. Visible signs of pollution by $NO_2$ are chlorosis and tips burn (Pyatt, 1973)

### 2.7.4 Ammonia

Ammonia ($NH_4$) is formed when nitrogen is in a high temperature in the presence of water. $NH_4$ affects plants either at low or high concentration depending on different plant species. Visible expression on affected vegetation usually takes several days to develop, and appears as irregular bleached or bifacial necrotic lesions. Tomato plant is one of the plants that are resistant to $NH_4$ (Gheorghe and *Ion, 2011*).

### 2.7.5 Carbon monoxide (CO)

Carbon monoxide (CO) gas affects plant at a high concentration level otherwise no visible injury has been reported as results of the gas. (Gheorghe and *Ion*, *2011*). This phenomena is true because carbon monoxide quickly is oxidized into $CO_2$ hence little is in the air.

### 2.7.6 Secondary pollutants and plants

Many of primary air pollution agents produced by vehicles as vehicles fumes are later on combined with other substances outside a plant to form complex compounds. Such compounds are Peroxyacetyl nitrate ($CH_3CO.O_2.NO_2$) referred in short as PAN. PAN causes chlorosis and necrosis in plant leaves. PAN affects plant photosynthesis and respiration processes. The primary air pollutants can also form ozone ($O_3$). The ozone gas is very lethal to plants. The effects by $O_3$ include, deformation of leaves, deformation of flower and pollination processes is altered. These affects lowers plant productivity (Fennelly, 1975).

## 2.8 Plant Stress

Plant stress is a result of restricted water movements in the plant. The water movement is restricted mainly because of water inlets or outlets closure. The unfavorable soil condition can cause inlet on the roots to stop functioning (either water logged or dry soil conditions) hence plant stress. This causes plant wilt (Idso and Idso, 1994). Unfavorable atmospheric condition cause water outlet not to function properly. This is due to stomata closure by either being occluded by PM or physiological closure due presence of harmful gases (Pourkhabbaz *et al.*, 2010). If the condition persists then leaf secessions takes place

# CHAPTER THREE: MATERIALS AND METHODS

## 3.0 Introduction

This chapter deals with the methods used in the study. It starts with study area description and climate. It then describes how the study area was chosen as experimental site. Experimental design and establishment are also detailed. Chemical and physical compositions of soils, choice of candidate specie are presented in the Chapter. Sowing of Seeds, management, and maintenance of experiments is also indicated. Plant stress determination process is explained in the chapter. Data collection is elaborated in the chapter.

## 3.1 Study Area

Waiyaki Highway is located in Nairobi County. Nairobi County covers an area of 696 $Km^2$. Its geographical coordinates are, latitude 1°16′59″ S and longitude 36°49′00″ E and has an elevation of 1684 m above sea level. It has a high network of roads with heavy traffic jams from time to time (Odhiambo *et al.*, 2010). Nairobi is the Capital City of Kenya and a transit city for heavy transport from Mombasa to other East African landlocked countries. The study focused on one of Nairobi County roads named Waiyaki Highway. The Waiyaki Highway is a road used by vehicles on transit to and from other countries to Mombasa port.

## 3.2 Climate of the Study Area

The temperature of Nairobi averages 23.4$^o$C. at day time and 18.6$^o$C. at night. In June and September are coldest months coupled by foggy conditions, with temperatures range from 10$^o$C and 15$^o$C. The city has rainfall of about 1,024.2 mm per year, which has bimodal characteristics occurring in April and October (Tripadvisor 2017). Nairobi is relatively not windy but sometimes having westerly breeze (Travel 2017).

## 3.3 Selection of Study Sites

The area selected for the study was along Waiyaki Highway road. The Highway stretches from University Way roundabout to Uthiru Flyover Bridge, about twelve kilometers in length (Figure 3.1). The road has a general gradient of less than 10%.

A reconnaissance survey was conducted by walking along Waiyaki Highway, from where it starts (at university way roundabout) in Nairobi City to where it end (at Uthiru Flyover). While walking, sites with tree leaves and tree trunks with dark color were marked. Dark color on trees was the main characteristic used to choose points for experiment placement. The observation was skewed to the left of Waiyaki Highway as one move from Nairobi towards Nakuru. Three main sites were identified, these included; a site at ICEA Building, immediately after a junction from Waiyaki Highway to Chiromo Campus of the University of Nairobi with coordinates, latitude - 1.26903$^{o}$ S and longitudes - 36.8055$^{o}$ E. A second site was at ABC Place with coordinates, latitude -1.25958$^{o}$ S and longitudes - 36.7757$^{o}$E. A third experimental site was at Kangemi Market along Waiyaki Highway with coordinates, latitude - 1.26431$^{o}$ S and longitude - 36.7493$^{o}$ E. A control site was identified within National Agricultural Research Laboratories (NARL) of Kenya Agricultural Livestock Research Organization (KALRO) with coordinates, Latitude - 1.25821$^{o}$ S and longitudes - 36.7727$^{o}$ E.

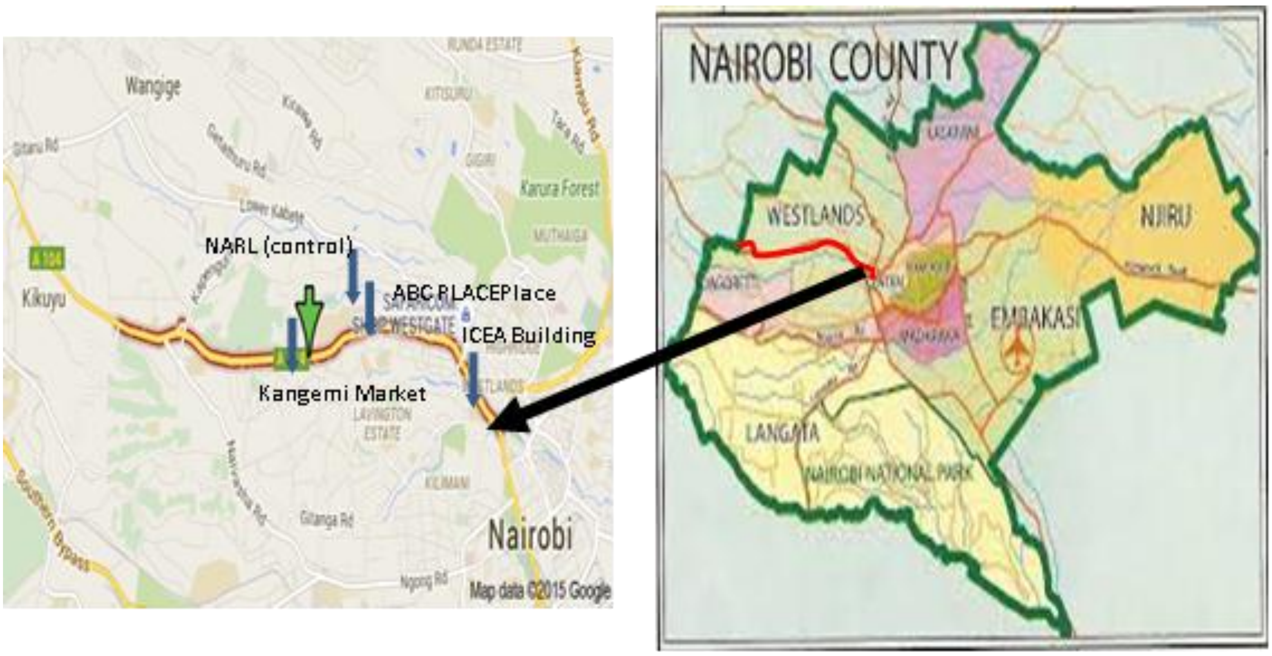


**Figure 3.1 Map of Nairobi County Showing Waiyaki Highway and selected sites**

### 3.4 Experimental Design

The actual position of experiments exposed to air pollution (test plants) was determined by equation below demonstrated by Ole Raaschou-Nielsen *et al.* (2013). This equation takes into account road bends, wind movements (speed and direction) while considering the height of experiment from the ground for best results.

$$\boldsymbol{x} = \int_{\circ}^{\infty} \frac{q}{\pi(ucdx^2)\cos\alpha} \left(exp\,\frac{y}{2c^2x^2}\right) dx$$ …………………….. **Equation (formula)**

Where:-

$\boldsymbol{x}$ Was the distance of the experiment to the roadway at each point,

$\boldsymbol{y}$ Height of stand that the experiment was placed on,

$\boldsymbol{u}$ Was mean wind speed at each point,

$\boldsymbol{q}$ Was expected standard deviation of results,

$\boldsymbol{\alpha}$ Was an angle of tilt of line source relative to a reference frame,

$\boldsymbol{c}$ And $\boldsymbol{d}$are standard deviations of horizontal and vertical wind directions (measured in radians) respectively.

This Equation was created and used by Ole Raaschou-Nielsen et al, (2013). From Equation, each experiment was positioned 4 meters away from any edge of tarmac.

### 3.5 How Plant Stress is Determined

A pressure bomb or pressure chamber or Scholander bomb is an instrument, which makes it possible to measure approximate water potential or possible water movements in plant tissues (Scholander *et al.,* 1965). If water potential is high then; the more difficult it is for water movements of water in plant's tissues. The high water potential in plants causes stress (Scholander *et al.*, 1965). The high water potential is due to unfavorable soil or environmental conditions. Unfavorable atmospheric conditions can be caused by many agents among them, air pollution. The unfavorable atmospheric condition then causes high negative water potential, which eventually restricts water movement causing plant stress (Zeiger, 2002).

A leaf attached to a stem is cut and placed inside a sealed chamber upside down and closed tightly before it is pressurized with air, which is allowed into the chamber slowly. As the pressure increases in the chamber, at some point, sap will be forced out of xylem and will be visible at the cut end of leaf stem or midrib. The pressure that is required to do so is equal and opposite to water potential of the leaf, according to Scholander *et al.* (1965). The pressure is measured by a gauge on the pressure bomb. This pressure is measured in psi (Ψ) units and the units are above the prevailing pressure at a given site.

### 3.6 Chemical and Physical Composition of Collected Soil

Soil used in the study was collected from Muguga forest under broad-leaved trees of *Croton megalocarpus*. The soil had uniform dark-brown color, an indication of well decomposed organic matter. The soil was of loam with well-formed crumb and brownish color, cattle manure was added at a rate of 1:10 (Starke, 2010), which agrees with Wikihow (2016). Wikihow (2016) details physical, chemical and pH requirements for proper tomato growth including how to add Manure, which was adapted. Chemical composition of the soil, was determined in KEFRI Muguga Laboratory using standard procedures (Wikihow, 2016).

### 3.7 Choice of Species of Plants

Tomato (*Lycopersicon esculentum* Mill 1768), was used in the study. This plant was chosen because of its sensitivity to air pollution being hairy and able to trap particulate matter both $PM_{10}$ and $PM_{2.5}$ (Holmes and Schultheis, 2001). Seeds and technical advice on management were given by Starke, (2012). The company supplies high-quality germplasm of horticultural plants to farmers. Seeds of *L. esculentum* had been coated with Thiram Chemical to check on fungal and insect attack. Three varieties of *L. esculentum* were acquired from the company. Cultivars chosen were: hybrid tomato Star 9065 reference number 3207/4, hybrid tomato Star 9037 reference number 4063/2 and hybrid tomato Samantha reference number 3741/1.

### 3.8 Establishment of Experiments

Raised platforms (0.5m above soil surface) were constructed (length of 3m by width of 0.3m) using timber strong enough to hold soil weighing about 60 kg. a wooden structure to carry experimental plants was placed on top. The Wooden structure was in rectangular form with closed bottom and top side open (Plate 2, in Appendix 6). Four structures were placed at pre-determined sites by use of GPS coordinates shown elsewhere in the script. The configuration of three platforms (those in air pollution at ICEA Building, ABC Place, and Kangemi Market and therein referred to as test plants) was parallel to the Highway and a distance from road determined by using Equation above. The platform was raised to just above 0.5 m (by use of formula) above ground to control in-situ soil interactions. Each platform or each study site had twelve bags containing soil (each pot had 5 kg of soil). Polythene bags were of size 25 cm x 30 cm lay flat (10 inches by 12 inches) and closed bottom. Each experiment had three tomato varieties replicated four times in a random fashion within the structure as shown in Figure 3.2.

## 3.9 Sowing of Seeds

Big pots, which had been filled with soil (size 25 cm x 30 cm lay flat) were placed in selected sites and directly sown with seeds of the tomato plant. Seeds were sown on November 7th, 2014 in all sites. To study on germination, three seeds of each variety were sown in each pot in all experiments. Complete Random Block Design (CRBD) was employed when laying pots in an experiment. Each variety was randomly replicated four times at each four sites.

## 3.10 Management and Maintenance of Experiments

Experiment upkeep involved security, watering, and weeding. The experiments were guarded by Nairobi County security officers. This was strengthened by daily visits to check on expected security. Watering frequency was done depending on weather condition, minimal irrigation during rainy conditions once a week or increased to several times in a week if dry conditions experienced (Wikihow, 2016). Water used was from one source of a borehole, which ensured uniformity of water nutrients. Weeding was done often as weeds came up. There was insecticide spray to keep off white flies in two replicates at ABC place and control. The insecticide was sprayed where damage to seeds by herbivorous animals had been noticed.

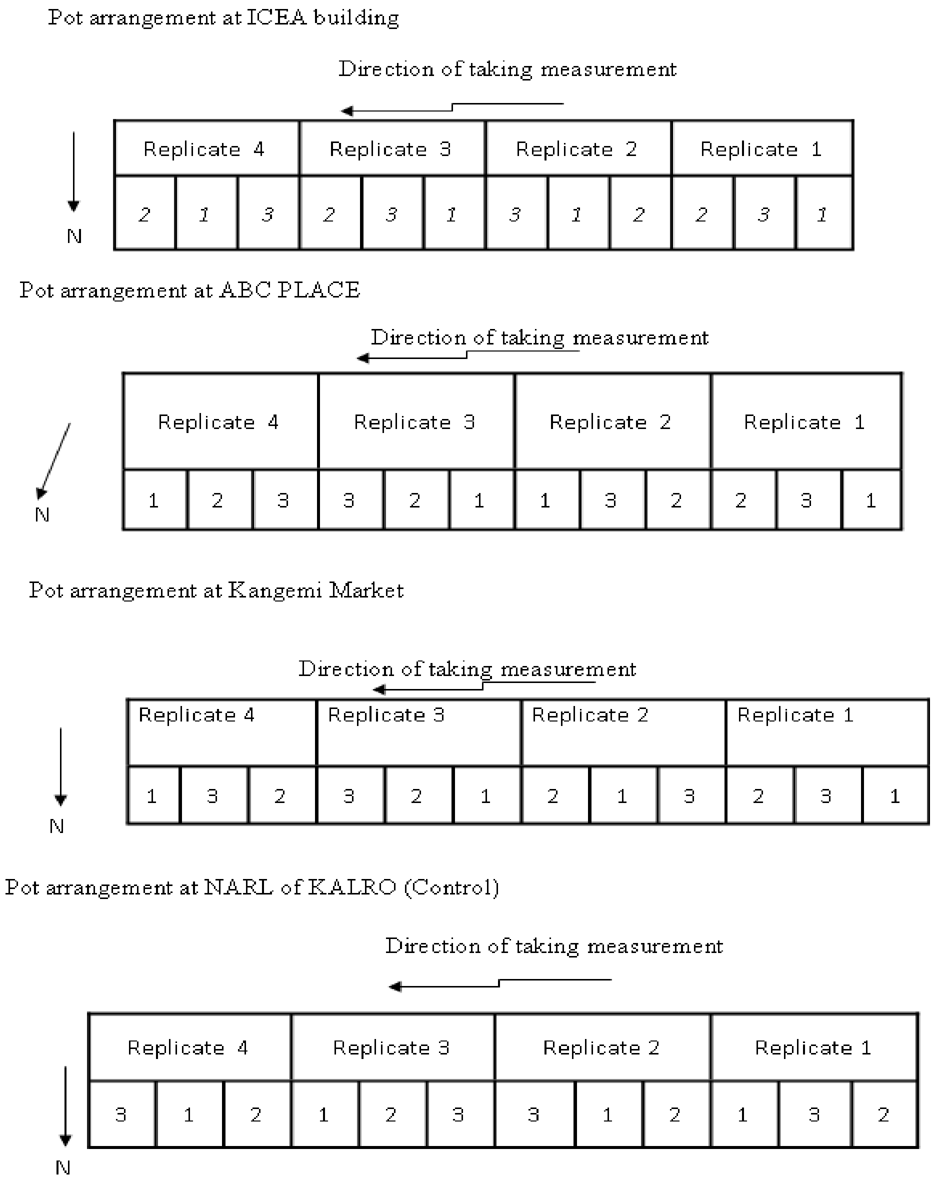


Figure 3 2 Experiments layout (complete Random block design)

## 3.11 Data Collection

### 3.11.1 Measurements of Air Pollution (Particulate Matter)

The measure of air pollution can be done in two ways, measuring of gases and measure particulate matter. In this study particulate matter has been used to estimate air pollution. Air pollution was measured in terms of Particulates Matter ($PM_{10}$ and $PM_{2.5}$). Particulates Matter ($PM_{10}$ and $PM_{2.5}$) at various sites of the study was determined by instruments acquired from Ministry of Labour, Occupation Health Services section. The instrument was known as

DustTrak Aerosol Monitor model 8520. Particulate matter instrument has a diaphragm, which was placed at experimental sites and sucked in air from the environment at the rate of one cubic meter per minute for twelve hours. This action was done three times, one at beginning of the study (at day 0 of the study), at day 45 of the study or a half way of the experiment and lastly at end of experiment (at day 90 of the study), (Appendix 1). The diaphragm in the Aerosol Monitor, has a well-designed paper sieve, which traps particles in the air passing through. The initial weight of sieve was taken (by a scale that measures weight in µgm) before and after twelve hours. Since the amount of air passing through the diaphragm was known, calculation of PM in µgm per cubic meter was performed. Appendix 1 shows how the calculation was done after measurement of particulate matter ($PM_{10}$ and $PM_{2.5}$). Results were used to calculated significant level. The PM was taken on days, which were dry and sunny.

### 3.11.2 Germination Rate of Seeds

*L. esculentum* seeds germinated after seven days from date of sowing. All germinates appeared in one day. Germination was uniform in all experiments. Assessment of germination was done by physical counting of germinated seeds. Data collected was recorded in Excel spread sheet.

### 3.11.3 Measurement of Plant Height

Height measurement was taken using a linear tape. The measurements were done on each tomato plant, from soil level to tip of the plant. These measurements started after ten days from date of germination, thereafter every week (for nine weeks) until end of study. Collected data were recorded in designed height data collecting sheet then transferred later to Excel spread sheet.

### 3.11.4 Measurement of Length of Leaves

Leaf length measurements were done using linear tape. This started off after juvenile leaves were replaced (36 days from seed germinating or 43 days from seeds sowing) by mature leaves and done every week (for five weeks). Tomato leaf is of compound structure with opposite leaflets. Measurement was from leaf attachment on main stem to leaf apex. One longest leaf, on each plant was measured each time. The measurement continued for five weeks or until conclusion of experiment. Data was recorded in a designed leaf data collecting sheet. The data was transferred into Excel spread sheet.

### 3.11.5 Number of Flowers

Number of flowers on each plant in the experiment was counted. The count started when flowers buds developed. This counting continued every week for six weeks (end of flowering). At each count all flowers would be counted. All plants in each treatment were considered and number of flowers taken. This was done by physically counting and recording all flowers in a data sheet. During analysis peak number of flowers on each tomato was used.

### 3.11.6 Determination of Plant Biomass

Plants were washed (shoot and roots) and put in labeled brown papers. Initial weight was determined gravimetrically by use of electronic balance. Samples were then loaded in pre-heated oven. The oven was pre-heated to $100^{o}$C. for 3 hours. Drying was done for 72 hours at constant temperature of $100^{o}$C. Total dry matter was determined gravimetrically by use of electronic weighing scale on ex-oven materials while hot. Weights of samples were recorded as biomass (gms) for each plant variety in Excel spread sheet. Method described above was adopted from Faichney and White (1983).

### 3.11.7 Determination of Plant Stress

Plant leaf stress was determined by a pressure bomb (Scholander *et al.,* 1965). The method is detailed in section 3.5 above. This was done at pre-dawn and Measurements were taken in psi ($\Psi$) units using pressure bomb. Data was recorded in a designed sheet and then entered in Excel spreadsheet.

### 3.1.1 Determination of Stomata Distribution and Behavior on Leaves

Stomata count (open or closed, on lower or upper epidermis) was used to determine physiological nature of plants exposed to air pollution. Leaves used to determine numbers of stomata were washed in clean water, both upper and lower sides. The Leaves were then painted with clear nail polish. After the paint dried, clear seal tape was laid on top of the portion of the leaf with clear nail paint. The clear seal tape was then peeled off. The seal tape carried on itself the nail polish. The nail polish had picked leaf surface impressions. The clear seal tape was placed in well-marked envelopes according to tomato variety and site. When the impressions on nail polish were put under view point of an electronic microscope, stomata and other features were seen. The microscope had a scale graduation showing micro-area on a graticule (a transparent glass with micro-readings or graduations). Number of stomata was determined by counting stomata impressions made on clear nail paint in a specific standardized area. Area was standardized to be 0.0016mm$^2$ in all stomata count procedure. Any stomata falling within this area were counted. This was repeated for upper leaf surface and lower leaf surface for each variety in the experiments. In each case, open and closed stomata were counted separately. Results were recorded in excel for further analysis. Number and state (open or open) of stomata on leaves were scrutinized in several ways. One was analysis of number of stomata on upper and lower epidermis of leaves, two, analysis of number of open stomata on upper and lower epidermis of leaves. Three, was analysis of number of closed stomata on upper and lower epidermis of leaves. The stomata analysis was based on a micro-area of (0.04mm x 0.04mm) 0.0016mm$^2$ on leaves, as described by Pourkhabbaz*et al.* (2010).

### 3.12 Data Analysis

Both inferential (tables) and descriptive (graphs) statistics were used depending on nature of data collected. Data collected on number of flowers and stomata were analysed using a Kruskal-wallis or Poisson distribution while data collected on particulate matter, seeds germinated, height of plants, leaf lengths, plant stress and dry weight measurements were subjected to one way analysis of variance (ANOVA) at significance level, $\alpha = 0.05$ using Genstat software. Stomata count was analysed using Log linear regression in Genstat software at level $\alpha = 0.05$. Least Significant Difference (LSD) was used to separate specific means on data which could be significantly different at $\alpha = 0.05$.

**CHAPTER FOUR: RESULTS**

## 4.0 Introduction

This Chapter deals with presentations of results obtained. Results are presented based on descriptive and inferential statistical analysis of data collected. The results start with data on Particulate Matter, then on chemical and physical composition of soil. Seeds germination rate, height of plants, length of leaves, number of flowers, and biomass of plant species are shown. Plant stress and stomata count data are also presented.

## 4.1 State of Air pollution at the Experimental Sites

The levels of air pollution as measured I the study sites are presented in appendix 1. Table 4.1 shows state of air pollution at chosen study sites, at the beginning of experiment, 45 days and 90 days of experimentation period. The actual readings of weight are found in appendix 1, which, were used to generate table 4.1. At each and every experiment, analysis of air pollution at 0 days, 45 days and 90 days had no significance difference at $\alpha$ =0.05 (Table 4.1). Air pollution between experimental sites was highly significant difference ($P< 0.05$). Throughout the study period, the air pollution at all experimental sites remained more or less constant ($P<0.05$) (Table 4.1)

Table 4.1 Particulate matter measurement levels at different stages of the experiments.

| Experimental Site | Air Pollution at beginning of experiment ($\mu gm^{-3}$) | Air Pollution after 45 days ($\mu gm^{-3}$) | Air Pollution after 90 days ($\mu gm^{-3}$) | p-value |
|---|---|---|---|---|
| ABC Place | $42.5\pm0.02^{a}$ | $42.2\pm0.01^{a}$ | $43.3\pm0.01^{a}$ | 0.078 |
| ICEA Building | $42.5\pm0.02^{a}$ | $42.4\pm0.03^{a}$ | $41.4\pm0.04^{a}$ | 0.086 |
| Kangemi Market | $43.6\pm0.00^{a}$ | $42.9\pm0.01^{a}$ | $41.5\pm0.01^{a}$ | 0.084 |
| NARL (Control) | $0.56\pm0.03^{b}$ | $0.61\pm0.03^{b}$ | $0.55\pm0.01^{b}$ | 0.099 |
| P-value | 0.001 | 0.000 | 0.002 | |

Numerical value followed by the same alphabetical letter(s) in a superscript version within columns and across rows show that figures are not significantly different from one another at $\alpha$ = 0.05 (least significant difference).

## 4.2 Germinating and Behaviour of *L. esculentum* Plants in Air Polluted Environment

### 4.2.1 Chemical Composition of Soil Used in the Experiment

Macro-elements, that is Nitrogen, Potassium and phosphorous in soils was at satisfactory level required by tomato plants. Micro-elements were present at normal level; pH level was slightly acidic, but conducive for tomato growth. Results of soil chemical analysis and composition are indicated in Table 4.2. Plants in nature require in macro-elements of nitrogen, phosphorous. If soils are poor in those elements NPK fertilizer is added and incase of this try, no fertilization was required. The nitrogen is required for plant cell buildup especially the nucleus which contains DNA and chlorophyll buildup. Plants also manufacture carbohydrates general fiber body building. The micronutrients are required by plants in smaller quantities for many other essential plant activities.

Table 4.2 Chemical composition, of soil used for growing experimental Plant species.

| Elements and Soil Acidity | Quantities | Limits of elements and soil acidity for tomato growth* |
|---|---|---|
| Total organic carbon % | 3.26 | 0 to 138 |
| Total Nitrogen (N %) | 0.32 | 0.05 to 3.0 |
| Available P (ppm) | 183 | Unavailable |
| Potassium (µg/mm) | 25.1 | 15 to 30 |
| Calcium (µg/mm) | 9.9 | 5 to 24 |
| Magnesium(ppm) | 6.42 | Unavailable |
| Manganese(ppm) | 130 | 40 to 140 |
| Copper (ppm) | 13.3 | 0 to 56 |
| Iron (ppm) | 122 | 0 to 129 |
| Zinc(ppm) | 34.4 | 0 to 35.8 |
| Soil pH | 6.01 | 5.0 to 7.0 |

Standard Quantities*set by Okalebo *et al.* (2002).

### 4.2.2 Germination Rate of Seeds of *L. esculentum*

At ABC Place, ICEA Building, Kangemi Market and NARL (control) experiments; hybrid Samantha recorded similar results of 91.7 % seed germination rate. Hybrid H9037 at ABC Place and ICEA Building recorded 100 % seed germination rate. Hybrid H9037 at Kangemi Market had 91 % germination rate, while at NARL (control) (58.3 %). Tomato variety of hybrid H9065 recorded results of (75 %) germination rate in all experimental sites. Germination of hybrid H9037 varied across experimental sites. Differences in average seed germination of *L. esculentum* in experimental sites, was not statistically different at $\alpha = 0.05$ as shown in Table 4.3. The germination rate of tomato varieties had significant differences within each experimental site ($P < 0.05$) (Table 4.3).

Table 4.3 Seed germination rate of *L. esculentum* in the experiments

| Experimental Sites | Hybrid H9065 (%) | Hybrid H9037 (%) | Hybrid Samantha (%) | P – values |
|---|---|---|---|---|
| ABC Place | 75±13.9 | 100±11.1 | 91.7±2.8 | 0.046 |
| ICEA Building | 75±13.9 | 100±11.1 | 91.7±2.8 | 0.046 |
| Kangemi Market | 75±11.1 | 91.7±5.6 | 91.7±5.6 | 0.048 |
| NARL (Control) | 75±0.1 | 58.3±16.6 | 91.7±16.8 | 0.044 |
| P value | 0.093 | 0.048 | 0.100 | |

Confidence level, 95%.

### 4.2.3 Height of *L. esculentum* Plants

At ABC Place, hybrid Samantha recorded highest height of 102.9 cm followed by hybrid H9037 (84.3 cm) and finally hybrid H9065 (80.7 cm). At ICEA Building, the tomatoes performed almost like at ABC place with hybrid Samantha recording highest height (80.4 cm) followed by hybrid H9037 (63.6 cm) and finally hybrid H9065 (59.4 cm). At Kangemi Market hybrid Samantha still was tallest (89.5 cm) followed by hybrid H9037 (76.6 cm) and finally hybrid H9065 (69.6 cm). At NARL (control) hybrid Samantha was tallest (92.1 cm) followed by hybrid H9037 (83.7 cm), and finally hybrid H9065 (78.7 cm). There was a significant difference in height growth between experimental sites along Waiyaki Highway and control at ($P < 0.05$). However results showed no significant differences between varieties of *L. esculentum* for every experimental site ($P < 0.05$), as shown in Table 4.4.

Table 4.4 Results of mean heights of *L. esculentum.*

| Experimental Sites | Hybrid H9065 (cm) | Hybrid H9037 (cm) | Hybrid Samantha (cm) | P – values |
|---|---|---|---|---|
| ABC Place | $80.7 \pm 7.9^{a1}$ | $84.3 \pm 10.4^{a1}$ | $102.9 \pm 8.2^{a1}$ | 0.472 |
| ICEA Building | $59.4 \pm 3.8^{a3}$ | $63.6 \pm 2.69^{a2}$ | $80.4 \pm 5.4^{a3}$ | 0.614 |
| Kangemi Market | $69.6 \pm 9.1^{a2}$ | $76.6 \pm 5.84^{a1}$ | $89.5 \pm 6.0^{a2}$ | 0.802 |
| NARL (Control) | $78.7 \pm 2.2^{a1}$ | $83.7 \pm 2.5^{a1}$ | $92.1 \pm 6.3^{a2}$ | 0.835 |
| P-value | 0.031 | 0.026 | 0.031 | |

Numerical value followed by the alphabetical letter(s) in a superscript version within rows and numerical values in superscript version within columns show that figures are not significantly different from one another at $\alpha = 0.05$ (least significant difference).

### 4.2.4 Height patterns of Plants at Experimental Sites

At ABC Place, hybrid H9065 had the highest growth rate pattern followed by hybrid Samantha (Figure 4.1, ABC Place Experiment). Hybrid H9037 had the lowest gradual growth rate patterns. The similar growth rate patterns were observed at ICEA Building (Figure 4.1, ICEA Building Experiment) and Kangemi Market (Figure 4.1, Kangemi Market Experiment). Trend patterns of two tomato hybrid varieties at NARL (control), H9065 and H9037 exhibited similar growth rate (Figure 4.1, NARL, Experiment). However, at NARL experimental site Hybrid Samantha had a trend that was different from the rest.

### 4.2.5 Length of Leaves of Plants in the Experimental sites

At ABC Place, hybrid H9065 recorded highest leaf length (42.6 cm) followed by hybrid H9037 (37.1 cm), and finally hybrid Samantha (35.0 cm). At ICEA Building, tomatoes performed almost like ABC place with hybrid H9065 recording highest leaf length (45.2 cm) followed by hybrid H9037 (40.9 cm), and hybrid Samantha (34.2 cm), respectively. At Kangemi Market, hybrid H9065 had highest leaf length (39.0 cm) followed by hybrid H9037 (37.3 cm), and hybrid Samantha (35 cm), respectively. At Control (NARL) site, hybrid H9065 had highest leaf length (41.6 cm) followed by hybrid H9037 (35.1 cm), and hybrid Samantha (29.8 cm). However these observed differences were not statistically significant ($P < 0.05$) (Table 4.5). Comparatively, tomato varieties did not record any significant differences between experimental sites ($P < 0.05$) (Table 4.5)

### 4.2.6 Number of Flowers on Test Plants

At ABC Place, hybrid Samantha had highest number of flowers (30) per plant, followed by hybrid H9037 with 18 flowers per plant, and finally hybrid H9065 with 16 flowers per plant. At ICEA Building, hybrid Samantha had highest number of flowers per plant (60), followed by hybrid H9065, which had 26 and finally hybrid H9037 had 24 numbers of flowers per plant. At Kangemi Market, hybrid Samantha had 54 flowers per plant followed by hybrid H9037, which had 19 flowers per plant, while hybrid H9065 had 18 flowers per plant. At NARL (Control), hybrid Samantha had highest number of flowers (43), followed by hybrid H9065, which had 27 and finally hybrid H9037 had 16. Test was run to determine whether number of flowers per plant between experiments for each tomato variety were same using Chi square ($\chi^2$) at $\alpha = 0.05$. Results showed that, there was significance difference at $P < 0.05$ (Table 4.6) between plants exposed to air pollution and control. Number of flowers per plant between varieties was significantly different ($P < 0.05$). When tested under least significant difference, all number of flowers per plant was significantly different from each other in all experimental sites. Number of flowers per plant within varieties was also significantly different ($P < 0.05$). These results are in table 4.6.

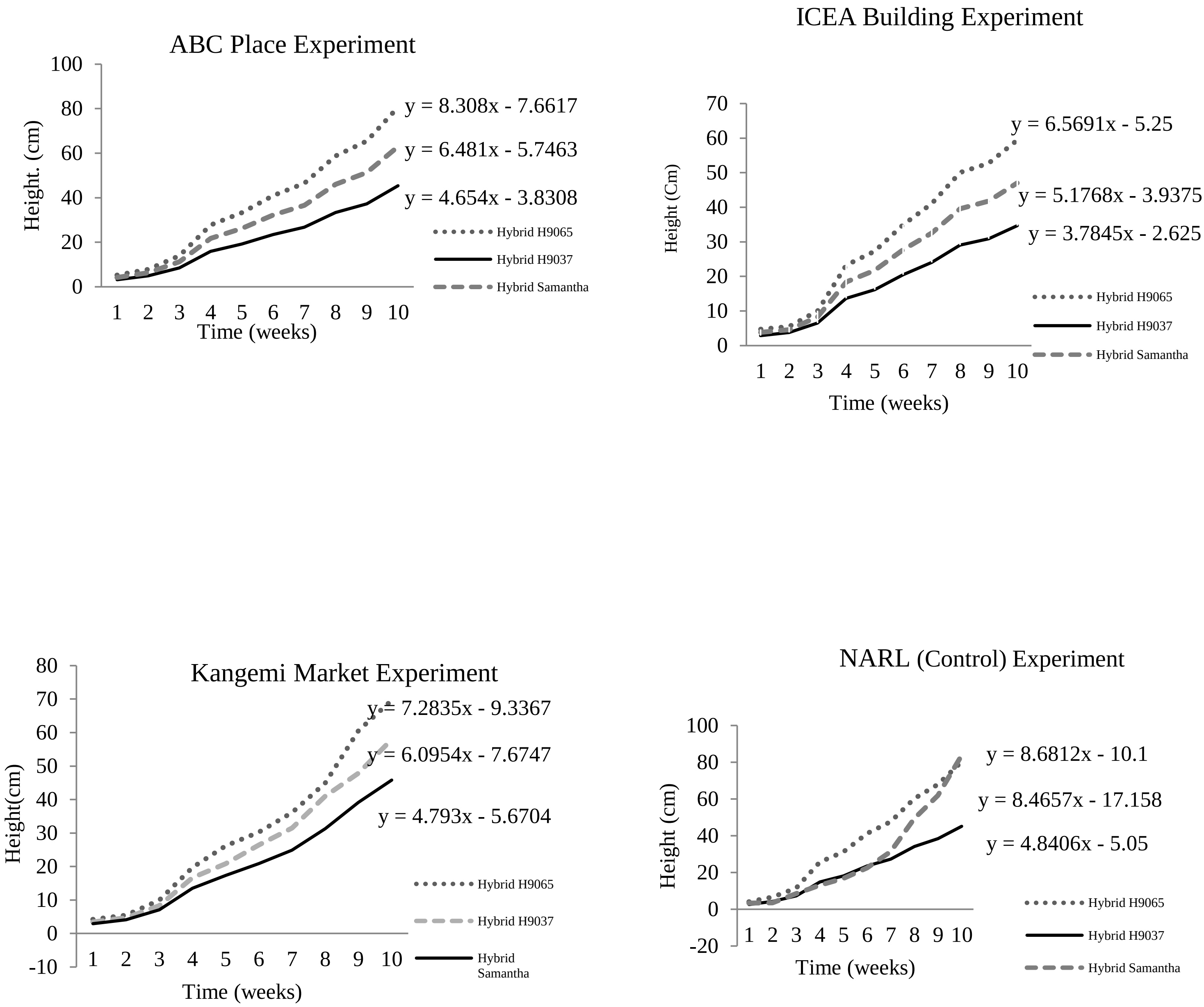


Figure 4 1 Growth rate and growth patterns of three tomato varieties grown at different experimental sites

Table 4.5 Measurement of leaf length of the test plants

| Experimental Sites | Hybrid H9065 (cm) | Hybrid H9037 (cm) | Hybrid Samantha (cm) | P – values |
|---|---|---|---|---|
| ABC Place | 42.6±3.6 | 37.1±1.4 | 35.0±1.6 | 0.064 |
| ICEA Building | 45.2±3.3 | 40.9±4.2 | 34.2±2.8 | 0.054 |
| Kangemi Market | 39.0±1.6 | 37.3±1.4 | 35±1.5 | 0.059 |
| NARL (Control) | 41.6±0.7 | 35.1±1.8 | 29.8±1.8 | 0.064 |
| P-value | 0.0553 | 0.0568 | 0.0745 | |

Confidence level, 95%

Table 4. 6 Number of flowers on test plants

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 16±1 | 18±0 | 30±1 | 0.011 |
| ICEA Building | 26±1 | 24±1 | 60±1 | 0.001 |
| Kangemi Market | 18±1 | 19±1 | 54±1 | 0.004 |
| NARL (Control) | 27±1 | 16±1 | 43±1 | 0.002 |
| Chi-square ($\chi^2$)value | 7.583 | 7.673 | 7.741 | |
| P-value | <0.001 | 0.049 | 0.018 | |

Confidence level, 95%

### 4.2.7 Biomass of Test Plants

At ICEA Building, hybrid H9065 had highest biomass of 0.12 gm., followed by hybrid Samantha with 0.10 gm. and lastly hybrid H9037 with 0.08 gm. (Table 4.7). At ABC Place, highest biomass was recorded in hybrid H9065 followed by other two hybrids (Samantha and H9037), which had same weight of 0.03 gm. Highest biomass at Kangemi Market was recorded in hybrid H9065 with 0.08 gm., followed by hybrid Samantha with 0.06 gm. and lastly hybrid H9037 with 0.04 gm. Highest biomass at NARL (control) was by hybrid H9065 and hybrid H9037 which scored same weight as 0.08 gm., followed by hybrid Samantha with 0.04 gm. However, these observed differences between tomato hybrids for every experimental site and between experimental sites for every tomato hybrid were not statistically significant (P < 0.05) (Table 4.7).

Table 4. 7 Biomass (gm) of test plants

| Experimental Sites | Hybrid H9065 (gm.) | Hybrid H9037 (gm.) | Hybrid Samantha (gm.) | P – values |
|---|---|---|---|---|
| ABC Place | 0.05±0.01 | 0.03±0.02 | 0.03±0.01 | 0.091 |
| ICEA Building | 0.12±0.03 | 0.08±0.01 | 0.11±0.02 | 0.095 |
| Kangemi Market | 0.08±0.02 | 0.04±0.02 | 0.06±0.02 | 0.057 |
| NARL (Control) | 0.08±0.01 | 0.08±0.01 | 0.04±0.01 | 0.092 |
| P-value | 0.085 | 0.057 | 0.085 | |

Confidence level, 95%

## 4.3 Effects of air pollution on stress levels on *L. esculentum*

At ICEA buildings, hybrid H9065 recorded 15.2 Ψ; hybrid H9037 had 11.2 Ψ and hybrid Samantha had 12.7 Ψ as stress levels (Table 4.8). At ABC Place, hybrid H9065 recorded 10.5 Ψ, hybrid H9037 had 10.2 Ψ and hybrid Samantha with 9.3 Ψ as stress level. At Kangemi Market site, stress levels were: hybrid Samantha scored 14.8 Ψ being highest followed by hybrid H9037 with 13.5 Ψ and finally hybrid H9065 12.80 Ψ. Highest stress level at NARL site (control) was scored by hybrid H9037 (10.3 Ψ) followed by hybrid Samantha (9.3 Ψ) and lastly by H9065 (9.0 Ψ). However, these observed differences between tomato hybrids for every experimental site where not statistically significant (P < 0.05). Nevertheless, there was a significant difference between experimental sites for every tomato hybrid (P < 0.05) (Table 4.8). Generally plants grown at control site recorded lowest stress whereas those plants at Kangemi Market and ICEA Building recorded the highest stress level.

Table 4.8 Stress status of Experimental Plants.

| Experimental Sites | Hybrid H9065 (Ψ) | Hybrid H9037 (Ψ) | Hybrid Samantha (Ψ) | P – values |
|---|---|---|---|---|
| ABC Place | $10.5 \pm 0.5^{a2}$ | $10.2 \pm 2.1^{a3}$ | $9.3 \pm 0.3^{a3}$ | 0.524 |
| ICEA Building | $15.2 \pm 2.8^{a1}$ | $11.2 \pm 2.6^{a2}$ | $12.7 \pm 1.5^{a1}$ | 0.597 |
| Kangemi Market | $12.8 \pm 1^{a2}$ | $13.5 \pm 0.5^{a1}$ | $14.8 \pm 1.6^{a1}$ | 0.583 |
| NARL (Control) | $9.0 \pm 1.3^{a3}$ | $10.3 \pm 0.6^{a3}$ | $9.3 \pm 1.5^{a3}$ | 0.522 |
| P –value | 0.023 | 0.041 | 0.031 | |

Numerical value followed by the alphabetical letter(s) in a superscript version within rows and numerical values in superscript version within columns show that figures are not significantly different from one another at $\alpha = 0.05$ (least significant difference).

### 4.3.1 Estimation of Number of Stomata on Upper Epidermis of Leaves on Test Plants

Number of stomata in upper epidermis of test and control experimental plants was not statistically different, at ($P < 0.05$) as shown in Table 4.9. Similarly, the observed differences between tomato hybrids for every experimental site and between experimental sites for every tomato hybrid were not statistically significant ($P < 0.05$) (Table 4.9).

Table 4. 9 Total number of stomata on upper epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 4±0 | 4±1 | 5±0 | 0.592 |
| ICEA Building | 6±0 | 6±1 | 8±1 | 0.709 |
| Kangemi Market | 3±1 | 5±1 | 4±0 | 0.715 |
| NARL (Control) | 5±1 | 6±1 | 6±1 | 0.935 |
| P/ value | 0.445 | 0.437 | 0.302 | |

Confidence level, 95%

### 4.3.2 Estimation of Number of Open Stomata on Upper Epidermis of Leaves on Test Plants

At ABC Place and Kangemi Market, no open stomata were on upper epidermis in all tomato varieties, while at ICEA Building and control had several open stomata. At ICEA building, hybrid H9065 had most number of open stomata on upper epidermis (2) other two tomato varieties (Hybrid H9037 and hybrid Samantha) had 1 open stomata (on an area of $0.0016mm^2$on a leaf). At NARL, hybrid H9037 recorded 4 open stomata on upper epidermis, followed by hybrid H9065 with a density of 3 opened stomata in upper epidermis; lastly hybrid Samantha recorded 2 opened stomata same side. Differences within experimental sites were not statistically different at ($P < 0.05$) (Table 4.10). The difference in number of open stomata in upper epidermis of experimental plants between experiments sites was highly significant at ($P < 0.05$) as shown in Table 4.10.

### 4.3.3 Estimation of Number of Closed Stomata on Upper Epidermis of Leaves on test Plants

At ABC Place, hybrid H9065 had most closed stomata (5) on upper epidermis (on an area of $0.0016mm^2$on a leaf), followed by hybrid Samantha with 4 closed stomata and lastly hybrid H9037 with average of 3. At ICEA Buildings, most closed stomata on upper epidermis were recorded in hybrid H9065 (6) while hybrid H9037 and Samantha had 5. At Kangemi Market hybrid H9037 had highest number of closed stomata (4) followed by hybrid Samantha and hybrid H9065 had 3. Control experimental plants at NARL had each with 3 numbers of closed stomata on upper epidermis for all tomato varieties. However, the observed differences between tomato hybrids for every experimental site and between experimental sites for every tomato hybrid were not statistically significant ($P < 0.05$) (Table 4.11).

Table 4. 10 Number of open stomata on Upper epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 0±0 $^{a2}$ | 0±1 $^{a2}$ | 0±1 $^{a2}$ | 0.444 |
| ICEA Building | 2±0 $^{a1}$ | 1±1 $^{a2}$ | 1±1 $^{a2}$ | 0.707 |
| Kangemi Market | 0±1 $^{a2}$ | 0±1 $^{a2}$ | 0±0 $^{a2}$ | 0.87 |
| NARL (Control) | 3±0 $^{a1}$ | 4±0 $^{a1}$ | 2±0 $^{a2}$ | 0.614 |
| P/ value | 0.003 | 0.002 | 0.058 | |

Numerical value followed by the alphabetical letter(s) in a superscript version within rows and numerical values in superscript version within columns show that figures are not significantly different from one another at α = 0.05 (least significant difference)

Table 4. 11 Number of closed stomata on Upper epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 5±0 | 3±1 | 4±1 | 0.484 |
| ICEA Building | 6±0 | 5±1 | 5±0 | 0.868 |
| Kangemi Market | 3±0 | 4±1 | 3±1 | 0.694 |
| NARL (Control) | 3±0 | 3±1 | 3±1 | 0.895 |
| P-value | 0.352 | 0.372 | 0.543 | |

Confidence level, 95%

#### 4.3.4 Estimation of Number of Stomata on Lower Epidermis of Leaves on Test Plants

The observed differences between tomato hybrids for every experimental site and between experimental sites for every tomato hybrid were not statistically significant ($P < 0.05$) (Table 4.12).

Table 4.12 Total number of stomata on lower epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 15±1 | 15±1 | 15±0 | 0.993 |
| ICEA Building | 16±1 | 15±1 | 16±1 | 0.936 |
| Kangemi Market | 14±0 | 15±1 | 17±0 | 0.645 |
| NARL (Control) | 14±1 | 12±0 | 12±1 | 0.612 |
| P/ value | 0.954 | 0.651 | 0.356 | |

Confidence level, 95%

### 4.3.5 Estimation of Number of Open Stomata on Lower Epidermis of Leaves on Test Plants

At ABC Place, hybrid Samantha had most open stomata (8) on lower epidermis followed by hybrid H9037 with 7, and lastly hybrid H9065 with 5 open stomata. At ICEA Buildings, most open stomata on lower epidermis were recorded by hybrid H9037 (22) while hybrid H9065 had 8, lastly hybrid Samantha had only 3. At Kangemi Market hybrid H9037 had highest open stomata number (10), followed by hybrid Samantha with 7 and lastly hybrid H9065, which had 4. NARL (Control) had highest number of open stomata on lower epidermis recorded for hybrid Samantha with 5 followed by hybrid H9065 and hybrid H9037, which had similar number of 4. These observed differences between experimental sites were not statistically significant at ($P < 0.05$) (Table 4.13). The number of open stomata on lower epidermis was significantly different at ICEA Building and Kangemi Market. There was no significant difference between experimental sites for ABC place and control at ($P < 0.05$) (Table 4.13).

Table 4.13 Number of open stomata on lower epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 5±0 | 7±0 | 8±0 | 0.338 |
| ICEA Building | 8±1 | 22±0 | 3±1 | <0.001 |
| Kangemi Market | 4±1 | 10±1 | 7±0 | 0.026 |
| NARL (Control) | 4±0 | 4±0 | 5±0 | 0.084 |
| P/ value | 0.445 | 0.437 | 0.302 | |

Confidence level, 95%

### 4.3.6 Estimation of Number of Closed Stomata on Lower Epidermis of Leaves on Test Plants

When Poisson distribution was used to analyse number (per area of 0.0016mm$^2$ on a leaf) of stomata status at lower epidermis in reference to closed number of stomata, in between experimental sites, there was no significant different at ($P < 0.05$) as shown in Table 4.14. The observed differences between tomato hybrids for every experimental site and between experimental sites for every tomato hybrid were not statistically significant ($P < 0.05$)

Table 4.14 Number of closed stomata on lower epidermis of leaves.

| Experimental Sites | Hybrid H9065 (No.) | Hybrid H9037 (No.) | Hybrid Samantha (No.) | P – values |
|---|---|---|---|---|
| ABC Place | 10±0 | 8±1 | 7±0 | 0.454 |
| ICEA Building | 7±0 | 9±1 | 13±1 | 0.845 |
| Kangemi Market | 10±1 | 6±1 | 10±0 | 0.112 |
| NARL (Control) | 10±1 | 7±1 | 6±1 | 0.297 |
| P-value | 0.41 | 0.40 | 0.41 | |

Confidence level, 95%

Stomata number, position and if open or closed on a leaf determines plant physiological behavior. All hybrids had almost similar number of stomata in lower and upper leaf epidermis.

Between experimental sites, control had more of open stomata on upper epidermis. In the experiment, plants in air polluted environment had more stomata underneath leaf than control as a pollution circumventing mechanism (Figure 4.2)

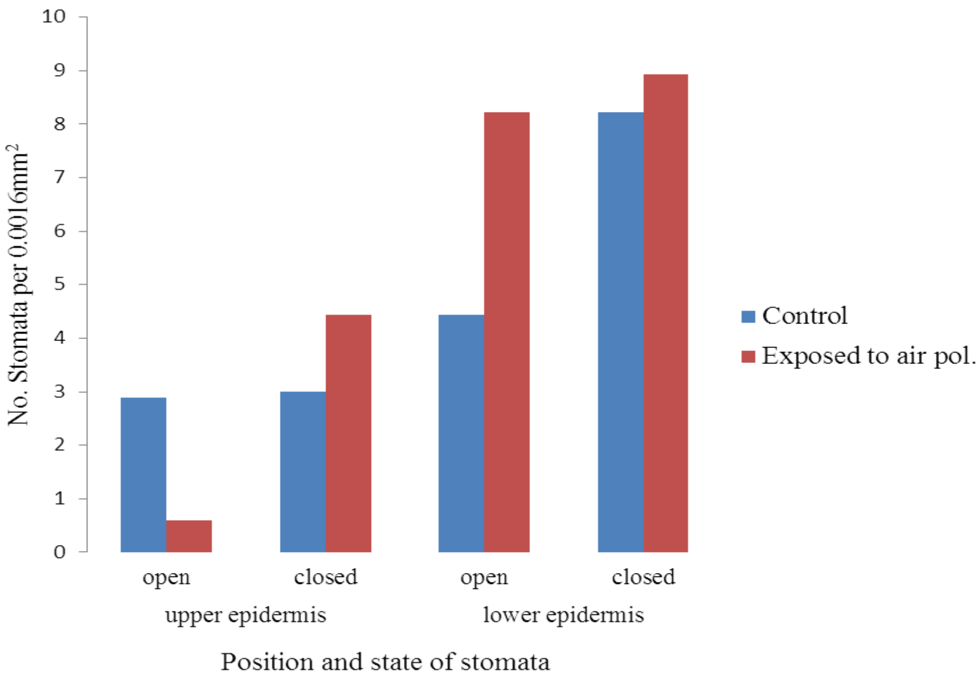


**Figure 4.2 State of stomata for plants exposed to pollution and control**

# CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 5.0 Discussions

Interpretation and commenting on key results was focused on in discussion. Results were then compared with previous work in existing literature. Practical use of some findings is shown in the recommendations.

### 5.1.1 Levels of Air Pollution at the Experimental Sites

The result demonstrates the presence and consistence of air pollution during the experimental period. The air pollution levels of the experimental sites compared well with Kinney *et al.* (2012) results (although slightly lower), where it was found that in NCBD, air pollution ranged from 50.7 to 128.7 $\mu g/m^3$. Accepted international safe level of air pollution is $50\mu g/m^3$ (WHO, 2006). Although the measured air pollution was below the WHO acceptable level it proved adequate for the study since some significance on parameter was experienced. Waiyaki highway has air pollution just like other roads in Nairobi as revealed by Kinney *et al.* (2012) and Odhiambo *et al.* (2010) on studies along University way and Haile Selassie Avenue. Odhiambo *et al.* (2010) confirmed air pollution on roads in Nairobi is from vehicle fumes. Odhiambo *et al.* (2010) did not give levels of air pollution in his study. Kinney *et al.* (2012) had findings that, sites a way from road side had less air pollution than those sites close to the roadsides. It was on the basis of these findings that further helped position the control site of the project. The results show contrary to the hypothesis that there was no air pollution along Waiyaki Highway. There were air pollution at experimental sites, but within accepted levels.

### 5.1.2 Germination of Seeds of *L. esculentum*

Air pollution did not affect tomato hybrid varieties seed germination rate. This is because the results of seed germination rates of all tomato varieties both at test experimental sites and control experiment are same. Lyanguzova (2011) findings also agree with this phenomenon that plants seeds germination is not affected by air pollution. It also agrees with Zeiger, (2006) who made observation in California, Los Angeles on numerous sites along roads, quarries, cement works, and other industrial areas with heavy air pollution and concluded that plant seeds germination is not affected by air pollution. This was because, seeds were in ground and seed embryos are enclosed in a seed coat which protected them from unfavourable atmospheric conditions like air pollution. This concurs with hypothesis that, exhaust fumes from moving vehicles along Waiyaki highway do not have effect on seed germination, of *L. esculentum* plants.

### 5.1.3 Effects of Exhaust Fumes on Height of Experimental Plants

The results show heights within test tomato plants were lower than tomatoes at control, which signifies effects of air pollution on tomato growth. This agrees with Sumitra *et al.* (2014) study that, air pollution does affect plants growth. It also agrees with Kurczynska *et al.* (1998), studies on Scots pine (*Pinus sylvestris* L.) in Poland. Kurczynska *et al.* (1998) reports, due chemical build-up in leaves and stem anatomy as influenced by air pollution, which disturbs function of cambium, phloem, and xylem), but do not agree with Patrick, (1985) and Schembari *et al.* (2014) who says plants have resistance to air pollution. The findings do not agree with hypothesis that, exhaust fumes from moving vehicles do not have effect on height growth of *L. esculentum* plants.

The height growth patterns of all the tomato hybrids were similar in plants grown along Waiyaki Highway and plants at the control. Only hybrid Samantha had a different patern at NARL (control)

### 5.1.4 Leaf Length of the Experimental Plants

Leaf length was same in tested plants and control experiments. These phenomena of leaves being unaffected supports findings of Schembari *et al.* (2014), who made a study on plants severely affected by air pollution in Barcelona. Cuza and Carol (2009) reported that structural characteristics of leaves in various species indicated a significant potential for resistance to air pollutants. This agrees with hypothesis that, exhaust fumes from moving vehicles along Waiyaki highway do not have effect on leaf development of *L. esculentum* plants.

### 5.1.5 Number of Flowers on the Test Plants

Test experimental plants had more number of flowers than control. However, only Hybrid H9065 variety recorded an average number flowers lower than control due to observed flower buds abortion before flower blossom (Plate 3 under Appendix 6). This production of high number of flowers produced by plants exposed to air pollution is confirmed by Winchester (2015), that plants produce a lot of flowers in environment polluted by agents related to vehicle fumes. However in such circumstances, scent of flowers is mixed with air pollution and confuses external pollutants like bees. Hypothesis that, exhaust fumes from moving vehicles along Waiyaki highway do not have effect on flower formation, of *L. esculentum* plants is refuted. This indicates that study of plant's flowers can give a paradigm of effects of air pollution.

### 5.1.6 Biomass of the Experimental Plants

The results of biomass of test plants, give unclear trend in relationship with control. In this study, test plants did not build substantial biomass although in elevated $CO_2$. This did not agree with Chandler and Page, (2007) who said that plant growth in elevated $CO_2$ can boost growth as much as a third of normal biomass accumulation. Productivity of *L. esculentum* in terms of biomass both above and below ground was not influenced by exhaust fumes from moving vehicles for a period of three months along Waiyaki Highway. However three months could not have been enough time to evaluate the effects of air pollution on biomass of test plant. Hypothesis is accepted that, exhaust fumes from moving vehicles along Waiyaki highway do not have effect on biomass of *L. esculentum* plants.

### 5.1.7 Determination of Stress on the Experimental Plants

Average stress for each of tomato varieties recorded from experimental sites were higher than stress recorded from tomato varieties at control. Thus, indicating that test plants indeed experienced stress due to air pollution along Waiyaki Highway. Hypothesis that exhaust fumes from moving vehicles do not contribute to stress of *L. esculentum* plants is been disapproved by this study. These agree with Kurczynska *et al.* (1998) findings and Blande, (2014) report that, in extreme conditions of air pollution, plants are so stressed that leaves have accelerated senescence caused by excess water stress. Stress is due to closure of stomata, which ensures reduced intake of harmful gases from air pollution environment.

Plants are stressed due to restricting water movements inside plants tissue and are achieved by closure of stomata. Plants close stomata to avoid intake of unwanted substances in air. Gaseous component of air pollution follow pathway of normal intake of carbon dioxide of leaves of plants. This is supported by Elstner and Osswald, (1994) who indicate harmful gases in air pollution were readily absorbed as a part of photosynthetic process. So a plant responds by stomata closure to reduce this effect. Similar studies by Elstner and Osswald, (1994) noted that there were various gases in air as pollutants that can affect plants but most notorious of them all was Sulphur dioxide. Sulphur dioxide after going through stomata and into leaf, can combine with other inorganic elements to produce lethal compounds like sulphuric acid. This acidic condition may make cells inactive and finally be killed. Williams *et al.* (1996) supported this idea, in their previous studies. Williams *et al.* (1996) noted that, there was an overall reduction in chlorophyll, ascorbic acid, earotinoid and protein contents for plants exposed to air pollution thereby indicating that such plants were not functioning properly hence under stress. In long run, stress affects plant productivity. The hypothesis that, exhaust fumes from moving vehicles along Waiyaki highway do not contribute to stress as shown by plant stress of *L. esculentum* plants is refuted.

### 5.1.8 Stomata Number, Position and Status on Experimental Plants

In normal environmental conditions, stomata density is more under leaf than upper epidermis (Pourkhabbaz *et al.*, 2010). The condition is for purpose of avoiding pollution, direct sunlight and heat. Upper epidermis (which acts as landing field for particulate matter) of plants exposed to air pollution, had fewer stomata compared to control for reason of avoiding PM. Test plants had more of open stomata in lower epidermis than control. Test plants had no open stomata in upper epidermis while control had open stomata on upper epidermis, which is supported by Pourkhabbaz *et al.* (2010) that, high density of open stomata was not found in upper epidermis but in lower epidermis. This physiological behavior is a natural protective resistance mechanism. This agrees with Barnes *et al.* (1999) who reported that plants have a natural inducement to resistance air pollution. The study has shown that plants in air pollution are enduring stressed due to the way stomata is distributed and state (open or closed) as compared to those in non-polluted environment. This does not contradicts hypothesis that, exhaust fumes from moving vehicles along Waiyaki highway do not contribute to stress as shown by stomata of *L. esculentum* plants, but open stomata on upper epidermis contracted the hypothesis.

## 5.2 Conclusions

From the foregoing discussions and results obtained from this study, following conclusions can be made:-

- The hypothesis that, there is no air pollution as a result of Exhaust fumes from moving vehicles along Waiyaki highway is rejected because in the study Waiyaki Highway has air pollution ranging from 41 to 43 $\mu g/m^3$
- The hypothesis that Exhaust fumes from moving vehicles do not have effect on seed germination, leaf length, growing, flower production and biomass of *L. esculentum* plants is rejected in case of Height, number of flowers and accepted in case of leave length and biomass.
- The hypothesis that Exhaust fumes from moving vehicles do not contribute to stress of *L. esculentum* plants is rejected. Tomato plants were stressed and plants physiological behavior of stomata on leaves exhibited stress.

## 5.3 Recommendations

Practical use of some findings and gaps requiring attention in the study, are depicted in recommendations below;-.

1. Air pollution is present with us, along roads and it affects organisms including people. It is recommended that future roads of a busy nature should be away from where people live and work. Policy on air pollution be instituted.
2. Plants are good indicators of air pollution and should be used to examine its presence by National Environmental Management Authority (NEMA) during environmental impact assessment (EIA),
3. Plants planted along roads should not be expected to have good productivity because stress, however can be used for beautification and trapping of Particulate matter
4. Further long term comprehensive studies (internal plant chemical build-up) to be undertaken with many plant species.

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## APPENDICES

Appendix 1 Particulate matter measurement

| Experimental Site | Filter paper initial wt.(µgm) | Filter paper final wt. (µgm) | Total $PM_{10}$ for 12 hrs. in (µgm) | Total air sucked thro' diaphragm ($M^3$) | $PM_{10}$ per µgm/$M^3$ |
|---|---|---|---|---|---|
| | B | C | D | E | F |
| | - | - | C-B | - | D/E |
| ICEA Building (i) | 8951.7 | 772982780.9 | 772973829.2 | 18273613 | 42.3±SE |
| ICEA Building(ii) | 8964.5 | 3300349681.7 | 3300340717.2 | 78207126 | 42.2±SE |
| ICEA Building(iii) | 8934.7 | 327056698.6 | 327047763.9 | 76923491 | 42.9 |
| ABC Place (i) | 7789.3 | 787673082.8 | 787665293.5 | 18446494 | 42.7 |
| ABC Place (ii) | 9173.5 | 632825739.1 | 632816565.6 | 14924919 | 42.4 |
| ABC Place (iii) | 4308.9 | 465712128.9 | 465707820 | 10983675 | 42.4 |
| Kangemi Market (i) | 9653.9 | 791348307.7 | 791357961.6 | 18489672 | 42.8 |
| Kangemi Market (ii) | 5189.0 | 1032577052.6 | 1032571863.6 | 20987233 | 42.9 |
| Kangemi Market (iii) | 6727.6 | 1090731044.1 | 1090724316.5 | 25543895 | 42.7 |
| NARL(i) | 8976.1 | 164535 | 155559 | 259265 | 0.63 |
| NARL(ii) | 4093.8 | 262399.52 | 258305.72 | 423452 | 0.61 |
| NARL(iii) | 1797.9 | 62628.87 | 60830.97 | 106721 | 0.57 |

Appendix 2 Vehicle exhaust fumes, darken leaves, and darken tree truck and moribund tree

Exhaust fumes from the engines of moving vehicles along Waiyaki highway

Darken leaves

**Darken leaves with darken**

**Moribund tree along Waiyaki**

Appendix 3 Measuring of PM along Waiyaki Highway

**Appendix 4 Published paper by JK Lugadiru**

1. **Lugadiru JK[1*]** Wycliffe Wanzala[2] Jacinta M. Kimiti[3] 2016 Distribution of stomata on *Lycopersicon esculentum* leaves for plants growing in air pollution related to vehicle fumes along Waiyaki Highway Nairobi County Kenya Journal of Natural Sciences Research www.iiste.org ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online) Vol.6, No.10, 2016 p86

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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.

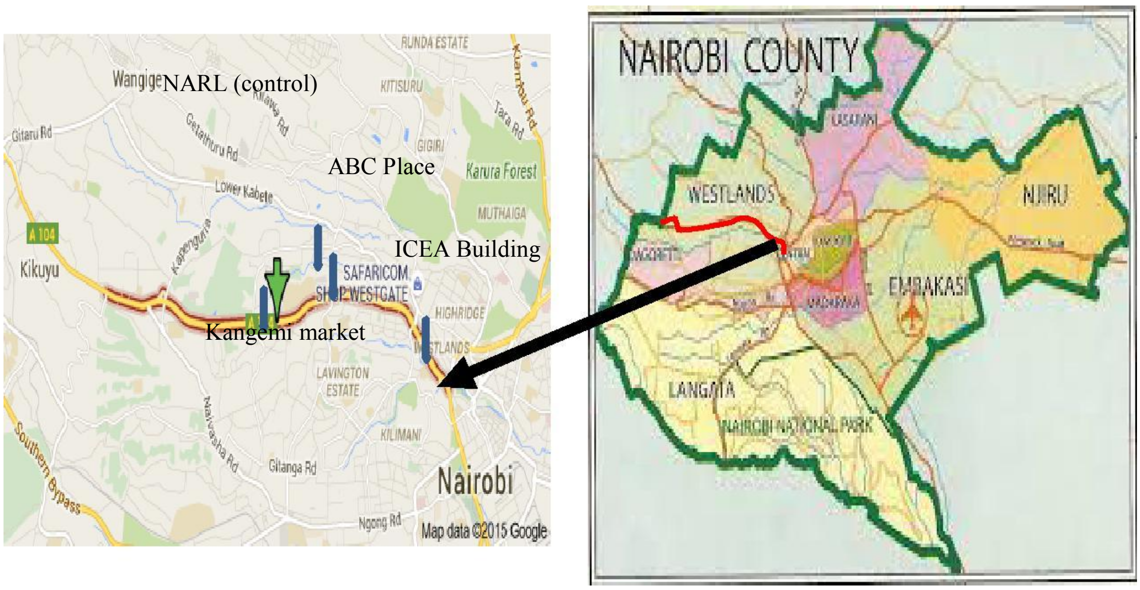


Figure 1 . Map of Nairobi County showing Waiyaki Highway and Experiments sites

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. Sitting 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 minute 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 waiyakihighway**

**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 $P<0.05$ and that there is strong significant difference ($P<0.05$). 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$^3$

In Table 1 are levels of air pollution at different times of the experiment. showing average pollution level between within experiments

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

| Experimental Site | Pollution at day 0 (µgm$^3$) | Pollution after 45 days (µgm$^3$) | Pollution after 90 days (µgm$^3$) | p-value |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| BC Place | 42.5±0.02[a] | 42.2±0.01[a] | 43.3±0.01 [a] | 0.078 |
| ICEA Building | 42.5±0.02 [a] | 42.4±0.03 [a] | 41.4±0.04 [a] | 0.086 |
| Kangemi Market | 43.6±0.00[a] | 42.9±0.01 [a] | 41.5±0.01 [a] | 0.084 |
| NARL (Control) | 0.56±0.03[b] | 0.61±0.03[b] | 0.55±0.01 [b] | 0.099 |
| P-value | 0.001 | 0.000 | 0.002 | |

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

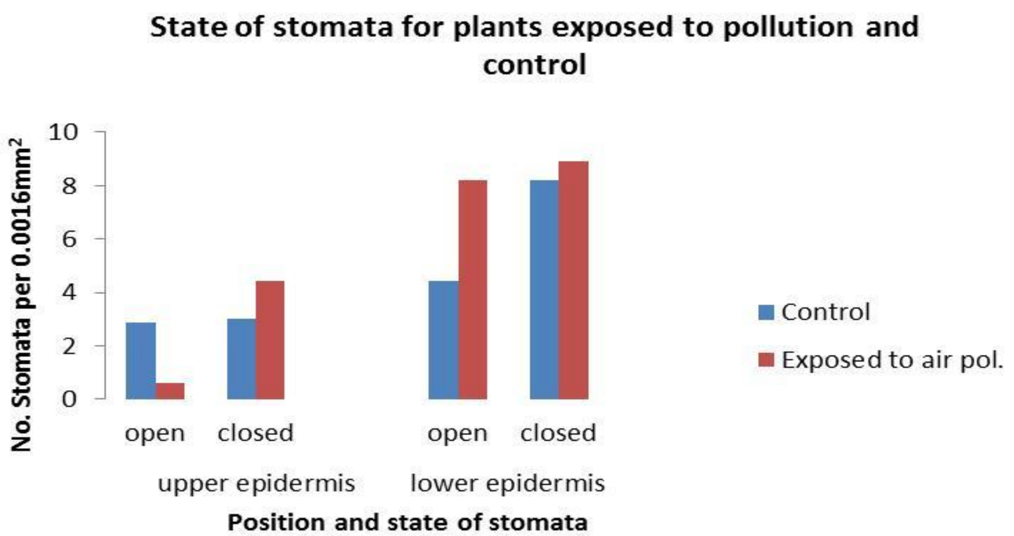


Figure 3 Number of stomata in either lower or upper of tomato leaves and if open or closed

**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 behavior, 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 behavior and also distribute of stomata when exposed to air pollution related to vehicle fumes along Waiyaki Highway of Nairobi County in Kenya.

**Acknowledgement**

I wish to acknowledge sincerely the following persons who contribute in a devotedly way, so as this study was a success, may Jehovah God reward them. Dr Gabriel Muturi PhD, Deputy Director, in charge of Forest Biodiversity and Dry Lands Research Programme in KEFRI for advice and sponsoring this project. Mr Ely Mwanza the Regional Director Central Eco-Region Research Programme KEFRI for giving me permission to be a way from work while under taking the study. There was permission from Nairobi County Environment Department for undertaking the project within their Adjudication County, which is much appreciated greatly for such corporation. Suitable seeds and technical advice on management on tomatoes, was availed from a 'Starke Ayres Pannar' Seed Company; a Southern Africa company operating in Kenya. I say thanks for such provision of good high quality germplasm tomatoes seeds. The Centre Director NARL of KARLO for permission to set control experiment in his office compound. A treated experiment was placed next to ICEA building in Westland with permission from the esteem Management of the building. The chief of Kangemi for allowing me place a treated experiment at Kangemi market. The third treated experiment was placed at ABC place with kind permission from entrepreneur of the premises. I have to thank two young persons, Mr LB Morgan and Mr Grand Mbogwa, for helping in data collection and general management of the experiments. I acknowledge the help received while undertaking stomata study from Mr Esitubi of biotechnology section KEFRI.. Particulate matter measurement was taken in conjunction with Mr Waweru of Occupational Health Services of Ministry of labour. I acknowledge Mr G. Mwaura for assisting in data analysis. Lastly nursery workers of KEFRI are hereby acknowledged for doing good work towards success this project.

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## Appendix 5 Traffic Jam on Waiyaki Highway

## Appendix 6 Qualitative Effects of Air Pollution during the Study

**Plate 1 Tomato leaf with partial PM as trapped by hairy condition**


**Plate 2 Raised platform used in the experiment**

**Plate 3 Flower Buds (yellowed) about to Aborted before Flower Bloom**

**Appendix 7** Exhaust fumes engulf a Nairobi street.