

**CHANGES IN EPIDERMAL CELL MORPHOLOGY OF *Eleusine indica*  
(L.) Gaertn. FOUND ALONG ROADSIDES IN BENIN CITY**

**BY**

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UNIVERSITY OF BENIN**

**MAY, 2021.**

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**BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF  
PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE  
SCIENCES AND SUBMITTED TO THE SCHOOL OF  
POSTGRADUATE STUDUES UNIVERSITY OF BENIN, BENIN CITY,  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
AWARD OF MASTERS DEGREE IN PLANT BIOLOGY AND  
BIOTECHNOLOGY.**

**MAY, 2021**

## CERTIFICATION

We the undersigned attest and declare that the thesis Mrs Vanessa Stephen Emonyon Titled “Changes in Epidermal Cells Morphology of Eleusine Indica Found Along Roadsides In Benin City”: has successfully passed the anti-plagiarism test and does not violate any copy right regulations.

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Supervisor Sign and Date

**Prof H. O. Shittu** .....  
PG Coordinator Sign and Date

**Prof F.I. Okungbowa** .....  
Head of Department Sign and Date

## DEDICATION

I dedicate this work to the almighty God for His readily available help, care and protection and to the Blessed Virgin Mary, the queen of our family.

## ACKNOWLEDGEMENT

I acknowledge the almighty God for his care, divine provision and sustainability.

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## ABSTRACT

Ruderals are plants that have capacity to thrive and grow in disturbed and polluted environment such as roadsides. *Eleusine indica* is an example of a ruderal found along the roadside. It is very widely distributed and very invasive weed. It has shown to have ability to withstand pollution created by vehicles. Automobiles usage in cities has greatly increase with development and urbanization. These pollutions include air pollutants such as NO<sub>x</sub>, SO<sub>2</sub>, O<sub>3</sub> and particulate matters like Pb, Hg and other heavy metals. The experiments were carried out to investigate the impact of these aggravated pollution as a result increase in traffic in Benin city, Edo state, Nigeria. In carrying out the experiment samples were collected along the major roads in Benin city. Ten sites were selected and these were Aduwawa market road sides, Auchi bypass roadside, Agbor road market roadside, Asaba bypass roadside, S and T Barracks market roadside, Lagos bypass roadside, Santana market roadside, Sapele bypass road, Ekenwan bypass roadside, Okhokhugbo market roadside. The comparative analysis between the sizes, numbers and structure of the stomata and epidermal cells of *Eleusine indica* found in polluted environment (roadsides) and less or zero polluted environment (farmland) was carried out. It was observed that an increase in the number of stomatal in the adaxial layer of the leave at sample site as against the control whereas in the abaxial layer of the cell there was a decrease in the number of stomata as compared to the control. The size of the guard cells diameter of the epidermal cells and thickness of the stomata found at both the lower and upper layers of the polluted samples decreased in relation to the samples from the less polluted environment. The anatomical modification of the epidermal cells are the potentials used by the *Eleusine indica* for extraction of pollution from automobile, improving the capacity for phytoremediation.

# CHAPTER ONE

## INTRODUCTION

There are different kinds of plants with capacities for surviving in areas where most plants cannot survive, these plants are usually called ruderals. Ruderals are very important in the Ecosystem because their presence in difficult terrains would warrant that at some point, they eventually would become primary colonisers of those areas, when they die their leaves and other dead parts would enhance humus content in the soil, thereby improving organic content in the soil and also help to improve the soil nutrients status. When that happens eventually other plants would migrate to such areas. Ruderals are also important in the Ecosystem because their presence in difficult terrains would to a large extent guarantee survival of herbivores present in those terrains. Finally, another importance of ruderals such as *Eleusine indica* is the fact that their capacity to survive in difficult terrains guarantee their utilisation in phytoremediation experiment and phytoremediation interventions. To remediate a particular soil, sensitive plants are not used but instead rugged plants are being used. That is, plants that would be able to survive those harsh environments conditions they are exposed to these is how important ruderals are to the Ecosystem. Suffice it to say that *Eleusine indica* which is a ruderal has been utilized in several occasions and listed by several researchers as a plant with significant capacities for remediation (Ikhajiagbeet *al* 2020, Garbaet *al* 2012 Amir *et al* 2017).

*Eleusine indica* also contains phytochemicals being used in the field of medicine for treating several illnesses. The plant is used for deworming, eliminate or reduce cough and lung troubles, dysentery, heart problems and high blood pressure, spleen and liver complaints, bladder and kidney stone and in case of sprains, dislocation of bones and lumbago (Desai *et al* 2017). Pattanayak and Maity (2017) stated that *Eleusine indica* are used as medicine for herbivores. They can also be used for erosion control and dune stabilizer.

Why are we looking at epidermal changes in morphology of these particular ruderal along the road sides? It must be understood first that plants that are directly at the banks of road sides are at the mercy of gaseous effluent from automobiles vehicles. And some of these gaseous effluents contain heavy metals that toxicants or contaminate that can destroy or damage plants or affect plants negatively. Usually these is possible because the gaseous contaminant passes through the plant stomates and eventually gets into the plant and impair their metabolism. However, the facts that a lot of plants still appear relatively 'ok' along roadsides particularly very busy road sides within the metropolitan areas like Benin City. One would want to think that the plant may have some adaptations for surviving in this harsh environment. One of these adaptations are usually anatomical, and no other important part of the plant that is primarily impacted other than the epidermal layer because that is the first layer of cells within the plants leaf that is exposed to the environment. The epidermal cell layer of all plants is composed primarily of pavement cells, relatively unspecialized morphologically, which fulfil a protective function for the tissue layers underneath and a spacing function for morphologically more specialized cells in the epidermis (Glover and Martin ,2000).

So, our question therefore is; Is it possibly that locating these plants along different roads but in the same metropolis would it imply any significant changes in their leaf epidermal morphology? If yes, then it goes a long way to say therefore that perhaps these modifications may either be genetic or physiological, that is depending on the plant's capacity to respond. It should be noted that the leaf epidermal characteristics of a plant are critical for the productivity of the plant because it is within the leaf epidermis that we have the stomata. If the stomates close or the stomata is malfunctional or the plant response to the contaminants in the environment affects stomata conductance then it definitely would affect the overall

performance of the plant. The possible differences in leaf epidermal presentations during exposure to gaseous effluents from automobiles particularly along the road is the reason for this study.

### **Justification for the research**

We have said that the capacity of a plant to survive in any given area depends on a whole lot of factors including its anatomical features. One of such anatomical features is the foliar epidermal structure of the plant. Important because the leaf is the most important structure of the plant that accounts for the plant productivity or nutrient assimilation capacity. Therefore, the anatomical structure within the leaf that are important for these particular features include the stomata for which CO<sub>2</sub> can get into the plant during photosynthesis and O<sub>2</sub> can come out that is important for aerobic processes in the Ecosystem. Other parts of the epidermal surface of the plant are the guard cells which control the stomata and other epidermal cell layers. The fact that plants occur within the road side of the metropolis perhaps may indicate the plant's capacity to survive accumulated gaseous contaminants present in automobiles that are always deposited on the road daily.

It is necessary to note that not all plants that have capacity for survival and tolerance. But for those plants particularly ruderals that have high capacity for tolerance. It is important to under study how they relate with the gaseous contaminants that are present in within their enclave.

*Eleusine indica* has been worked by several researchers and has been found to have capacities for remediation. Incidentally a lot of plants are found to grow along the pavement and road sides within the Benin City metropolis. Amongst the plant growing on the road sides this plant have been found to be the one of the most populous plants according to the studies of

Anoliefo *et al*, 2008 and Anoliefo *et al*, 2006. Having known that these plants are surplus along the road sides the question is whether their capacity for tolerance differ from location to locations these is important because changes in anatomy is critical for the plants productive capacity. The studies are necessary to confirm first whether there were significant changes in plant anatomic features when you move from one location to another. If those changes existed then they could either be genetic or psychological.

### **Aims and objectives**

The aim of the study was to determine the changes in the epidermal cell morphology within the leaves of *Eleusine indica* found along the road sides in Benin City.

Specific objectives of the study were to

1. To determine the changes in leaf epidermal features and also root anatomical features of the plant
2. To determine the capacity of *Eleusine indica* as a bioremediator.

## CHAPTER TWO

### LITERATURE REVIEW

#### **Road sides conditions**

The soil found along the road sides can be degraded and very different from those found in farm lands and other fields (Mills *et al.*, 2020). This is because along the road sides, the soil has been known to be contaminated due to deposition of heavy metals from the exhaust fumes of vehicles. Road sides have the higher compaction, electrical conductivity and pH. It also has low water infiltration and macro aggregation which exceed threshold levels for plants. The use of automobiles is growing fast globally especially in developing countries (Yunus *et al.*, 1996). 70% of air pollution in the world is due to automobiles which is most apparent in city centers due to the growing population of vehicles and subsequently increase in emission (Swami, 2018). Atmospheric pollutants have a negative effect on the plants; they can have direct toxic effects, or indirectly by changing soil pH followed by solubilization of toxic salts of metals like aluminium. The particulate matters have a negative mechanical effect. They cover the leaf blade reducing light penetration and blocking the opening of stomata. These impediments influence strongly the process of photosynthesis which rate declines sharply (Gheorghe and Ion, 2011).

Some substances that are normally present in the atmosphere in a certain concentration can be considerate pollutants because their concentration is much higher than usual concentration. Many forms of pollution are human-made. Industrial plants, power plants and vehicles with internal combustion engines produce nitrogen oxides, VOCs, carbon monoxide, carbon dioxide, sulphur dioxide and particulates that is heavy metals. In most mega-cities, cars are the main source of these pollutants (Abechi *et al.*, 2010).

## **Mechanism of the decomposition of the pollutant**

There are several ways pollutant gets deposited along the road sides. The major pollution is from vehicle exhaust and oil leakages.

Traffic related emissions are produced either as gases, particles, or in soluble form. Road vehicle interaction produces several emissions including organic and inorganic substances, starting from those related to road wear and maintenance to materials that are produced from vehicle-related oils and tire and brake wears. Heavy metals as well as large amounts of mineral compounds are moving from the driving lanes into the roadside environment (Aljazzar and Kocher, 2016).

The main air pollutants are represented by gases forms, particles in suspension, different ionizing radiation and noise. Burning of fossil fuels and organic matter produce huge quantities of fine carbon particles which form the soot pollution. Soot can be dispersed over a quite wide area and transported to great distances by blowing winds (Singh and Verma, 2007).

Recent finds have linked the emission of heavy metal contaminants such as Pd, Cd Zn, Ni from the use of anti-knock from automotive fuel (Nakayama *et al.*, 2011).

Non-exhaust emissions are mainly generated due to leakage of vehicle-related fluids, abrasion of vehicle parts and road surface. Worn out tyres, the oil leakage, the corrosion of metals in roadside soil and plants (Çelenk and Kiziloğlu, 2015).

Air and water carry traffic pollutants from the road into the surrounding environment. Heavy metals also get to soil and plant cover through precipitation (Nasar *et al.*, 2012)

## **Contaminants found along road sides**

Pollutants found along road sides are basically aerosols from transport and constructions NO<sub>x</sub>, CO, HCl, Lead and other heavy metals (Uka *et al.*, 2019).

The gases forms are: oxidized and reduced forms of carbon (CO<sub>2</sub>, CO, CH<sub>4</sub>), of nitrogen (NO<sub>2</sub>, NO, N<sub>2</sub>O<sub>4</sub>, NH<sub>3</sub>, NH<sup>4+</sup>), SO<sub>2</sub>, O<sub>3</sub>, C<sub>6</sub>H<sub>6</sub> vapours, Hg, volatile phenols and Cl (Rai, 2016).

Hydrocarbons and nitrogen dioxide present in auto emission react with oxygen in the presence of sunlight to form ozone and photochemical smog (PAN- peroxyacetyl nitrate). (Marx, 1975)

The particulate forms are heavy metals with toxic effect (Pb, Ni, Cd, As), polycyclic aromatic hydrocarbons PAHs, etc. Heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), iron (Fe), manganese (Mn) and zinc (Zn) are found on roadside soil with lead having a very high significant value concentration. (Lagerwerff and Specht 1970, Aslam *et al.*, 2013, Alina-Maria *et al.*, 2016, Hiller *et al.*, 2021)

### **Effect of these contaminant found along the road sides in plants**

Pollutant found along road sides can damage cell membranes of plants, accumulation of harmful compounds in tissues of plant such as leaves, fruits and roots. Also blocking of different metabolic processes. In average quantity heavy metals are essential microelement but excess uptake has detrimental effect on the plant.

Pollutants may cause primary damage, with direct identifiable impact on the plant or secondary damage in the form of perturbations in the delicate balance of the biological structure of soil affecting the growth of plants.

The leaves are usually the site of injury by gaseous contaminants. They enter the leaves through the small opening that are necessary for normal exchange of gases by leaves (Marx, 1975)

Apart from screening out sunlight, dust on leaves blocks stomata and lowers their conductance to CO<sub>2</sub>, simultaneously interfering with photosynthesis and invariable plant growth and productivity. Polluting gases such as SO<sub>2</sub> and NO<sub>x</sub> enter leaves through stomata just like CO<sub>2</sub>. The uptake of the nitrogen dioxide is predominantly by foliar deposition. NO<sub>x</sub> dissolves in cells and gives rise to nitrite ions (NO<sub>2</sub><sup>-</sup>, which are toxic at high concentrations) and nitrate ions (NO<sub>3</sub><sup>-</sup>) that enter into nitrogen metabolism as if they had been absorbed through the roots. (Darral,1989).

Two of the most widespread air pollutants, SO<sub>2</sub> and O<sub>3</sub> disrupt translocation substantially reducing root growth invariably water uptake may also reduce and availability of nutrients such as in mycorrhizae and root nodules are also affected. (Kasana and Mansfield, 1986)

SO<sub>2</sub> has been attributed to destruction of epidermal cells adjacent to stomata and accumulation of sulphur with guard cells this is because the route from the stomata and epidermal cell is very short as reported by Black and Unsworth (1980). Several studies have shown that the disturbances caused by SO<sub>2</sub> to biochemical functions (Li *et al.*, 2007) and cell structure (Jutawong and Suwanwarer, 1997; Tripathi and Gautam, 2007)

Ozone is also a serious pollutant causing injury to vegetation by necrosis of the leaves especially the upper layer. Ozone alters cellular and sub cellular membrane permeability. It oxidizes the constituents of the cells such as lipids and sulfhydryl group in enzymes causing in balance in cell nutrients leading to the death of the cell. (Crittenden and Read, 1978 and Marx, 1975)

Soot deposited on the surface of leaves inhibits light penetration, increased surface temperature due to absorption of heat and clogging of stomata. The result of these is reduced gaseous exchange, reduced photosynthesis and general weakening of the plant growth. Necrotic spots also develop in many species due to soot deposition. (Taylor, 1974 and Armburst, 1986).

Most of the reduction in growth parameters of plants growing on polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzyme (Kabata-Pendias, 2001).

Middle aged leaves and young plants are more sensitive to ozone. This pollutant interacts with SO<sub>2</sub>, NO<sub>2</sub>, PAN and heavy metals in complex manner. The deposition of particulate could inhibit gas exchange through stomata thus consequently affect the photosynthesis (Larcher, 1995).

Magnesium oxide deposited on the soil these compounds can soon increase the soil pH to levels injurious to plants. Deposition of these substances on the soil prevents germination of seedlings. The seedlings that are able to emerge usually have yellow/brown tips of leaves and their roots are stunted. In areas of heavy pollution, composition of the vegetation changes completely.

Impact of boron pollution is more severe on older leaves than on younger leaves. Boron is also accumulated in the leaves and produces injury symptoms quite similar to fluoride pollution.

Sodium sulphate dust can cause necrosis of leaves of the plants. The damage increases in moist condition.

The effect of heavy metal toxicity on the growth and development of plants differs according to the particular heavy metal for that process. Heavy metals in plant causes a significant increase in peroxidase activity and decrease in photopigments (MacFarlane and Burchett, 2001)

Stomatal closure protecting the leaf against further entry of the pollutant but also curtail photosynthesis.

### **Tolerance capacity of plants to pollutant**

Levels of air pollution tolerance vary from species to species, depending on the capacity of plants to withstand the effect of pollutants without showing any external damage (Shannigrahi *et al.*, 2004).

Plants grow on heavy metal polluted soils resultant in reduction in growth due to changes in their physiological and biochemical activities especially true when the heavy metal involved does not play any beneficial role towards the growth and development of plants. There are two aspects on the interaction of plants and heavy metals, one hand; heavy metals show negative effects on plants and other hand, plants have their own resistance mechanisms against toxic effects and for detoxifying heavy metal pollution (Asati *et al.*, 2016).

Foliar surface of urban roadside plants acts as a sink for particulate matter deposition and through their deposition they show specific morphological, physiological, and biochemical responses. Deposition of PM pollutants on a leaf surface induces structural and functional changes (Panda and Rai, 2015). Plant's ability to tolerate air pollutants depends on its biochemical, physiological and morphological characteristics.

Plants employ different mechanisms in the remediation of heavy metal polluted soils (Asati *et al.*, 2016). Plants have different inherent morphological traits like phyllotaxy, leaf shape, size and texture, presence or absence of hairs, stomatal frequency, surface roughness, stomatal density, and trichome length that determines their dust collecting capacity (Li *et al.*, 2019).

Also, urban roadside plants demonstrate a wide array of responses when exposed to pollutants in the form of photosynthesis, respiration, enzymatic reactions, stomatal behaviour, membrane disruption, senescence, and ultimately death (Rai and Panda, 2014)

The plants response to air pollutants is hypothetically measured using air pollution tolerance index (Singh and Rao, 1983). Furthermore, it is known that proteins directly take part in plant stress responses, and plant adaptations to heavy metal stress are always accompanied with deep proteomic changes. Therefore, technique of proteomics can be exploited for deciphering the possible relationships between proteins abundance and plant stress adaptation.

It can contribute to better understanding of physiological mechanisms under heavy metal stress such as perception of stress and further signaling cascade that leads to changes in the expression of huge numbers of genes at transcriptional level and in metabolite profile, which could be used for an acquisition of an enhanced plant tolerance under heavy metal toxicity (Kosová *et al.*, 2011).

### **Mechanism of tolerance**

Plant resistance to stress is a consequence of two possible mechanisms. stress avoidance or stress tolerance. In stress avoidance plant is manner to avoid facing stress using either metabolic process or energy. Stress tolerant plant can resist a particular stress condition. Plant become stress tolerant through adaptation: heritable modifications to increase fitness and acclimation: non heritable physiological and biochemical gene expression. Tolerance is a

resistant reaction to reduce or repair injury with morphology, structure physiology biochemistry or molecular biology, when plant encounters stresses.

Due to the abundance of stomata in leaves, they are most susceptible parts of a plant to acute injury, because the stomata pores allow penetration of the pollutants into the sensitive tissues. The first barrier of gaseous air pollutant after the cuticle boundary layer resistance on leaves known as the epidermal cells (Heath *et al.*, 2009).

The diffusion pathway of absorbed pollutant molecules is through the stomata, and consequently resistance to flow is determined by the frequency and distribution of stomata available for entry and by the dimensions of their apertures (Meidner and Mansfield, 1968).

Leaf resistance to gaseous flux is offered by four components: boundary layer, stomatal apparatus, cuticle and mesophyll cell surface. Sestak, Catsky and Jarvis (1971), Bennet *et al.* (1973) Since leaf morphology can exert an effect on leaf resistance by altering the depth of the boundary layer, it is logical to suggest that disparate susceptibilities to equivalent pollutant dosage between plant species, conspecific individuals and leaves of the same plant result in part from variation in leaf morphology.

Physiological response was showed in stomata control mechanism by closing response to non-injurious levels of SO<sub>2</sub> and opening response at non injurious levels (Olszyk and Tibbitts, 1981). Acclimatization of plants to air pollutants might change their morphological structure such as thicker epidermal cells and longer trichomes (Rangkuti, 2003)

### **Examples of plants tolerant to roadside pollution**

*Polygonum spp.*, *Elymus repens*, *Digitaria ischaemum*, *Echinochloa crusgalli*, *Bromus inermis*, *Plantago major*, *Portulaca oleracea*, *Panicum maximum*, and *Eleusine indica*

Ogbe and Bamidele (2006), Aigbokhian *et al.* (2011) Okereke and Mbaekwe (2011) Pal *et al.* (2002).

### ***Eleusine indica***

*Eleusine indica* (L.) Gaertn. belongs to the Family Poaceae or Gramineae. It has a number of common names, including bullgrass, crab grass, crabgrass, crowfoot grass, crow's foot, crowfoot, crow's-foot grass, crowsfoot grass, dog grass, fowlfoot grass, grass, goose foot, goose grass, goose-grass, goosegrass, Indian goose grass, Indian goosegrass, iron grass, silver crabgrass, wild finger millet, wire grass, wiregrass, yard grass

### **Habitats**

It is a typical weed of disturbed urban areas. Habitats include lawns and gardens, cracks in city sidewalks and pavement, margins of building foundations, vacant lots, construction sites, areas along railroads and roadsides, fields, and waste areas. Less often, Goose Grass is found in very degraded floodplain areas where there is scant ground vegetation.

### **Origin**

This species is widespread throughout tropical and sub-tropical regions of the world. The geographical origin of *E. indica* is uncertain due to its worldwide expansion, but it is thought to have come from Africa and Asia.

### **Naturalised Distribution**

Crowsfoot grass (*Eleusine indica*) is common and spread widely in several countries. Reported from Europe, Asia and Africa. It is naturalised in Queensland, New South Wales, Victoria, the Northern Territory and Western Australia. It is also naturalized in the southern parts of South Australia, in the ACT, on several offshore islands (i.e. Lord Howe Island,

Norfolk Island, Christmas Island and the Cocos Islands) and is sparingly naturalized in Tasmania. Dominant in Costa Rica; Egypt (Egypt (African part)); El Salvador; France; Guatemala; India; Lao People's Democratic Republic; Madagascar; Malaysia; Mexico; Morocco; New Zealand; Panama; Papua New Guinea; Poland; Portugal (Azores); Sao Tomé and Príncipe; Saudi Arabia; Slovakia; South Africa; Spain (Canary Is.); Taiwan, Province of China; United States; Viet Nam; Yemen; Zimbabwe (Hansen 1980)

### **Description**

*Eleusine indica* has a tufted base with alternate leaves annual plant growing about 0.5m; Its habit is prostrate and spreading, culms ascending or erect to about 40 cm. Leaves are globous and bright, fresh green in colour, common roadside plant and of disturbed sites. The plant is a common weed of cultivation. Usually, a pioneer plant colonizing bare sites.it has a deep root and tough fibrous structure. While still young it is a valuable source of fodder. It tolerant of heavy disturbance like trampling and pollution (Dítě *et al.*, 2019 Ecocrop, 2019).

### **Uses**

The leaves, root and seeds are edible. The seeds are used as millet that is why they are referred to as wild finger millet.

Fodder for animals. It is used as forage; the young plant is soft and palatable although the older part becomes tough and coarse.

Medicinal uses; all part of the plant is been used in several countries for traditional medicine. They are used for the treatment of liver diseases influenza hypertension. The root is diuretic, laxative and depurative. Pain killers and eye and ear treatment. (Al-Zubairi *et al.*, 2009)

The stems are used to make mats basket and also suitable for paper manufacture

Land conservation through stabilization of sandy soils and erosion prone lands because of its strong extensive rooting system.

Special Features: they are unique for being Invasive and Naturalizing in new sites, disturbed environment and polluted sites. serious annual weed in the world, has evolved resistance to several herbicides including paraquat, a non-selective herbicide (Chen *et al.*, 2017)

### **Propagation**

The Eleusine plant is propagated by Seed. The seeds are dispensed by wind, water and animals.

### **Epidermal cells**

These are different kind of cells that make up the epidermis of plants. Amongst the numerous functions of the epidermis, the main function is to protect the plant from stress (biological, chemical, physical and anthropogenic such as pollution). Their compacted cells prevent water loss in plants. They are found in all part of the plant from tip to root. These epidermal cells undergo various differentiation to specialized cells to perform the range of functions (Glover, 1999). Epidermal cells protect the plant from herbivores while others attract pollinating animals. Epidermal cells have fewer or no chloroplast except the guard cells.

### **Type of epidermal cells**

Pavement cell is the most frequently occurring cell type. Their basic function is to protect the tissue layers. These cells have different shapes in different organs and plant depending on their function. In leaves these form the mechanical strength which is important due to the large air spaces in the mesophyll layer. They are also shaped to accommodate either vertical or lateral growth.

Stomatal guard cells are essential for gaseous exchange required for photosynthesis. The epidermis of most leaves shows dorsoventral anatomy: the upper (adaxial) and lower (abaxial) surfaces. The guard cells make up the stoma complex, they are bean shaped creating the pore opening. These cells have chloroplast for manufacturing of sugar. Stomatal cells affect water retention and gaseous exchange.

Trichomes are also known as epidermal hairs. They are mostly found in the root as root hairs which is used for absorption of nutrients and water from the soil. Trichomes grow out of the epidermis.

## CHAPTER THREE

### MATERIALS AND METHODS

#### Study Area

The investigation was carried out at Benin City, Edo State, Nigeria. During the month of January 2020. Benin city is fourth largest city in Nigeria, located in the south part with a total population of 1,782,000. Benin City has a borderline tropical savanna climate. Benin City is situated between Latitude and longitude coordinates of 6.339185, 5.617447 with the gps coordinates of 6° 20' 21.0660" N and 5° 37' 2.8092" E. In Benin City, the wet season is warm, oppressive, and overcast and the dry season is hot, muggy, and partly cloudy. Over the course of the year, the temperature typically varies from 67°F to 88°F and is rarely below 60°F or above 91°F.

Five major roads leading in and out of the city was selected. Two points each of the five main roads were selected for sampling because these major roads experience extreme congestion, representing extreme, heavy and severe traffic congestion traffic flows respectively were considered as experimental road sites, while a farm land far off from the road was selected as a control site.

Along these roadsides a major road market point, towards the city central was selected as they are usually associated with serious traffic congestion. As well as the bypass point leading out of the city these are also associated with high traffic volume because of the road leads to a large part of the country. A lot of heavy-duty vehicles are also associated with these roads.

Ten (10) different sites selected for this present study of the *Eleusine indica*. are (1) Aduwawa market road sides (2) Auchi bypass roadside (3) Agbor road market roadside (4) Asaba bypass roadside (5) S and T Barracks market roadside (6) Lagos bypass roadside (7)

Santana market roadside (8) Sapele bypass road (9) Ekenwan bypass roadside (10) Okhokhugbo market roadside. The control site was a farmland located kilometers away from the any major road with traffic.

### **Sample collection**

The whole plant was uprooted at each sample site, it was wrapped in a brown paper and foil paper to preserve the plant integrity. These specimens were taken to University of Ibadan plant anatomical laboratory, Oyo state, Nigeria; for the epidermal cell analysis photomicrographs of the specimens were taken from the permanent slides using a Leitz Wetzler ortholux microscope fitted vivitar-V-335 camera. Quantitative characters measured were epidermal cell length and width, number of epidermal cells per field of view, epidermal cell wall thickness and stomata length.

### **Epidermal Preparations**

Small pieces of each leaf sample (5-8mm<sup>2</sup>) taken from a standard central position, usually midway between the apex and the base of the lamina, were soaked in concentrated trioxonitrate (v) acid in capped specimen bottles for about 30min to 72hrs, depending on the nature of the leaf. Specimens that were not well macerated within this period were transferred to water bath at 60<sup>0</sup>C for 60min. Abaxial and Adaxial epidermises were then teased apart using fine forceps and dissecting needle. Any mesophyll tissue adhering to the membrane was cleared out in the water by means of a camel brush. Each epidermal membrane was transferred into 50% ethylalcohol for 2 minutes to harden and then stained in safranin for 5 minutes before dehydrating in ethyl alcohol series. Each membrane was mounted in Canada balsam.

Photomicrographs were taken using a Zeiss 9901 Research Photomicroscope. One leaf sample per specimen was examined and the number of specimens studied in each species ranges from two to six listed above, depending on the geographical distribution of the various taxa and the availability of specimens.

The epidermal features that were observed include; size of guard cell, number of stomata, thickness of stomata, and number of vascular bundles. The stomata apparatus (SI) was estimated based on Metcalfe and Chalk (1979). The mean and standard deviation were calculated using Microsoft excel 2010

### **Root Preparations**

Fresh roots were collected from the 10 different sites in Benin City, Edo State, Nigeria. The healthiest roots were collected and fixed in FAA (1:1:18); glacial acetic acid: 40% formaldehyde: 70% ethanol (v/v), for 48 - 72 h. The roots were washed several times in distilled water then with two changes of 30% ethanol and dehydrated in the order 30, 50, 70, 95% and absolute alcohol. To infiltrate wax into the specimens, they were placed for 3 h in each of the following solutions containing a ratio of absolute alcohol to pure chloroform (v/v: 3:1, 1:1, 1:3) and then pure chloroform. At the stage of pure chloroform, wax pellets at 60°C melting point were added and the wax changed with new ones at intervals. The specimens were left in the oven for 2 - 7 days to remove the chloroform. To embed in wax, the contents of the vials were transferred into molds and the specimens kept in place with hot needles. As the wax solidified, it was transferred to a cold-water bath for hardening and later stored for two days in a refrigerator.

For sectioning, a reichert rotary microtome was used and 10 - 20mm thick sections were made. The ribbons were placed on clean slides smeared with a thin film of Haupt's albumen, allowed to dry and drops of water added prior to mounting.

The slides were placed on a hot plate at 40°C for few minutes for the ribbons to expand and were stored overnight. The slides were immersed in pure xylene for 2 - 5 min in a solution of xylene and absolute alcohol with 1:1 ratio (v/v) for few minutes. The slides were then transferred to another solution of xylene and alcohol in the ration 1:3 (v/v) for few minutes, to 95, 90, 70 and 50% alcohol. Drops of alcian blue were added on the specimens, washed off with water and counter strained with safranin for two minutes, then dehydrated in 50% alcohol, 70, 80, 90% xylene/alcohol solution and mounted in Canada balsam. The slides were dried on a hot plate at 30°C. Then photomicrographs of the specimens were taken from the permanent slides using a LeitzWetzler ortholux microscope fitted vivitar-V-335 camera (Cutler, 1978).

## CHAPTER FOUR

### RESULTS

#### **Control**

Adaxial: Adaxial cells with thin to moderately thick guard cells, 60-8 $\mu$ m long, 396 stomatal rows. Stomatal thickness: 19 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 19 $\mu$ m wide. The xylem and phloem thickness ranges from 15-20 $\mu$ m and 8-9 $\mu$ m. Micro hairs absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 28-8 $\mu$ m long, 625 stomatal rows. Stomatal thickness: 20 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 20 $\mu$ m wide. Macro hairs absent. trichome absent.

#### **Lagos Bypass roadside**

Plate 1 and Plate 2 shows the structure of the stomata, guard cells and epidermal cell of leaves while the vessels of the root is shown in Plate 3 in comparison to the control. Adaxial: Adaxial cells with thin to moderately thick guard cells, 20-7 $\mu$ m long, 475 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. The xylem and phloem thickness ranges from 9-11 $\mu$ m and 7-8 $\mu$ m. Micro hairs absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 20-7 $\mu$ m long, 477 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Micro hairs absent.

### **Asaba Bypass Roadside**

This is indicated in Plate (4-6) in comparison with the control. Adaxial: Adaxial cells with thin to moderately thick guard cells, 20-7 $\mu$ m long, 423 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. The xylem and phloem thickness ranges from 9-11 $\mu$ m and 7-8 $\mu$ m. Microhairs absent. Macrohairs absent. Hooks absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 20-7 $\mu$ m long, 433 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Microhairs absent. Hooks absent.

### **Aduwawa market roadside**

Plates (7-9) shows Adaxial: Adaxial cells with thin to moderately thick guard cells, 26-8 $\mu$ m long, 465 stomatal rows. Stomatal thickness: 14 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 13 $\mu$ m wide. The xylem and phloem thickness ranges from 10-15 $\mu$ m and 8-11 $\mu$ m. Microhairs absent. trichome absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 26-8 $\mu$ m long, 476 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Microhairs absent. trichome absent.

### **Agbor Road market roadside**

In Plates (10 -12) Adaxial: Adaxial cells with thin to moderately thick guard cells, 23 $\mu$ m long, 485 stomatal rows. Stomatal thickness: 12 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 15 $\mu$ m wide. The xylem and phloem thickness ranges from 11-12.2 $\mu$ m and 8.9-9.0 $\mu$ m. Microhairs absent. Hooks absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 22 $\mu$ m long, 203 stomatal rows. Stomatal thickness: 11 $\mu$ m wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 14 $\mu$ m wide. Microhairs absent. Hooks absent.

### **Ukhuokhugbo market roadside**

As indicated by Plate (13 -14) Adaxial: Adaxial cells with thin to moderately thick guard cells, 25-5.6 $\mu$ m long, 515 stomatal rows. Stomatal thickness: 15 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 17 $\mu$ m wide. The xylem and phloem thickness ranges from 10-14 $\mu$ m and 7-9 $\mu$ m. Microhairs absent. Macrohairs absent. Hooks absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 23 $\mu$ m long, 528 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 15 $\mu$ m wide. Microhairs absent. Macrohairs absent. Hooks absent.

### **Auchi Bypass roadside**

Adaxial: Adaxial cells with thin to moderately thick guard cells, 15 $\mu$ m long, 480 stomatal rows. Stomatal thickness: 15 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 17 $\mu$ m wide. The xylem and phloem thickness ranges from 11-15 $\mu$ m and 8-9 $\mu$ m. Microhairs absent. Macrohairs absent. Hooks absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 34 $\mu$ m long, 515 stomatal rows. Stomatal thickness: 15 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 17 $\mu$ m wide. Microhairs absent. Macrohairs absent. Hooks absent. These is represented in Plate (16-18).

### **S & T Barrack market roadside**

Plate 19 and Plate 20 shows the structure of the stomata, guard cells and epidermal cell of leaves while the vessels of the root are shown in Plate 21 in comparison to the control

Adaxial: Adaxial cells with thin to moderately thick guard cells, 24 $\mu$ m long, 466 stomatal rows. Stomatal thickness: 15 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 14 $\mu$ m wide. The xylem and phloem thickness ranges from 9-11 $\mu$ m and 7.5-9 $\mu$ m. Microhairs absent.

Abaxial: Abaxial cells with thin to moderately thick guard cells, 29 $\mu$ m long, 457 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Microhairs absent.

### **Santana market roadsides**

In Plate 23 Adaxial: Adaxial cells with thin to moderately thick guard cells, 26 $\mu$ m long, 465 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Plate 24 shows the xylem and phloem thickness ranges from 11-15 $\mu$ m and 9-10 $\mu$ m. Microhairs absent. Macrohairs absent. Hooks absent.

In Plate 22 Abaxial: Abaxial cells with thin to moderately thick guard cells, 26 $\mu$ m long, 486 stomatal rows. Stomatal thickness: 13 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 12 $\mu$ m wide. Microhairs absent. Hooks absent.

### **Benin Sapele Road Bypass**

Adaxial: Adaxial cells with thin to moderately thick guard cells, 27 long, 432 stomatal rows. Stomatal thickness: 14 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in

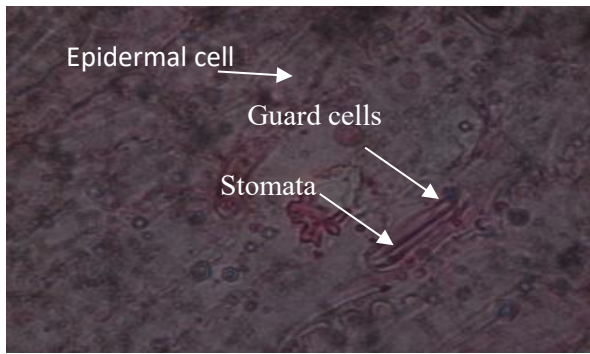
shape. Subsidiary cells 13 $\mu$ m wide represented in Plate 26. Whereas the xylem and phloem thickness ranges from 9-14 $\mu$ m and 8-12 $\mu$ m. Microhairs absent. trichome absent in Plate 27.

In Plate 25, Abaxial: Abaxial cells with thin to moderately thick guard cells, 29 $\mu$ m long, 441 stomatal rows. Stomatal thickness: 17 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 13 $\mu$ m wide. Microhairs absent. trichome absent

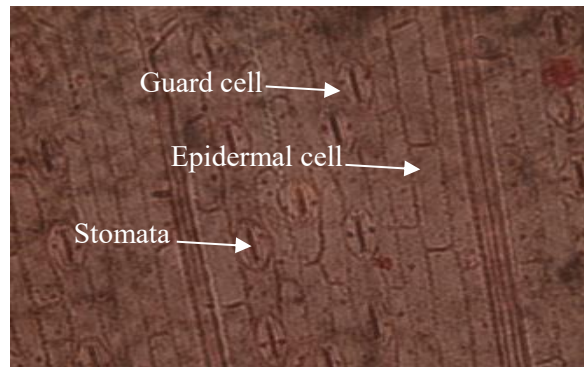
### **Ekenwan Bypass roadside**

Plate 29 indicate, Adaxial: Adaxial cells with thin to moderately thick guard cells, 25 $\mu$ m long, 481 stomatal rows. Stomatal thickness: 16 $\mu$  wide, guard cells dumb bell shaped subsidiary cells triangular in shape. Subsidiary cells 16 $\mu$ m wide. The xylem and phloem thickness ranges from 6-12 $\mu$ m and 11-13 $\mu$ m. Microhairs absent. trichome absent as represented in plate 30

Whereas in Plate 28, Abaxial: Abaxial cells with thin to moderately thick guard cells, 20 $\mu$ m long, 436 stomatal rows. Stomatal thickness: 17 $\mu$  wide, guard cells dumb bell-shaped subsidiary cells triangular in shape. Subsidiary cells 15 $\mu$ m wide. Microhairs absent. trichome absent.

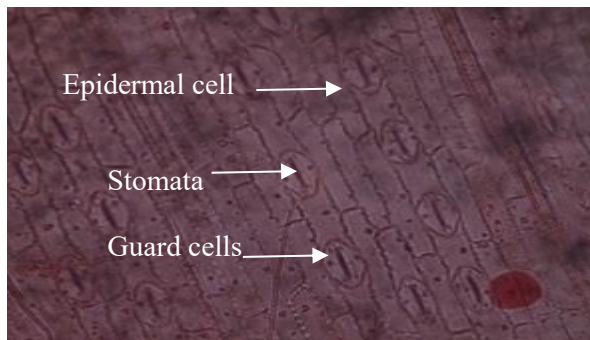


Adaxial of *Eleusine indica* Lagos Bypass

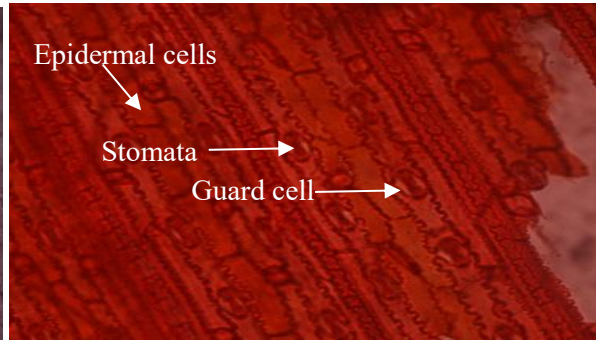


Adaxial of *Eleusine indica* control

**Plate 1: Adaxial of *Eleusine indica* (Lagos Bypass and control)**

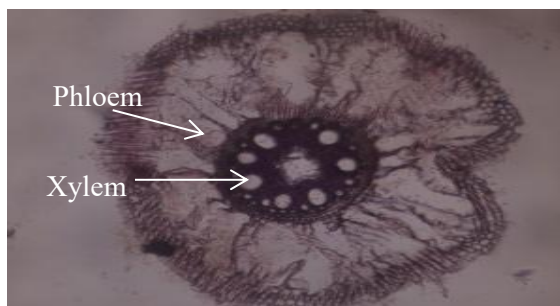


Abaxial of *Eleusine indica*

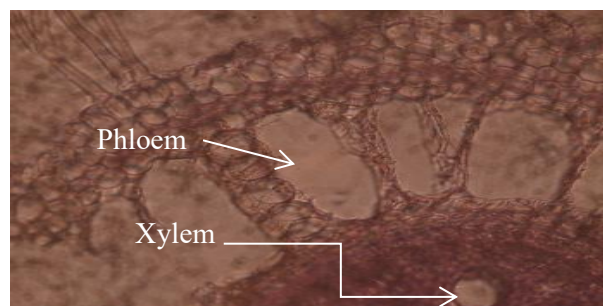


Abaxial of *Eleusine indica* control

**Plate 2: Adaxial of *Eleusine indica* (Lagos Bypass and control)**

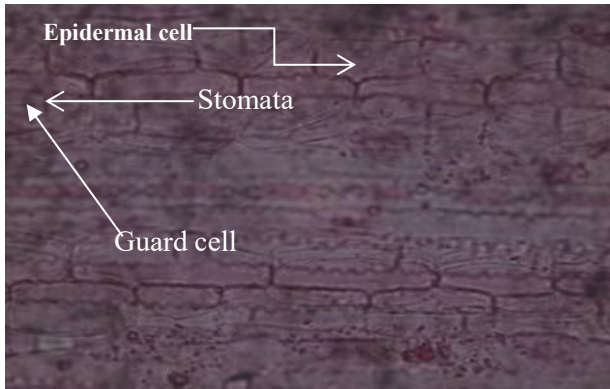


Root of *Eluesine indica*

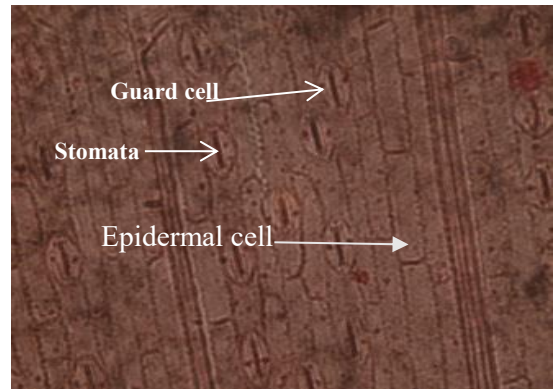


Root of *Eluesine indica* control

**Plate 3: Root of *Eluesine indica* (Lagos Bypass and control)**

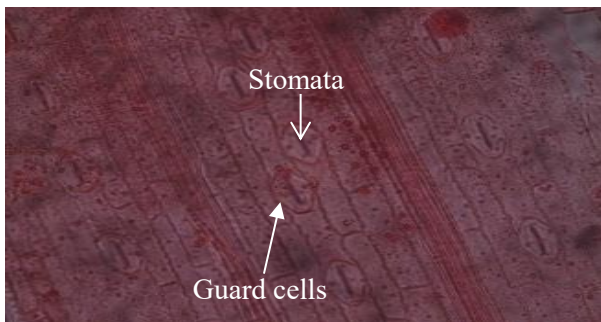


Adaxial of *E. indica* Asaba Bypass

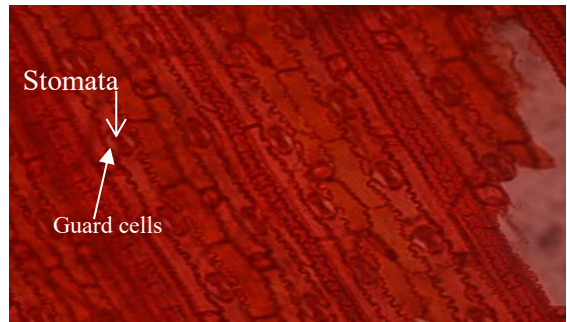


Adaxial of *E. indica* control

**Plate 4: Adaxial of *Eluesine indica* (Asaba Bypass and control)**

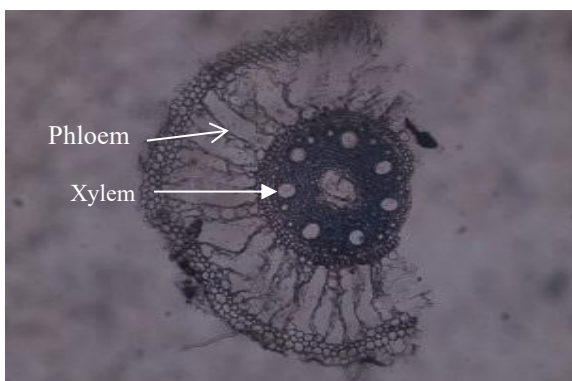


Abaxial of *E. indica* Asaba Bypass

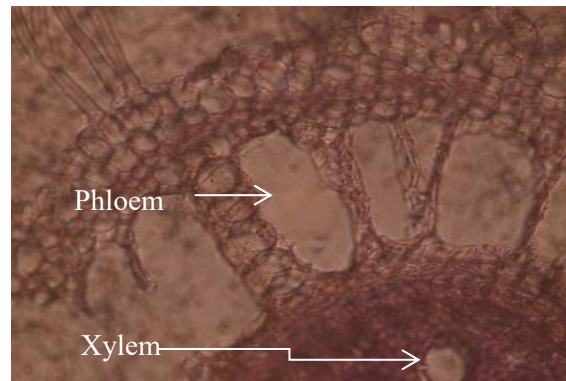


Abaxial of *E. indica* control

**Plate 5: Abaxial of *Eluesine indica* (Asaba Bypass and control)**

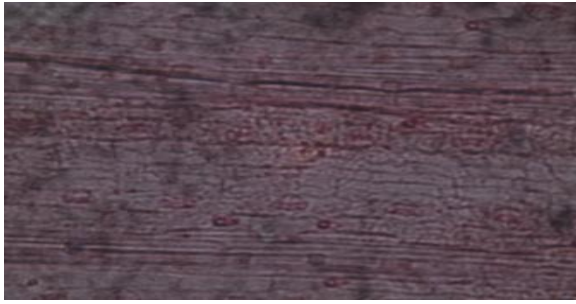


Root of *Eluesine indica*



Root of *Eluesine indica* control

**Plate 6: Root of *Eluesine indica* (Asaba Bypass and control)**

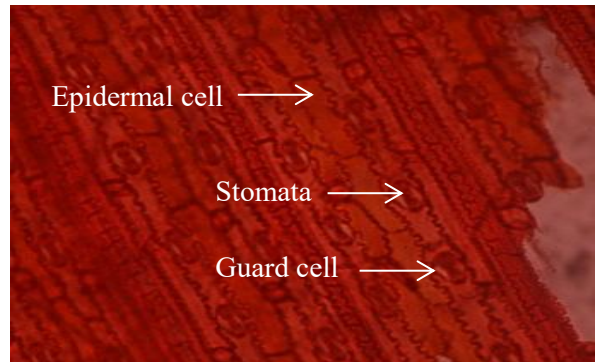
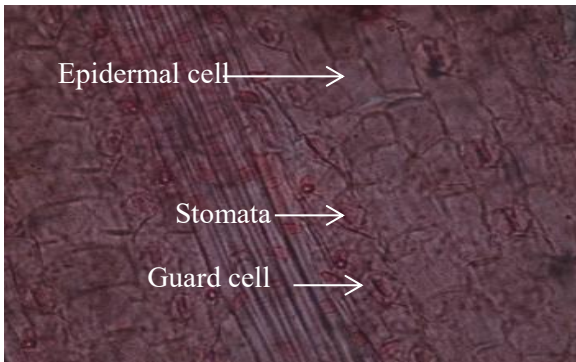


Adaxial of *Eluesine indica* Aduwawa market

Adaxial of *Eluesine indica* control

**Aduwawa market**

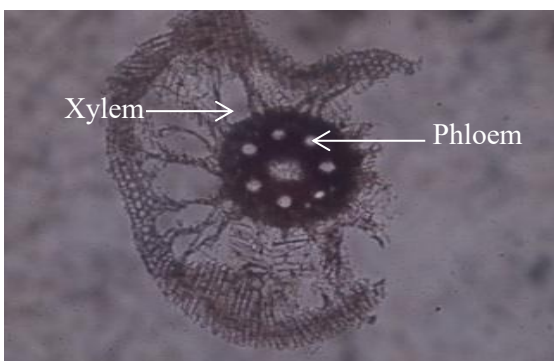
**Plate 7: Adaxial of *Eluesine indica* at Aduwawa market and control**



Abaxial of *Eluesine indica*

Abaxial of *Eluesine indica* control

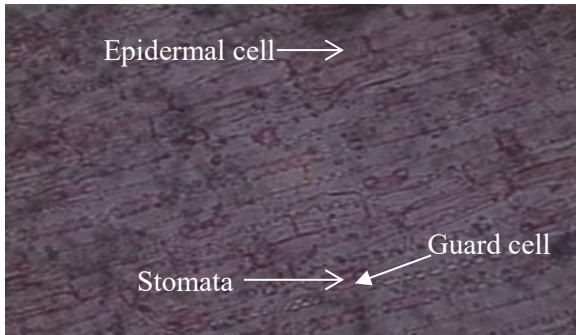
**Plate 8: Abaxial of *Eluesine indica* at Aduwawa market and control**



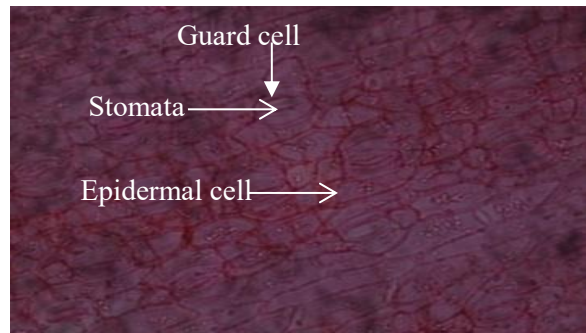
Root of *Eluesine indica*

Root of *Eluesine indica* control

**Plate 9: Root of *Eluesine indica* at Aduwawa market and control**



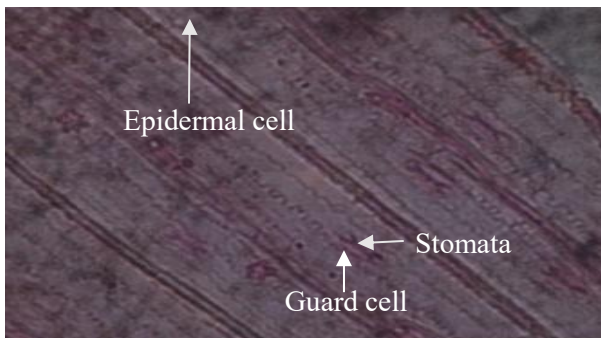
Adaxial of *Eluesine indica*



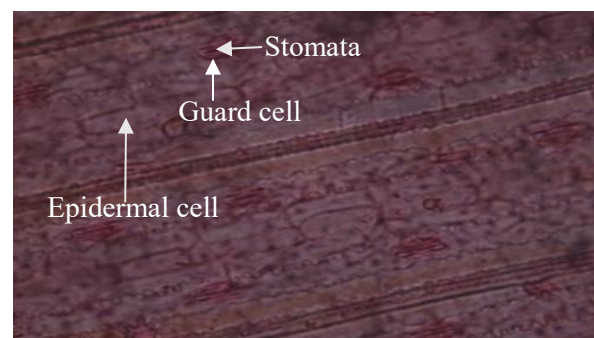
Adaxial of *Eluesine indica* control

**Agbor road market**

**Plate 10: Adaxial of *Eluesine indica* (Agbor road market and control)**

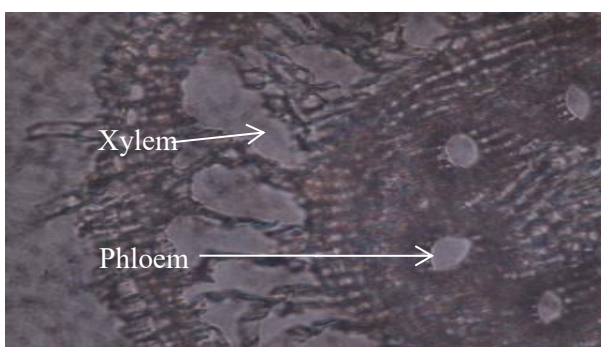


Abaxial of *Eluesine indica*

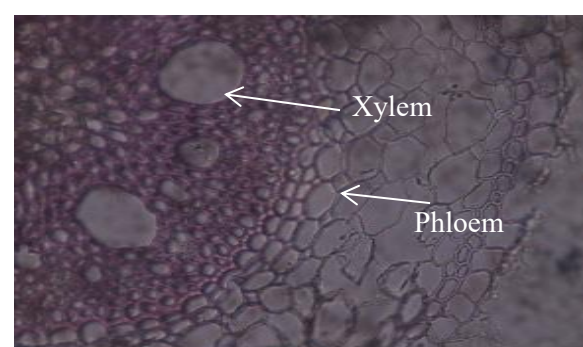


Abaxial of *Eluesine indica* control

**Plate 11: Abaxial of *Eluesine indica* (Agbor road market and control)**

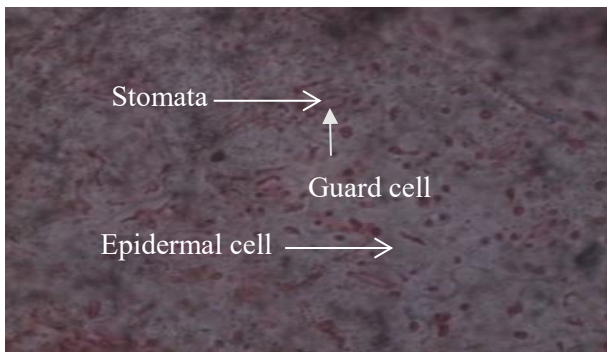


Root of *Eluesine indica*

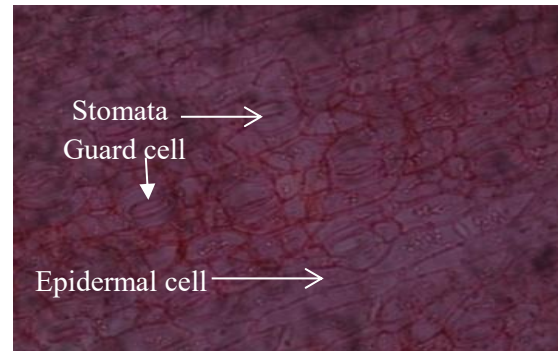


Root of *Eluesine indica* control

**Plate 12: Root of *Eluesine indica* (Agbor road market and control)**

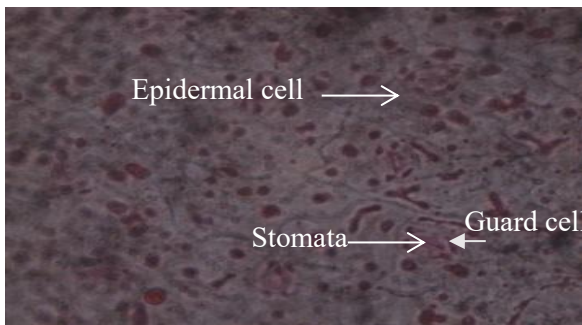


Adaxial of *Eluesine indica*

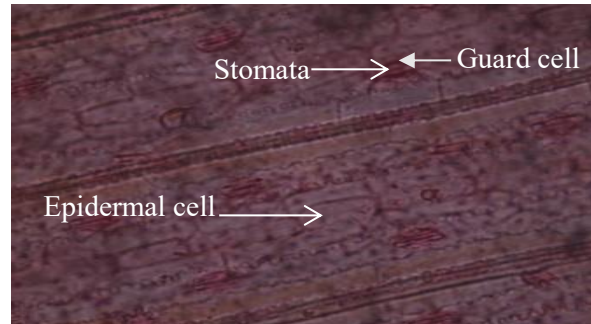


Adaxial of *Eluesine indica* (control)

**Plate 13: Adaxial of *Eluesine indica* (Ukhuokhugbo and control)**

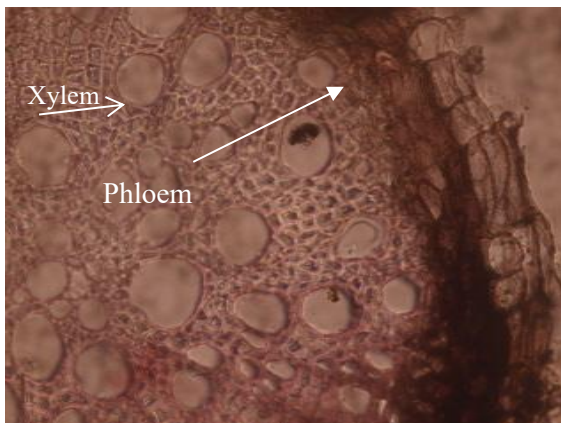


Abaxial of *Eluesine indica*

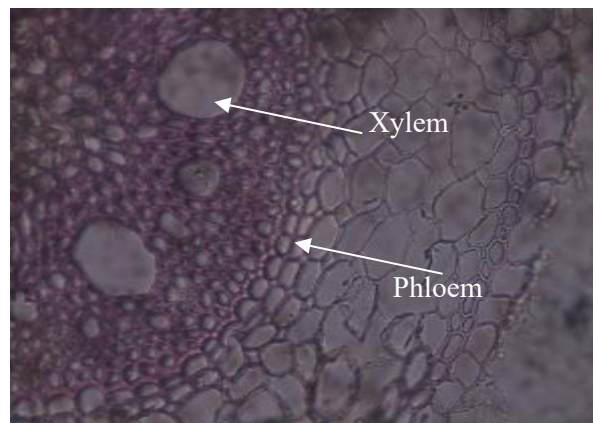


Abaxial of *Eluesine indica* (control)

**Plate 14: Abaxial of *Eluesine indica* (Ukhuokhugbo and control)**

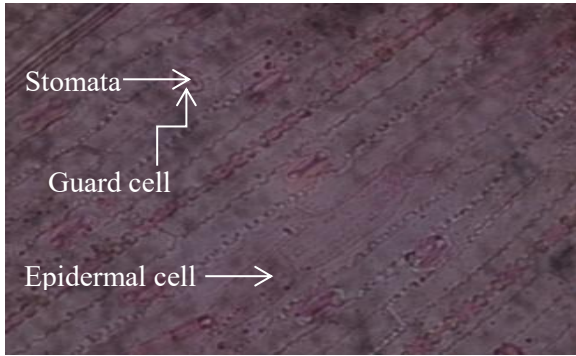


Root of *Eluesine indica*

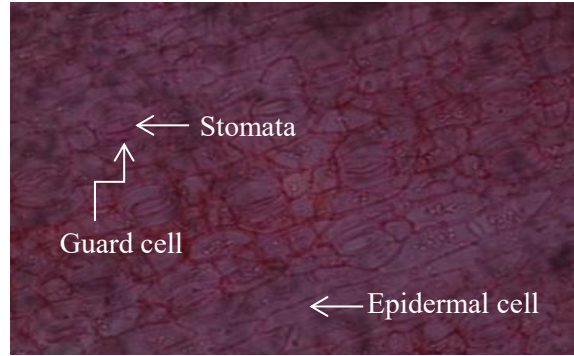


Root of *Eluesine indica* (control)

**Plate 15: Root of *Eluesine indica* (Ukhuokhugbo and control)**



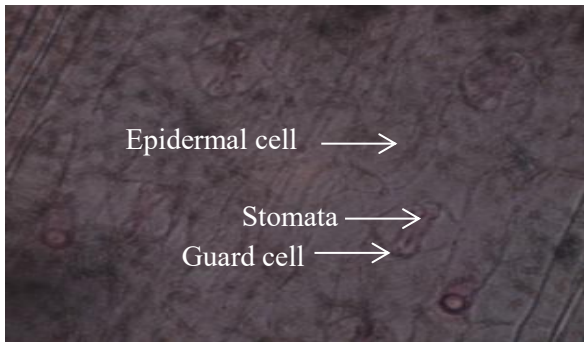
Adaxial of *Eluesine indica*



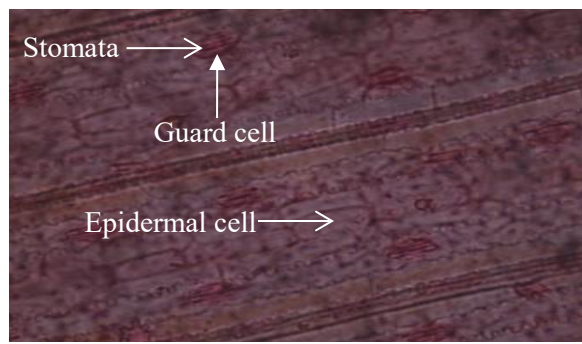
Adaxial of *Eluesine indica* (control)

***Auchi Bypass***

**Plate 16: Adaxial of *Eluesine indica* ( *Auchi Bypass* and control)**

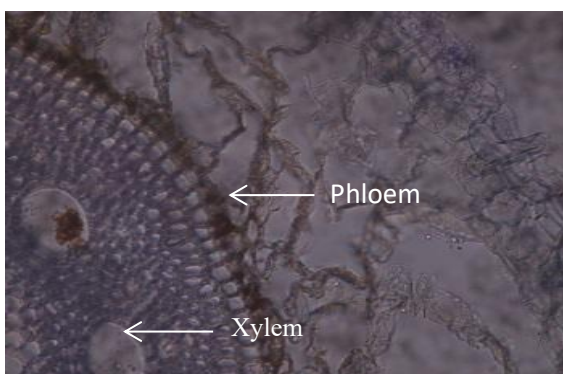


Abaxial of *Eluesine indica*

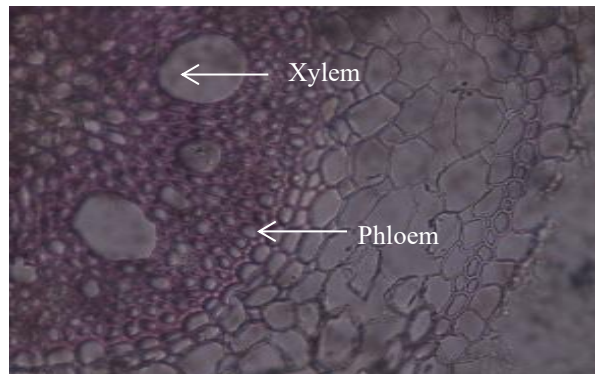


Abaxial of *Eluesine indica* (control)

**Plate 17: Abaxial of *Eluesine indica* ( *Auchi Bypass* and control)**

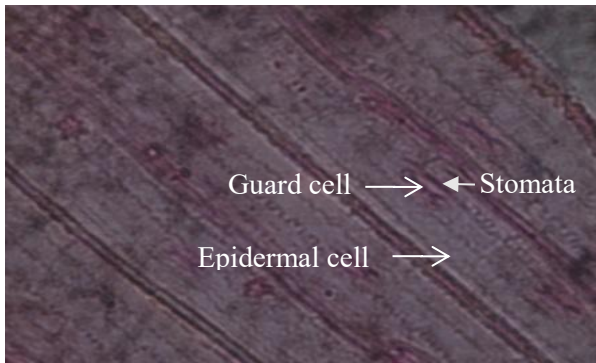


Root of *Eluesine indica*

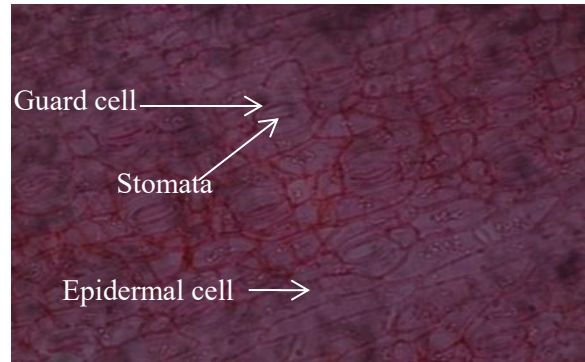


Root of *Eluesine indica* (control)

**Plate 18: Root of *Eluesine indica* ( *Auchi Bypass* and control)**

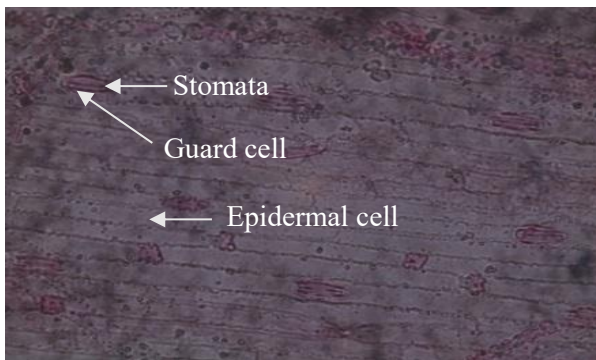


Abaxial of *Eluesine indica*

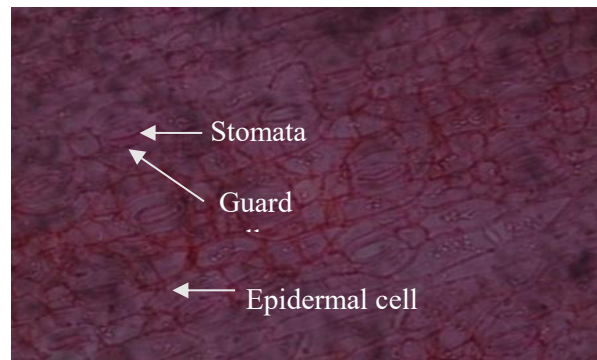


Abaxial of *Eluesine indica* control

**Plate 19: Abaxial of *Eluesine indica* which is at S & T Barrack market roadside and control**

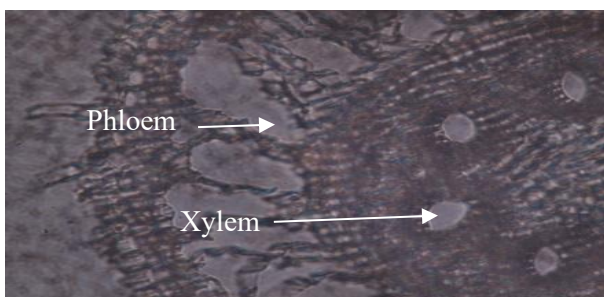


Adaxial of *Eluesine indica*

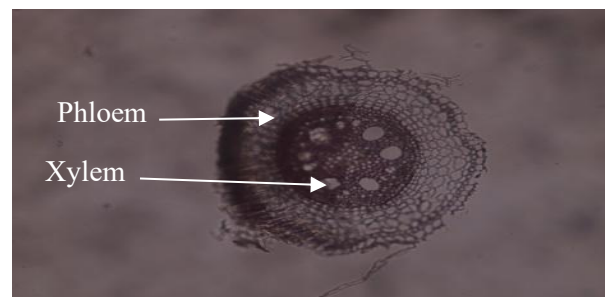


Adaxial of *Eluesine indica* control

**Plate 20: Adaxial of *Eluesine indica* which is at S & T Barrack market roadside and control**

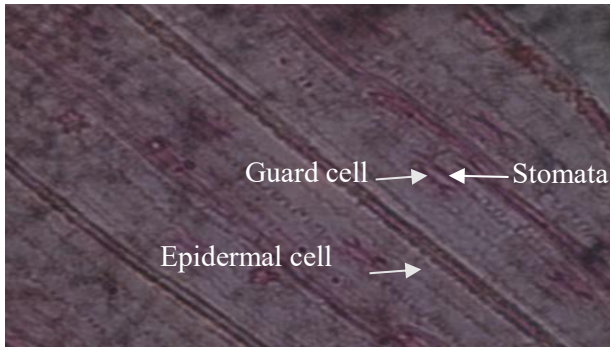


Root of *Eluesine indica*

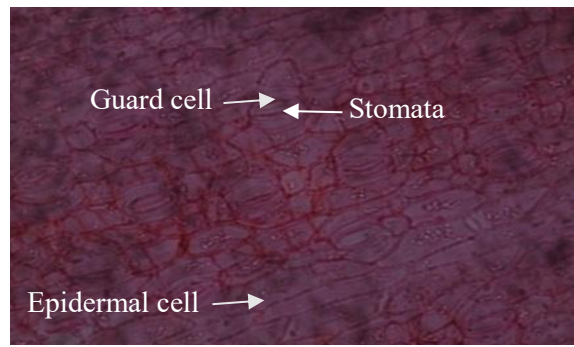


Root of *Eluesine indica* control

**Plate 21: Root of *Eluesine indica* which is at S & T Barrack market roadside and control**



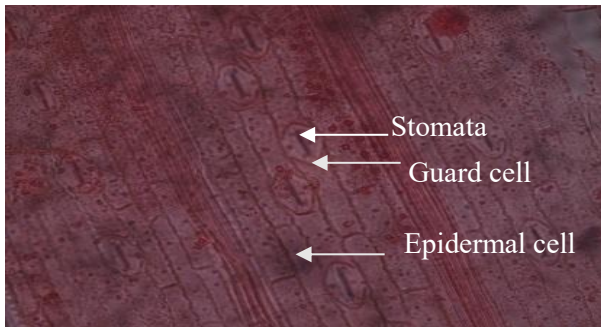
Abaxial of *Eluesine indica*



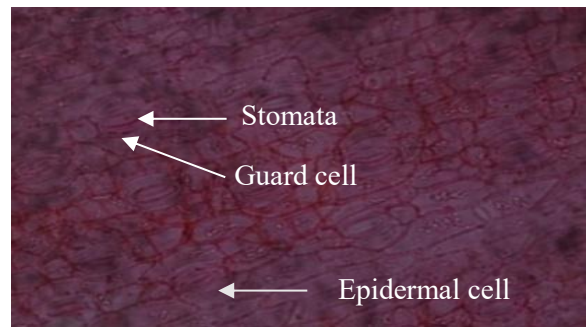
Abaxial of *Eluesine indica* control

**Santana market**

**Plate 22: Abaxial of *Eluesine indica* which is at Santana market and control**

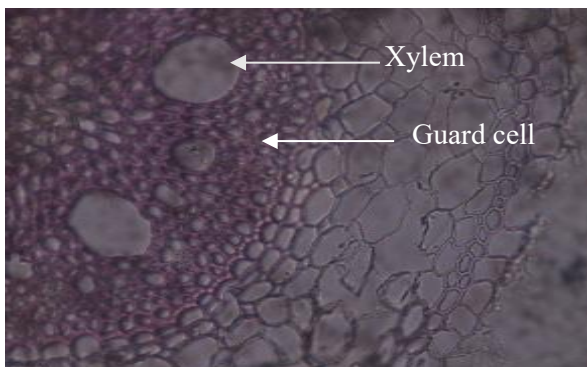


Adaxial of *Eluesine indica*

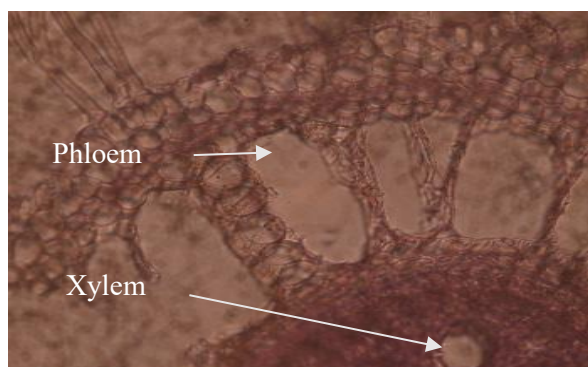


Adaxial of *Eluesine indica* control

**Plate 23: Adaxial of *Eluesine indica* which is at Santana market and control**

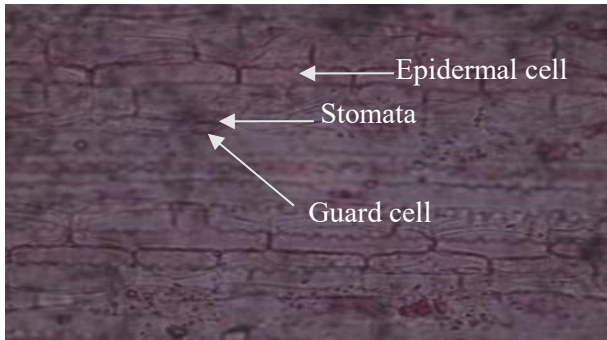


Root of *Eluesine indica*

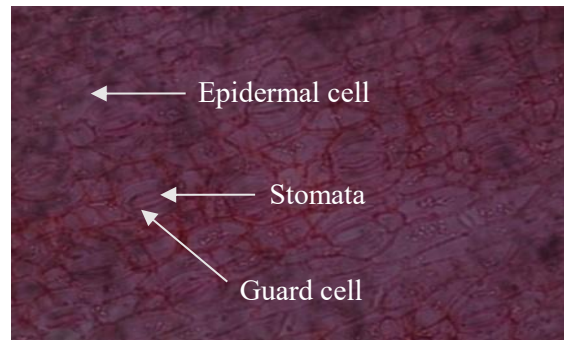


Root of *Eluesine indica* control

**Plate 24: Root of *Eluesine indica* at Santana market and control**



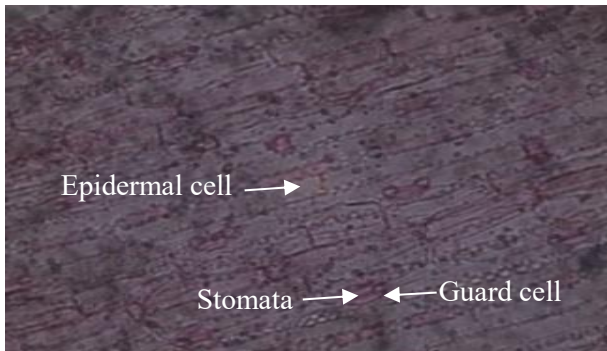
Abaxial of *Eluesine indica*



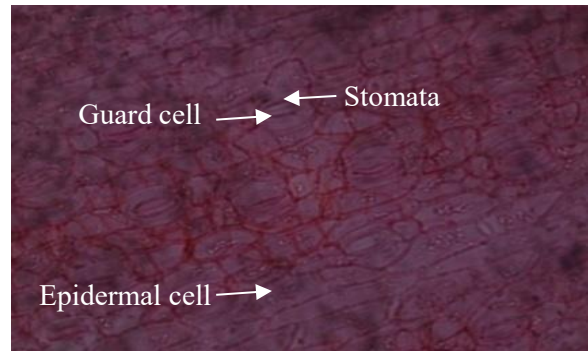
Abaxial of *Eluesine indica* control

**Benin Sapele Road Bypass**

**Plate 25: Abaxial of *Eluesine indica* which is from Benin Sapele Road Bypass and control**

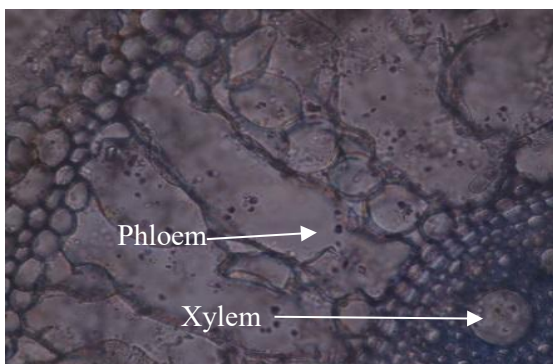


Adaxial of *Eluesine indica*

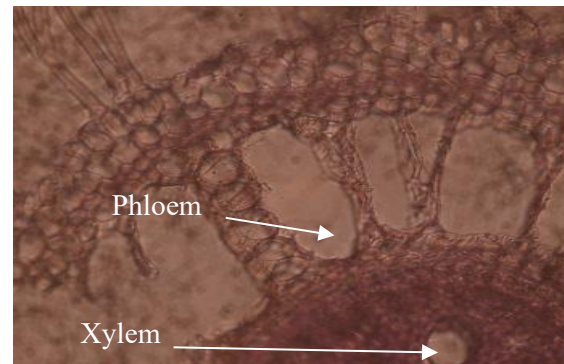


Adaxial of *Eluesine indica* control

**Plate 26: Adaxial of *Eluesine indica* which is from Benin Sapele Road Bypass and control**

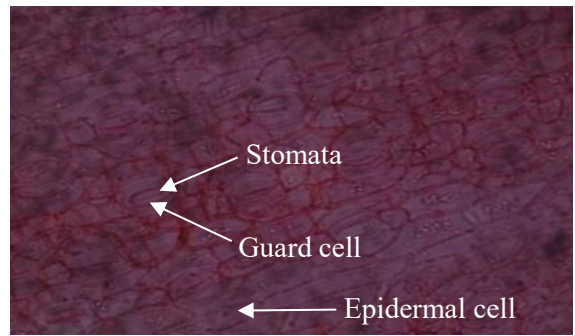
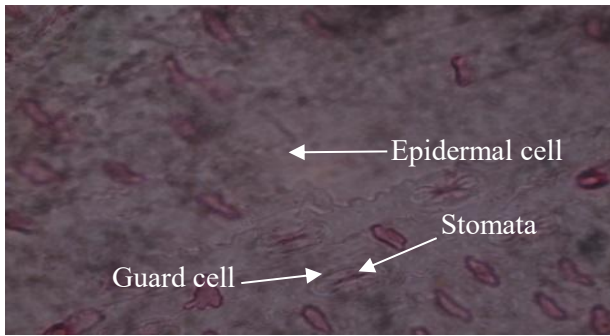


Root of *Eluesine indica*



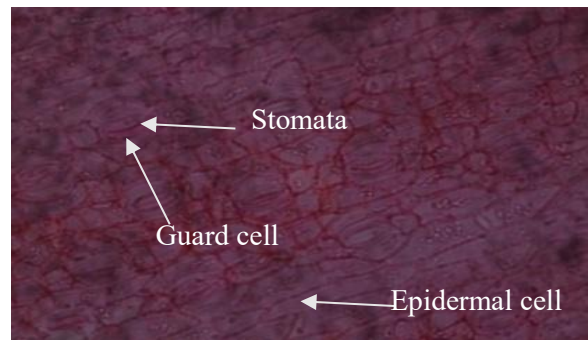
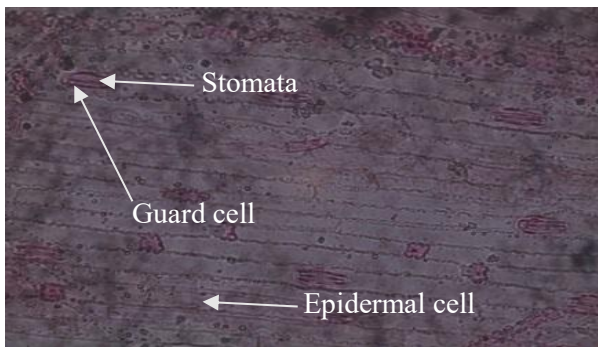
Root of *Eluesine indica* control

**Plate 27: Root of *Eluesine indica* at Benin Sapele Road Bypass and control**



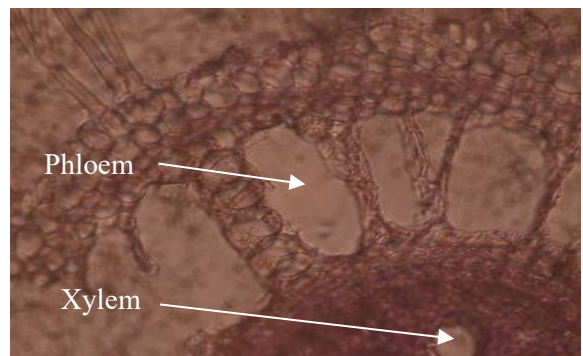
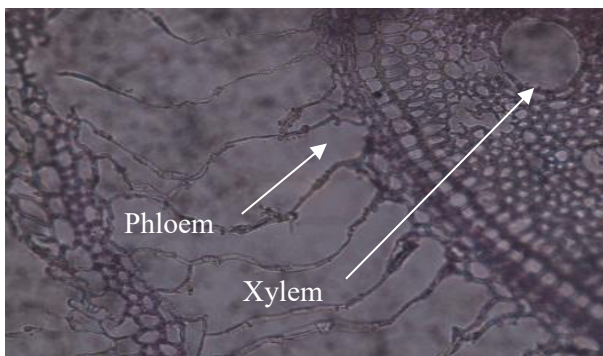
Abaxial of *Eluesine indica* from Ekenwan bypass    Abaxial of *Eluesine indica* control

**Plate 28: Abaxial of *Eluesine indica* which is from Ekenwan bypass and control**



Adaxial of *Eluesine indica* from Ekenwan bypass    Adaxial of *Eluesine indica* control

**Plate 29: Adaxial of *Eluesine indica* which is from Ekenwan bypass and control**



Root of *Eluesine indica* at Ekenwan bypass

Root of *Eluesine indica* control

**Plate 30: Root of *Eluesine indica* at Ekenwan bypass and control**

**Table1: showing epidermal feature of *Eluesine indica***

	Numbers of Stomata		Size of Guard cell( $\mu\text{m}$ )		Diameter of epidermal cell ( $\mu\text{m}$ )		Thickness of Stomata	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Control	396	625	60	28	19	20	19	20
Lagos Bypass roadside	475	477	20	20	12	12	13	11
S & T Barracks market roadside	466	457	24	29	14	12	15	11
Aduwawa Market roadside	465	476	26	26	14	14	14	13
Auchi Bypass roadside	480	515	27	27	16	14	17	17
Asaba bypass roadside	423	433	27	27	13	12	14	14
Agbor-park market	485	505	23	22	14	14	14	14
Okhokhugbo Market	515	528	24.4	23	15	13	15	16
Ekenwa bypass roadside	418	436	25	20	16	15	16	17
Santana market roadside	465	475	24	26	13	15	14	11
Sapele road bypass	432	441	27	29	13	13	14	17

**Table2: showing Root Anatomical studies of *Eluesine indica***

	Xylem thickness	Phloem thickness
Control	15.0-20.0	8.0-13.2
Lagos Bypass roadside	9.0-11.0	7.0-8.0
S & T Barracks market roadside	9.0-11.0	7.5-9.0
Aduwawa Market roadside	10.0-15.0	8.0-11.0
Auchi Bypass roadside	11.0-15.0	8.0-9.0
Asaba bypass roadside	12.0-17.0	9.0-10.0
Agbor-park market	11.0-12.2	8.9-9.0
Okhokhugbo market	10.0-14.0	7.0-9.0
Ekenwa bypass road	6.0-12	11.0-13.0
Santana market roadside	11.0-15.0	9.0-10.0
Sapele road by pass	9.0-14.0	8.0-12.0

Table 1 shows the interrelationship between the morphological characters of polluted and non-polluted *Eleusine indica*. The epidermal cells size, guard cell sizes, stomata thickness and number of stomata identified on the adaxial and abaxial sides of the leaves from all the polluted sites showed no significant difference from one another. Stomata number was more on the abaxial surface than adaxial surface in all the plants. There were more stomates numbers per squared millimeter on abaxial and less stomates number on the adaxial surfaces in both the polluted and the control.

The sizes of the guard cells of the different road sides tend to decrease than those from the farm land as shown in table 1. Also, for the thickness of the stomata present at both the adaxial and abaxial layers, they also show relative decrease in the plants from the roadsides than the polluted.

The diameter of the epidermal cells of *Eleusine indica* found along the road and influenced by pollution tend to decrease in comparison to the ones found on the farmland with less pollution factors as shown from table 1.

A comparison of the cells in the plates is visible (Plates 1 – 30). There were distortions or reduction in stomata, guard cells and epidermal cells per unit area of the leaf also in root vessels. This suggests the effects of the pollution in the soil, perhaps by automobile deposits or other petroleum-related contaminants.

## CHAPTER FIVE

### Discussion

The epidermal cells of plant are known to express some level of plasticity, because of their multifunctionality they are often less specialized. Leaf area expansion is affected by environmental conditions because of differences in cell number and/or cell size (Cookson *et al* 2006). The epidermal cells form, basically have strong thickening walls which limit the harmful effect of environmental factors. The change in epidermal cells is one of plants reactions to air pollution.

Auto-pollution which include air pollutant and particulate matter emitted from automobiles are the greatest source of pollution in urban areas especially roadsides. Plant growing along the roadside bear the brunt by absorbing the pollutants (Verma and Charg 2014).

Common pollutants found along the roadsides are NO<sub>x</sub>, SO<sub>2</sub>, O<sub>3</sub> and heavy metals (Uka *et al.*, 2014). Plants respond differently to these pollutant in the environment. These changes can be physiological or morphological. The leaf is the most dominant pollutant acceptor of pollution from vehicle exhaust along roadside. Plants detect and react to environmental stresses in a number of ways, including anatomical adaptations.

Ruderals are different kinds of plants with capacities for surviving in areas where most plants cannot survive. Shrub species that grow along city roadsides act as a filter for land deposition, reducing the toxic effects of pollutants and mitigating the impact of other stress factors in such an environment. Plants respond to pollution-induced stress with a variety of active morphological and anatomical responses, including changes in the number and thickness of stomata, epidermal cells, and guard cells (Samal and Santra 2002; Raina and Sharma 2006; Raina and Agrawal 2004; Raina and Bala 2011; Tiwari, 2001).

In light of the above, the current study aims to determine the impact of contaminant on the anatomical structure of *Eleusine indica*. The findings on anatomical traits of these species growing at contaminated sites were compared to those of respective plant species growing at a farm (monitor), which was used as the study's control site

*Eleusine indica* is one of the most abundant weeds found along roadsides and pavements. They are known to be very invasive (Ikaijagbe *et al.*, 2020).

In this study, increase in the number of the stomatal at the adaxial levels of plant found in polluted sites were observed as compared to the control. In the lower layer there is a decrease in the number of stomatal in the abaxial layer.

The size of guard cell in the lower and upper layer was observed to decrease in size as compared to the control. This is similar to the report by Sharma and Roy (1995) and Jahan and Zafar (1992).

The reduction of the stomatal size could be considered as an adaptive response of these plants to avoid entry of harmful constituents of the automobile pollutant (Salgare and Ihorat., 1990).

The number of epidermal, stomata, and guard cells on the dorsal and ventral surfaces of leaves of all the selected plant species growing at different polluted sites were found to be lower than on farm land (control). Various workers have observed a similar decrease in stomata size and epidermal cells at contaminated sites relative to unpolluted sites (Kulshreshtha *et al.*, 1980, 1994; Sharma and Roy, 1995; Aggarwal, 2000; Snehlata *et al.*, 2010; Saadabi, 2011; Saadabi and Al-NurEl-Amin 2011; Rai and Mishra, 2013; Shrivastava and Mishra, 2018).

Many authors determine the index of the number of stoma in a unit, area as the most reliable and often used indicator of species reaction to air pollution.

There were distortions or reduction in the number of stomata per unit area of the leaf. this suggests the effects of the pollution in the soil, perhaps by automobile deposits or other petroleum-related contaminants. This further confirms earlier report of (Omoriegic *et al.*, 2016)

Vessel walls thickness was reduced in all species in the polluted environment with death of some vessel members resulting in reduction of vessel members and ultimately leading to the loss of structural integrity.

This reduction in stomata size may be viewed as a plant's adaptive response to prevent harmful constituents of various land pollutants from entering the plant, which could otherwise have negative consequences (Salgare and Thorat, 1990). The distorted shapes of stomata found in the leaves of the investigated plants may have resulted from a decrease in pH in the cytoplasm of guard cells, resulting in a shift in turgor relations of the stomata complex (Kondo *et al.* 1980), as well as physiological injury inside the leaf (Kondo *et al.*, 1980). (Ashenden and Mansfield, 1978). Metal pollutants penetrate the leaves through the stomata in the same way as CO<sub>2</sub> does. This may be an adaptation of these plants to increase the area available for proper gas exchange, as Raina and Chand Bala (2011) and other researchers have suggested (Shrivastava and Prakash, 2017; and Shrivastava and Mishra 2018). The wall thickness of both xylem and phloem was found to be reduced across various contaminated environment sites.

Vessel wall thickness was reduced in all organisms in the contaminated atmosphere as a result of the death of certain vessel members, resulting in a reduction in the number of vessel members and, as a result, a loss of structural integrity.

The leaves of non-polluted plants grew normally while the polluted plants suffered anatomical disfigure. Cell disruption in leaves was eminent. In the study, emphasis was on leaf epidermal changes.

This is even more predicated on the fact that plant productivity relies, to a very large extent, on the activities of leaves around the dermal region, including stomatal opening and closure, implicated during plant-water relations and for CO<sub>2</sub> intake for photosynthesis.

## **Conclusion**

It is concluded from the study that due to environmental pollution; many injurious and toxic materials are being release into atmosphere continuously which is eventually absorbed by the plants along the roadside. These pollutants enter into the plant through the leaves, root and plant body causing modifications in their epidermal cells. *Eleusine indica* have shown to exhibit minimal physical structural changes in polluted environment making them suitable for phytoremediation of polluted environment.

## REFERENCE

- Abechi, E, Okunola, O S. Zubairu, A. Usman, E. Apene, (2010). Evaluation of heavy metals in roadside soils of major streets in Jos metropolis, Nigeria. *Journal of Environmental chemistry and Ecotoxicology* 2, 98-102.
- Aigbokhian E. I., Osazuwa-Peters O. L. and Ilubon K. O. (2011) Range and Distribution of *Mimosa diplotricha* in Nigeria and effects of fire on seed germination. *Nigerian Journal of Botany* 24(1): 142-151.
- Aggarwal, P. (2000). The effect of auto-exhaust pollution on leaf surface of *Cassia siamea*(L.), a road side tree. *ActaEcologica*, 22: 101-106
- Al-Zubairi A. S, Abdul A B, Abdelwahab, S I, Peng Y C, Mohan S, and Elhassan M. M.,(2011) "*Eleusine indica* Possesses Antioxidant, Antibacterial and Cytotoxic Properties", *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 965370, 6 pages, 2011. <https://doi.org/10.1093/ecam/nep091>
- Hamzah A, Hapsari I R. and Priyadarshini R. (2017). The potential of wild vegetation species of *eleusine indica* L. and *Sonchus arvensis* L. for phytoremediation of Cd-contaminated soil. *J. Degrade. Min. Land Manage.* pp. 797-805.
- Anoliefo G.O. and Vwioko D.E. (1995). Effects of spent lubricating oil on growth of *Capsicum annum* L. and *Lycopersicon esculentum*, Mill. *Environmental Pollution*. 88:361–364.

- Anoliefo, G. O., Ikhajiagbe, B, Okonofhua, B. O. and Diafe, F. V. (2006) Eco-taxonomic distribution of plant species around motor mechanic workshops in Asaba and Benin City, Nigeria: Identification of oil tolerant plant species. *African Journal of Biotechnology*. 5 (19), pp. 1757-1762.
- Armburst D.V. (1986). Effect of particulate (dust) on cotton growth, photosynthesis, and respiration. *Agron Journal* **78**:1078-1081
- Ashenden, T.W. and Mansfield, T.A. (1978). Extreme pollution sensitivity of grasses when SO<sub>2</sub> and NO<sub>2</sub> are present in atmosphere together. *Nature*. **273**: 142-143.
- Asati A., Pichhode M. and Nikhi K. (2016) Effect of Heavy Metals on Plants: An Overview. *International Journal of Application or Innovation in Engineering & Management*. **5**(3); 2319 - 4847
- Alina-Maria A., Cuciureanu A., Kim L. and Batrinescu G. (2016) heavy metal contamination of roadside soil near sun highway in Romania. *IncdEcoind – International Symposium – Simi 2016 “The Environment and the Industry”*, *Proceedings Book*. 351-358pp.
- Black, V.J. and Unsworth, M.H., (1980). Stomatal responses to sulphur dioxide and vapor pressure deficit. *Journal of Experimental Botany* **31**:667- 677.
- Çelenk F and Kiziloğlu T. (2015), Distribution of Lead Accumulation in Roadside Soils: A Case Study from D 100 Highway in Sakarya, Turkey. *International Journal of Research in Agriculture and Forestry*. **2**(5):1-10.

- Chen J, Huang H, Wei S, Huang Z, Wang X, Zhang C. (2017) Investigating the mechanisms of glyphosate resistance in goosegrass (*Eleusine indica* (L.) Gaertn.) by RNA sequencing technology. *Plant Journal*. **89**(2):407-415. doi: 10.1111/tpj.13395.
- Cookson, S.J., Radziejwoski, A. and Granier, C. (2006), Cell and leaf size plasticity in *Arabidopsis*: what is the role of endoreduplication?. *Plant, Cell & Environment*, **29**: 1273-1283. <https://doi.org/10.1111/j.1365-3040.2006.01506.x>
- Crittenden P. D. and Read D. J. (1978) The effects of air pollution on plant growth with special reference to sulphur dioxide. *New phytologist*. **80**:49-62
- Darrall, N.M. (1989). The effect of air pollutants on physiological processes in plants. *Plant, Cell and Environment*. **12**:1-30.
- Desai A.V., Patil V. M., Patil S.S., Yeligar C. V. and Patil S.V. (2017) Phytochemical investigation of *Eleusine indica* for In-vivo diuretic and in-vitro anti-urolithiatic Activity. *World Journal on Pharmaceutical Research*. **6**(8):1292 – 1304.
- Dítě Z., Dítě D. and Feráková V. (2019) *Eleusine indica* (L.) Gaertn., new species of the adventive flora of Slovakia Thaiszia. *Journal of Botany*. **29**(1):077-085 <http://www.bz.upjs.sk/thaiszia>
- Garba, S. T., Osemeahon, A. S., Maina, H. M. and Barminas, J. T. (2012) Ethylenediaminetetraacetate (EDTA)-Assisted phytoremediation of heavy metal contaminated soil by *Eleusine indica* L. Gaertn. *Journal of Environmental Chemistry and Ecotoxicology*. **4**(5):103-109.

Glover B. J. and Martin C. (2000) Specification of Epidermal Cell Morphology. *Advances in Botanical Research*.**31**:193 -217.

Glover B. J. (2000) Differentiation in plant epidermal cells, *Journal of Experimental Botany*. **51**(344):497–505, <https://doi.org/10.1093/jexbot/51.344.497>

Gheorghe J. F. and Ion B. (2011). The Effects of Air Pollutants on Vegetation and the Role of Vegetation in Reducing Atmospheric Pollution. In:*The Impact of Air Pollution on Health, Economy, Environment and Agricultural Sources*, Khallaf M. K(ed), IntechOpen, DOI: 10.5772/17660. Available from: <https://www.intechopen.com/books/the-impact-of-air-pollution-on-health-economy-environment-and-agricultural-sources/the-effects-of-air-pollutants-on-vegetation-and-the-role-of-vegetation-in-reducing-atmospheric-pollu>

Hansen A. (1980): Eleusine Gaertner. – In:*Flora Europaea*. Tutin T.G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M. and Webb D. A. (eds.): Volume 2, p258–259. – Cambridge University Press, Cambridge., 489pp.

Hiller, E., Pilková, Z. and Filová, L. (2021) Concentrations of selected trace elements in surface soils near crossroads in the city of Bratislava (the Slovak Republic). *Environmental Science and Pollution Research* **28**:5455–5471. <https://doi.org/10.1007/s11356-020-10822-z>

Ikhajiagbe B., Igiebor F.A., Omoregie G.O., Ekhatior P.O. and Anoliefo G.O. (2020) Growth and remediation performance of *Eleusine indica* obtained from oil-polluted soils in Benin metropolis after exposure to grey water. *Journal of Materials and Environmental Science*.**11**(8)1304-1314.

- Isichei A. O. and Akin- Fajiyeye M. A.(2013) Plant Invasions in Nigeria. (Chapter 20) In: *Nigerian Forests: Protection and Sustainable Development*. Okon, E, Bown, D. and Isichei, A. (eds). *JABU Environmental Symposium Series*. 320pp.
- Jahan S.and Iqbal M. Z. (1992) Morphological and Anatomical Studies of Leaves of Different Plants Affected by Motor Vehicles Exhaust. *Journal of Islamic Academy of Sciences*5(1):21-23.
- Junaid A., Khan A. S., Khan S. H., (2013) Heavy metals contamination in roadside soil near different traffic signals in Dubai, United Arab Emirates. *Journal of Saudi Chemical Society*, **17**(3):315-319, ISSN 1319-6103, (<https://www.sciencedirect.com/science/article/pii/S1319610311000846>)
- untawong, N. and Suwanwarer, P. (1997). Effects of SO<sub>2</sub> on leaf anatomy, chlorophyll content and sulphur accumulation. *Journal of the National Research Council*29:1-13
- Kabata-Pendias A. (2001) “Trace Elements in Soils and Plants”, CRC Press, Boca Raton, Fla, USA, 3rd edition. 403pp.
- Kasana M S and Mansfield T A (1986) Effects of air pollutants on the growth and functioning of roots. *Proceedings. Indian Academia of. Science.* **96**(6) 429-441.
- Kosová, K., Vítámvás, P., Prášil, I. T., and Renaut, J. (2011). Plant proteome changes under abiotic stress- contribution of proteomics studies to understanding plant stress response. *J. Proteomics* **74**:1301–1322. doi: 10.1016/j.jprot.2011.02.006.
- Kulshreshtha, K., Farooqui, A., Srivastava, K., Singh, S.N., Ahmad, K.J. and Behl, H.M. (1994a). Effect of diesel pollution on cuticular and epidermal features of *Lantana*

*camara* Linn. And *Syzygiumcumini* Linn. (Skeels). *Journal of Environmental Science and Health*. **29** (2): 301-308.

Kulshreshtha, K.; Srivastava, K. and Ahmad, K. J.(1994b). Effect of automobile exhaust pollution on leaf surface structures of *Calotropis procera*L. and *Nerium indicum* L. *Journal of Botanical Taxonomy and Geological Botany*, **105** :185-189.

Kulshreshtha, K., Yunus, M., Dwivedi, A.K. and Ahmad, K.J. (1980). Effect of air pollution on the epidermal traits of *Jasminum sambac*Ait. *New Botanist*, **7**: 193-197.

Lagerwerff J.V. and Specht A. W. (1970) contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc. *Environmental Science & Technology* **4** (7)583-586  
DOI: 10.1021/es60042a001

Larcher W. (1995). *Physiological Plant Ecology*. Berlin: Springer. 514pp.

Li, B., Xing, D., Zhang, L., (2007). Involvement of NADPH oxidase in sulfur oxide- induced oxidative stress in plant cells. *National Product Reports***6**: 628- 534.

Li YM, Wang SJ, Chen QB. Potential of thirteen urban greening plants to capture particulate matter on leaf surfaces across three levels of ambient atmospheric pollution. *Int J Env Res Pub Health* 2019;**16**:402.

MacFarlane G.R, M.D Burchett, Photosynthetic Pigments and Peroxidase Activity as Indicators of Heavy Metal Stress in the Grey Mangrove, *Avicennia marina* (Forsk.) Vierh., *Marine Pollution Bulletin*, Volume 42, Issue 3, 2001, Pages 233-240, ISSN

0025-326X,

[https://doi.org/10.1016/S0025-326X\(00\)00147-8](https://doi.org/10.1016/S0025-326X(00)00147-8).

(<https://www.sciencedirect.com/science/article/pii/S0025326X00001478>)

Marx J. L.(1975) Air pollution: Effects on plants. *Science***187** (4178): 731- 739.

Naser, H., Sultana, S., Gomes R. and Noor, S. (2012). Heavy Metal Pollution of Soil and Vegetable Grown Near Roadside at Gazipur. *Bangladesh Journal of Agricultural Research*, **37**(1):9-17. <https://doi.org/10.3329/bjar.v37i1.11170>

Ogbe F.M.D. and Bamidele J. F. (2006) Incidence and Spread of an invasive weed *Mimosa invisa* Mart. in Benin City Metropolis, Nigeria. *International Journal of Botany***2**(3): 336-339

Okereke C. N. and E. I. Mbaekwe (2011) Some aspects of the Biology and Ecology of *Mimosa invisa* Mart. around Agu-Awka, Anambra State Nigeria. *Nigerian Journal of Botany***24**(1): 67- 80

Olszyk DM, Tibbitts TW. (1981). Stomatal response and leaf injury of *Pisum sativum* L. with SO<sub>2</sub> and O<sub>3</sub> exposures. *Plant Physiology***67**:539-544.

Omoriegie, G.O., Ikhajiagbe, B. and Sunday, C.S (2017). Leaf Epidermal Changes in Three Common Crop Plants Found in A Gasflared Community in Oben Village, Edo State Nigeria. *Ethiopian Journal of Environmental Studies & Management* **10**(2): 241 – 250.

Pal A, Kulshreshtha K, Ahmad K. J. and Behl H. M., (2002) Do leaf surface characters play a role in plant resistance to auto-exhaust pollution?. *Flora - Morphology, Distribution, Functional Ecology of Plants*, **197**(1):47-55. <https://doi.org/10.1078/0367-2530-00014>.

- Pattanayak S, Maity D (2017) Use of *Eleusine indica* (L.) Gaertn. (Kechilaghas) as an antipyretic medicine of herbivores. *Exploratory Animal and Medical Research*.7(1): 94-96.
- Rai, P. and Mishra, R.M. (2013). Effect of urban air pollution on epidermal traits of road side tree species, *Pongamiapinnata* (L.) Merr. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 2(6):04-07.
- Rai P. K. (2016) Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. *Journal of Asia-Pacific Biodiversity*9(1):47-55.
- Rai P. K. and Panda L. S.(2014) Leaf dust deposition and its impact on biochemical aspect of some roadside plants in Aizawl, Mizoram, India: an Indo-Burma hot spot region. *Air quality atmosphere and health*.7:93 -101.
- Rai pk and Panda L S 2015: roadside plants as bio indicators of air pollution in an industrial region, Rourkela, India. *International journal of advancements in Research and Technology* 4:19-41.
- Raina, A.K. and Sharma, A. (2006). Assessment of air pollution and its impact on the leaves of some plant species. *Pollution Research*. 25:543-547.
- Raina, A.K and Bala, C. (2011). Effect of vehicular pollution on *Durantarepens* L. in Jammu city. *Journal of Applied and Natural Science*, Vol. 3(2), pp. 211-218, 2011
- Raina, A.K. and Aggrawal, B. (2004). Effect of vehicular exhaust on some trees in Jammu. *Journal of Industrial Pollution Control*, Vol. 20(2), pp. 229-232.

- Rangkuti MN. 2003. Kemampuanmenjeraptimbel (Pb) pada daunbeberapajenistanamanpenghijauanjalantolJagorawi: analisisstruktur anatomi dan histokimia [Thesis]. Bogor: BogorAgricultural Univ
- Saadabi, A. M. A. ( 2011). Effects of auto-exhaust pollution on the micro-morphology and leaf epidermal features of ornamental plants in Khartoum, Sudan. *Journal of Applied Sciences Research*, v. 7, n. 3, p. 270-273.
- Saadabi, A.M.A., El-Amin, A. (2011). Effects of Environmental Pollution (Auto-Exhaust) on the MicroMorphology of Some Ornamental Plants from Sudan. *Environmental Research Journal*, 5(2): 38-41.
- Salgare, S.A. and Thorat, V.B. (1990). Effect of auto exhaust pollution at Andheri (West), Bombay on the micromorphology of some trees. *Journal of Ecobiology*, 2(4): 267-272. [24].
- Samal, Alok and Santra, Subhas. (2002). Air quality of Kalyani township (Nadia, West Bengal) and its impact on surrounding Vegetation. *Indian journal of environmental health*. 44. 71-6.
- Singh SK, Rao DN (1983) Evaluation of plants for their tolerance to air pollution. In: *Proceedings of the Symposium on Indian Association for Air Pollution Control*, New Delhi, pp 218–224

Sharma, M. and Roy, A.N. (1995). Effect of automobile exhaust on the leaf epidermal features of *Azadirachta indica* and *Dalbergiasissoo*. *Int. Journal of Mendel*, 12 (1-4): 18-19

Shannigrahi A S., Fukushima T and Sharma R C 2004 Anticipated Air Pollution Tolerance of Some Plant Species Considered for Green Belt Development In And Around An Industrial/Urban Area In India: An Overview *international journal of environmental studies* vol 61 issue 2 Pages 125-137

Shrivastava, R. and Mishra, A. (2018). Air pollution induced changes in foliar micro-morphology of road side shrub species *Thevetiaperuviana* and *Plumeriaalbain* Rewa City, MP, India. *International Research Journal* Vol. 7(3), 1-7. [28].

Shrivastava, R. and Prakash, J. (2017). Effect of Vehicular Pollution on Micro-Morphological Structure of *Hibiscus Rosa Sinensis*. *International Journal of Engineering Technology Science and Research* Volume 4(12), 521-527. [29].

Singh SN, Verma A. Phytoremediation of air pollutants: a review. In: Singh SN, Tripathi RD, editors. *Environmental bioremediation technologies*. Berlin, Heidelberg: Springer; 2007:293-314.

Snehlata, D. Shah and Poonam (2010). Comparative ecomorphological studies on *Daturaalbalinn*. Effects of aiming along roadsides and railway tracks. *Environ. We Int. Sc. Tech.* 5 : 156-162.

- Swami, Abhishek. (2018). Impact of Automobile Induced Air Pollution on roadside vegetation: A Review. *ESSENCE International Journal for Environmental Rehabilitation and Conservation*. 9. 101-116. 10.31786/09756272.18.9.1.113.
- Taylor 1974 Air pollution effects Influenced by Plant-Environmental Interaction. A Symposium Series; American Chemical Society: Washington, DC
- Tiwari, S. (2012). Air Pollution Induced changes in Foliar Morphology of two shrub species at Indore city, India. *Research Journal of Recent Sciences*, Vol.2, 195-199.
- Tripathi, A.K., Gautam, M., 2007. Biochemical parameters of plants as indicators of air pollution. *Journal of Environmental Biology* 28, 127- 132.
- Uka, U.N., Belford, E.J.D. &Hogarh, J.N. Roadside air pollution in a tropical city: physiological and biochemical response from trees. *Bull Natl Res Cent* **43**, 90 (2019).  
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