

TOXICOLOGICAL EFFECTS OF DIESEL-CONTAMINATED SOIL ON THE INTERNAL STRUCTURE OF LEGUME CROPS

Chinedu Emmanuel Okafor

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria

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Abstract: The effect of diesel on the leaf, stem and root anatomy of Pigeon pea (*Cajanus cajan*), African Yam bean (*Sphenostylis stenocarpa*) and Bambara groundnut (*Vigna subterranean*) was investigated. Seeds of the plants were cultivated on soil contaminated with diesel. 5kg of the soil was treated with 50ml, 100ml, 150ml, and 200ml of diesel to obtain 1%, 2%, 3%, and 4% level of contamination while untreated soil (0%) served as the control. Epidermal peels of the leaves were obtained by boiling parts of the fresh leaves of the legume plant samples in concentrated nitric acid in a water bath for about 2-3 minutes. Plant parts (stem and root) were washed in water and sectioned with a sipcon rotaty microtome. The contaminant affected the number of stomata on the leave s of the plants. It led to the distortion of the epidermal cells of the leaves, and the mesophyll cells hence affecting its function. These effects gradually increased as the percentage of the pollutant increased in the soil. On the stem, the pollutant aff ected arrangement of the vascular bundles and the pith and this also had deleterious consequences on the functions of these tissues. The effect of the diesel fuel pollution on the root anatomy was noticed in the reduction of the air spaces in the root as the percentage of the pollutant increased. This observation confirms earlier reports tha t crude oil and its derivatives has deleterious effect on the plants.

Keywords: Diesel, Leaf, Stem, Root, Anatomy, Polluted Soil.

Introduction

Pollution from petrochemical hydrocarbons is one of the serious problems all around the world. Alkio *et al* (2005) reported that the study of abiotic stress responses in plants has become increasingly more important in agriculture, forest management, and ecosystem restoration strategies because of continuous increase in environmental pollution. At relatively low concentrations, petroleum derivatives in general are phytotoxic to plants (Zarinkamar *et al.*, 2013). Petroleum hydrocarbons can reach the soil from many sources including oil spillages by transportation vessels, pipeline leakages, automobile servicing stations, fuel stations and other anthropogenic activities (Eze *et al.*, 2013). The increasing use of diesel in diesel engines of cars, industrial trucks and generators has led to a marked increase in the demand for diesel in Nigeria (Ogbo, 2009). It can affect vegetation growth by stimulating the soil bacteria to consume soil nutrients and reduce soil oxygen (Gill *et al.*, 1992). Contamination by diesel can kill the roots, and this prevents the plant from taking up water and other nutrients (Ogbo, 2009). Omosun *et al.* (2008) reported irregular epidermal cell, increased sinuosity of the anticlinal cell, and tangentially flattened stem and root cortical cells of *Amaranthus hybridus* as a result of contamination

of the soil by huge amounts of crude oil. Abdulrahman *et al.* (2011) reported reduction in stomatal frequency due to DPK contamination of the soil on *Ipomoea aquatic*. Ghasemi *et al.* (2014) reported that contamination of soil by crude oil caused deposition of oil droplets in the cells of *Vicia ervilia*. Omosun *et al.* (2009) reported that contamination of soil by crude caused reduction in stomatal frequency, tissue damage and breakdown in the stem and root of four *Mucuna* species. Naidu (2001) has reported that oil refinery effluents causes four types of symptoms in plants which are typical of nutrient deficiencies – yellowing of foliage, chloro-necrosis, wilting and defoliation. Pigeon pea (*Cajanus cajan* L.) is predominantly grown and consumed in India. It is also known as red gram, arhar, tur dal which belongs to the family of Leguminosae (Ghadge *et al.*, 2008). Pigeon pea was long considered to be one of the two species of the genus *Cajanus*. However, this genus is now thought to be congeneric with *Atylosia* and *Endomallus*, and also includes species of *Rhynchosia* and *Dunbaria*. *Cajanus* is now recognised as having 32 species. It is a leguminous shrub that can attain a height of 5 m. Pigeon pea probably evolved in South Asia and appeared around 2000 BC in West Africa, which is considered a second major centre of origin (Sharma *et al.*, 2011). African yam bean (*Sphenostylis stenocarpa*) is a vigorously climbing herbaceous vine whose height can reach 1.5-3 metres or more depending on the height of the stakes and cultivar (Adewale and Odoh, 2013). The crop produces many branches which also twine strongly on available stakes. The vegetative growing stage is noted with profound production of trifoliolate leaves. Four to ten flowers are arranged on long peduncles, which are usually on the primary and the secondary branches. Its inflorescence is raceme and exhibits acropetal mode of floral maturation (Adewale, 2011). The large and excellently attractive flowers blends pink with purple, the standard petals slightly twist backward on itself at anthesis. According to the observation of Popoola *et al.* (2011) using 25 AYB accessions, the pollen grains had tricolporate, fenestrate and scabrate exine. The stem of the plant produces small underground tubers of various sizes and shapes (Adewale and Dumet, 2011). Bambara groundnut (*Vigna subterranean* L.) a herbaceous, intermediate annual plant, with creeping stems at ground level which belongs to the family Leguminosae and subfamily Papilionoideae and it is of African origin (Borget, 1992) with subterranean fruit-set which is widespread south of the Sahara (Ocran *et al.*, 1998). The general appearance of the plant is bunched leaves arising from branched stems which form a crown on the soil surface. Stem branching begins very early, about 1 week after germination, and as many as 20 branches may be produced and each branch is made up of internodes (Akpalu, 2010). It serves as an important source of protein in the diets of a large percentage of the population in Africa, particularly to poorer people who cannot afford expensive animal protein (Bamshaiye *et al.*, 2011). Though, grown extensively in Nigeria, it is still one of the lesser utilized and unexploited legume (Oguntunde, 1985; Enwere, 1998).

Materials and Methods

Study Area

The study was conducted in a potted plant and was kept in the botanical garden of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. Umudike is located approximately at latitude 05° 29' N, longitudes 07° 32' E.

Umudike falls within the rainforest zone of southeastern Nigeria with a mean altitude of

123m. daily mean temperature ranges from 23° C to 32°C. The field work was carried out between August and November, 2015.

Design of the Experiment

A factorial arrangement (5x3) was used in a completely randomized design (CRD). The treatments were various diesel concentration in the soil (0%, 1%, 2%, 3%, and 4%), 0% was used as the control. Each treatment was replicated three times.

Collection of Samples

The soil used in this study had no previous history of diesel contamination. The soil sample was obtained from the top soil (0-15 cm) at College of crop and soil sciences (CCSS) demonstration farms within the campus of Michael Okpara University of Agriculture, Umudike. The seeds of *C. cajan*, *V. subterranean* and *S. stenocarpa* used were obtained from the Seed Bank of National Root Plant Research Institute, located in Umudike, Ikwuano LGA of Abia state.

Soil Treatment

Soil samples were homogenized and sieved. The contamination with diesel was done by thoroughly mixing the soil in their respective 5 litre plastic buckets. Soil of 5kg was treated with 50ml, 100ml, 150ml, and 200ml of diesel to obtain 1%, 2%, 3%, and 4% diesel contamination. Each treatment including the control (0%) was replicated three times.

Anatomical Studies

Anatomical sections were obtained using the modified method described by Edeoga *et al.*, (2007). Some mature and parts of the leaves, stems and roots of the three legume species (*C. cajan*, *V. subterranean* and *S. stenocarpa*) were collected. These plant parts were fixed in FAA (Formalin, acetic acid and alcohol in ratio of 1:1:18 respectively). These plant parts were washed in water and sectioned with a Sipcon Rotaty Microtome. The sections (25nm) were first stained with 2 drops of alcian blue for three minutes. The alcian blue stain is washed off and the sections were counter stained with safranin solution in about two minutes, and then dehydrated with pure xylene at intervals for few seconds. The sections were finally mounted on slides using Canada balsam. A hot plate at 40 °c was used to dry the slides.

Epidermal Peels

The method of Edeoga *et al.* (2008) was used. Epidermal peels were obtained by boiling parts of the fresh leaves of the legume plant samples in concentrated nitric acid in a water bath for about 2-3 minutes. The plant samples were then carefully washed in water and the lower and upper epidermis teased from the mesophyll using dissecting needles and forceps. The epidermal peels were stained with safranin solution for about three minutes and washed off with water before mounting in glycerine.

Photomicrography

Observations and photomicrographs of the slides of the anatomical sections and the epidermis were taken using Novel Digital microscope (Scope Image 9.0) at the Histology Laboratory, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Results

Results of the study are shown in plates 1and 2 for leaf anatomy, plates 3-5 for stem anatomy and plates 6-7 for root anatomy.

The pollutant affected the number of stomata on the leaves of the plants. This pollution also led to the distortion of the epidermal cells of the leaves, and the mesophyll cells hence affecting its function. These effects gradually increased as the percentage of the pollutant increased in the soil. On the stem, the pollutant affected arrangement of the vascular bundles and the pith and this also had deleterious consequences on the functions of these tissues. These effects it was observed increased as the percentage of the contaminant increased.

The effect of the diesel fuel pollution on the root anatomy was noticed in the reduction of the air spaces in the root as the percentage of the pollutant increased.

Plate 1a: Leaf epidermis of *S. stenocarpa* growing on 0% (control) soil showing the stomata and proper arrangement of the epidermal cells (x400).

Plate 1b: Leaf epidermis of *S. stenocarpa* growing on 1% diesel fuel polluted soil showing lesser number of stomata and partial disorder in the arrangement of the epidermal cells (x400).

Plate 1c: Leaf epidermis of *S. stenocarpa* growing on 2% diesel fuel polluted soil showing lesser number of stomata and obvious disorder in the arrangement of the epidermal cells (x400).

Plate 1d: Leaf epidermis of *S. stenocarpa* growing on 3% diesel fuel polluted soil showing few numbers stomata and a very serious disorder in the arrangement of the epidermal cells (x400). **Plate 1e:** Leaf epidermis of *S. stenocarpa* growing on 4% diesel fuel polluted soil showing very few numbers of stomata and complete disorder in the arrangement of the epidermal cells (x400).

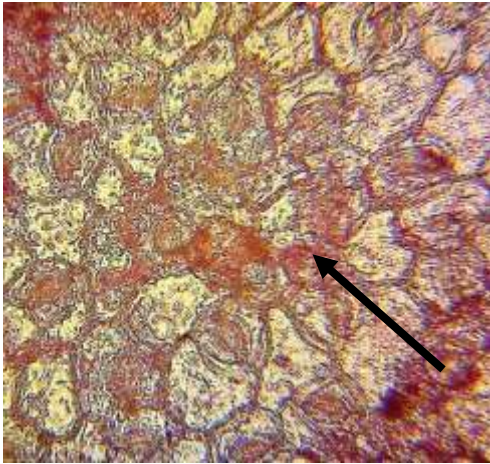


Plate 2a

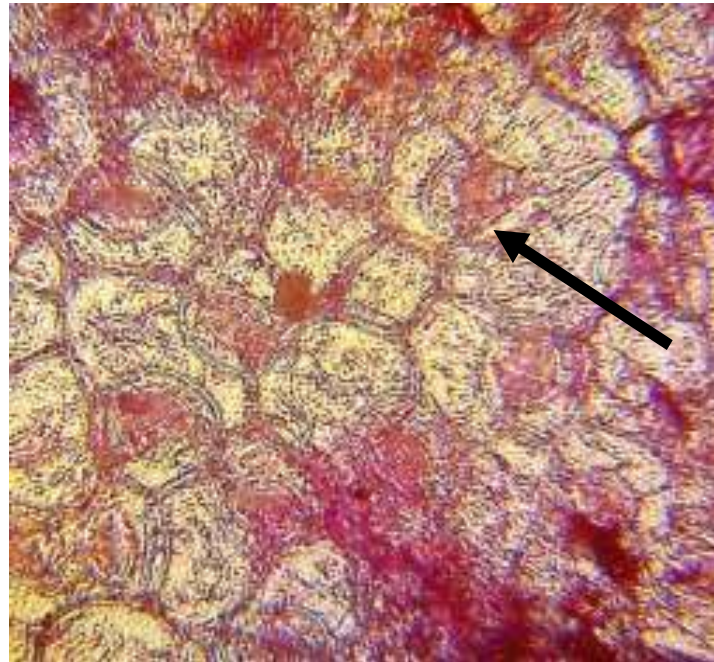


Plate 2b

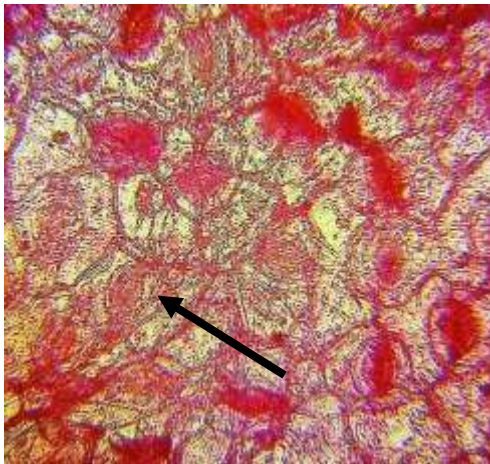


Plate 2c

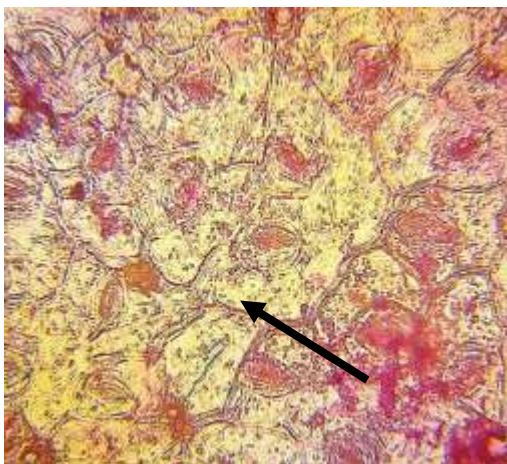


Plate 2e

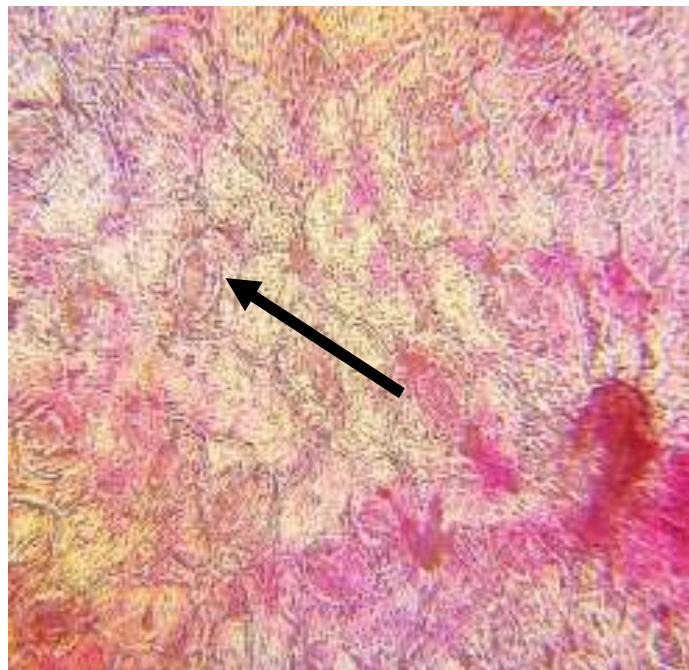


Plate 2d

Plate 2a: Leaf epidermis of *V. subterrean* growing on 0% (control) soil showing the stomata and proper arrangement of the epidermal cells (x400). **Plate 2b:** Leaf epidermis of *V. subterrean* growing on 1% diesel fuel polluted soil showing lesser number of stomata and partial disorder in the arrangement of the epidermal cells (x400). **Plate 2c:** Leaf epidermis of *V. subterrean* growing on 2% diesel fuel polluted soil showing lesser number of stomata and obvious disorder in the arrangement of the epidermal cells (x400). **Plate 2d:** Leaf epidermis of *V. subterrean* growing on 3% diesel fuel polluted soil showing few numbers stomata and a very serious disorder in the arrangement of the epidermal cells (x400). **Plate 2e:** Leaf epidermis of *V. subterrean* growing on 4% diesel fuel polluted soil showing very few numbers of stomata and complete disorder in the arrangement of the epidermal cells (x400).



Plate 3a

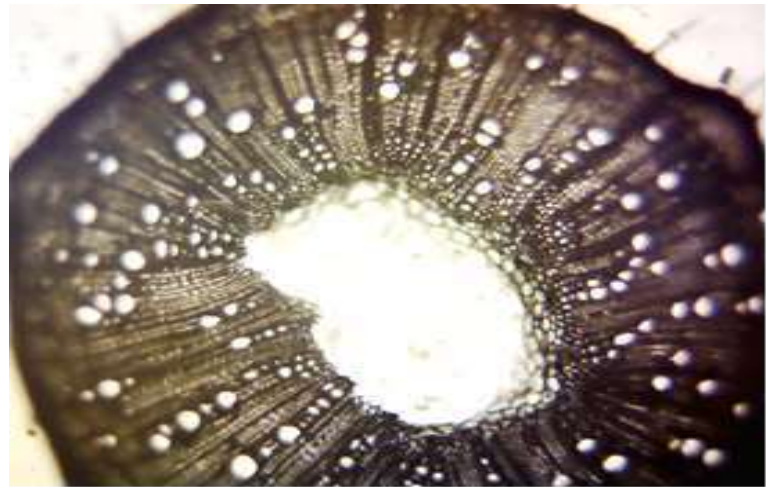


Plate 3b



Plate 3c

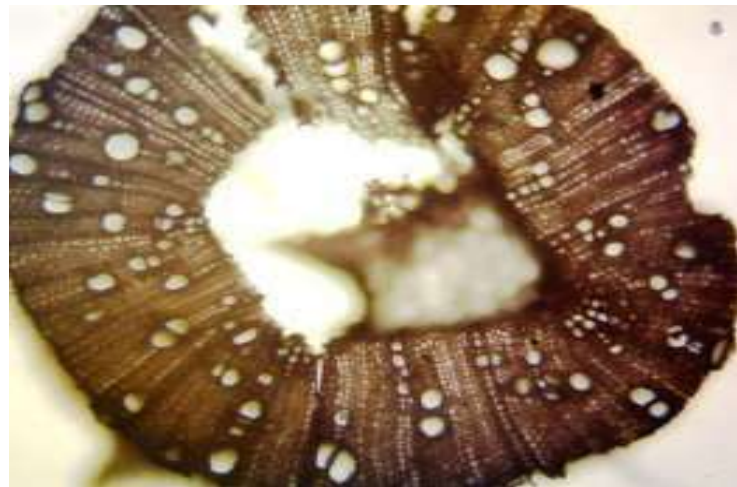


Plate 3d

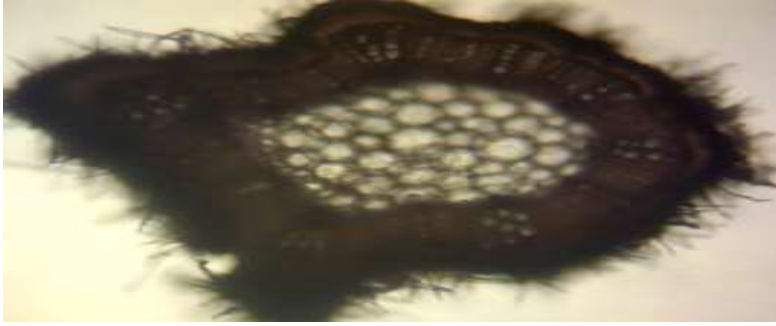
**Plate 3e**

Plate 3a: T/S of *C. cajan* stem growing on 0% (control) soil showing normal arrangement of the vascular bundles and air spaces (x100). **Plate 3b:** T/S of *C. cajan* stem growing on 1% diesel fuel polluted soil showing partially distorted mid-rib (x100). **Plate 3c:** T/S of *C. cajan* stem growing on 2% diesel fuel polluted soil showing vascular bundle and air spaces greatly reduced (x100). **Plate 3d:** T/S of *C. cajan* stem growing on 3% diesel fuel polluted soil showing distorted mid-rib and vascular bundle (x100). **Plate 3e:** T/S of *C. cajan* stem growing on 4% diesel fuel polluted soil showing clogged vascular bundle, mid-rib and greatly reduced air spaces (x100).

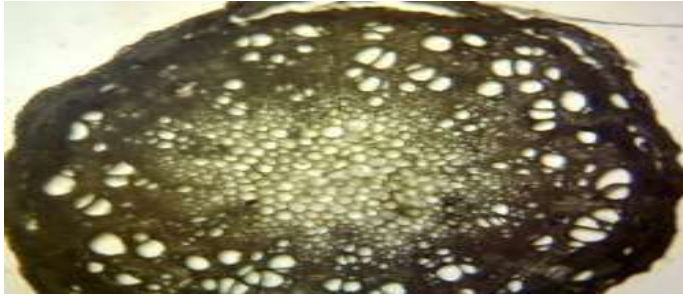


Plate 4a



Plate 4c



Plate 4e

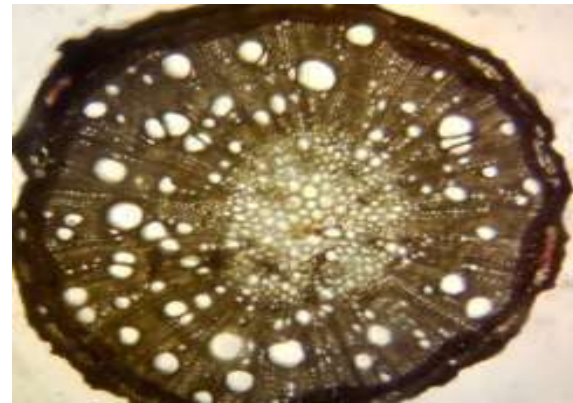


Plate 4b

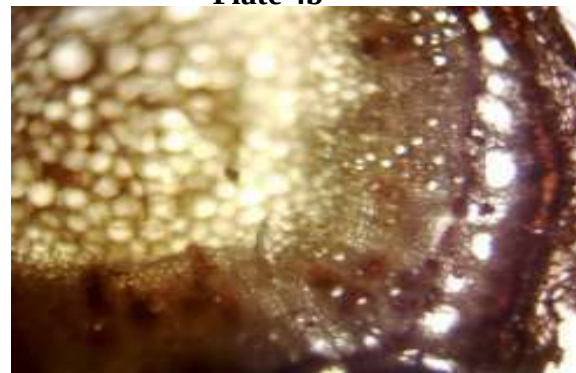


Plate 4d

Plate 4a: T/S of *S. stenocarpa* stem growing on 0% (control) soil showing normal arrangement of the vascular bundles and air spaces (x100). **Plate 4b:** T/S of *S. stenocarpa* stem growing on 1% diesel fuel polluted soil showing partially distorted mid-rib (x100). **Plate 4c:** T/S of *C. cajan* stem growing on 2% diesel fuel polluted soil showing vascular bundle and air spaces greatly reduced (x100). **Plate 4d:** T/S of *S. stenocarpa* stem growing on 3% diesel fuel polluted soil showing distorted mid-rib and vascular bundle (x100). **Plate 4e:** T/S of *S. stenocarpa* stem growing on 4% diesel fuel polluted soil showing clogged vascular bundle, mid-rib and greatly reduced air spaces (x100).

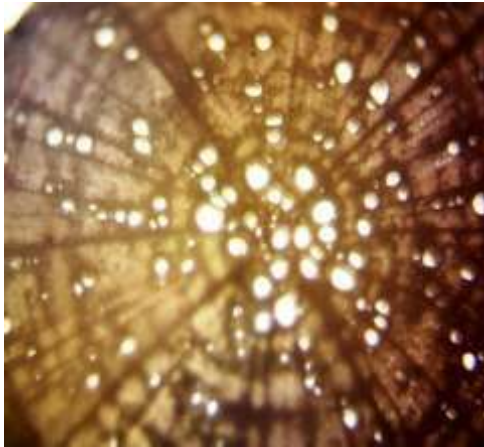


Plate 5a

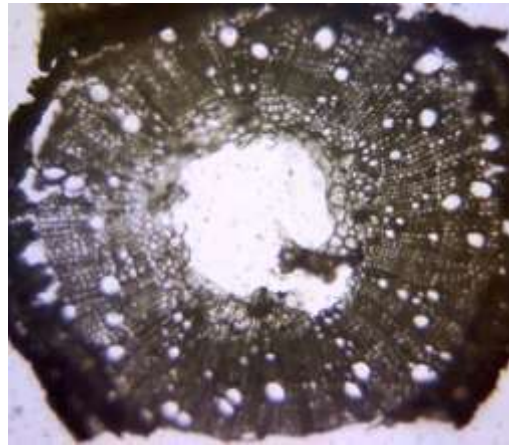


Plate 5b



Plate 5c



Plate 5d



Plate 5e

Plate 5a: T/S of *C. cajan* root growing on 0% (control) soil (x100). **Plate 5b:** T/S of *C. cajan* stem growing on 1% diesel fuel polluted soil showing rib (x100). **Plate 5c:** T/S of *C. cajan* root growing on 2% diesel fuel polluted soil showing reduced intercellular air spaces (x100). **Plate 5d:** T/S of *C. cajan*

root growing on 3% diesel fuel polluted soil showing distorted pit, and reduced intercellular air spaces (x100). **Plate 5e:** T/S of *C. cajan* stem growing on 4% diesel fuel polluted soil showing greatly reduced intercellular air spaces (x100).

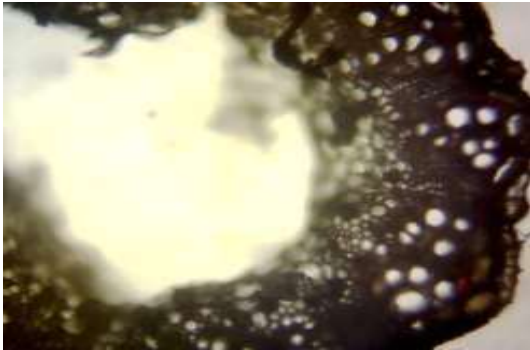


Plate 6a

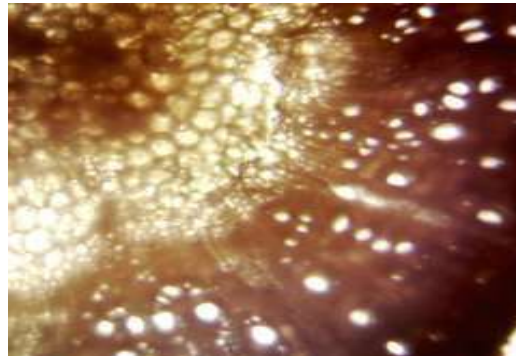


Plate 6b



Plate 6c



Plate 6d

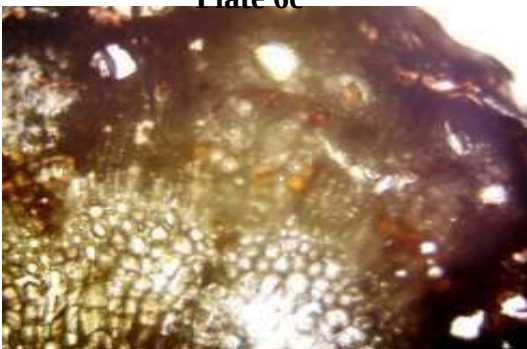


Plate 6e

Plate 6a : C/S of *S. stenocarpa* Root growing on 0% control soil (x100). **Plate 6b :** C/S of *S. stenocarpa* root growing on 1% diesel fuel polluted soil showing numerous intercellular air spaces (x100). **Plate 6c:** C/S of *S. stenocarpa* root growing on 2% diesel fuel polluted soil showing reduction in intercellular air spaces (x100). **Plate 6d:** C/S of *S. stenocarpa* root growing on 3% diesel fuel polluted soil showing distorted pit, and reduced intercellular air spaces (x100). **Plate 6e:** C/S of *S. stenocarpa* root growing on 4% diesel fuel polluted soil showing greatly reduced intercellular air spaces (x100).

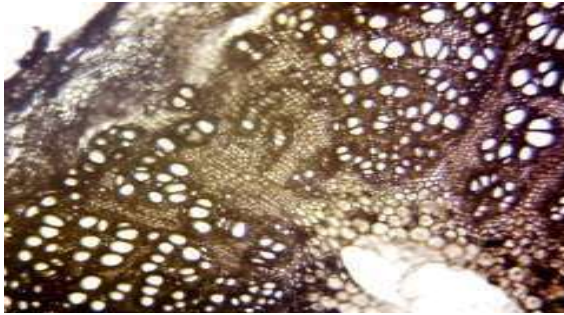
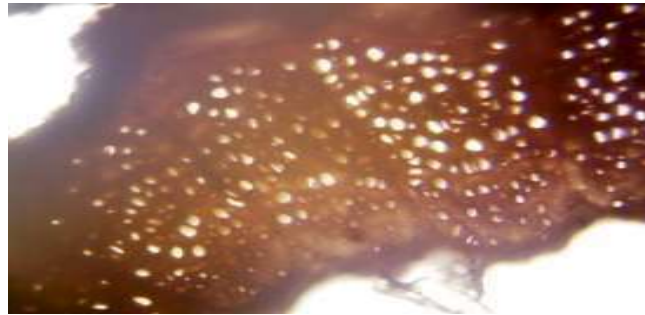
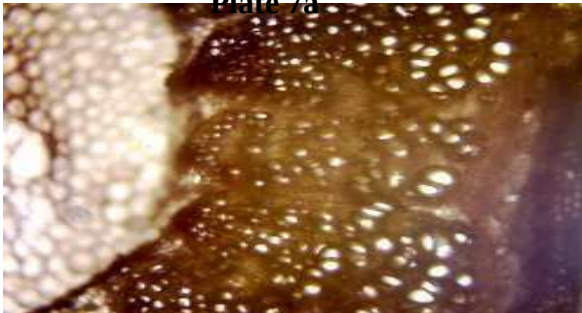
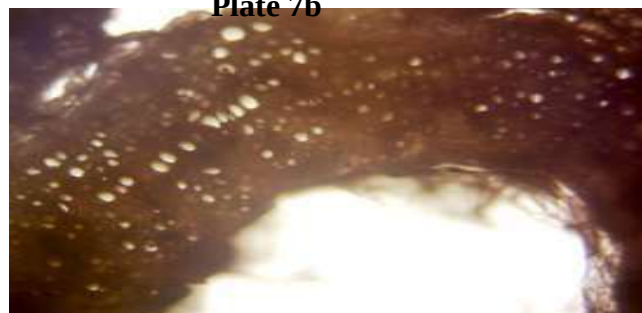
**Plate 7a****Plate 7b****Plate 7c****Plate 7d****Plate 7e**

Plate 7a : C/S of *V. subterrean* Root growing on 0% control soil (x100). **Plate 7b** : C/S of *V. subterrean* root growing on 1% diesel fuel polluted soil showing reduced intercellular air Spaces (x100). **Plate 7c**: C/S of *V. subterrean* root growing on 2% diesel fuel polluted soil showing reduction in intercellular air spaces and distortion in the epidermal cells (x100). **Plate 7d**: C/S of *V. subterrean* root growing on 3% diesel fuel polluted soil showing distorted pit, distortion in the epidermal cells and reduced intercellular air spaces (x100). **Plate 7e**: C/S of *V. subterrean* root growing on 4% diesel fuel polluted soil showing greatly reduced intercellular air spaces, severe damage in the epidermal cells (x100).

Discussion

The reduction in the number of stomata in the leaves as the level of the contamination increased was evident in the leaves of the plants used for this study. This observation agrees with that made by Omosun *et al.* (2009) who reported reduction in the number stomata of *Mucuna* species as a result of crude oil pollution. Reduction in the number of stomata on the leaf epidermis as the levels of pollution by crude oil its derivatives increases (Gill *et al.*, 1992; Vwioko and Fashemi, 2005; Omosun *et al.*, 2008). This observation can be attributed to an adaptation mechanism by the plants to reduce water loss through transpiration (Omosun *et al.*, 2009). It was also observed a distortion in the structure of the

epidermal cells as the pollution level increased and this is in agreement with Gill *et al.* (1992) observation as well as Omosun *et al.* (2009) in the case of crude oil contamination on *Mucuna* species. Distortion, clogging of the vascular bundles and damage of other tissues like the pith were the most noticeable effect the contaminant had on the stem anatomy of the plants. Lorestani *et al.* (2014) reported sensitivity of *Vicia ervilia* stem to different levels of light crude oil pollution and such sensitivity include distortion of the vascular bundles. Omosun *et al.* (2008) reported that as the level of crude oil contaminant increased, the stem cortical cells of *Amarantus hybridus* were flattened. This can be attributed to the response of the plants to decrease in nutrients and nitrogen supply from the soil (Ghasemi *et al.*, 2014).

In the roots, it was observed that the intercellular air spaces reduced gradually as the level of contamination of diesel fuel increased. It was also observed that increased level of diesel contamination caused breakdown of cells and tissues of the root. Similar observations were made by Omosun *et al.* (2009) and Gill *et al.* (1992) and it can be attributed to a phytotoxic response of these plants because of their contact with diesel fuel (Sridhar and Diehl, 2005).

Conclusion and Recommendation

All three plants used for this study showed sensitivity to the contamination from diesel. This sensitivity was observed as various degrees of cell and tissue damage on the epidermal layers of the leaves, stem and root. It was observed that rate at which these plants reacted to this pollution increased with the increase in the level of contamination confirming earlier reports that crude oil and its derivatives have very deleterious effect on plants.

Since pollution from these petroleum hydrocarbons is very common these days, efforts should be made to save the environment, soil and these plants from the irreversible damage caused by these contaminants. Finally, controlled distribution and use of diesel to avoid it spilling uncontrollably will reduce whatever deleterious effect it will have on the environment in general and plants in particular.

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