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Anatomical Response of *Digitaria horizontalis* and *Eluesine indica* Grown in Waste Engine Oil Contaminated Soil

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ABSTRACT: The ability of *Digitaria horizontalis* and *Eluesine indica* grass species to phytoremediate waste engine oil contaminated soil was investigated. Four (4) kg of air dried soil was measured into four (4) kg perforated plastic buckets. Waste engine oil was added to four (4) kg different soil samples to obtain different concentrations on weight basis: 0 % (control), 2 %, 4 %, 7 % and 10 % v/w oil-in-soil and allowed to stand for seven days before transplanting. At the end of 8 weeks of transplanting, the plants were harvested. There were observed changes in some anatomical structures of the two grass species, which could be attributed to the effects of the waste engine oil in the soil in which they were grown. Epidermal cells of the leaves showed that both all the two grass species exhibited irregular epidermal cell shape and sinuous cell walls. In stem anatomy, there was reduction in cell size, the increase in cell shape irregularity, increased thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as the waste engine oil concentration increased. Similarly, there was reduction in root cell size, the increase in cell shape irregularity and increased thickness of cell wall of the parenchyma tissues and break down of cortex region as the waste engine oil concentration increased. In conclusion, the two grass species studied were sensitive to the toxic effects of waste engine oil in the soils and their sensitivity varied with different concentrations of waste engine oil used.

Keywords: *D. horizontalis, E. indica,* Epidermal cells, Sinuous cell walls, Vascular bundles

Introduction

Waste engine oil is defined as any oil that has been produced from crude oil or any artificial oil that has been contaminated by chemical or physical impurities as a result of usage (Ogedegbe *et al*., 2013). Waste engine oil may contain some toxic materials including heavy metals that could affect growth, yield and general performance of plants (Agbogidi and Egbuchua, 2010). Contamination of soil with waste engine oils affects the overall flora and fauna (Ogedegbe *et al*., 2013). Soil polluted with waste engine oils have low oxygen concentration, reduced fertility and reduced microbial composition (Ogedegbe *et al*., 2013).

Heavy metals are important environmental pollutants and many of them are very toxic even in small amount (Abdussalam *et al.,* 2015). Heavy metal toxicity is one of the major abiotic stress leading to hazardous effects in plants (Hossain *et al.,* 2012). Additionally, the commonest soil contaminant in the rural areas where agriculture/farming forms the mainstay of the rural inhabitants is waste engine oil (Agbogidi, 2011). Anoliefo and Edegbai (2006) reported complete inhibition on the seed germination of maize and bean grown on waste pit soil from drilling waste dumps in Kutchalli oil drilling area of Nigeria. The values of plants grown in uncontaminated soil were higher than the values of waste pit soil in combinations with different properties of Kutchalli soil which resulted in growth (germination, height of plants, number of leaves, leaf area) retardation. The waste pit soil was concluded to be toxic to plant growth and development.

Plants have been observed to be far more sensitive to pollution than animals and man and are therefore used as environmental pollution indicators (Rani *et al*., 2006). Studies have recorded changes in plants due to all kinds of environmental pollutants, and most of these works refer to physiological alterations (Patra and Sharma, 2000; Kabata- Pendias and Pendias, 2001). Furthermore, the use of plants to remove pollutants from the environment or render them harmless is phytoremediation (Raskin *et al*., 1996). The phytoremediation mechanisms and efficiency depends on the bioavailability, pollutant type and properties of the soil (Cunningham and Ow, 1996). Before phytoremediation starts, USEPA recommends a study to determine whether plants grown to clean-up pollutants, can or cannot be harmful to people. Most researchers have concluded that grasses and legumes are the best candidates for the phytoremediation process owing to their multiple root ramifications (grasses) and improved rhizhospheral zone (Adams and Duncan, 2002). The grasses and legumes phytoremediation potentials offer increased rhizospheral zone and encourage microbial growth and activity (Merkl *et al*., 2004, 2005). The technology is advantageous to tropical environments due to its low cost component where requisite funds for alternative clean-up measures may be lacking. Investigations of the tropical plants potential for phytoremediation however, are scarce (Oyibo, 2013). The aim of the study was to determine the effects of waste engine oil on the anatomical structures of *D. horizontalis and E. indica* grown in waste engine oil in the soil.

Materials and Methods

Soil samples: The soil samples used were collected from the experimental farm of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria. The waste engine oil was obtained as pooled engine oil from two different major mechanic workshops in the Mechanic Village, Ohiya, Umuahia, Abia State. The plant materials used were collected from bush fallows within Umuahia metropolis. Top soil (0-10 cm) were collected from a marked area, air dried and sieved through a 2 mm mesh gauge (Ogedegbe *et al*., 2013). Samples of the soil (4 kg each) were introduced into different 4-litre perforated plastic buckets after which different concentrations (2%, 4%, 7% and 10%) of waste engine oil were added and labeled as T_1, $T_{2,}$ T_3 and T_4. The mixing was done gradually to ensure thorough and even mixing and each treatment was in three (3) replicates. An untreated soil sample with 0% waste engine oil served as a control (Tc) (Adenipekun *et al.*, 2009). After mixing, the soil samples were left under shade for a period of seven days without planting to ensure uniformity of oil, moisture content, air content, constant temperature and effective activities of soil micro-organisms (Oyibo, 2013). They were artificially irrigated with water before the transplanting of the plant species and left for natural irrigation.

Plant materials: The plant species being investigated were propagated by tiller. The tillers of the different plants were separated and the same height (shoot 15 cm) was selected; the roots were soaked in water for 2 days to improve their rooting ability (Brandt, 2003). The tillers were transplanted into different treated soil samples, each with three tillers and allowed to stand for eight weeks. The plant samples were harvested and soil was washed off with water after which they were separated from the shoot and placed in labeled separate envelops for heavy metal analysis.

Assessment of experimental plant for anatomical changes: Anatomical characters of the leaves, stems and roots and epidermal peel of the leaves were assessed. Anatomical sections were obtained using the modified method described by Edeoga *et al* (2007). Some matured and fresh parts of the leaves, stem and roots of the two grass species were collected. These plants were fixed in FAA (formalin, acetic acid and alcohol in the ratio of 1:1:18). These plant parts were washed in water and sectioned with a Sipcon Rotary Microtome. The sections (25 nm) were first stained with two drops of alcian blue for three minutes. The alcian blue stain was washed off and the sections were counter stained with safranin solution for 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 ^{o}C was used to dry the slides.

Epidermal peels were obtained using the method of Edeoga *et al* (2008). Epidermal peels were obtained by boiling parts of the fresh leaves of the different grass species samples in concentrated nitric acid in a water bath for 2-3 minutes. The plant samples were then carefully washed in water and the lower and upper epidermis teased from the mesophyll using dissecting needle and forceps. The Epidermal peels were stained with safranin solution for three minutes and washed off with water before mounting in glycerin. Observations and photomicrographs of the anatomical sections and the epidermal peels were taken using Novel Digital Microscope (scope image 9.0) at the histology laboratory, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Statistical Analysis: The results were summarised using Descriptive Statistic Package of Microsoft Excel while one-way ANOVA was used to test for statistical differences among the treatments and Tukey's pairwise comparisons test was performed to determine the location of significant difference ($P<0.05$).

Results

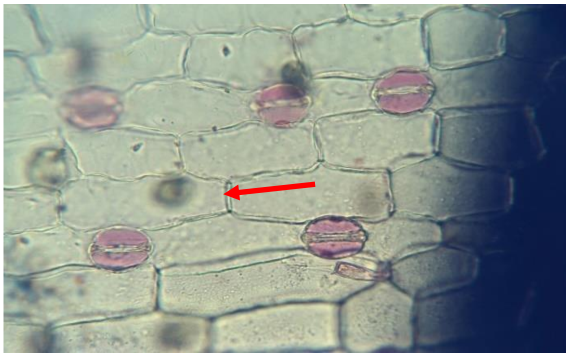
The result of the stomatal frequency of upper and lower epidermis of the two grass species revealed that the value of the grass species grown in the control soil surpassed the value of other plants grown in higher waste engine oil contaminated soil (Table 1). In the leaves of *D. horizontalis*, the stomatal frequency of the lower epidermis was the highest (33.33) at the 0% concentration and least (19.22) at 10% concentration. The stomatal frequency of the upper epidermis was also the highest (27.53) at the 0% concentration and least (17.50) at 10% concentration. In the leaves of *E. indica*, the stomatal frequency of the lower epidermis was the highest (32.76) at the 0% concentration and least (20.98) at 10% concentration. The stomatal frequency of the upper epidermis had the highest (26.00) at the 0% concentration and the least (19.35) at 10% concentration (Table 1).

Table 1: Stomatal Frequency of Upper and Lower Epidermis of the two grass species grown in different waste engine oil polluted soil

Grass species/Concentration (%)	Stomatal Frequency		Mean
D. horizontalis	**Lower epidermis**	**Upper epidermis**	
0	33.33	27.53	30.43
2	30.10	26.50	28.30
4	27.27	24.90	26.09
7	22.61	21.42	22.02
10	19.22	17.50	18.36
Mean	**26.51**	**23.57**	
E. Indica			
0	32.76	26.00	29.38
2	30.00	23.83	26.92
4	29.72	22.59	26.16
7	25.00	20.42	22.71
10	20.98	19.35	20.17
Mean	**27.692**	**22.438**	

Anatomical Changes

Leaf Anatomy: The effects of different concentrations of waste engine oil contamination on the epidermis of the leaves of *D. horizontalis* showed slightly curved epidermal wall of the grasses grown on 0 % waste engine oil contaminated soil (Plate a). The curved epidermal walls of the grasses grown on 2% and 4% waste engine oil contaminated soils were shown in Plates b and c. The slightly sinuous epidermal walls of the grasses grown on 7% and 10% waste engine oil contaminated soils were shown in Plates d and e. On the other hand, the effects of different concentrations of waste engine oil contamination on the epidermis of the leaves of *E. indica* showed slightly sinuous epidermal wall of the grasses grown on0 % waste engine oil contaminated soil (Plate a).The sinuous epidermal walls of the grasses grown on 2% and 4% waste engine oil contaminated soils were shown in Plates b and c. The strongly sinuous epidermal walls of the grasses grown on 7% and 10% waste engine oil contaminated soils were shown in Plates d and e.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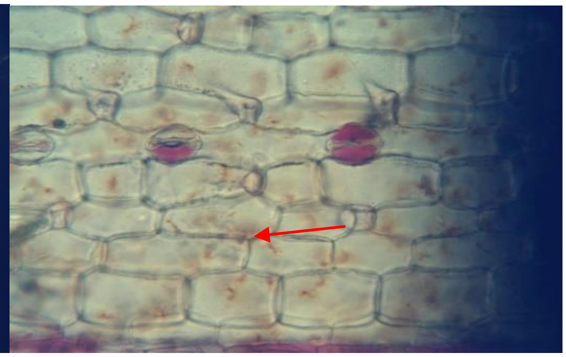
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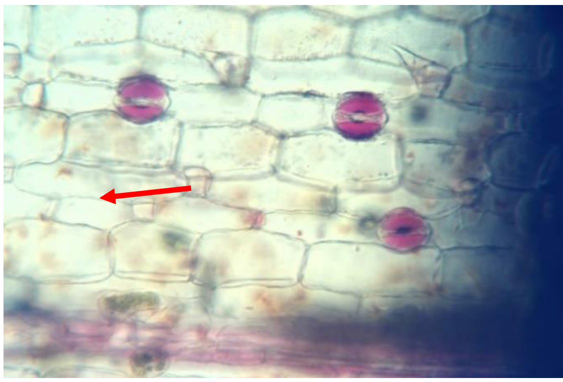
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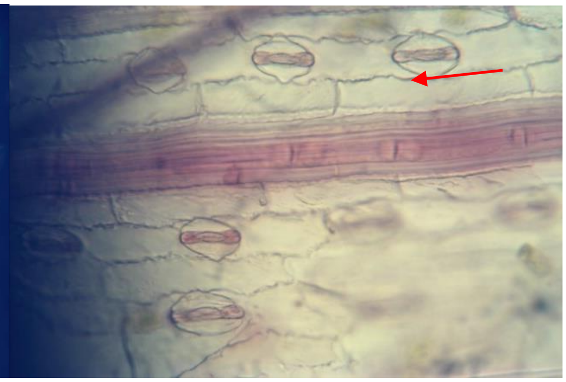
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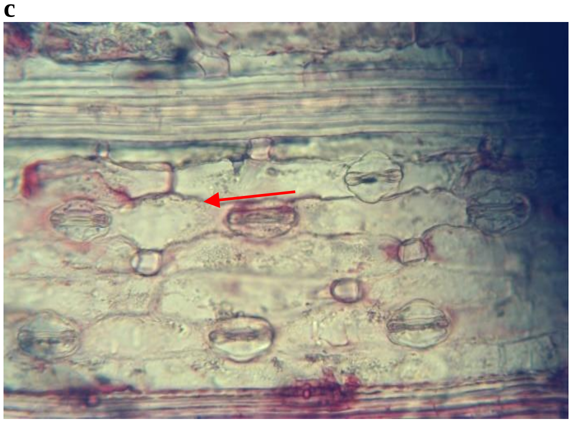
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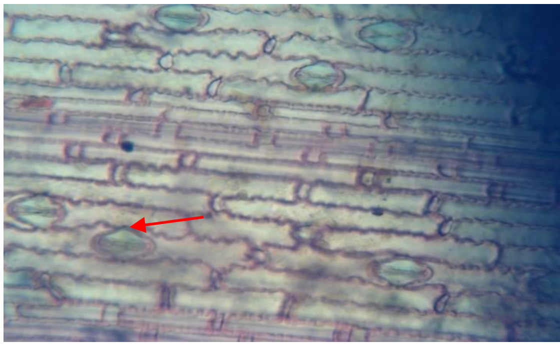
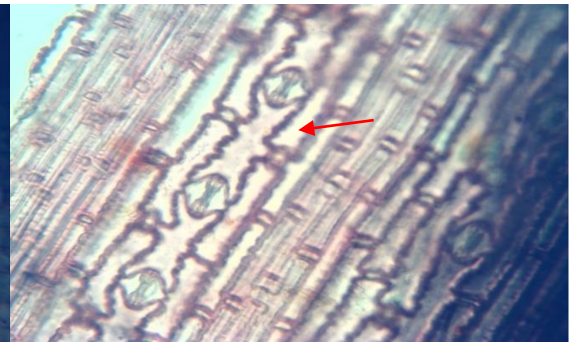
Plate 1: Effects of different concentrations of waste engine oil contamination on the epidermis of the leaves of *D. horizontalis*

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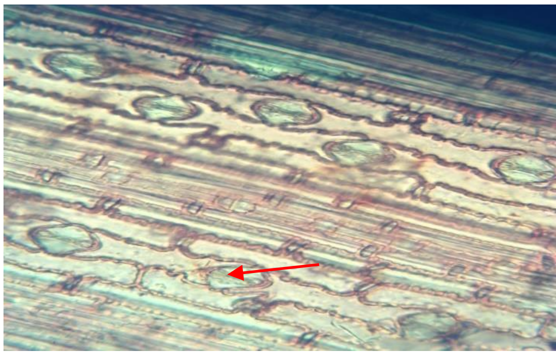
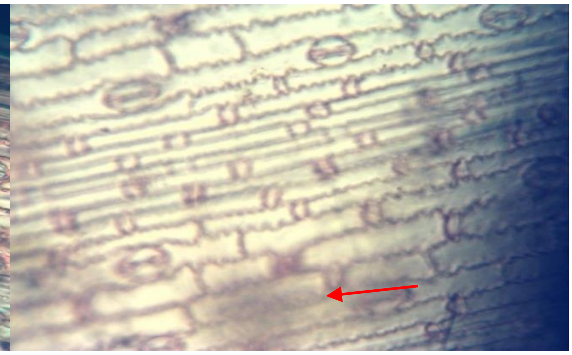

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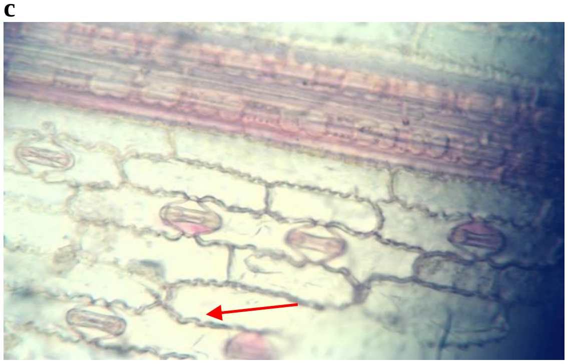

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Plate 2: Effects of different concentrations of waste engine oil contamination on the epidermis of the leaves *E. indica.*

Stem Anatomy: The effects of different concentrations of waste engine oil soil contamination on the T/S of stems of *D. horizontalis* showed large parenchyma cells and intercellular air spaces in the T/S of stem of the grasses grown on 0 % and 2% waste engine oil contaminated soils (Plate a and b)**.** The T/S of stem of the grasses grown on 4% waste engine contaminated soil showed reduced parenchyma cells and intercellular air spaces (Plate c). The T/S of stem of the grasses grown on 7% waste engine contaminated soil showed thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased (Plate d). The T/S of stem of the grasses grown on 10 % waste engine contaminated soil showed increased thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased (Plate e),

The effects of different concentrations of waste engine oil soil contamination on the T/S of stems of *E. indica* showed large parenchyma cells and intercellular air spaces in the T/S of stem of the grasses grown on 0 % waste engine oil contaminated soil (Plate a)**.** The T/S of stem of the grasses grown on 2 % waste engine contaminated soil showed slightly reduced parenchyma cells and intercellular air spaces (Plate b). The T/S of stem of the grasses grown on 4 % waste engine oil contaminated soil showed reduced parenchyma cells and intercellular air spaces (Plate c). The T/S of stem of the grasses grown on 7% waste engine contaminated soil showed thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased (Plate d).The T/S of stem of the grasses grown on 10% waste engine contaminated soil showed increased thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased (Plate e).

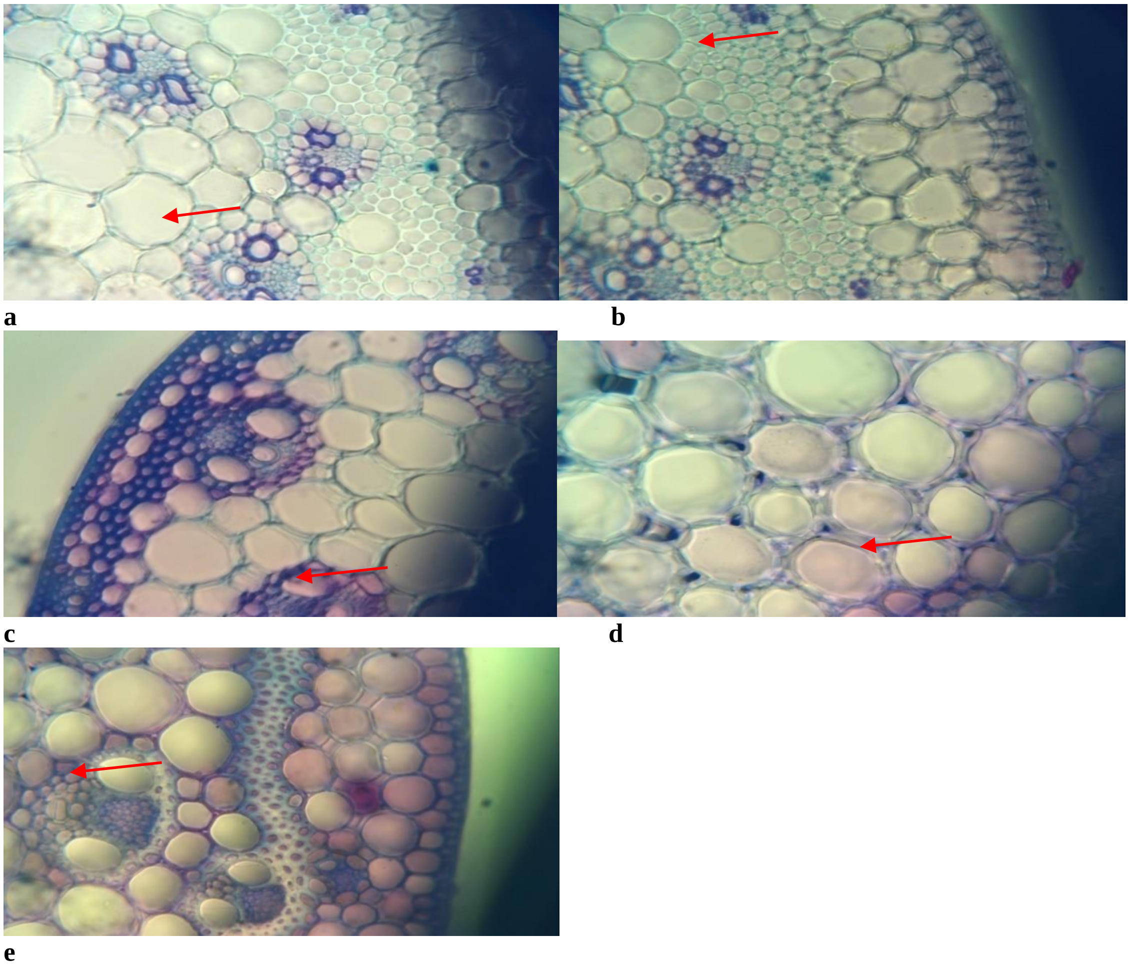
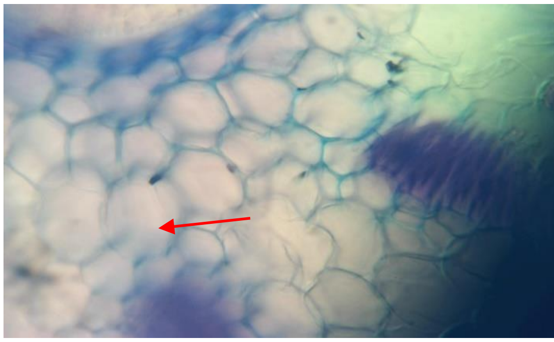
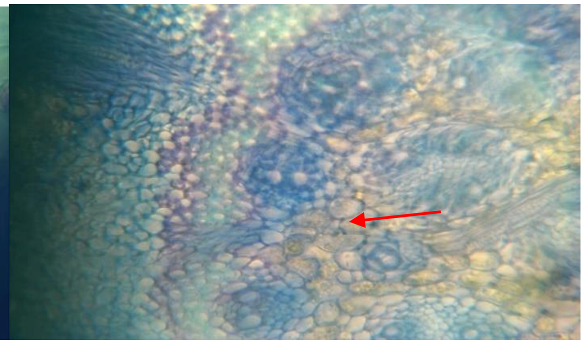
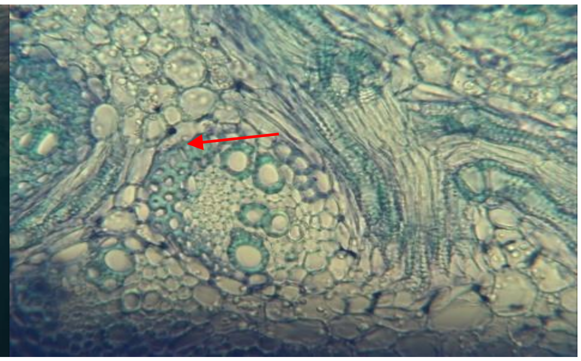


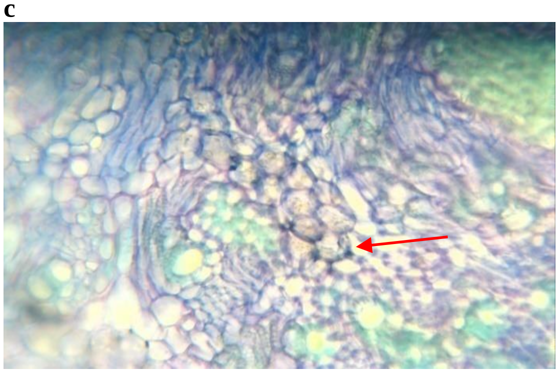
Plate 3: Effects of different concentrations of waste engine oil soil contamination on the T/S of stems of *D. horizontalis*

a **b**

c **d**



e

Plate 4: Effects of different concentrations of waste engine oil soil contamination on the T/S of stems of *E. indica.*

Root Anatomy: The effects of different concentrations of waste engine oil soil contamination on the T/S of roots of *D. horizontalis* showed normal parenchyma cells of pith in the T/S of root of the grasses grown on 0 % waste engine oil contaminated soil (Plate a). The T/S of root of the grasses grown on 2 % waste engine contaminated soil showed slightly reduced parenchyma cells of pith (Plate b). The T/S of root of the grasses grown on 4 % waste engine contaminated soil showed slightly tissue breakdown of cell in the cortex region and parenchyma cells of pith (Plate c). The T/S of root of the grasses grown on 7% waste engine contaminated soil showed increased breakdown of cell in the cortex region as oil concentration increased (Plate d). The T/S of root of the grasses grown on 10 % waste engine contaminated soil showed increased breakdown of cell in the cortex region as oil concentration increased (Plate e).

The effects of different concentrations of waste engine oil soil contamination on the T/S of roots of *E. indica* showed normal parenchyma cells of pith on the T/S of root of the grasses grown on 0% waste engine oil contaminated soil (Plate a). The T/S of root of the grasses grown on 2 % waste engine contaminated soil showed slightly reduced parenchyma cells of pith (Plate b). The T/S of root of the grasses grown on 4 % waste engine contaminated soil showed slightly tissue breakdown of cell in the cortex region (Plate c). The T/S of root of the grasses grown on 7 % waste engine contaminated soil showed increased breakdown of cell in the cortex region as oil concentration increased (Plate d). The T/S of root of the grasses grown on 10 % waste engine contaminated soil showed increased breakdown of cell in the cortex region and parenchyma cells of pith as oil concentration increased (Plate e).

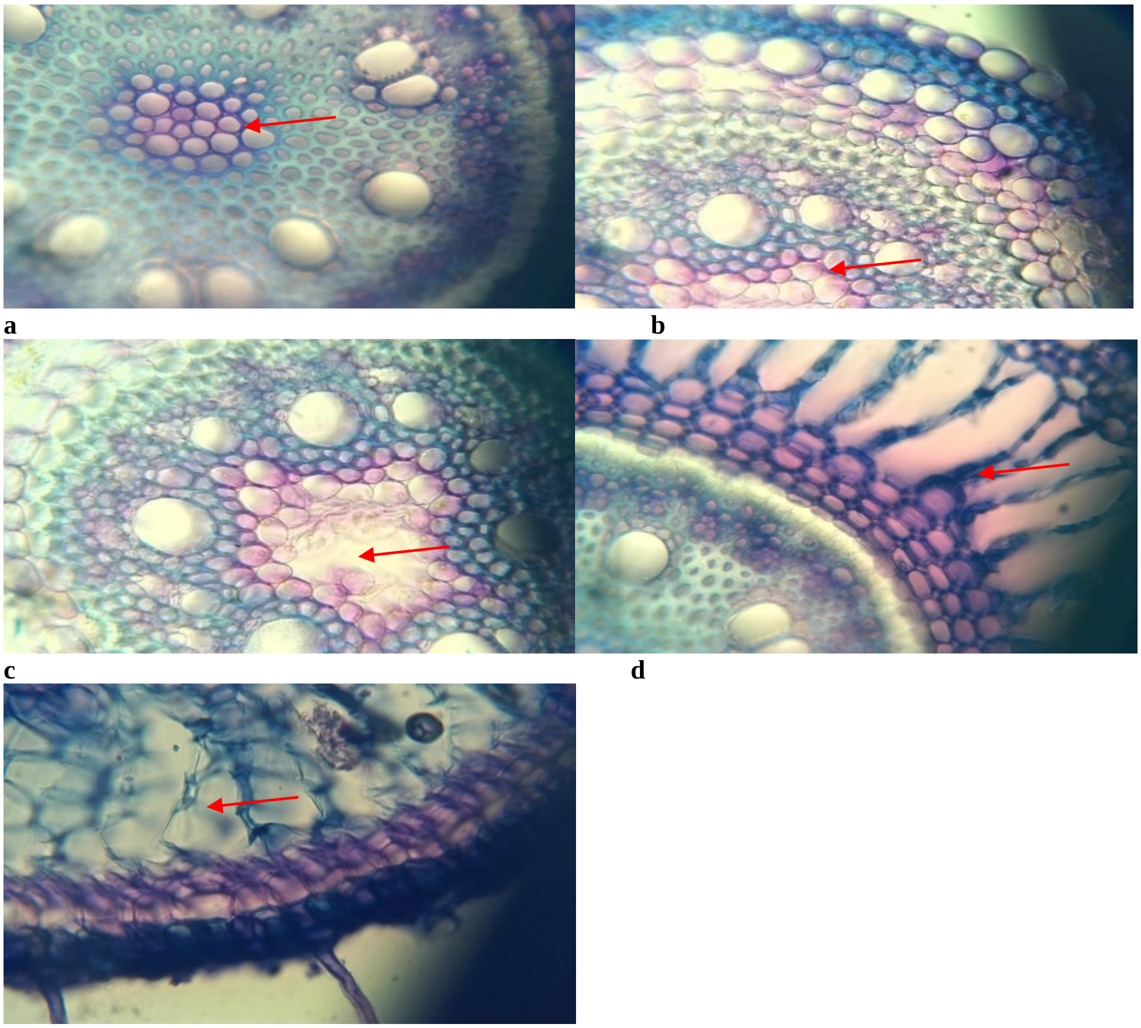


Plate 5: Effects of different concentrations of waste engine oil soil contamination on the T/S of root of *D. horizontalis***.**

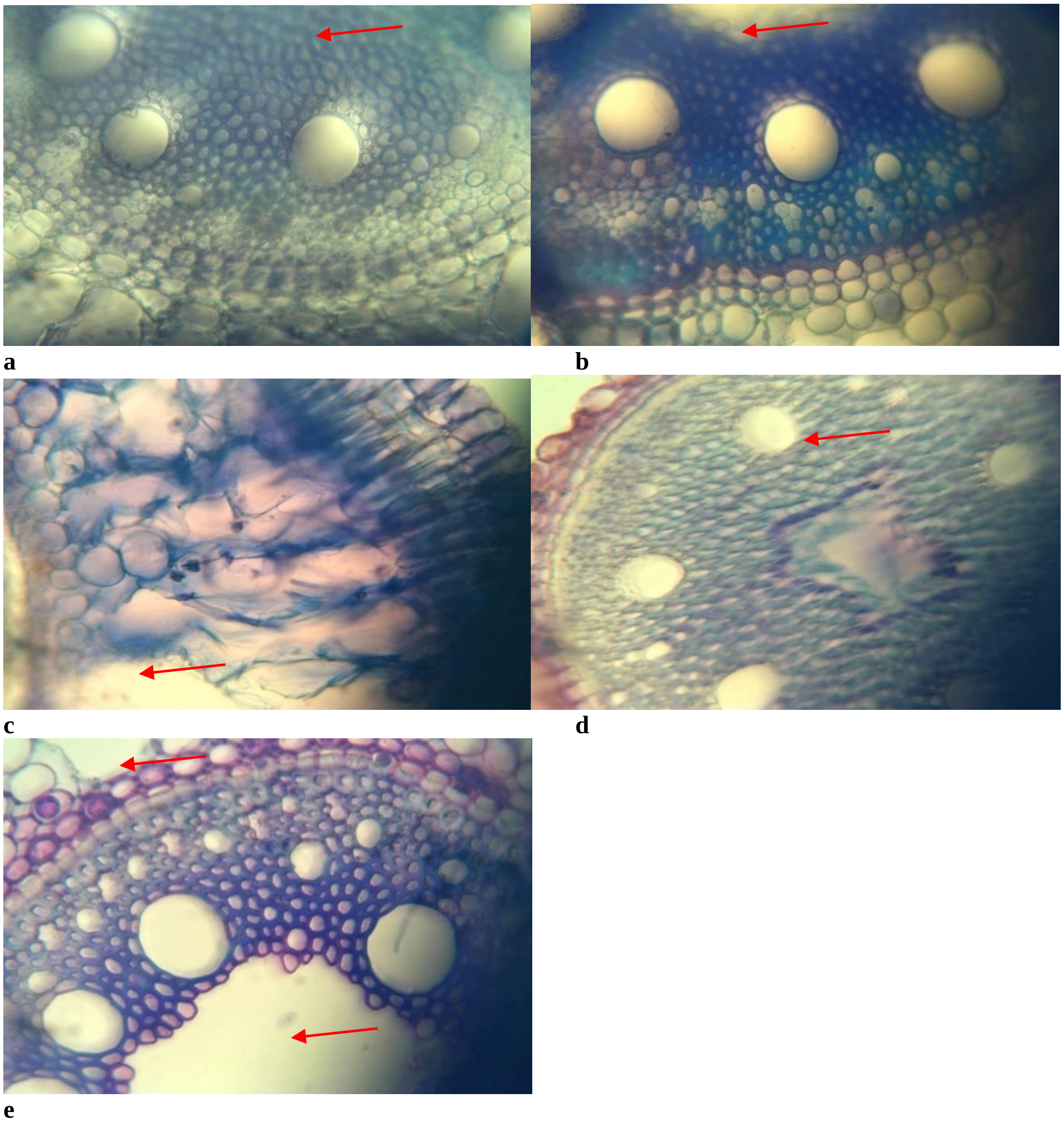


Plate 6: Effects of different concentrations of waste engine oil soil contamination on the T/S of root of *E. indica.*

Discussion

Changes in the anatomy of the two grass species observed could be attributed to effect of the waste engine oil on the soil in which they were grown. These observed changes in anatomy were however uniform across the two grass species. The reduction in the number of stomata in the leaves of the two grass species as the level of the waste engine oil contamination increased was evident. The observed reduction in the stomatal frequency in the epidermis as the level of contamination increased, were reported for other plants (Gill *et al*., 1992; Vwioko and Fashemi, 2005; Omosun *et al.,* 2008; Omosun *et al.,* 2009).

In the leaf anatomy, epidermal cells of the leaves showed that all the two grass species exhibited irregular epidermal cell shape and sinuous cell walls. The epidermal cell walls exhibited a gradation of sinuosity, being strongly sinuous in *E. indica* and slightly sinuous in the *D. horizontalis* species. It was also observed that as the concentrations of the waste engine oil increased, the sinuosity of the epidermal cell wall increased. This observation was also reported by

Omosun *et al*. (2008), that observed that the irregular epidermal cell shape and increasing level of sinuosity in the 1-3% treated *A. hybridus* were due to morphological aberrations.

In stem anatomy, there were reduction in cell size, increase in cell shape irregularity, increase in thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased. Maruthi *et al* (2007) showed that the SEM micrographs of stems of ZnT4 treated barley plants showed breakdown of the cell walls of vascular bundle compared to controls. Also the xylem vessels dilated and lost their shape in the stem of ZnT4 treated barley plants. Barley plants can accumulate significant amounts of Zn and Cd without showing phytotoxicity or reduction in plant growth when the environmental metal concentrations are low.

In root anatomy, several changes were observed in the root tissues of plants exposed to waste engine oil contaminations. There were reduction in cell size, the increase in cell shape irregularity and increase in the thickness of cell wall of the parenchyma tissues and break down of cortex region as oil concentration increased. These observations could be attributed to a condition of physiological drought (Omosun *et al*., 2009). This condition caused parenchyma cells to become smaller and have thicker cell walls as a drought avoidance mechanism to reduce water loss (Omosun *et al*., 2009). Roots of plants cultivated in contaminated soil also presented changes in size, shape and arrangement of cortical parenchyma cells (Marcelo *et al*., 2011).

Conclusion

The anatomical changes observed in the leaves, roots and the shoots of *D. horizontalis* and *E. indica*, showed responses to environmental pollutants. The *E. indica* could withstand waste engine oil-polluted soil at 10 % treatment for eight weeks; making it a potential candidate for phytoremediation. *Eluesine indica* could be potential candidates for phytoremediation of waste engine oil polluted sites and indicator for environmental pollutants. However, *Digitaria horizontalis* could be used only in soil with low concentrations of waste engine oil.

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