African Scientist Vol. 22, No. 1 March 31, 2021
Printed in Nigeria

1595-6881/2021 $10.00 + 0.00
© 2021 Nigerian Society for Experimental Biology
http://www.niseb.org/afs

AFS2021001/22102

Phytotoxicity Study on the Effects of Waste Engine Oil on the Anatomy of *Sataria barbata* (LAM.) KUNTH and *Brachiaria deflexia* (SCHUMMACH.) C.E. HUBB. EX ROBYNS

Nonyelum Helena Ifediora*, Hilary Odo Edeoga, Garuba Omosun, and Oluomachi Marvellous Obi

Department of Plant Science & Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria**.**

*Corresponding author; Email: ifedioranonyelum@gmail.com Tel: +234 (0) 8064328449

(Received February 26, 2021; Accepted in revised form March 19, 2021)

ABSTRACT: The present study explored the use of anatomical characters of *Sataria barbata* and *Brachiaria deflexia* as indicators of waste engine oil pollution**.** Four (4) kg of air dried soil was measured into perforated plastic buckets and treated with waste engine oil to obtain different concentrations [0% (control), 2%, 4%, 7% and 10%] on volume to weight basis. The experiment was set up in 3 replicates. The soil samples were allowed to stand for seven days before seedlings were planted. The plants were harvested after 8 weeks of transplanting. Anatomical investigations of the leaves, stems and roots and the epidermal peel of the leaves were carried out. There was reduction in cell size, increase in cell shape irregularity, increased thickness of cell walls of the parenchyma tissues and breakdown of vascular bundles as oil concentration increased in stem anatomy. There was reduction in root cell size, increase in cell shape irregularity and increased thickness of cell wall of the parenchyma tissues and break down of cortex region as oil concentration increased. The leaves epidermal cells showed that both grass species exhibited irregular epidermal cell shape and sinuous cell walls. Waste engine oil contaminated soil had adverse effect on the grass species studied. The effect was significantly different at ($p<0.05$).

Keywords: *Sataria barbata, Brachiaria deflexia,* Anatomy, Indicators, Pollution**.**

Introduction

The United States Environmental Protection Agency (USEPA, 2001) defined waste engine oil as any oil that has been produced from crude oil or any artificial oil and that has been contaminated by chemical or physical impurities as a consequence of usage (Ogedegbe *et al*., 2013). Engine oil is transformed chemically by nitration, oxidation, decomposition of organometallic compounds, cracking of polymers, etc., during motor operation. These changes occur because of the high temperature and the high mechanical strains that the oil is subjected to during motor operation (Severinski, 1997). Engine oil in another way, pile different pollutants which include antifreeze, water, insoluble particles and fuel (petrol or diesel). The sources of these particles include heavy metals, atmospheric dust, combustion products and metal oxides (Severinski, 1997). The difference between engine oil and waste engine oil is that waste engine oil contains additional chemicals that are manufactured or accumulated in the oil, as a result of its use as lubricant in engine at high temperatures and pressures as it runs inside an engine (Dorsey *et al.,* 1997). Heavy metals like tin, chromium, iron, manganese, aluminum, nickel, lead, silicon and copper are waste engine oil contents which come from engine parts, as they degrade (Keith and Telliard, 1979). Chronic pollution due to waste engine oil reaches several millions of tons annually. Also, in contrast to petroleum pollution, waste engine oil contamination is a world-wide problem as industrial activities and automotive traffic are the main sources (Vazquez-Duhalt, 1989). Polycyclic aromatic hydrocarbons (PAH) are types of compounds found in waste engine oil which are highly toxic to human health by reason of some

have been proved to be mutagenic and carcinogenic (IARC, 1983). Moreover, aside heavy metals and petroleum hydrocarbons, waste engine oil has compounds like lubrication additives which are toxic to the ecosystem.
Anoliefo and Edegbai (2006) investigated the effect of waste engine oil on the growth of *Solanum melongena* and *S. incanum* and found that 4 and 5 % oil amended soil showed that the emergence of *S. incanum* was completely inhibited, while at oil concentrations above 3 %, height and leaf values of both plants were depressed. Adenipekun and Kassim, (2008) reported that waste engine oil at all concentrations delayed the germination of *Corchorus olitorius* by 2 days (compared to control) and caused a significant reduction in all the growth parameters in plants grown on contaminated soil compared to control plants. In another work, Adenipekun *et al.* (2009), also showed that waste engine oil pollution had adverse effects on Okra plants, though the plants could tolerate low levels of contamination (<0.2%).
Many plants species have been shown to have the potentials to grow in polluted soils and actually extract the contaminants from the growth media. They function in several different ways. Some plants can hyperaccumulate toxic compounds and volatilize them (Oyibo, 2013). Some aquatic plant roots can filter contaminants from water (Brooks and Robison, 1998). Studies on phytoremediators has been on their use in cleaning up volatile organic chemicals, heavy metals and organic compounds such as petroleum compounds (Cunningham and Ow, 1996). If effective, because the treatment can be done *in situ*, the cost of using plants is lower than most other current methods and it is relatively environmentally safe, phytoremediation can be an attractive alternative to current remediation methods. Using this technology lowers the total cost of the clean-up project and minimizes the disturbance the remediation will cause in the environment (Oyibo, 2013).
Lee *et al.,* (2001) observed that the use of phytoremediation could enhance habitat recovery through the stimulation of vigorous vegetative growth of plant and not only as a clean-up option that degrade pollutants. It also has the added advantage of being applied without the need for removal and transportation of contaminated soil. Also, it has the ability to remediate a wide range of pollutants in various environments. It is less expensive, less labour-intensive, relies on solar energy to give lower carbon footprint, and has got a high level of public acceptance (McGuiness and Dowling, 2009). It can be used at sites with low to moderate levels of contamination. It entails more than just planting and allowing the foliage to grow. The site must be constructed to prevent erosion and flooding in order to maximize pollutant uptake (DreemEssays.com 2009).
Most reports on the phytoremediation of the petroleum hydrocarbon polluted soils showed that legumes (Leguminosae) and grasses (Poacae) were used (Schwab *et al.,* 2006; Kirkpatrick *et al.,* 2006; Merkl *et al.,* 2005; Qui *et al.,* 1997; Gunther *et al.,* 1996; April and Sims, 1990). Plants from the grass family are considered to be particularly suitable for phytoremediation as they encourage increased rhizospheral zone because grasses possess multiple ramified root systems. This allows increased growth and activity of microbes around the rhizosphere of root (April and Sims, 1990). The aim of this study was to investigate the phytoremediation potentials of *Sataria barbata* and *Brachiaria deflexia* grown in waste engine oil polluted soil.

Materials and Methods

Soil Sample: Soil samples used for this study 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 used was obtained as pooled engine oil from two different major mechanic workshops located in the Mechanic Village, Umuahia, Abia State. The plant materials used were collected from bush fallow located at Umuahia metropolis. Top soil of 0-10cm depth which was collected from a marked area was air dried, sieved through a 2mm mesh gauge (Ogedegbe *et al.*, 2013). 4kg of the soil sample was introduced into different 4 litre perforated plastic buckets after which different concentrations (2%, 4%, 7% and 10%) of waste engine oil were added to each 4kg soil samples; denoted as T_1, T_2, T_3 and T_4. The mixing was done gradually to ensure thorough and even mixing and the treatment was replicated three times. The untreated soil with 0% waste engine oil served as a control (Tc) (Adenipekum *et al.*, 2009). After thorough mixing, the soil sample were left under the 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) after which they were artificially irrigated with water in the experimental farm before the transplanting of the studied plant species and left for natural irrigation.
Plant materials: The plant species being investigated were propagated by tiller. The tillers of the plants were separated differently and the same height (shoot 15cm) 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: The following parameters were measured:
Anatomical character of the leaves, stems and roots: 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 respectively). 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 peel of the leaves: 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.
Photomicrography- 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 grown in different waste engine oil polluted soil revealed that the values of the grass species grown in the control soil surpassed the values of other plants grown in higher waste engine oil contaminated soil (Table 1). In the leaves of *S. barbata*, the stomatal frequency of the upper epidermis was highest in the control (18.42±3.46) and lowest in the 10% (5.35±2.34) concentration of waste engine oil. The stomatal frequency of the lower epidermis had the highest value (10.01±2.08) in control while the lowest (7.13±0.63) in the 10% concentration of waste engine oil (Table 1). In the leaves of *B. deflexia* the stomatal frequency of the upper epidermis had the highest value (7.02±0.56) in the 0% concentration of waste engine oil while the lowest value (5.56±0.65) was recorded in the 10% concentration of waste engine oil. The stomatal frequency of the lower epidermis had the highest at the 0% (7.62±0.31) concentration of waste engine oil while the lowest was observed in the 10% (5.00±0.58) concentration of waste engine oil (Table 1).

Table 1: Stomatal frequency of upper and lower epidermis of the two grass species grown in different waste engine oil polluted soil

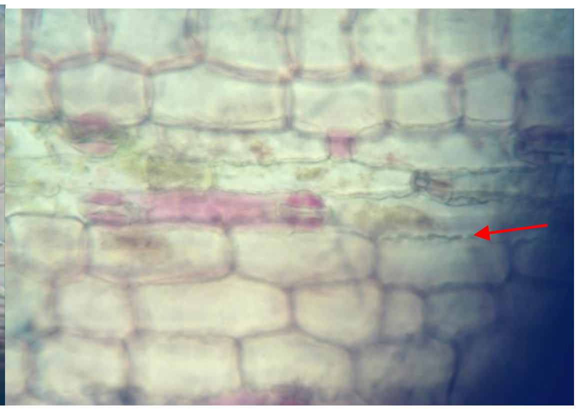
	Stomatal Frequency	
Treatment	**Upper epidermis**	**Lower epidermis**
	S. barbata	
0	$18.42±3.46^{a}$	10.01±2.08
2	$12.47±1.80^{ab}$	9.42±2.26
4	$11.59±0.71^{ab}$	9.04±0.55
7	$10.67±0.33^{ab}$	9.49±1.41
10	$5.35±2.34^{b}$	7.13±0.63
	B. deflexia	
0	7.02±0.56	7.62±0.31
2	7.69±0.88	7.09±0.79
4	7.53±1.34	6.84±1.28
7	6.67±0.88	5.26±1.63
10	5.56±0.65	5.00±0.58

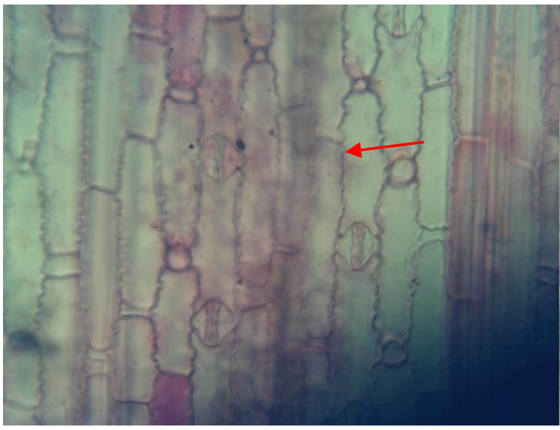
a, b = Means with different superscripts across the columns are significantly different at $p<0.05$

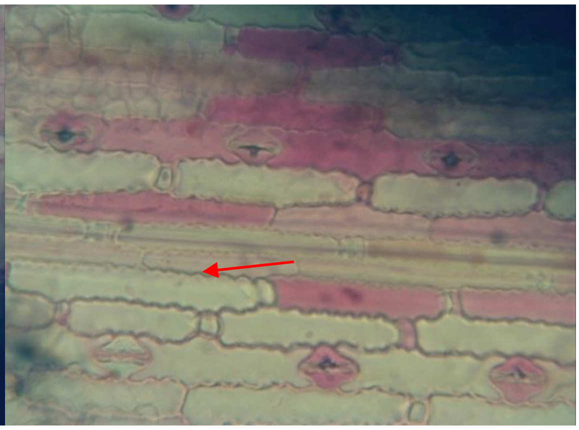
Anatomical changes: The effect of various treatments of waste engine oil on the epidermal structure of the leaves of *S. barbata* ranged from slightly sinuous in the control (0%) (Plate a), to sinuous in the 2% and 4% treatments (Plates b and c), to strongly sinuous in the 7% and 10% treatments (Plates d and e).

The effect of various treatments of waste engine oil on the epidermal structure of the leaves of *B. deflexia ranged* from slightly sinuous in the control (0%) (Plate a), to sinuous in the 2% and 4% treatments (Plates b and c), to strongly sinuous in the 7% and 10% treatments (Plates d and e).


a


b


c

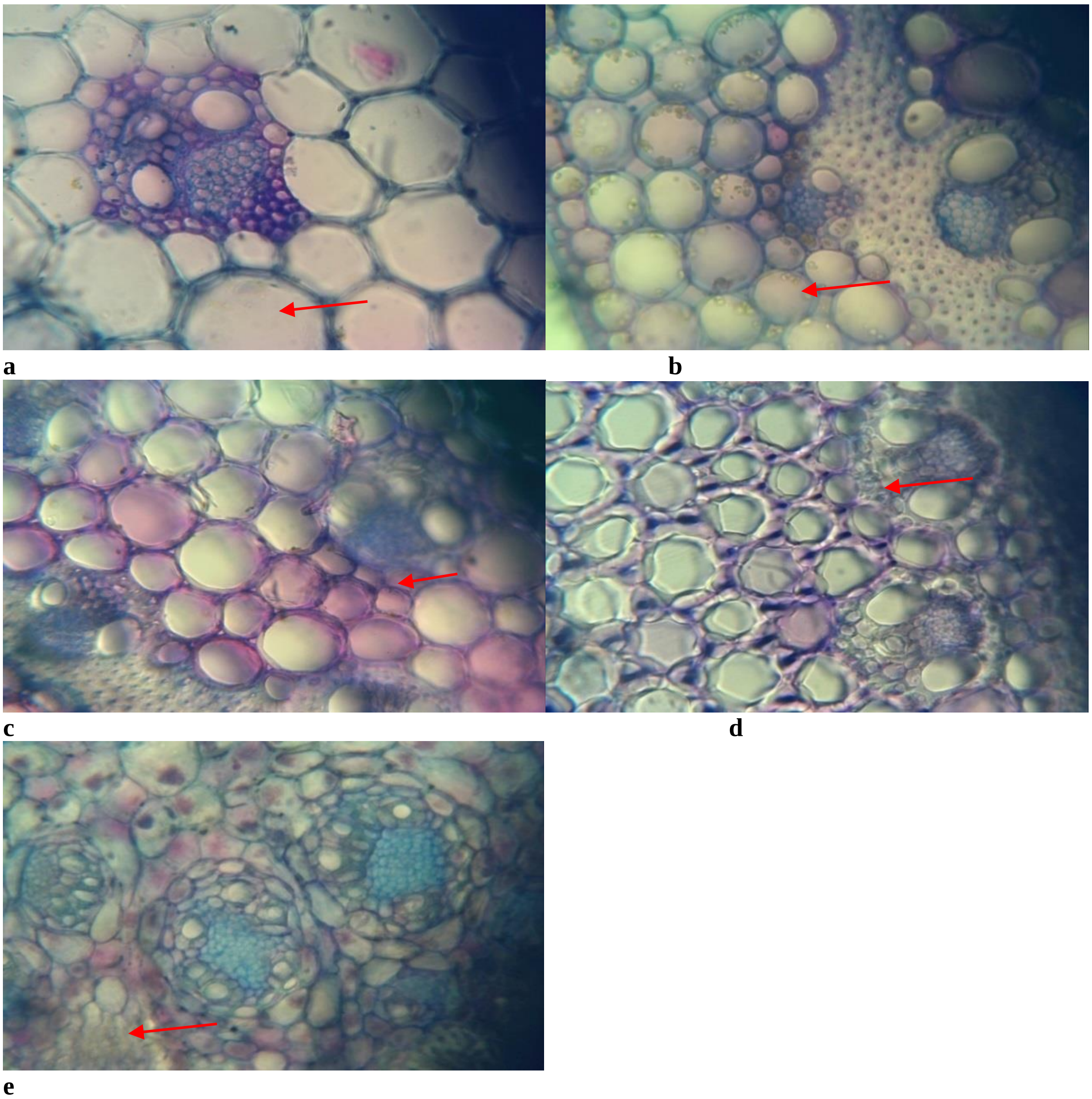

d


e

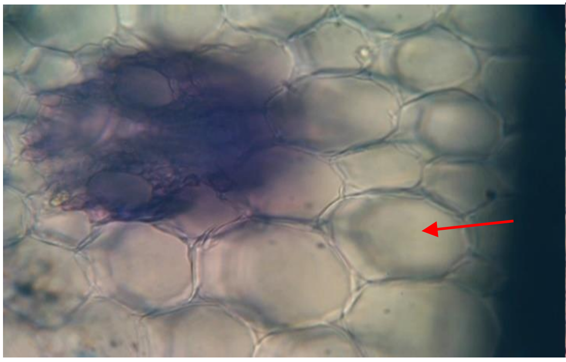
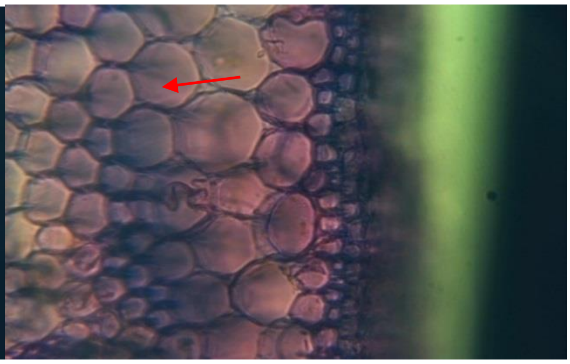
Plate 1: Effects of various percentages of waste engine oil contamination on the epidermal studies of the leaves of *S. barbata.* (x400)

Plate 2: Effects of various percentages of waste engine oil contamination on the epidermal studies of the leaves of *B. deflexia* (x400).

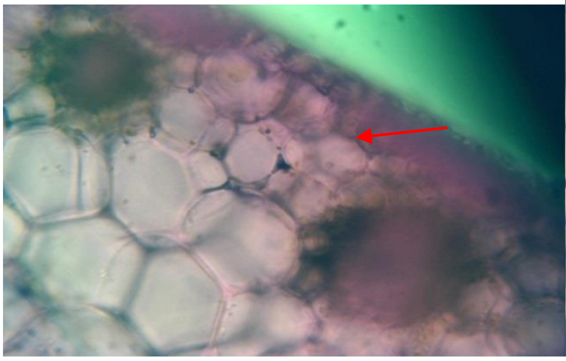
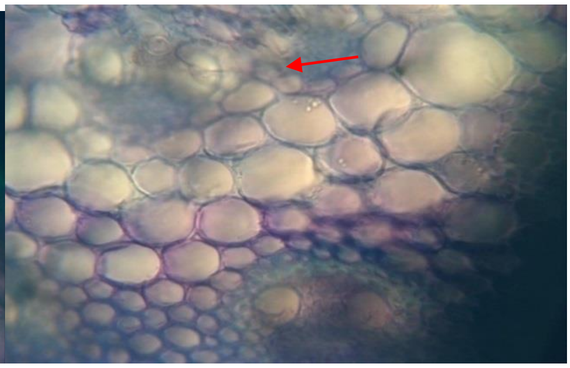
Stem Anatomy: The effect of various treatments of waste engine oil on the epidermal structure of the T/S of stems of *B. deflexia ranged* from large parenchyma cells and intercellular air spaces in the control (0%) (Plate a), slightly reduced parenchyma cells and intercellular air spaces in the 2% and 4% treatments (Plates b and c), to increased thickness of cell wall of the parenchyma tissues and breakdown of vascular bundles in the 7% and 10% treatments (Plates d and e).



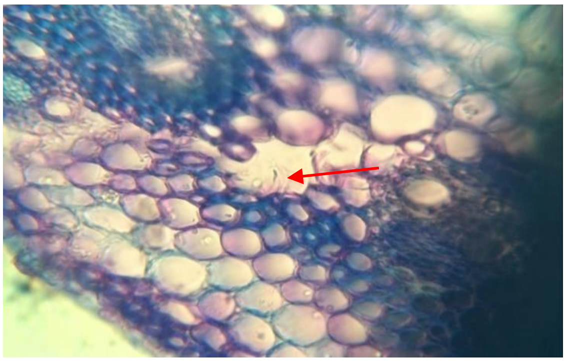
-Plate 3: Effects of various percentages of waste engine oil soil contamination on the T/S of stems of *S. barbata* (x400)

a b

c **d**



e

Plate 4: Effects of various percentages of waste engine oil soil contamination on the T/S of stems of *B. deflexia* (x400)

Root Anatomy: The effect of various treatments of waste engine oil on the epidermal structure of the T/S of root of *S. barbata* normal parenchyma cells of pith in the control (0%) (Plate a), slightly reduced parenchyma cells slight tissue breakdown of cell in the cortex region intercellular air spaces in the 2% and 4% treatments (Plates b and c), to increased breakdown of cell in the cortex region and increased breakdown of cell in the cortex region in the 7% and 10% treatments (Plates d and e).

The effect of various treatments of waste engine oil on the epidermal structure of the T/S of stems of *B. deflexia ranged* from large parenchyma cells and intercellular air spaces in the control (0%) (Plate a), slightly reduced parenchyma cells and slightly tissue breakdown of cell in the cortex region in the 2% and 4% treatments (Plates b and c), to increased tissue breakdown of cell in the cortex region and parenchyma cells of pith in the 7% and 10% treatments (Plates d and e).

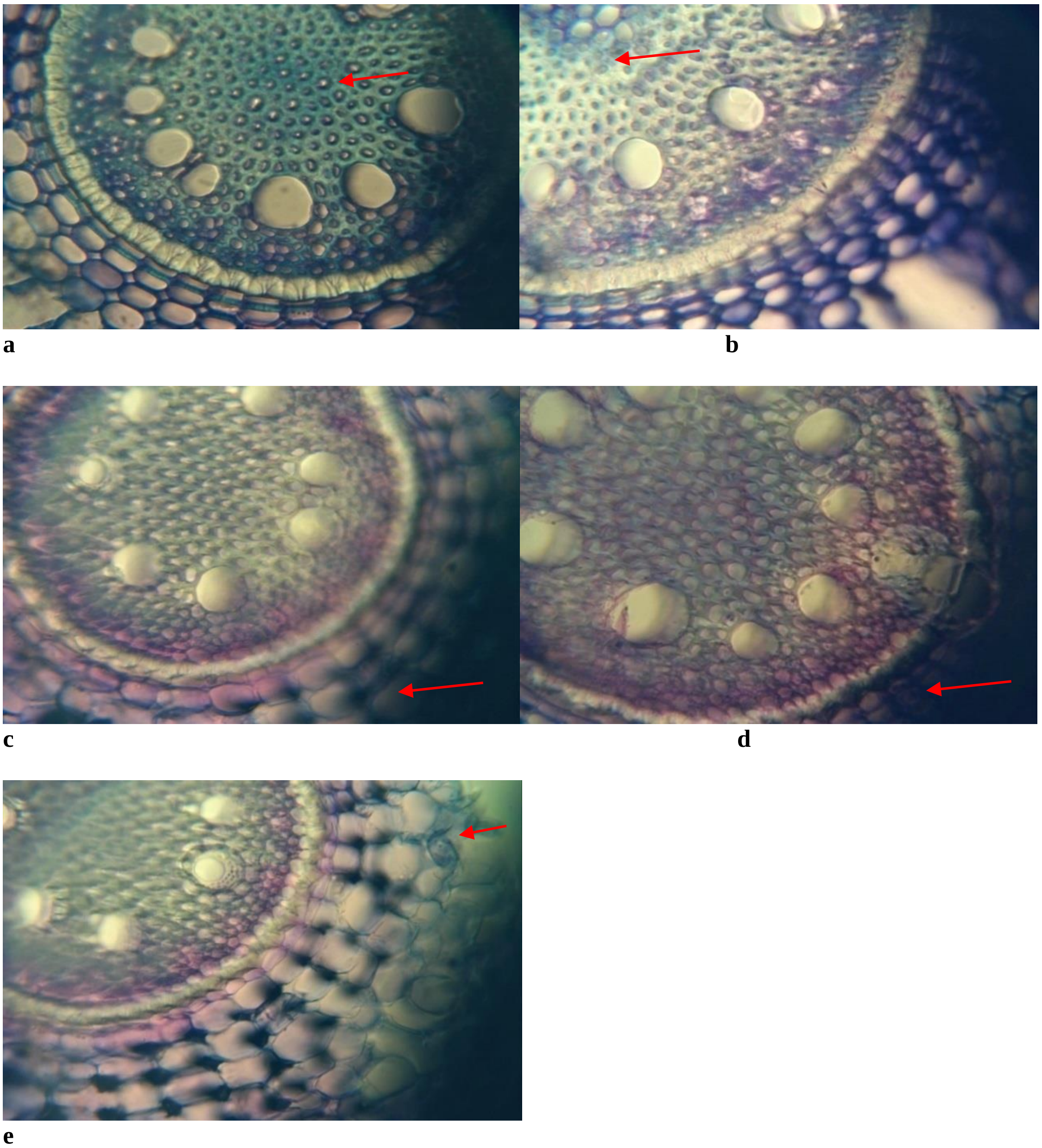


Plate 5: Effects of various percentages of waste engine oil soil contamination on the T/S of root of *S. barbata* (x400).

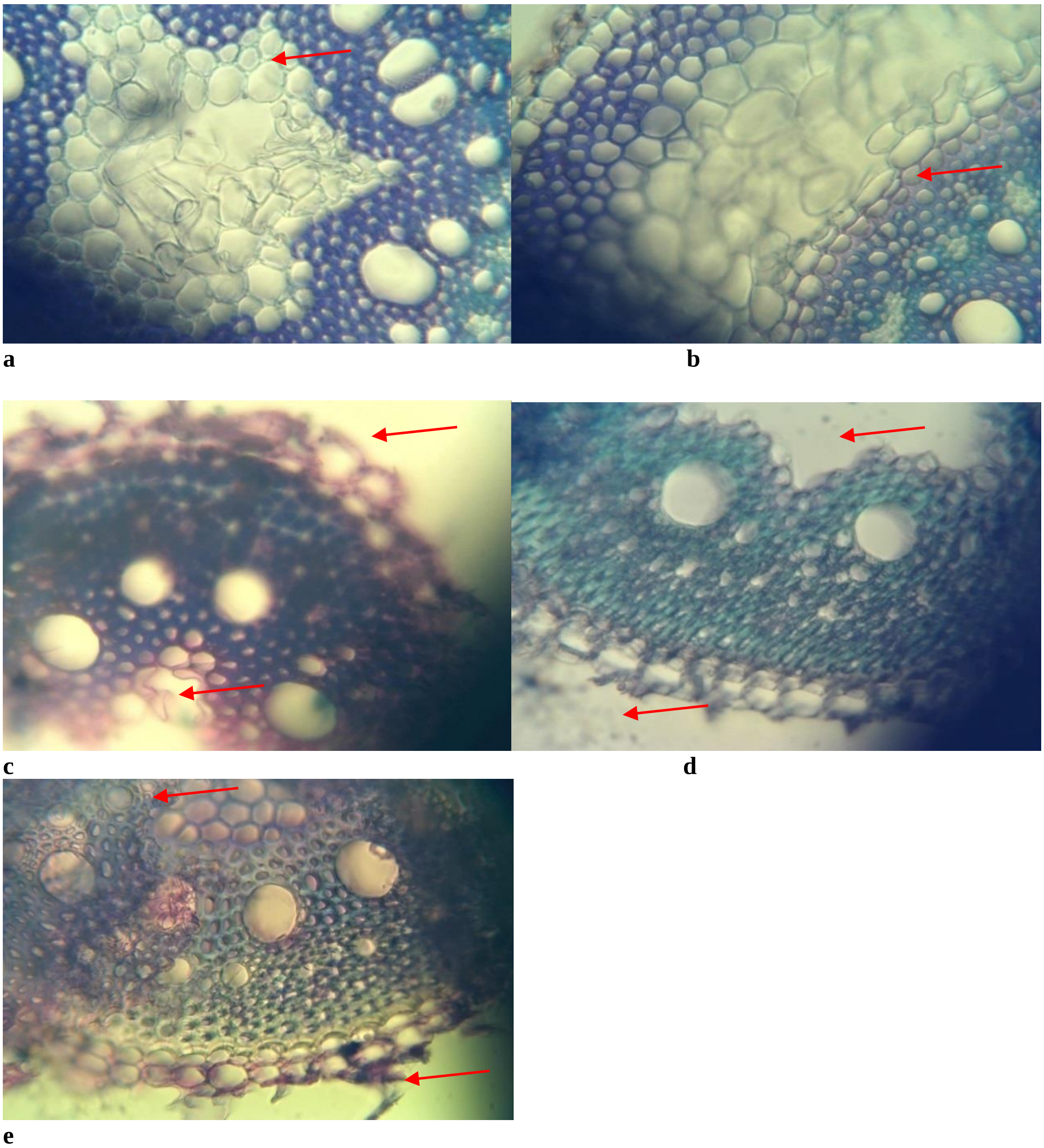


Plate 6: Effects of various percentages of waste engine oil soil contamination on the T/S of root of *B. deflexia* (x400).

Discussion

There were observed changes in anatomy and were however uniform across the two grass species studied. Agbogidi, and Ohwo, (2013) Stated that there was a buildup of trace metals (Pb, Fe, Zn and Cu) in soils contaminated with spent lubricating oil when compared with values recorded in soils of no oil impact. Decrease in the number of stomata in the leaves as the level of the contamination increased was evident in the leaves of the two grass species studied. Stomata play a pivotal role in controlling the balance between water loss and biomass production (Omosun *et al.,* 2009). The effect was significantly different at ($p<0.05$)
The leaf anatomy of the 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 exhibit a gradation of sinuosity, being strongly sinuous in *B. deflexia* followed by *S. barbata* species. This could be as a result of physiological drought due to the stressful polluted environment. The effects of various treatments of waste engine oil on the epidermal structure of the leaves of *D. horizontalis* ranged from slightly curved epidermal wall in the control (0%) (Plate

a), to curved epidermal wall in the 2% and 4% treatments (Plates b and c), to slightly sinuous epidermal structure of the walls in the 7% and 10% treatments (Plates d and e) was also noted by Ifediora *et al.,* (2019) a. On the other hand, the effects of various treatments of waste engine oil on the epidermal structure of the leaves of *E. indica* ranged from slightly sinuous epidermal wall in the control (0 %) (Plate a), to sinuous epidermal wall in the 2% and 4% treatments (Plates b and c), to slightly sinuous epidermal structure of the walls in the 7% and 10% treatments (Plates d and e was in line with Ifediora *et al.,* (2019) a. This could be as a result of adaptation changes by the plants. The adverse effect of environmental pollution on plant growth include morphological aberrations and stomata abnormalities (Sharma *et al*., 1980).

Furthermore, in the stem anatomy of the two grass species, as oil concentration increased, there were observed cell size reduction, the parenchyma tissues increase in thickness of cell wall, vascular bundles breakdown and increased irregularity in the cell shape. Ifediora *et al.,* (2019) b made similar observations of 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 in stem anatomy of *P. scrobiculatum* and *A. compressus*. Higher Zn concentration will result in structural changes in roots, stem and leaves and altered physiological and morphological characteristics was observed by Maruthi *et al.,* (2007).

Finally, the root anatomy showed reduction in 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 oil concentration increased. Roots of plants cultivated in contaminated soil presented changes in size, shape and arrangement of cortical parenchyma cells (Marcelo *et al*., 2011). Particularly, plants of the treatment with more contamination had widened cell spaces in the cortex that were virtually always present where death of parenchyma cells was observed. Intercellular spaces formation and changes in the cellular arrangement are common in *Brachiaria* resulting from root maturation (Marcelo *et al.,* 2011). Besides the cell degeneration induction, changes in cell shape and organization suggests a heavy metal interference in the root maturation rate, probably due to the ability of heavy metal disrupt the hormonal balance (Barceló *et al*., 1990; Sandalio *et al.,* 2001). heavy metals including cadmium (Cd) and lead (Pb) are toxic metal and influence the plant growth adversely by affecting the leaves and root growth and inhibit enzymatic activities and resulted in reduce production (Zeng *et al*., 2008; Lai *et al.*, 2012).

Conclusion

In conclusion*, S. barbata and B. deflexia* plant species studied showed adaptation response to pollutants in the environment which were observed as anatomical changes that were present in the leaves, roots and the shoots. Therefore, these plants studied could be used for phytoremediation of waste engine oil polluted soil.

References

Adenipekun CO, Kassim LQ: Effects of spent engine oil on the growth parameters and moisture content of *Celosia argentea*. Proceeding of the 15th Annual Botanical Society of Nigeria Conference, University of Uyo, Uyo. pp. 108-111. 2008.

Adenipekun CO, Oyetunji OJ, Kassim LQ: Screening of *Abelmoschus esculentus* L. Moench for tolerance to spent engine oil. J Appl Biosci, 20:1131-1137. 2008.

Agbogidi OM, Ohwo AO: 2013). Trace metal profile of *Moringa oleifera* (Linn.) seedlings sown in spent lubricating oil contaminated soils. J Curr Res Sci, 1(4):242-246. 2013.

Anoliefo GO, Edegbai BO: Effect of crude oil as a soil contaminant on the growth of two egg plant species, *Solanum melongena L.* and *S. incanum.* J Agric, For Fish, 1: 1-25. 2006.

April W, Sims RC: Evaluation of the use of prairie grass for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere, 20:253-265. 1990.

Barceló J, Vázquez, MD, Poshenrieder, C. H. (1990). Plant water relations as affected by heavy metal stress: a review. J Plant Nutr, 13: 1-37.

Brandt R: Potential of Vetiver (*Vetiveria zizanioides* (L.) Nash) for the use in phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. Westfälische Wilhelms-Universität Münster Institut für Landschaftsökologie. 2003.

Brooks RR, Robinson N: Phytomining, Trends in Plant Sci, 3**:** 359-362. 1998.

Cunningham SD, Ow DW: Promises and prospect of phytoremediation. Plant Physiology, 110:715-719. 1996.

DreamEssays.com: Term paper on phytoremediation. Available at; http://www.DreamEssays.com[Retrieved on 7/7/2015] 2009.

Doersey Jr AS, Rabe C, Thampi S: *Toxicological profile for used mineral based crankcase oil*. Agency for Toxicological Substances and Disease Registry, U.S. Department of Health and Human Services. 1997.

Edeoga HO, Omosun G, Ako CP: Trichomes of *Mormodica* species with Calcium oxalate crystals. Environ Ecol, 25(2): 399-402. 2007.

Edeoga HO, Omosun G, Osuagwu, GGE, Emezue OO: Micromorphology of the leaf cuticle in *Mimosa* species (Leguminoseae-Mimosoideae). Asian J Plant Sci, 7(4): 424-426. 2008.

Gill LS, Nyawuame HGK, Ehikhametalor AO: Effect of crude oil on the growth and anatomical features of *Chromolaena odorata* (L). Chromolaena Odorata Newsletter, 6: 1-6. 2005.

Gunther T, Dornberger U, Fritsche W: Effects of ryegrasss on biodegradation of hydrocarbons in soil. Chemosphere, 33:203-215. 1996.

Ifediora NH, Edeoga HO, Omosun G: Phytoremediation of Waste Engine Oil Polluted Soil in Southeast, Nigeria. Afr J Agric Food Sci, **2**(2), 1-9. 2019.

Ifediora NH, Edeoga HO, Omosun G, Mbadiugha GE: Impact of accumulation and deposition of some heavy metals in the tissues of leaves, shoots and roots of *Paspalum scrobiculatum* and *Azonopus compressus,* Afr Sci, 20(4):181-191. 2019.

International Agency for Research on Cancer (IARC): Polynuclear aromatic hydrocarbons: part 2. Carbon black, mineral oils and some nitroarenes. IARC Monographs on the Evaluation of the carcinogenic risk of chemical to Human. 33. World Health Organization, Lyo, France. 1983.

Keith L, Telliard W: ES & T special report: priority pollutants: I-a perspective view. Environ, Sci Technol, 13: 416-423. 1979.

Kirkpatrick WD, White PM, Wolf DC, Thoma GJ, Reynolds CM: Selecting plants and nitrogen rates to vegetate crude oil contaminated soil. Int J Phytoremediation, 8: 285-297. 1979.

Lai Y, Xu B, He L, Lin M, Cao L, Wu Y, Mou S, He S: Cadmium uptake by and translocation within rice (*Oryza sativa* L.) seedlings as affected by iron plaque and Fe_2O_3. Pak J Bot, 44: 1557–1561. 2012

Lee KC, Cunningham BA, Paulson GM, Liang GH, Moore RB: Effects of cadmium on respiration rates and activities of several enzymes in soybean seedlings. Plant Physiol, 36:4–6. 2013.

Marcelo PG, Lara TC, de Sá e Melo L, Marques M, de Oliveira GN, de Castro EM, Ângela MS: Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens.* Sci Agric (Piracicaba, Braz.), 68(5): 566-573. 2011.

Maruthi SBB, Han X, Diehl SV, Monts DL, Su Y: Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants. Braz J Plant Physiol, 19(1):15-22. 2007.

McGuinnes M, Dowling D: Plant associated bacteria degradation of toxic organic compounds in soils. Int J Environ Res Public Health, 2226-2235. 2009.

Merkl N, Schultze-Kraft R, Infante C: Assessment of tropical grasses and legumes for phytoremediation of petroleum contaminated soils. Water, Air and Soil Pollut, 165:195-209. 2005.

Ogedegbe AU, Ikhajiagbe B, Anoliefo, GO: Growth response of *Alternanthera brasiliana* (L.) Kuntze in a waste engine oil-polluted soil. J Emerg Trends Eng Appl Sci, 4(2): 322-327. 2013

Omosun G, Markson AA, Mbanasor O: Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. Am-Eurasian J Sci Res, 3 (1): 70-74. 2008

Omosun G, Edeoga HO, Markson AA: Anatomical changes due to crude oil pollution and its heavy metals component in three *Mucuna* species. Recent Res Sci Technol, 1(6): 264-269. 2009.

Oyibo C: Phytoremediation of some tropical soils contaminated with petroleum crude oil. Ph.D. Thesis, University of Ghana, Legon. Ghana, 158. 2013.

Qui X, Leland T W, Shah SI, Sorensen DL, Kendall EW: Field study: grass remediation for clay soil contaminated with polycyclic aromatic hydrocarbons. In: phytoremediation of soil and water contaminants. E. L. Kruger, T. A. Anderson and J. R. Coats. (Eds). American Chemical Society: Washington, D.C. ACS Symposium Series 664. pp186-199. 1997.

Sandalio LM, Dalurzo HC, Gómes M, Romero-Puertas MC, Del Rio LA: Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot, 52: 2115-2126. 2001

Schwab P, Banks MK, Kyle WA: Heritability of phytoremediation potential for the alfalfa cultivar Riley in petroleum contaminated soil. Water, Air Soil Pollut, 177:239-249. 2006.

Severinski J: What can the I. R. spectroscopy say about a motor oil during services? Nafta (Katowici, Polland), 28:726-731. 1977.

Sharma GK, Chandler C, Salemi: Environmental pollution and leaf curticular variation in *Puerreria lobata* Willd. Ann Bot, 45: 77-80. 1980.

United States Environmental Protection Agency (USEPA): Analysis of composting as an environmental remediation technology. Solid Waste Emergency Response (5305W). 2001.

Vazquez-Duhalt R: Environmental impact of used motor oil. Sci Total Environ, 79:1-23. 1989.

Zhang MK, Liu Y, Wang H: Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. Commun Soil Sci Plant Anal, 41(7):820–831. 2010.