African Scientist Vol. 20, No. 4 December 31, 2019 | 1595-6881/2019 $10.00 + 0.00

Printed in Nigeria

http://www.niseb.org/afs

AFS 2019076/20404

Impact of Accumulation and Deposition of Some Heavy Metals in the Tissues of Leaves, Shoots and Roots of *Paspalum scrobiculatum* and *Azonopus compressus*

Nonyelum Helena Ifediora*, Hilary Odo Edeoga, Garuba Omosun, and George Ezenwa Mbadiugha

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

*Corresponding author; Email: ifedioranonyelum@gmail.com, Tel: +234 (0) 806 432 8449

(Received December 10, 2019; Accepted in revised form December 20, 2019)

ABSTRACT: *Paspalum scrobiculatum* and *Azonopus compressus* were used to study the accumulation and deposition of lead in tissues of roots, rhizomes and leaves, as well as their impact on changes in the anatomy of their tissues. 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. The plants were harvested after 8 weeks of transplanting. The following parameters were measured, anatomical characters of the leaves, stems and roots and the epidermal peel of the leaves. The observed changes in the anatomy of the two grass species studied may be due to the waste engine oil polluted soil in which they were grown. The leaves epidermal cells showed that all the two grass species exhibited irregular epidermal cell shape and sinuous cell walls. 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 oil concentration increased in stem anatomy. 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 oil concentration increased. The two grass species studied in conclusion, were sensitive to waste engine oil contaminated soil which were determined by the five different concentrations of waste engine oil used.

Keywords: *Paspalum scrobiculatum, Azonopus compressus,* Anatomy, Indicators, Pollution**.**

Introduction

Waste engine oil is product that arises as result of servicing of the vehicles engines, generating set and other types of engines at automobile mechanic shops, mechanical or electrical engine repairers' shops (Anoliefo and Vwioko, 2001). Waste engine oil which is harmful to the soil environment, has dark brown to black color (Adedokun and Ataga, 2007). Its toxicity is due to presence of a mixture of different chemicals including low to high molecular weight (C15-C21) compounds, heavy metals, decomposition products, additives and lubricants which have been found to be harmful to the soil and human health (Duffus, 2002). Through the direct ingestion of contaminated soils, consumption of crops and vegetables grown on the contaminated lands or drinking water that has percolated through such soils humans and the ecosystem may be exposed to chemical hazards such as heavy metals (lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel) (McLaughlin *et al.*, 2000). Kayode *et al.,* 2009, reported that the presence of waste engine oil in soil increases bulk density, decreases water holding capacity and aeration propensity. The authors also noted reduced nitrogen, phosphorus, potassium, magnesium, calcium, sodium and increased levels of heavy metals in soils contaminated with waste engine oil. Vwioko *et al.* (2006) in contrast, noted buildup of essential elements such as organic carbon and organic matter and their eventual translocation to plant tissues. Odjegba and Sadiq (2002) reported low yield and decreased growth of plant grown in waste engine oil contaminated soil. According to Adams and Duncan (2002) this effect could be because the oil acts as a physical barrier preventing or reducing access of the seeds to water and oxygen. Phytoremediation is an *in situ*, solar driven technique, which limits environmental disturbance and reduces costs (Shimp *et al.*, 1993). Moreover, it is particularly well-suited to the treatment of large areas of surface contamination, when other methods may not be costly effective (Schnoor, 1999). It is a green technology and when properly implemented is both environmentally friendly and aesthetically pleasing to the public (Raskin and Ensley, 2000). As plants take up pollutants (mainly water soluble) through their roots and transport/translocate them through various plant tissues where they can be broken, volatilized or sequestered, they act as solar-driven pumping and filtering systems (Cunningham *et al*., 1996; Greenberg *et al*., 2006; Abhilash, 2007; Doty *et al*., 2007). Because of high growth rate, more adaptability to stress environment and high biomass, grasses have been more preferable in use for phytoaccumulation than shrubs or trees (Malik *et al*., 2010). For their ability to tolerate and accumulate metals in their shoots, several researches have screened high-biomass-accumulating, fast-growing plants plus agronomic crops (Banuelus *et al*. 1997). Native plants provides a link between the objectives of site cleanup and habitat restoration with phytoremediation (Aprill and Simms., 1990). The plants whose rhizospheres promote degradation of hydrocarbons will at the same time provide food and habitat for rehabilitated ecosystems as the transition is made from brownfield to greenspace. (Aprill and Simms, 1990).

Materials and Methods

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 0-10 cm depth which was collected from a marked area was air dried, sieved through a 2mm mesh gauge (Ogedegbe *et al.*, 2013). Four (4) kg of the soil sample was introduced into different four (4) litre perforated plastic buckets after which different concentrations (2%, 4%, 7% and 10%) of waste engine oil were added to each four (4) kg 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) (Adenipekun *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. The plant species being investigated were propagated by tiller. The tillers of the plants were separated differently 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.

The following parameters were measured: anatomical character of the leaves, stems and roots and epidermal peel of the leaves. 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 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, Collage 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 and Discussion

The result of the stomatal frequency of upper and lower epidermis of the five grass species grown in different waste engine oil polluted soil 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 *P. scrobiculatum*, the stomatal frequency of the lower epidermis had the highest at the 0% (22.12) concentration of waste engine oil while the lowest at the 10% (14.51) concentration of waste engine oil. The stomatal frequency of the upper epidermis had the highest at the 0% (18.18) concentration of waste engine oil while the lowest at the 10% (10.18) concentration of waste engine oil. In the leaves of *A. compressus*, the stomatal frequency of the lower epidermis had the highest at the 0% (29.40) concentration of waste engine oil while the lowest at the 10% (19.26) concentration of waste engine oil. The stomatal frequency of the upper epidermis had the highest at the 0% (25.10) concentration of waste engine oil while the lowest at the 10% (15.14) concentration of waste engine oil.

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

Grass species grown on waste engine oil polluted soil (%)	Stomatal Frequency		Mean
	Lower epidermis	Upper Epidermis	
P. scrobiculatum			
0	22.12	18.18	20.15
2	20.93	17.91	19.42
4	17.76	14.66	16.21
7	15.66	12.32	13.99
10	14.51	10.18	12.35
Mean	**18.196**	**14.65**	
A. Compressus			
0	29.40	25.10	27.25
2	26.70	24.80	25.75
4	23.55	20.32	21.93
7	20.19	18.29	19.24
10	19.66	15.14	17.40
Mean	**23.90**	**20.73**	

The reduction 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. The observed reduction in the stomatal frequency in the epidermis as the level of contamination increased, were reported by Omosun *et al.*, 2009 who observed that the reduction in stomatal frequency as concentration of crude oil increased in the soil could be an adaptation mechanism to reduce water loss through transpiration.

The effect of different concentrations of waste engine oil contamination on the epidermis of the leaves of *P. scrobiculatum* 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 effect of different concentrations of waste engine oil contamination on the epidermis of the leaves of *A. compressus* showed slightly sinuous epidermal wall of the grasses grown on 0 % 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. 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 exhibit a gradation of sinuosity, being strongly sinuous in *A. compressus* and slightly sinous in *P. scrobiculatum.* The degree of sinuosity of epidermal cells is usually caused by the degree of force exerted on the stomata in their course of development (Esau, 1997).

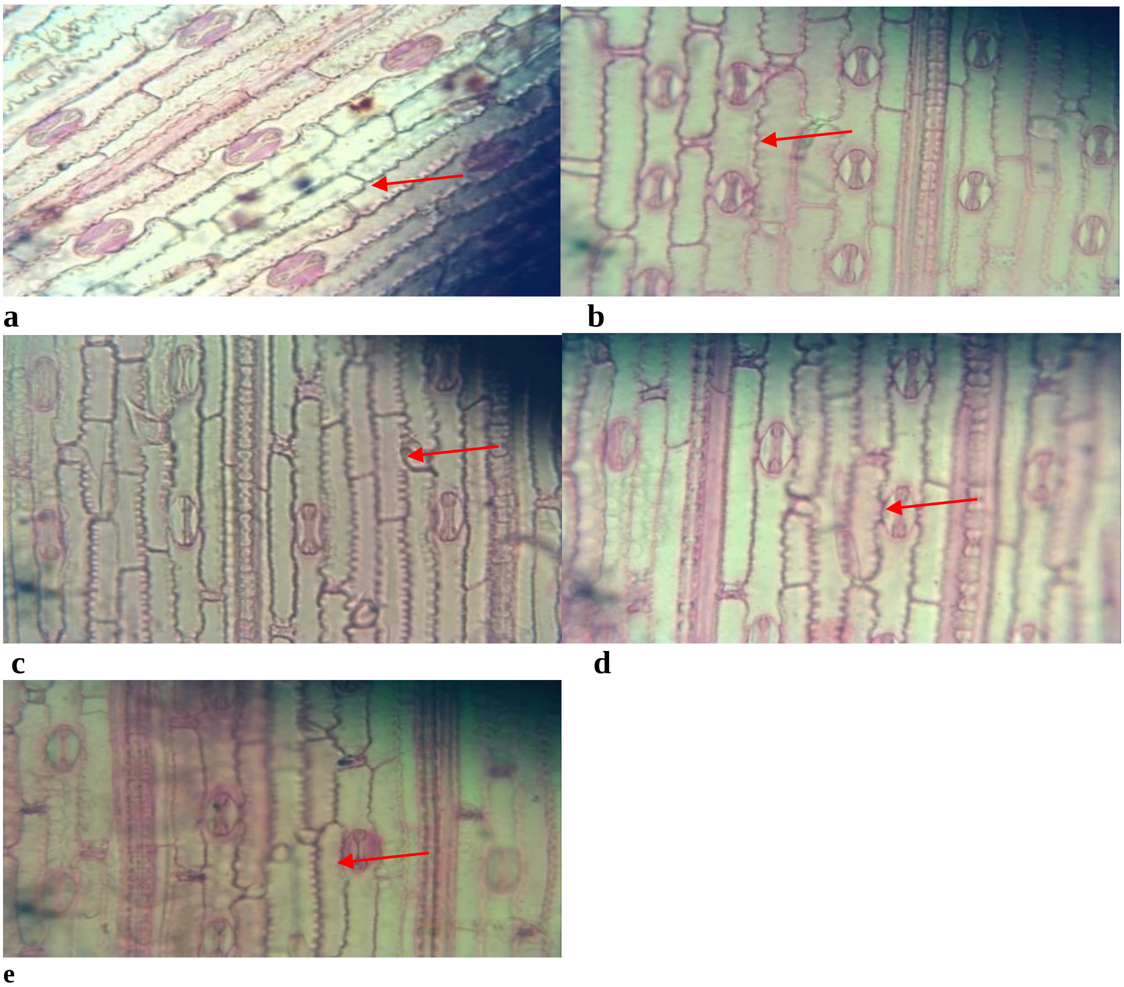


Plate 1: Effects of various percentages of waste engine oil contamination on the epidermal studies of the leaves of *P. scrobiculatum.*

The effect of different concentrations of waste engine oil soil contamination on the T/S of stems of *P. scrobiculatum* 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 (Plates 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)**.**

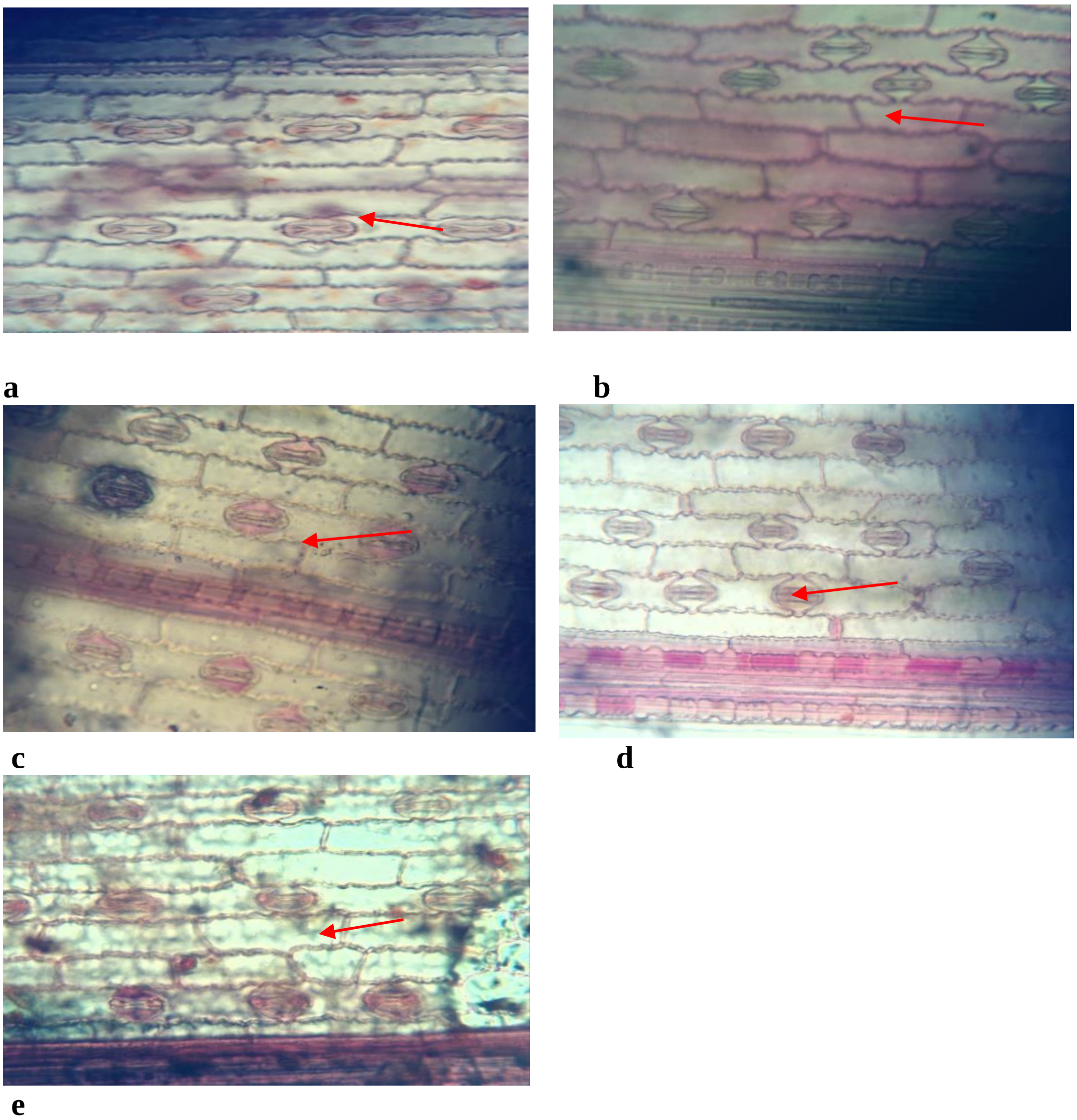


Plate 2: Effects of various percentages of waste engine oil contamination on the epidermal studies of the leaves of *A. compressus.*

The effect of different concentrations of waste engine oil soil contamination on the T/S of stems of *A. compressus* 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). 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) observed higher Zn concentration will result in structural changes in roots, stem and leaves and altered physiological and morphological characteristics.

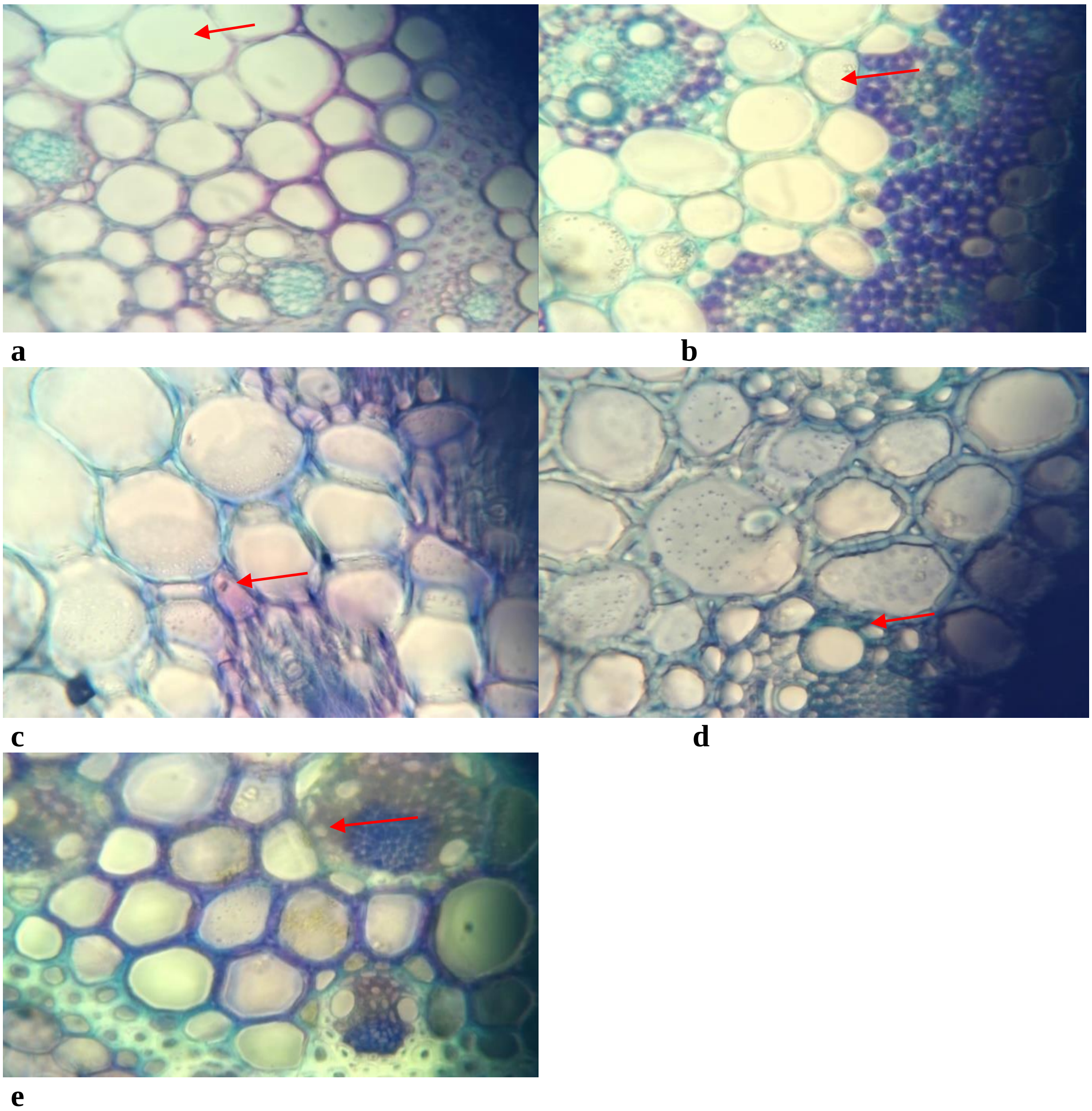


Plate 3: Effects of various percentages of waste engine oil soil contamination on the T/S of stems of *P. scrobiculatum.*

The effects of different concentrations of waste engine oil soil contamination on the T/S of roots of *P. scrobiculatum* 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).

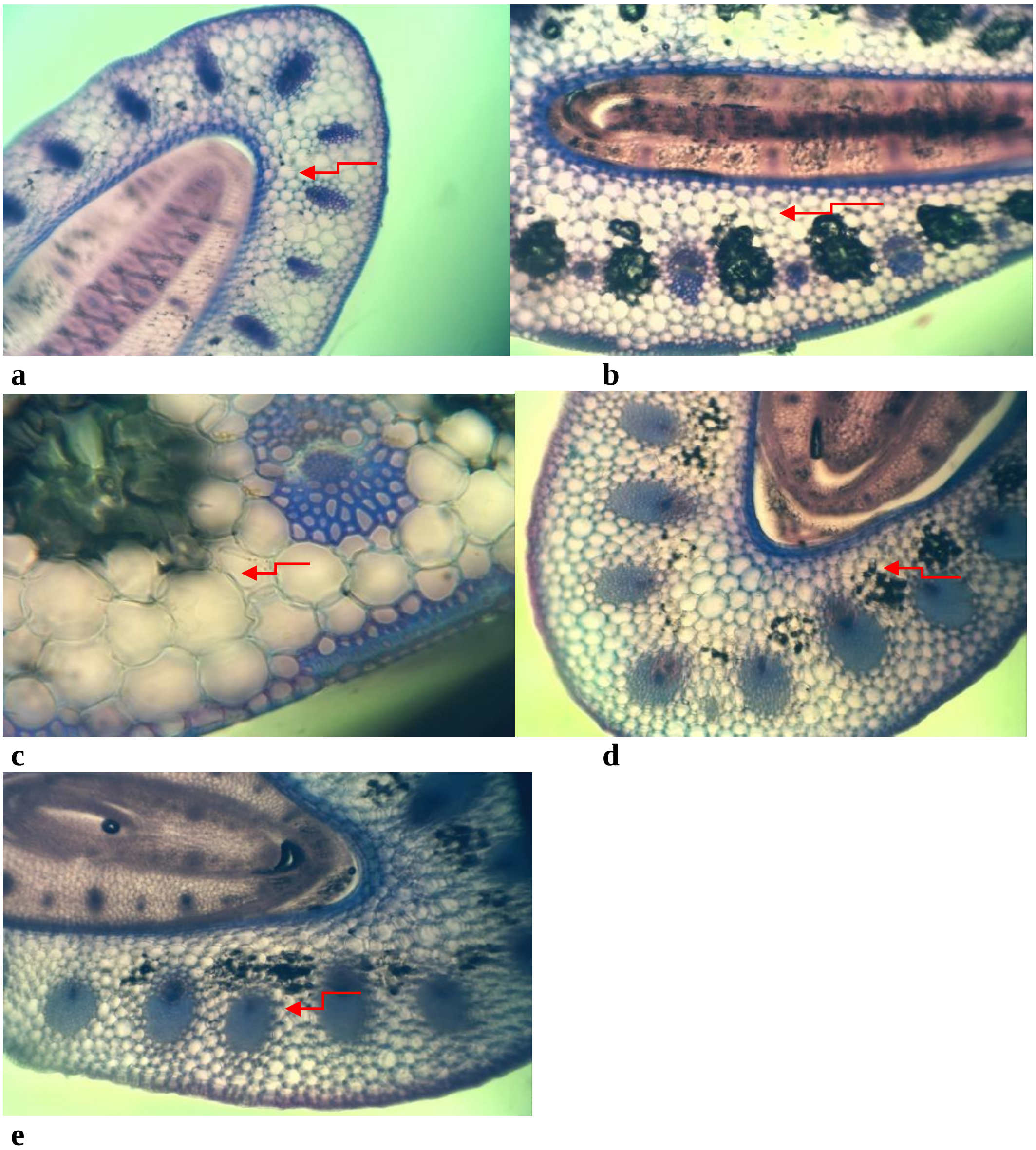
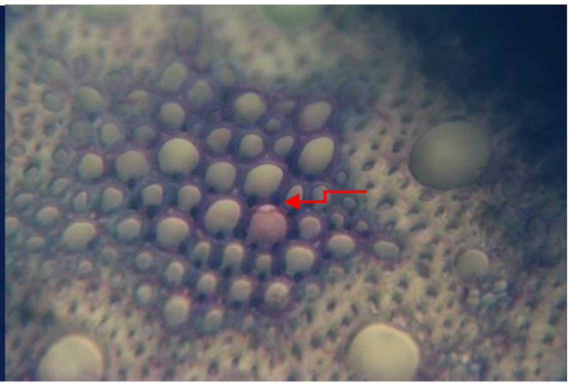
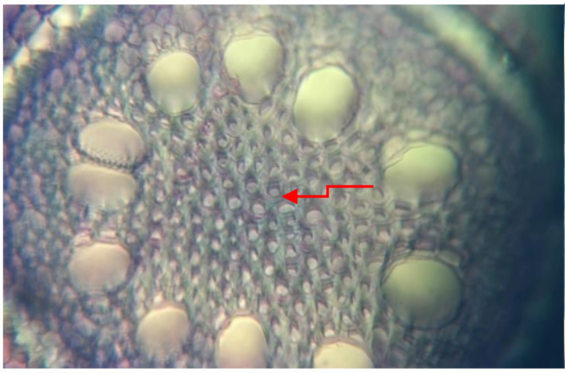
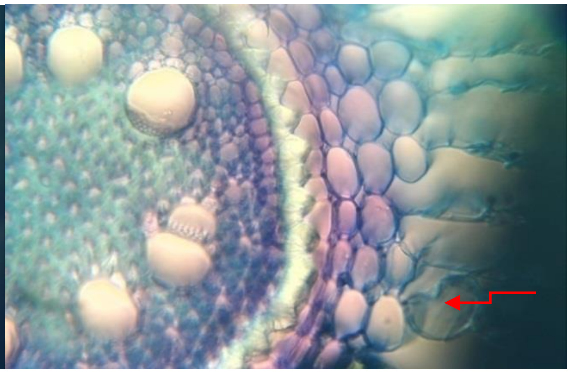


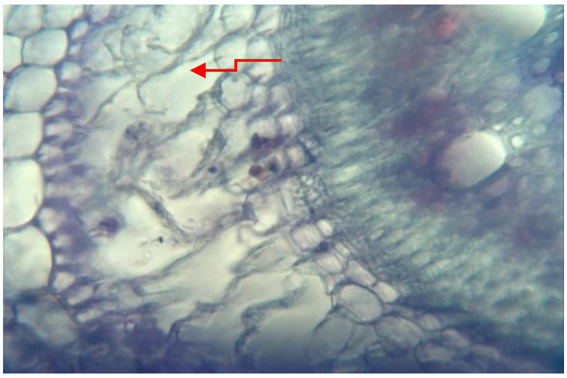
Plate 4: Effects of various percentages of waste engine oil soil contamination on the T/S of stems of *A. compressus*

a **b**

c d



e

Plate 5: Effects of various percentages of waste engine oil soil contamination on the T/S of root of *P. scrobiculatum.*

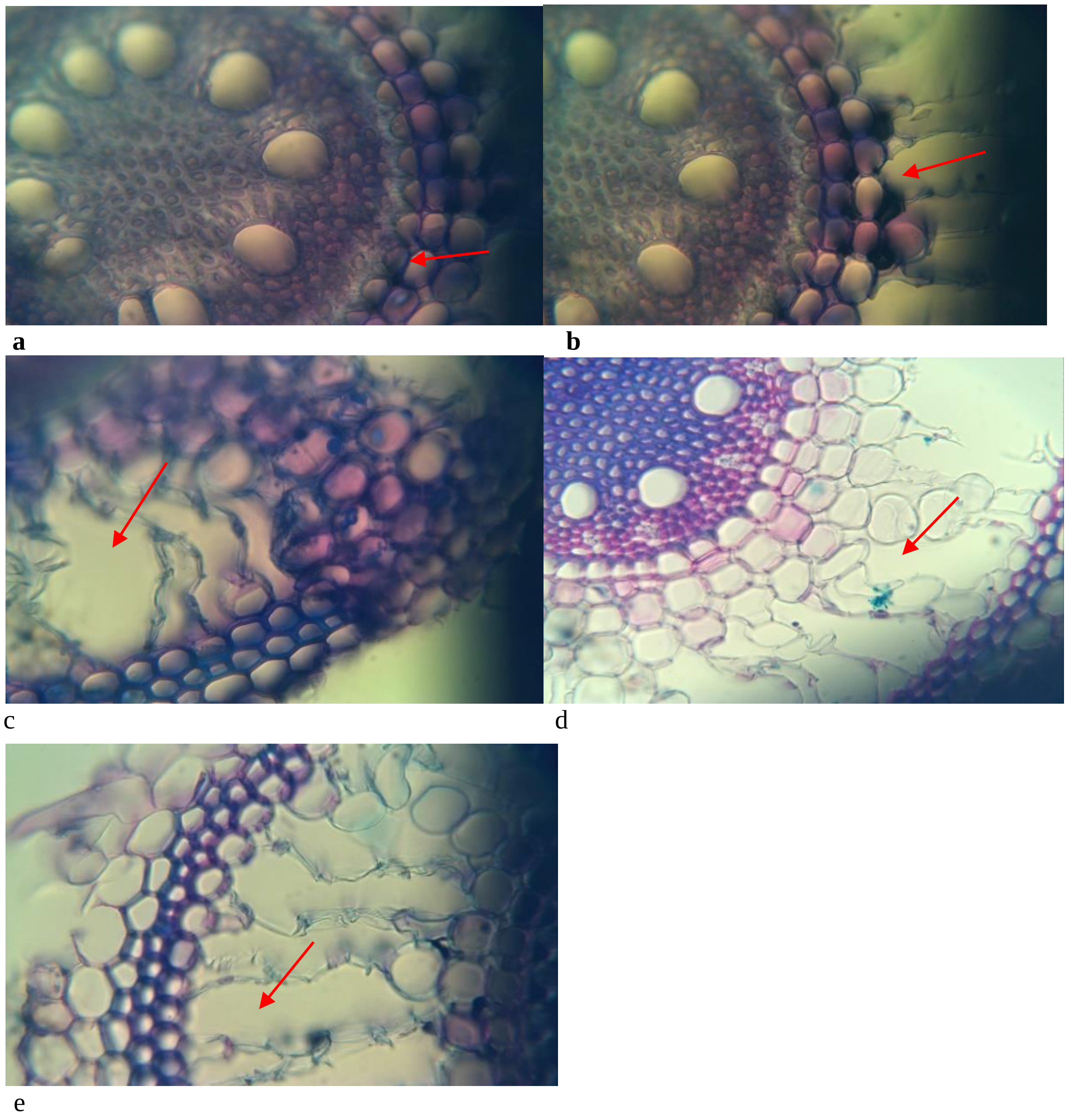


Plate 6: Effects of various percentages of waste engine oil soil contamination on the T/S of root of *A. compressus.*

The effect of different concentrations of waste engine oil soil contamination on the T/S of roots of *A. compressus* 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). In root anatomy, this study revealed several changes in the root tissues of plants exposed to waste engine oil contaminations. There was 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. 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). In *B. decumbens* the wall thickening of both xylem elements and cortical parenchyma of roots is another anatomical adaptation to heavy metals toxicity (Marcelo *et al.,* 2011). Some authors suggest that in plant, the capacity to bind heavy metal in the cell wall has a protective action against the deleterious effect of heavy metals by reducing the amounts of cytosolic heavy metals (Vázquez *et al*., 1992; Wójcik *et al*., 2005).

Conclusion

Paspalum scrobiculatum and A. compressus showed observed anatomical changes in their leaves, roots and the shoots as signs of their response to environmental pollutants. The *P. scrobiculatum* and *A. compressus* could withstand waste engine oil-polluted soil at 10 % treatment for eight weeks; making them potential candidate for phytoremediation. For phytoremediation of waste engine oil polluted sites and indicator for environmental pollutants, *P. scrobiculatum* and *A. compressus* could be potential candidates.

References

Abhilash PC: Phytoremediation: An innovative technique for ecosystem clean up. Our Earth. 4: 7-12. 2007.

Adedokun OM, Ataga AE: Effect of amendment and bioaugumentation of soil polluted with crude oil automotive gasoline and spent engine oil on the growth of cowpea (*Vigna unguiculata* L. walp). J Sci Resour. 2(5):1147-1149. 2007.

Adenipekun CO, Oyetunji OJ, Kassim LQ: Screening of *Abelmoschus esculentus* L. Moench for tolerance to spent engine oil. J Appl Biosci, 20:1131-1137. 2009.

Anoliefo GO, Vwioko DE: Effect of spent lubricating oil on the growth of *Capsicum annum* L. and *Lycopersicon esculentum* Mill. Environ Pollut, 99: 361–364. 2001.

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

Adams G, Duncan H: Influence of diesel fuel on seed germination. Environ Pollut, 120: 363-70. 2002.

Banuelos GS, Ajwa HA, Mackey B, Wu L, Cook C, Akohoue S, Zambruzuski S: Evaluation of different plant species used for phytoremediation of high soil selenium. J Environ Qual. 26: 639-646. 1997.

Brandt R: Potential of Vetiver (*Vetiveria zizanioides* (L.) Nash) for the use in phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. 2003. https://pdfs.semanticscholar.org/44db/3b3b8a40ce9c0bf6f71ed170bfda49a6878d.pdf. Accessed on 25/10/2017

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

Cunningham SD, Anderson TA, Schwab AP, Hsu TS: Phytoremediation of soils contaminated with organic contaminants. Advance Agronomy, 56: 55-114. 1996.

Doty SL, Shang QT, Wilson AM, Moore AL, Newman LA, Strand SE: Enhanced metabolism of halogenated hydrocarbons in transgenic plants contain mammalian P450 2E1. Proc Natl Acad Sci USA. 97: 6287–91. 2007.

Duffus JH: Heavy metal—a meaningless term? Pure Appl Chem, 74: 793-807. 2002.

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.

Esau K: *Anatomy of Plants*. 2[ed] John Wiley and Sons, New York. 550p. 1997.

Greenberg BM, Hunag XD, Gurska Y, Gerhardt KE, Wang W, Lampi MA: Successful field tests of a multi-process phytoremediation system for decontamination of persistent petroleum and organic contaminants. Proceedings of the twenty-ninth Artic and Marine Oil Spill Program (AMOP). Technical Seminar Vol. 1. Environment Canada. P: 389–400. 2006.

Kayode J, Oyedeji AA, Olowoyo O: Evaluation of the effect of pollution with spent lubricant oil on the physical and chemical properties of soil. Pac J Sci Technol. 10(1):387-391. 2009.

Malik RN, Husain SZ, Nazir I: Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak J Bot, 42(1): 291-301. 2010.

McLaughlin MJ, Zarcinas BA, Stevens DP, Cook N: Soil testing for heavy metals. Commun Soil Sci Plant Anal 31(11-14): 1661– 1700. 2000.

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.

Odjegba VJ, Sadiq AO: Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. The Environmentalist, 22: 23-28. 2002.

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

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, 158p. 2013.

Raskin I, Ensley BD: *Phytoremediation of toxic metals: Using plants to clean up the environment.* John Wiley and Sons, Inc. Publishing, New York. 304p. 2000.

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.

Schnoor JL, Light LA, McCutcheon SC, Wolfe NL, Carriera LH: Phytoremediation of organic and nutrient contaminants. Environ Sci Technol, 29:318–23. 1995.

Shimp JF, Tracy JC, Davis LC, Lee E, Huang W, Erickson LE, Schnoor JL: Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Crietal Rev Environ Sci Technol, 23:41-77. 1993.

Vázquez MD, Poschenrieder C, Barceló J: Ultra structural effects and localization of low cadmium concentrations in bean roots. New Phytologist, 140: 350-355. 1992.

Vwioko DE, Fashemi DS: Growth response of *Ricinus cummunis* L. (castor oil) in spent lubricating oil polluted soil. J Appl Sci Environ Manage, 9(2): 73-79. 2005.

Wójcik M, Vangronsveld J, D´Haen J, Tukiendorf A: Cadmium tolerance in *Thlaspi caerulescens*. Environ Experiment Bot, 53: 163-171. 2005.