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Full Length Research Article

Evaluation of the phytoremediation potential of *Gmelina arborea* Roxb. Seedlings on spent engine oil-stressed soil.

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Abstract

Waste oil finds its way into the environment through engine use, spills, leaks from exhaust systems and indiscriminate disposal. This study aimed at phytoremediation of spent engine oil (SEO) contaminated soil by using *Gmelina arborea* seedlings. Pollution was simulated by using four SEOconcentrations:0 ml (Control), 30 ml (T1), 150 ml (T2, and 750 ml (T3) to contaminate vegetated (VG)and unvegetated (UVG) soils. Using 2 × 4 factorial completely randomized design of 15 replicates, data were collected at 57 and 163 days after planting (DAP), and subjected to analysis of variance using SPSS version 23.Mean concentrations of heavy metals in VG soil decreased significantly (Pb: 0.031;Zn:1400; Cu:0.8; Cd: 0.011; Al:4.7 mg/100g) compared to the significant increase in UVG(Pb: 0.046; Zn:1787.5; Cu:2.8; Cd:0.042; Al:5.05mg/100g) soil and showed variation in the mean accumulation in the plant tissues. Pb (0.08), Al (6.88), Cu (0.83) and Cd (0.02) produced highest accumulation in the T3 root, Zn was highest (3,200mg/100g) in the T2 leaves while stem had least accumulations at higher concentrations. Results showed low adverse effects on the plant morphological characterizations but stomatal width and chlorophyll contents of the leaves had variations. Based on the results, *G. arborea* had a higher tolerance for SEO pollution. Therefore, the plant's ability to germinate, grow, survive in polluted soil and build-up of heavy metals showed its potency as hyperaccumulator and usefulness as a phytoremediator.

Keywords: Phytoremediation, heavy metal, accumulation, chlorophyll content, stomata, hyperaccumulator.

Introduction

Increase in the use of automobiles, crude oil and refined products especially engine oil and diesel, all over the world is resulting to an increased environmental contamination (Briffa *et al.,* 2020). Environmental pollution becomes escalated through leaks released from exhaust systems of operating engines and automobiles, crude oil spills and uncontrolled discharge of engine oil, more especially is the spent lubricant usually

referred to as “used”, “condemn” or “waste” engine oil. As pollutants, these released wastes alter the natural flow processes in the ecosystems (Stankovic and Devetakovic, 2016), contaminate food chain and may result in diversified adverse effects on human health (Thompson and Darwish, 2019). Gradual or severe accumulation of these pollutants contribute to an enormous ruin of the ecosystem. Though, many pollutants can be recycled into useful secondary products, but some including used engine oils cannot easily be recycled into a useful secondary product (USEPA, 2021).

Spent Engine Oil (SEO) is a waste petroleum-based oil mixed with different chemicals and lubricative additives like dirt, ash, metal scraping, heavy metal contaminants picked up from operating engine as they wear away (Wang *et al.,* 2000; Adedokun and Ataga, 2007; Ayoola and Akaeze, 2012; USEPA, 2021). The polycyclic aromatic hydrocarbon, aliphatic hydrocarbon and heavy metal compositions of SEO are toxic persistent environmental contaminants which pose a threat to both the surrounding environment and human health (Hui *et al.*, 2020; Hui *et al.*, 2022). Again, SEO is a common example of a hydrophobic organic compound which possesses toxic characteristics that long accumulates and contaminates the environment (Idemudia *et al.,* 2014). As a waste, SEO is not a natural contaminant but finds its way into the environment through indiscriminate disposals after servicing and subsequent draining and changing from machines like automobiles and power-generating engines by mechanics. In Nigeria, environmental impacts associated with spent engine oil is more observed and rampart than crude oil effects because of its common disposal practices into gutters, water drains, open vacant plots and farms (Odjegba and Sadiq, 2002). Such disposed oil is injurious to plants, micro-organisms, and aquatic lives since it usually contains hydrocarbons and very toxic polycyclic aromatic hydrocarbons in large quantities (Nwachukwu *et al.*2020).

Gmelina arborea is an important species of Verbenacea family, recognized as fast-growing popular commercial timber which inter-grows in mixed forests or can be planted elsewhere on a large scale (Kidulan, 2017). It has many reported uses in medicine (Agbogidi *et al.,* 2007; Munira *et al.*, 2013). Also, *G. arborea* is widely grown as a component of agroforestry systems, useful as windbreak, hedge and an ideal choice for large-scale reforestation programmes (Verma *et al.,* 2017). Therefore, there is a need to further explore the tolerance potential of *G. arborea* to a spent engine oil pollution. Hence, this study aimed at phytoremediation of spent engine oil (SEO) contaminated soil by using *Gmelina arborea* seedlings. This will investigate the plant characteristics, survival ability and heavy metals accumulation potentials in SEO polluted soil.

Materials and Methods

Study location

This study was conducted in the Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka (UNN), Enugu State, Nigeria, found above sea level with geographical location of Longitude $7^0$24’22’’E and Latitude $6^0$51’56’’N (Uguru *et al*., 2012).Nsukka annual rainfall ranges from 1845 mm to 2000 mm. Enugu is in tropical region with two major seasons, wet (April - October) and dry seasons ranges between (November – March). The annual mean temperature in Nsukka is28°C (Osugiri *et al*., 2019) and 60% humidity. The topography is characterized as undulating and the vegetation type is a mosaic of lowland rainforest and derived savanna. The soil is a mixture of different types including ferrallitic soils, also known as red earth or acid sands and hydromorphic soils.

Experimental Design

The experiment used a 2 × 4 factorial completely randomized design and replicated 15 times. Vegetated (Part A) and unvegetated (Part B) soils were considered. The vegetated soil was expected to absorb, accumulate and/or volatilize the heavy meals via the plants, unlike the unvegetated soil. Each part,

A and B of the experiment had four treatments; control (Control), T1 (low pollution), T2 (moderate pollution) and T3 (high pollution), and each treatment had fifteen bags. To simulate engine oil spillage, each of the 15 soil bags was polluted with 0 ml (Control), 10 ml (T1), 50 ml (T2, and 100 ml (T3) of spent engine oil. The experimental bags were watered, kept under the sun and rain-fed when it rained.

Collection of plant materials, species confirmation and seed viability test.

Mature fruits of *Gmelina arborea* used for this study were collected from the ground beside the trees within University of Nigeria Nsukka campus. The plant species was identified and authenticated at the University of Nigeria Herbarium, domiciled in the Department of Plant Science and Biotechnology by a renowned plant taxonomist, Mr Felix Nwafor (Voucher number: UNH/13180). The fruits were de-pulped mechanically and were soaked for12 hours in cold water for quick germination. The seeds germinated within 20 - 50 days under ideal conditions; the average rate for a healthy seed lot is 60%. More plants were easily raised by transplanting, which was done in the rainy season and watering early hours in the morning when there was a little or delay in rainfall.

Preparation of soil samples

Ten (10) centimeter depth of top garden and humus soil samples were obtained from the Botanic Garden and analyzed in the Department of Soil Science, UNN. The soils were mixed with poultry manure at a ratio of 3:3:2 (top soil: humus: poultry manure) and was watered for two weeks with adequate turning (Nwadinigwe and Uzodimma ,2005). Polythene bags (n = 120) were filled with 20 kg of the mixture and were separated into parts: A (60 bags for vegetated soil) and B (60 bags for unvegetated soil). The polythene bags were perforated underneath to avoid water logging and allow water drainage. Ten seeds of *G. arborea* were sown in each bag of part A while part B had no seeds. Observations were made daily, for the days of first seedling emergence expressed as the initial germination.

Transplanting

The plantlets were transplanted forty-three days (43) after germination into the polythene bags each containing 20 kg of the Control and amended (T1 to T3) soil. The transplanting was done in the evening to avoid the excessive effect of transpiration on the plantlets. The plants were irrigated with tap water at an interval of two days for two weeks for the plantlets to stabilize before the application of spent engine oil. When transplanted plants were stable, (14 days later) that is 57 days after planting (DAP), the pollution was repeated with 20 ml, 100 ml, and 650 ml, which gave a total number of 30 ml (T1), 150 ml (T2), and 750 ml (T3) respectively of spent engine oil used. Both parts A and B were polluted in the same manner. The control had no spent engine oil.

Data Collection

The morphological characterization of the plant such as plant height, stem length, number of leaves per plant, petiole height, leaf length, leaf width, stem circumference, number of roots, root length, and root diameter was determined at 57 days after planting (DAP) and 163 DAP that is106 days after pollution. Plant height, root length, leaf length, and width were measured with a meter rule. The leaf area was determined using the planimetry method (Piyush, 2012). Stem and root circumference were measured by using a Vernier caliper, thread, meter rule and recorded in centimeters. Three plants were randomly selected from each treatment and analyzed for heavy metal contents (Omosun *et al.,* 2008). The number of leaves and leaf scars from fallen leaves was determined visually by counting. Fresh weight of the plant samples (leaves, stems, and roots) was determined on a weighing balance (Gallenkamp 80). The plant samples dried in an electronic oven at 80^{o}C for 24 h were reweighed to get the dry weight.

Heavy Metal Analysis of soil and plant tissues (leaf, stem and root).

Heavy metals analysis was carried out with the help of UV-Vis Spectrophotometer as adopted by AOAC, (2002) method using pH meter, porcelain crucibles and muffle furnace with slight modifications. Determination of Lead (Pb) was based on the principle that when a sulphide is added to a solution containing lead ions, a brown colour due to the collection of colloidal lead sulphide is produced. Zinc was assayed by mixing 5 ml of acetate buffer and 10 ml of sodium thiosulphate solution into 10 ml of zinc filtrate and shaking with 10 ml Dithizone solution II. Then standard curve and blank were prepared before measuring the absorbance at 535 nm in a 721 VIS spectrophotometer. Nickel content of soil was quantified by application of the principle that nickel (II) in the presence of acetate buffer, forms a red or pale purple complex with 2-hydroxy-5-nitrobenzylidene isonicotinoylhydrazone (HNBISNH) and 2-(4-fluoro benzylideneamino) benzenethiol (FBBT) at pH 4.0 and 4.7 respectively. The solution was allowed to develop colour and the absorbance was measured at 490 nm. Cadmium was determined using xylenol orange indicator. Aluminum and Iron were quantified by the standard Spectrophotometric method (AOAC, 2002; Siriangkhawut, *et al.,* 2013). Soil copper contents were quantified by mixing thoroughly 10 ml of the sample, 5 ml of hydroxylamine hydrochloride and 10 ml of sodium citrate solution. Then the extract was made up to fifty (50 ml) with methanol followed by standard curve and blank preparation and measurement of the absorbance taken at 457 nm.

Chlorophyll analysis and anatomical studies

The chlorophyll contents of the samples were determined as described by Sumanta *et al.* (2014). In anatomical studies, matured fresh parts of the leaves, stems and roots of *G. arborea* were collected. These plant parts were immersed in formalin, acetic acid and alcohol in the ratio of 1:1:18 respectively for 48 h. The plant parts were washed in water and sectioned with a Reichert Rotary Microtome. The transverse sections were placed in Petri dishes containing 70% alcohol. The sections were mounted on slides, observed under a light microscope and photomicrographs were taken (Omosun *et al*., 2008).

Stomata studies were obtained by washing the fresh plant samples carefully in water, followed by peeling the upper and lower surfaces with dissecting needles and forceps. The epidermal peels were stained with Safranin solution and mounted on glycerin for microscopic observation. The following parameters were determined: number of epidermal cells and stomata, number of vascular bundles, stomata frequency, stomatal length and width were measured with a compound microscope at ×100 magnification using Image J software. All observations and photomicrographs were obtained with Moti B_3 compound microscope. Area of the stomata was measured in per mm^2.

The stomatal index (S. I.) was determined as follows;

$$\text{S.I.} = \frac{S \times 100}{E + S}$$

S= the number of stomatal present per unit area of leaf;

E = the number of epidermal cells per unit area of leaf.

Statistical analysis

The data were analyzed using Statistical Product and Solution Service (SPSS) version 23 (IBM Corporation, Armonk, USA). It was subjected to one-way analysis of variance (ANOVA). The Least Significant Differences (LSD) test was used to separate the means at ($p \leq 0.05$) level of significance. The result is presented as mean ± standard error (SE).

Results

Heavy metal concentration in soil before the study

The results of the heavy metals concentration in the amended soils are presented in Table 1. The result showed that the control soil was significantly lower compared to the spent engine oil polluted soils. Results observed that all the heavy metals in the soils polluted

with SEO increased with an increase in SEO concentration across the treatments. Pollution of the soil with SEO at 100 ml (T3) and 50 ml respectively produced the highest heavy metals in the soil before planting.

Table 1. Soil heavy metals after SEO introduction

Metal (mg/100g)	**T1**	**T2**	**T3**	**LSD**
Lead (Pb)	0.09 ± 0.01^{a}	0.04 ± 0.01^{b}	0.10 ± 0.02^{a}	0.05
Zinc (Zn)	5600.00 ± 57.74^{c}	12700.00 ± 115.47^{b}	16000.00 ± 144.34^{a}	386.89
Nickel (Ni)	23.66 ± 0.38^{c}	30.00 ± 0.29^{b}	36.00 ± 1.15^{a}	2.5
Aluminum (Al)	5.80 ± 0.35^{b}	10.32 ± 0.52^{a}	10.50 ± 0.58^{a}	1.7
Copper (Cu)	0.92 ± 0.03^{c}	1.48 ± 0.23^{b}	5.24 ± 0.23^{a}	0.65
Cadmium (Cd)	0.16 ± 0.05^{c}	0.48 ± 0.03^{b}	0.64 ± 0.02^{a}	0.12
Iron (Fe)	0.41 ± 0.03^{c}	1.79 ± 0.03^{b}	10.08 ± 0.58^{a}	1.16

Means with different alphabet superscripts along each row represents significant differences across treatments separated using LSD- least significant difference at $P < 0.05$; NS- not significant.

Heavy metal accumulation in vegetated and unvegetated soil

Results of the heavy metal accumulation showed significant differences ($p < 0.05$) across the treatments. Mean concentrations of heavy metals in vegetated soil samples decreased significantly when compared to the significant increase in the unvegetated soil samples (Figure 1). The unvegetated soil had the highest mean Lead, Aluminum, copper and cadmium concentrations at Treatment T3 compared to the vegetated soils. The mean concentrations of cadmium and zinc in unvegetated soils were significantly higher in treatments T2 and T3 but showed no significant difference. There was a very high concentration of nickel in the control of the unvegetated soil, followed by T2 and T3. The trend repeated itself on the T1 as well as the vegetated soils. Mean concentration of Zinc was highest in the unvegetated soil in SEO increased concentration manner.

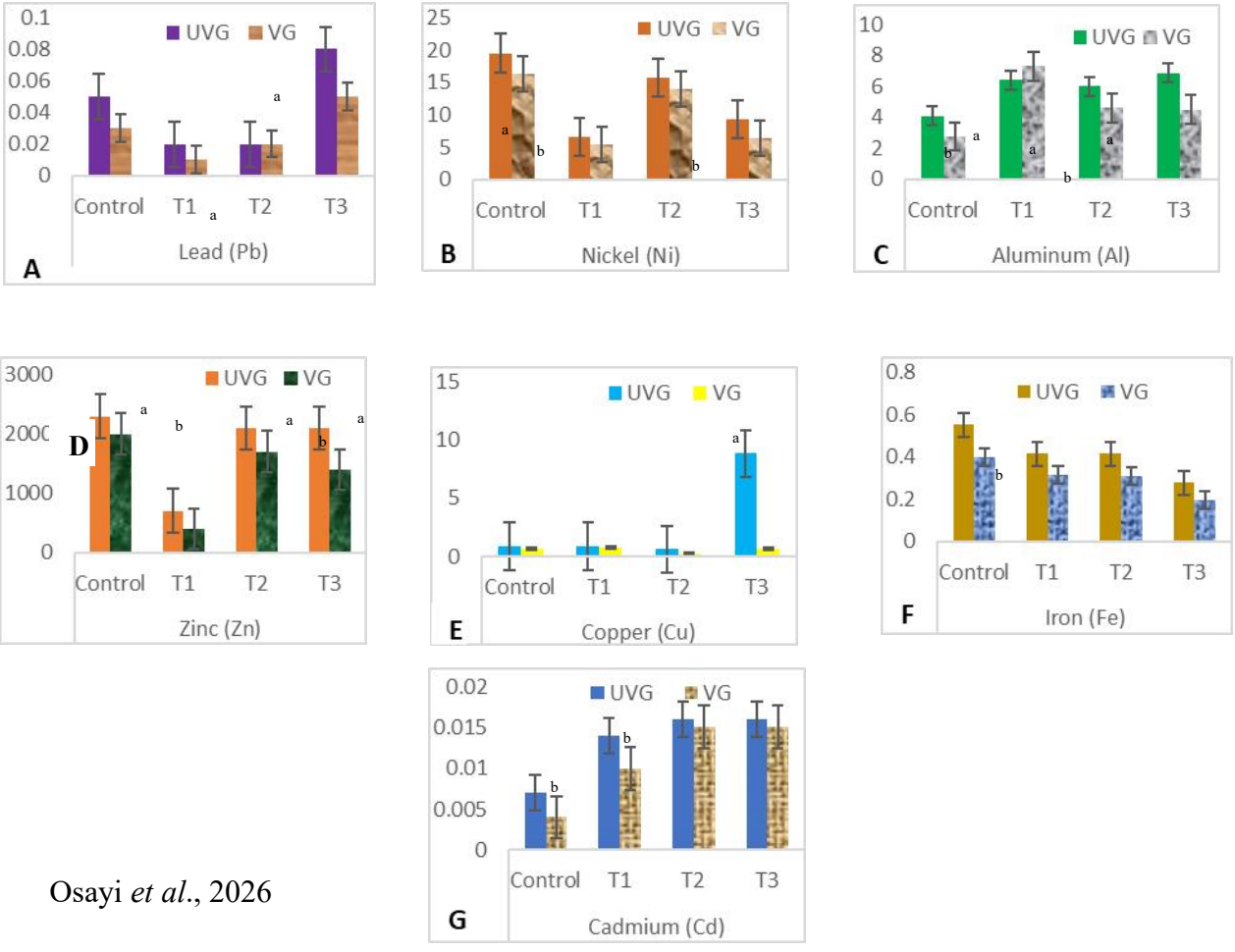


Figure 1: Mean comparison of the heavy metals accumulated in vegetated and unvegetated soils at 163 days after planting. **Key:** A-lead, B-nickel, C-aluminum, D-zinc, E- copper, F-iron, G-cadmium, UVG-Unvegetated and VG-Vegetated

Heavy metal accumulation in plant tissues (leaf, stem and root)

In Table 3, Lead (Pb) was not detected in the leaf and stem of SEO polluted plants and the control; but was detected in the root. Zinc concentrations were detected in the stem and root but were significantly highest in T2 leaves and stem while their concentrations in T1 decreased across the plant tissues. Nickel decreased significantly in the stem and root of T1, T2, and T3, unlike in the leaf. Aluminum concentrations in the leaf, stem and root of T1 to T3 were significantly higher than in the control ($p < 0.05$). Cadmium and iron contents of the leaf, and stem of T1 to T3 were not different from the control but cadmium content on the T2 and T3 roots differed significantly. Copper increased significantly in the root of T1, T2, and T3, unlike in the leaves, stem and control (Table 3).

Morphological characterization

Some of the leaves of plants in the heavily polluted soils (T3) curled upwards from their tips and withered, resulting in the death. The morphological characterizations are presented in Table 4. At 163 DAP, growth in T3 was stunted and showed significant ($p < 0.05$) low mean plant height, stem length, leaf length, root length, leaf diameter and petiole length as well as the leaf number when compared to T2, T1 and the Control. Plant growths were generally in the order of magnitude: Control > T1 > T2 > T3. However, diameter was highest in T3, and in the order of magnitude: T3 > Control > T2 > T1. The numbers of roots, the root length, and root diameter were lowest in T3. At 57 DAP, mean plant growth was highest in control, followed in T1 and lowest in T2.

Chlorophyll contents and stomata dimensions

Chlorophyll 'a' increased significantly in T3 compared to T1, T2, and the control; but decreased significantly in T2 ($p < 0.05$). T2 had the highest mass of Chlorophyll 'b', followed by T3 (Figure 2A). Stomata of T1 to T3 and the control were not different, but stomata length of T3 was significantly short compared to Control and T1 (Figure 2B).

Epidermal studies of Leaf, stem and root

Number of vessels per row in the upper epidermis of the leaf was highest in T3, but it was not significantly different ($P<0.05$) from other treatments and control. Epidermal layer thickness, parenchyma cell diameter, palisade mesophyll diameter, and leaf blade thickness were all of highest magnitude in T2 followed by T3, and significantly different from T1 and the control (Table 5).

Epidermis diameter of stem in T1, T2 and T3 were significantly shorter than the control ($p < 0.05$). Cortex diameter was however, lowest in the control and significantly highest in T3; followed by T1 than in T2. Mean xylem vessel diameter in T1, T2, and T3 were significantly lower than the control (Table 6).

Root vessel diameter was largest in T2, followed by T3 and Control. The difference in order of significance was T2 >T3 and Control > T1 (Figure 3).

Table 3. Concentration of metal deposition in leaves, stems and roots of *Gmelina arborea* on spent engine oil (SEO) polluted soil

Metal (mg/100g)	Treatment	Leaves	Stem	Root

Lead (Pb)	Control	$0.00 \pm 0.00^{a,2}$	$0.00 \pm 0.00^{a,2}$	$0.05 \pm 0.01^{b,1}$
	T1	$0.00 \pm 0.00^{a,2}$	$0.00 \pm 0.00^{a,2}$	$0.02 \pm 0.01^{b,1}$
	T2	$0.00 \pm 0.00^{a,2}$	$0.00 \pm 0.00^{a,2}$	$0.02 \pm 0.01^{b,1}$
	T3	$0.00 \pm 0.00^{a,2}$	$0.00 \pm 0.00^{a,2}$	$0.08 \pm 0.03^{a,1}$
Zinc (Zn)	Control	$2700.00 \pm 14.43^{b,1}$	$2300.00 \pm 86.60^{c,2}$	$2300.00 \pm 57.74^{a,2}$
	T1	$1666.67 \pm 88.19^{c,2}$	$2000.00 \pm 57.74^{d,1}$	$700.00 \pm 28.87^{c,3}$
	T2	$3200.00 \pm 28.87^{a,1}$	$3000.00 \pm 23.09^{a.2}$	$2100.00 \pm 28.87^{b.3}$
	T3	$1300.00 \pm 57.74^{d,3}$	$2500.00 \pm 86.60^{b,1}$	$2100.00 \pm 28.87^{b,2}$
Nickel (Ni)	Control	$19.21 \pm 0.12^{a,1}$	$19.53 \pm 0.23^{a,1}$	$19.53 \pm 0.23^{a,1}$
	T1	$19.56 \pm 0.23^{a,1}$	$12.79 \pm 0.40^{c,2}$	$6.54 \pm 0.23^{d,3}$
	T2	$19.53 \pm 0.23^{a,1}$	$16.91 \pm 0.40^{b,2}$	$15.75 \pm 0.40^{b,3}$
	T3	$19.54 \pm 0.22^{a,1}$	$16.54 \pm 0.29^{b,2}$	$9.31 \pm 0.40^{c,3}$
Aluminum (Al)	Control	$2.36 \pm 0.17^{c,2}$	$2.36 \pm 0.18^{c,2}$	$4.08 \pm 0.03^{c,1}$
	T1	$4.53 \pm 0.23^{a,3}$	$6.00 \pm 0.17^{a,2}$	$6.83 \pm 0.23^{a,1}$
	T2	$3.88 \pm 0.12^{b,2}$	$4.09 \pm 0.23^{b,2}$	$6.03 \pm 0.15^{b,1}$
	T3	$4.25 \pm 0.12^{ab,3}$	$6.27 \pm 0.32^{a,2}$	$6.88 \pm 0.17^{a,1}$
Copper (Cu)	Control	$0.41 \pm 0.03^{a,2}$	$0.41 \pm 0.03^{b,2}$	$0.79 \pm 0.05^{a,1}$
	T1	$0.42 \pm 0.03^{a,3}$	$0.53 \pm 0.04^{a,2}$	$0.82 \pm 0.04^{a,1}$
	T2	$0.22 \pm 0.02^{b,3}$	$0.41 \pm 0.03^{b,2}$	$0.60 \pm 0.04^{b,1}$
	T3	$0.41 \pm 0.03^{a,2}$	$0.31 \pm 0.02^{c,3}$	$0.83 \pm 0.04^{a,1}$
Cadmium (Cd)	Control	$0.01 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$
	T1	$0.00 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$
	T2	$0.01 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$	$0.02 \pm 0.00^{a,1}$
	T3	$0.01 \pm 0.00^{a,1}$	$0.01 \pm 0.00^{a,1}$	$0.02 \pm 0.00^{a,1}$
Iron (Fe)	Control	$0.28 \pm 0.05^{a,1}$	$0.28 \pm 0.05^{a,11}$	$0.55 \pm 0.03^{a,1}$
	T1	$0.28 \pm 0.05^{a,1}$	$0.28 \pm 0.05^{a,1}$	$0.41 \pm 0.03^{a,1}$
	T2	$0.28 \pm 0.05^{a,1}$	$0.28 \pm 0.05^{a,1}$	$0.41 \pm 0.03^{a,1}$
	T3	$0.28 \pm 0.05^{a,1}$	$0.28 \pm 0.05^{a,1}$	$0.28 \pm 0.05^{a,1}$

Means with different alphabet superscripts along a column for each metal represents significant differences across concentrations while means with different number superscripts along a row represents significant differences across plant parts using LSD (0.01) - least significant difference at $P < 0.05$.

Table 4: Morphological characterization of *Gmelina arborea* on spent engine oil (SEO) polluted soil

Plant parts (cm)	**DAP**	**Control**	**T1**	**T2**	**T3**	**LSD**
Plant Height	57 DAP	110.09 ± 11.28^{a}	101.60 ± 8.68^{a}	79.08 ± 7.36^{a}	91.71 ± 5.98^{a}	NS
	163 DAP	224.20 ± 6.60^{a}	194.93 ± 4.98^{b}	143.61 ± 7.56^{c}	34.97 ± 5.11^{d}	17.44
Stem Length	57 DAP	104.97 ± 11.29^{a}	97.54 ± 8.64^{a}	74.13 ± 7.35^{a}	86.44 ± 5.98^{a}	NS
	163 DAP	222.13 ± 6.60^{a}	186.32 ± 4.96^{b}	138.95 ± 7.73^{c}	33.17 ± 5.06^{d}	17.55
Stem	57 DAP	2.50 ± 0.32^{a}	2.31 ± 0.25^{a}	1.73 ± 0.20^{a}	2.27 ± 0.14^{a}	NS

Diameter	163 DAP	5.13 ± 0.39^{b}	4.35 ± 0.31^{b}	4.49 ± 0.51^{b}	13.07 ± 3.53^{a}	5.11
No. of	57 DAP	39.87 ± 5.40^{a}	35.33 ± 4.82^{ab}	22.33 ± 3.79^{c}	24.60 ± 4.09^{bc}	12.94
Leaves	163 DAP	129.80 ± 33.79^{a}	98.87 ± 18.63^{b}	24.63 ± 2.71^{c}	3.09 ± 0.72^{c}	54.80
Petiole	57 DAP	26.85 ± 3.97^{a}	18.83 ± 1.71^{b}	15.50 ± 1.80^{b}	17.52 ± 1.43^{b}	6.94
Length	163 DAP	17.59 ± 0.60^{a}	17.07 ± 0.69^{a}	14.38 ± 1.16^{b}	6.35 ± 1.04^{c}	2.56
Leaf Length	57 DAP	46.50 ± 7.16^{a}	34.63 ± 2.49^{b}	28.29 ± 2.46^{b}	34.33 ± 1.83^{b}	11.58
	163 DAP	22.04 ± 0.87^{a}	21.95 ± 0.72^{a}	19.47 ± 0.65^{b}	5.52 ± 1.01^{c}	2.34
Leaf	57 DAP	44.83 ± 7.20^{a}	34.74 ± 2.32^{a}	29.42 ± 2.47^{a}	34.76 ± 1.99^{a}	NS
Diameter	163 DAP	20.96 ± 0.83^{a}	20.33 ± 0.78^{a}	18.97 ± 0.60^{a}	1.59 ± 0.23^{b}	4.15
No. Roots	57 DAP	22.13 ± 1.29^{a}	21.73 ± 1.11^{a}	17.87 ± 1.04^{b}	19.60 ± 0.83^{ab}	3.06
	163 DAP	34.47 ± 0.94+a	25.73 ± 0.72^{b}	24.07 ± 0.65^{b}	7.93 ± 0.81^{c}	2.23
Root length	57 DAP	30.45 ± 0.73^{a}	29.80 ± 0.68^{a}	28.43 ± 0.51^{a}	29.58 ± 0.38^{a}	NS
	163 DAP	35.05 ± 0.54^{a}	17.99 ± 0.22^{b}	15.95 ± 0.32^{c}	6.03 ± 0.49^{d}	1.16
Root diameter (mm)	57 DAP	2.23 ± 0.14^{a}	2.15 ± 0.13^{ab}	1.79 ± 0.12^{c}	1.87 ± 0.11^{bc}	0.35
	163 DAP	4.55 ± 0.17^{a}	3.63 ± 0.06^{b}	2.60 ± 0.07^{c}	1.57 ± 0.11^{d}	0.32

Means with different alphabet superscripts along each row represents significant differences across treatments separated using LSD- least significant difference at $P < 0.05$; NS- not significant.

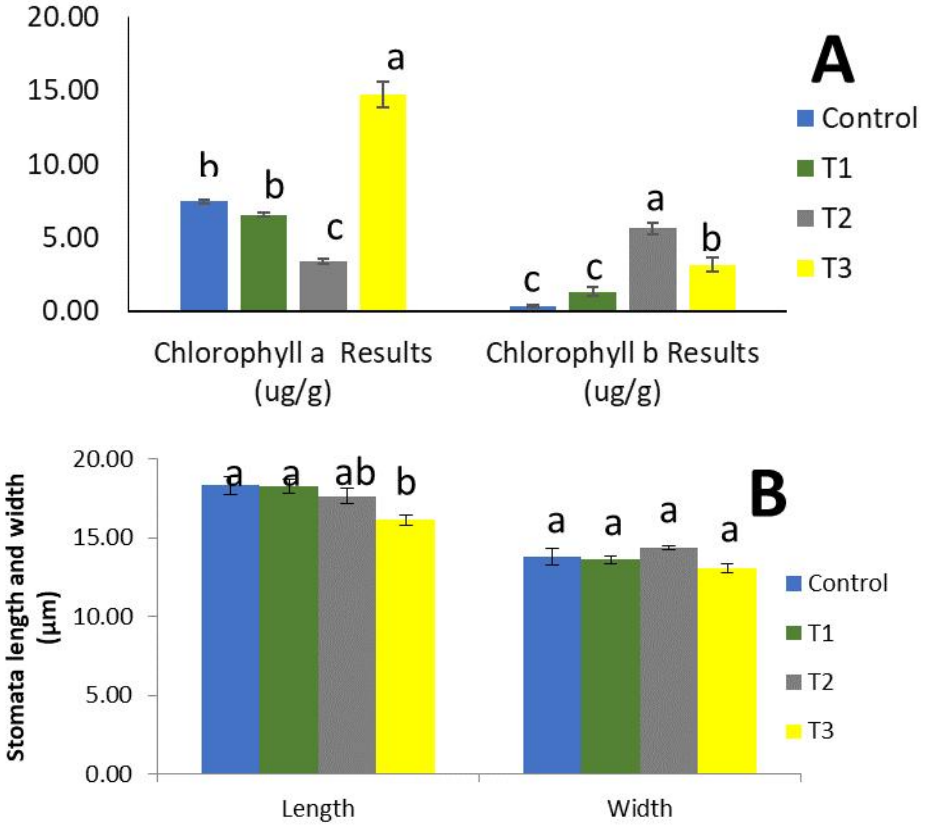


Figure 2: Effect of spent engine oil (SEO) on (A) Chlorophyll a and b, (B) stomata dimension.

Table 5: Meristematic values of upper epidermis of *G. arborea* on spent engine oil (SEO) polluted soil

Epidermal parts (µm)	Control	T1	T2	T3	LSD
N	3.33 ± 0.33 ^{a}	2.67 ± 0.33 ^{a}	3.33 ± 0.33 ^{a}	4.00 ± 0.00 ^{a}	NS
ELT	10.49 ± 0.33^{b}	8.11 ± 0.63^{b}	31.78 ± 3.75^{a}	28.53 ± 3.05^{a}	7.97
PCD	28.63 ± 0.97^{c}	33.31 ± 2.64^{c}	112.76 ± 8.72^{a}	41.32 ± 0.82^{b}	14.99

PMD	28.78 ± 0.80^{c}	39.88 ± 7.26^{c}	213.76 ± 24.43^{a}	126.83 ± 2.14^{b}	41.72
LB	116.73 ± 5.75^{d}	168.85 ± 7.39^{c}	576.59 ± 22.43^{a}	206.61 ± 13.05^{b}	44.97

Means with different alphabet superscripts along each column represents significant differences across treatments separated using LSD- least significant difference at $P < 0.05$; NS- not significant. Key: N – Number of vessels per row; ELT – epidermal layer thickness; PCD – parenchyma cell diameter; PMD – Palisade mesophyll diameter; LB – leaf blade thickness diameter.

Table 6: Meristematic values of transverse section of stem of *G. arborea*on spent engine oil (SEO) polluted soil.

Stem section(µm)	Control	T1	T2	T3	LSD
ED	25.43 ± 1.75^{a}	18.74 ± 0.91^{b}	17.65 ± 1.42^{b}	14.60 ± 0.51^{b}	4.05
CD	106.70 ± 2.78^{c}	274.79 ± 8.99^{b}	251.08 ± 15.28^{b}	371.25 ± 18.19^{a}	41.66
XD	44.29 ± 0.70^{a}	24.07 ± 0.17^{c}	25.01 ± 1.15^{c}	31.94 ± 1.03^{b}	2.78

Means with different alphabet superscripts along each column represents significant differences across treatments separated using LSD- least significant difference at $P < 0.05$; NS- not significant. Key: ED -Epidermis diameter, CD - Cortex diameter and XD - Xylem vessel diameter

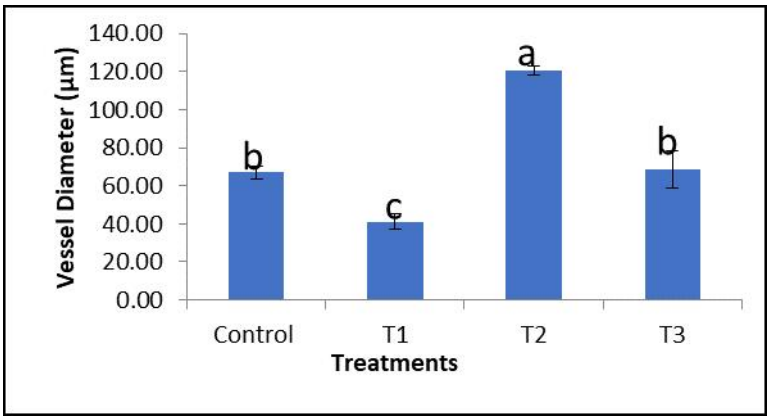


Figure 3: Vessel Diameter of the Root of *Gmelina arborea* on spent engine oil (SEO) polluted soil.

Anatomical changes

Results (Plates 1 to 7) showed different anatomical changes that were observed in the experimental plants. Length of the stomata of the leaves of *G. arborea* grown reduced upon the increased SEO pollution but stomatal width significantly increased across the treatments and control (Plate 1).

(A)

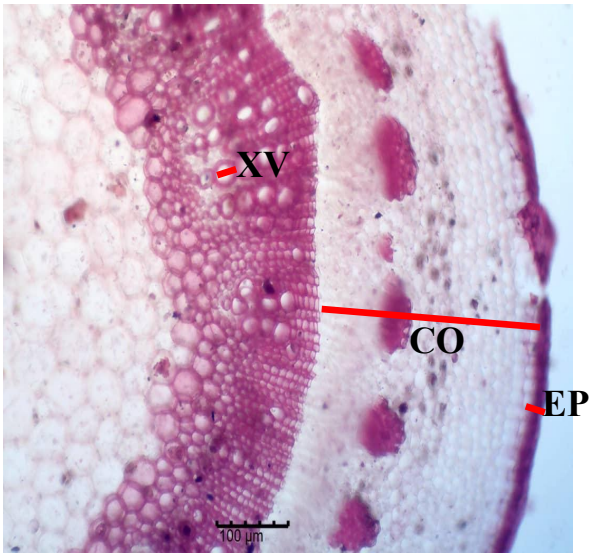


Plate 1: Cross section of upper epidermis of *G. arborea* stem showing the distribution of epidermis (EP), Cortex (CO), and Xylem vessel (XV).

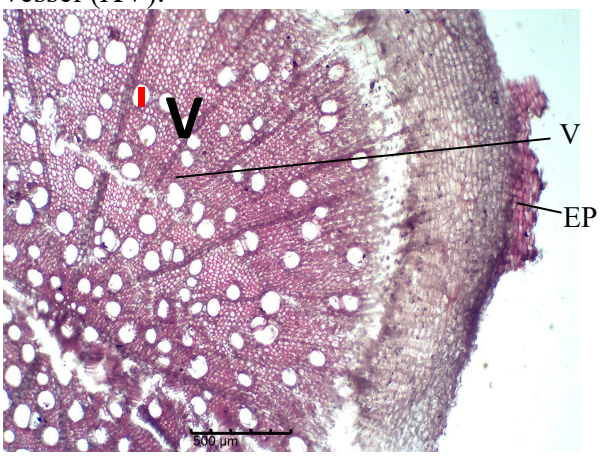


Plate 3. Transverse section of *G. arborea* root showing the distribution of vessel element (VE), parenchyma cell (PC), and the epidermis (EP).

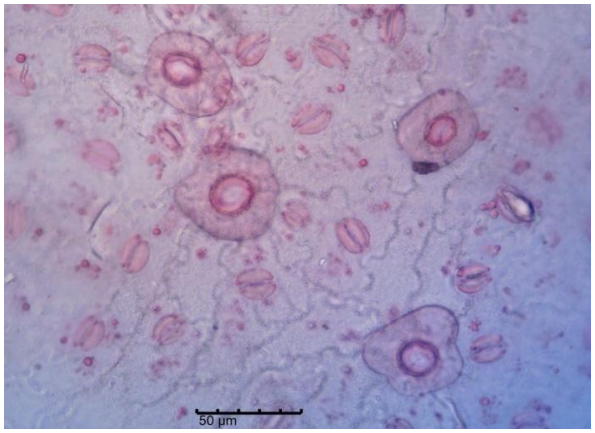


Plate 2: Transverse section of *G. arborea,* showing the distribution of stomata (St);

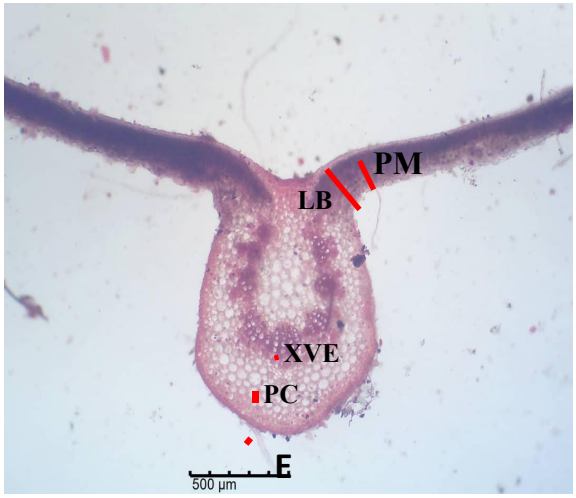
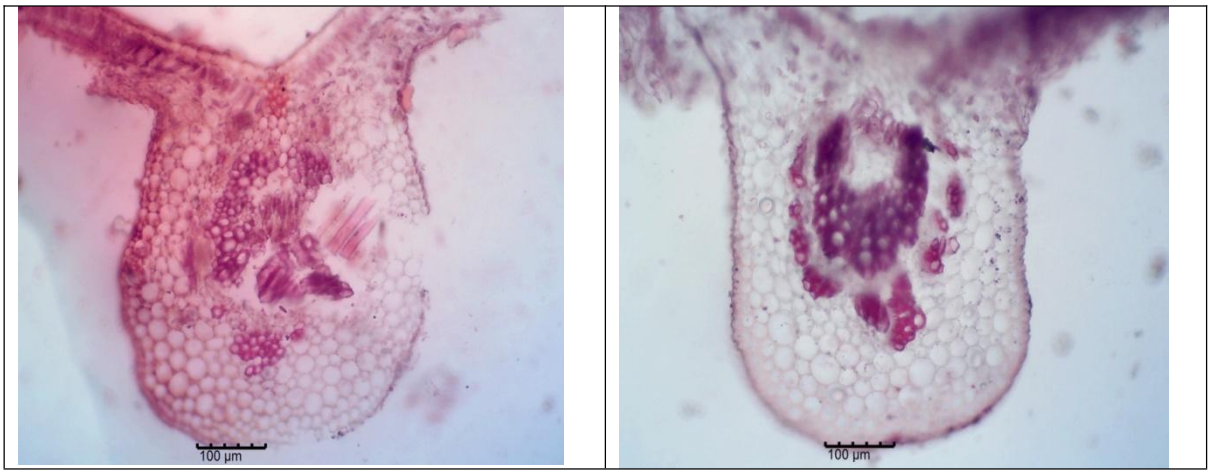


Plate 4. Transverse section of *G. arborea* leaf showing, palisade mesophyll (PM): Cortex (CO), Leaf blade thickness (LB), Xylem vessel elements (XVE).



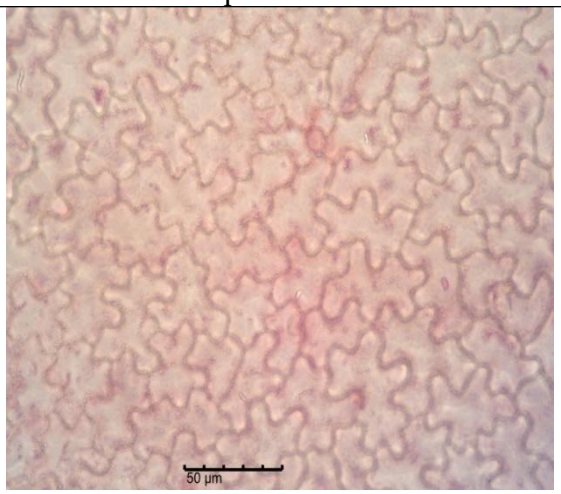
Control	**T1:** Insignificant changes in the distribution of tissues as compared with control.
 **T2:** Increased parenchyma cells, leaf blade thickness, and length of stomata.	 **T3:** Increased parenchyma and palisade cells, and reduced length of stomata,

Plate 5. Transverse section of *Gmelina arborea* leaf showing the distribution of tissues.

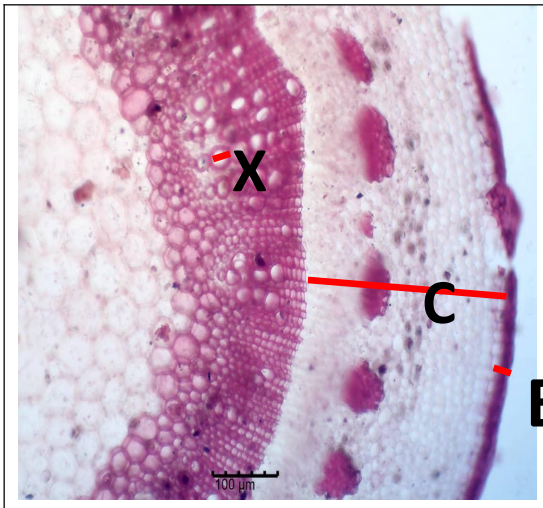
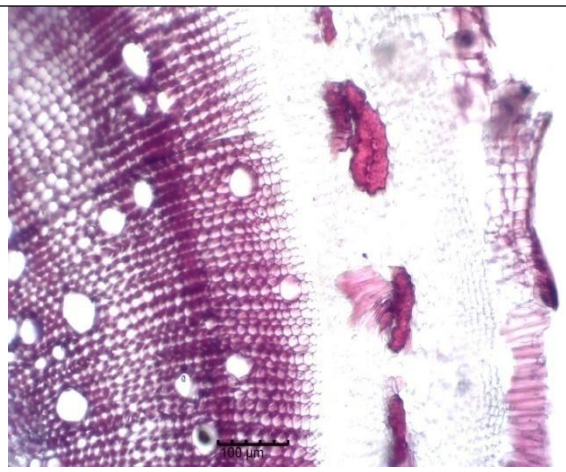
 **Control**	 **T1:** Increased cortex diameter
 **T2:** Increased vessel diameter, parenchyma cells and cortex diameter	 **T3:** Increase in the diameter of parenchyma, epidermal thickness, xylem vessels and cortex.

Plate 6: Transverse section of *Gmelina arborea* stems showing the distribution of tissues.



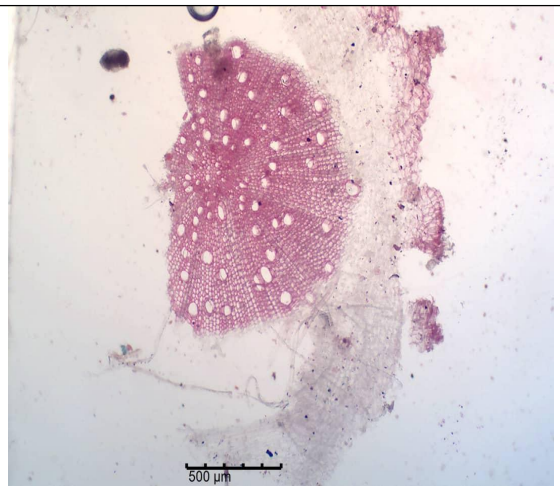
Control



T1: increased cortex diameter**.**



T2: increased diameter of vessel elements, epidermal thickness and cortex.



T3: increased diameter of codex and epidermal thickness; reduced xylem vessels diameter,

Plate 7: Transverse Section of *Gmelina arborea* roots showing the distribution of tissues.

Discussion

Phytoremediation efficacy studied showed that *Gmelina arborea* seedlings have the ability to clean up spent engine oil (SEO) polluted soils. Results of the heavy metals – Lead (Pb), Zinc (Zn), Nickel (Ni), Aluminum (Al), Copper (Cu), Cadmium (Cd) and Iron (Fe) recorded in this study showed that heavy metals are common compositions of SEO and can inhibit plant growth (Odjegba and Sadiq, 2002). All the heavy metals observed in the soils polluted by SEO increased with an increase in SEO concentration. Ogunlanan *et al* (2020) on assessing the level of heavy metals concentration in soil around transformer at Akoko Community of Ondo State, Nigeria reported that when heavy metal concentrations in the soil are high, it can interfere with plant growth and yield. According to Nkwocha *et al*. (2021), the effects of heavy metals on plant start in the rhizosphere where metalliferous minerals interact with root exudates. Osubor and Anoliefo, (2003) outlined the negative effects of SEO on plants to include metabolic malfunctions like physiological and biochemical processes, inhibition of photosynthesis and respiration.

The mean values of heavy metals detected in the vegetated soils were lower than those detected in the unvegetated soils. The reductions in heavy metal composition of vegetated soils imply that *G arborea* accumulated a number of metals from the vegetated soil. The present results are in agreement with the findings of Nkwocha *et al.* (2011) who also reported heavy metal uptake by crops in their work and noted that these heavy metals were stored in crop parts. Moreover, the noticeable decrease in some heavy metals in the vegetated soil than in the unvegetated soil could be attributed to heavy metals being absorbed and stored or volatilized by the plant's vegetative components. This was equally supported that plants were able to take up heavy metals from the soil and transport them to their aerial parts (Ogunlana *et al,*2020)

Results observed that plant tissues accumulated heavy metals from the SEO contaminated soils, though the concentration in the plant tissue was below accepted limit (WHO, 1996). The plant roots accumulated higher content of Pb, Al, Cu and Cd from the soil with the highest SEO pollution. The results were within the range of heavy metals obtained by Ubochi *et al.* (2019). Cadmium increased in concentration with increased spent engine oil concentration (Ogunlana *et al,*2020). This supported our present findings that some increase in heavy metals was concentration dependent. Nwite and Alu (2015) reported significantly higher uptake of lead (Pb) and cadmium (Cd) by maize grains in poly bags germination which was attributed to the release of heavy metals into the soil during application of SEO. Okonokhua *et al.* (2007) reported that heavy metals from used engine oil are retained in the soil even after drainage. In the present study, the observed range for Al (2.36 to 6.88 mg/100g) is in agreement with Ugwu *et al.* (2019) who recorded a similar range of aluminum concentrations on the plant parts. The mean value for copper (Cu) clearly shows that the values conformed to the standard (WHO 1996). The concentrations of Cu found in this present study were lower than the concentration of Cu previously reported by Ubochi *et al.* (2019). Ugwu *et al.* (2019), reported that Cu concentration was more on the stem, which is in contrast with the present study where Cu was higher on the root parts of the plant.

Concentrations of Zn were detected more in leaves and stem tissues at high SEO contaminated treatments. This agrees with the report of Chibowski (2000) that zinc is an essential element for plants, animals and humans but long exposure to high Zn-contaminated soil would lead to a wide range of health problems related to food chain toxicity. Ubochi *et al.* (2019) also reported that some activities of fauna like earthworm are negatively influenced when the concentrations of Zn are high in the soil. Zn accumulations on the plant tissues as observed in this research also agree with the reports of Garba *et al.* (2018) which recorded similar trend of Zn accumulation on the leaf part of the *Amaranthus hybridus* and *Chorchorius olitorius.* The observed Nickel (Ni) values in the plant tissues and across the treatments were low compared to the permissible limit of 35 mg/kg for Ni in soil (WHO, 1996). The mean values of Fe accumulated in the plant tissues in this SEO pollution were lower than those reported by Okonokhua *et al.* (2007) on the effects of SEO on soil properties and growth of maize (*Zea mays*). The implication is that human beings are at risk of heavy metal toxicity due to their uptake in the plant parts.

Spent engine oil significantly stunted the growth of plants in T3 and some of T2. The decrease in plants height was in line with Nwachukwu *et al.* (2020) who reported that high concentration of SEO noticeably decreased the height of plants. The decrease in plant height was attributed to the high oil concentration in the soil. There was an exceptional increment in the stem diameter in T3. This is comparable to the findings of Ogbuehi *et al.* (2011), who found that *Arachis hypogea* plants' height, number of leaves, percentage of germination, and leaf area all significantly decreased as a result of exposure

to used engine oil. Agbogidi and Eruotor (2012) also noted decreases in the plant height, leaf area, and quantity of *Jatropha curcas* leaves that were contaminated with used motor oil. *Telfairia occidentalis* and *Zea mays* planted on soil contaminated with used motor oil experienced a decrease in the number of leaves and a suppression of radicle and plumule growth during early seedling growth. With an increase in the concentration of applied waste engine oil, the height and petiole length both dropped. This outcome is comparable to that of Otunne and Kinako (2010), who showed that seedlings of *Telfaria occidentalis* and *Zea mays* experienced greater growth inhibition as used engine oil concentration increased. Plants growing in soil contaminated with oil usually experience difficulty in water penetrating the soil because of hydrophobicity nature of oil, as reported by Ajuziogu *et al.* (2019). Some of the leaves of plants in the heavily polluted soils curled upwards from their tips and withered, resulting in the death of such plants. This shows that, in addition to pollution levels, the age of the plant has a significant impact on its ability to survive oil pollution. As suggested by Agbogidi *et al.* (2007) the higher resistance of the older plants to spent oil pollution may be due to the presence of already cutinized tissues in such plants.

Chlorophyll a was significantly high in T3, but significantly low in T2, and T1 was not different from the control. Chlorophyll b mass was significantly high in T2 and T3, while T1 was not different from the control. Thus, it appears that the higher the concentration of the SEO pollution, the higher the chlorophyll content. This was in disagreement with the report of Odjegba and Sadiq (2002), but in line with Chandawat *et al.* (2011). The leaves of the control and the polluted plants across the treatments were green and dark. There was no paleness with increasing spent engine oil concentration. This was in contrast with Agbogidi and Ejemeta (2005) and Agbogidi and Ilondu (2013) as reported in *Moringa oleifera* and *Jatropha curcas,* respectively. Agbogidi and Ejemeta (2005) reported chlorosis that could be as a result of nutrient immobilization. Oil pollution could be a cause for the unavailability of some essential nutrient elements, while the toxic ones may be more readily available. Results obtained from Han *et al.* (2016) and Arellano *et al.* (2017) revealed that there was increase in the content of photosynthetic pigments in the assimilating organ of plants grown in oil-contaminated soil, which implies that it contains some essential nutrient elements. Baruah *et al.* (2014) supported their claims because an increase in photosynthesis plays a key role in the physiology of plants and their productivity. This implies that *G. arborea* can tolerate high level of SEO pollution. These types of plants that are highly tolerant to pollution can absorb pollutants, gaseous emissions, heat and have the tendency to trap and accommodate dust particles, hence improving environmental quality. Therefore, *G. arborea* can be said to be a powerful phytoremediator.

The results of the effects of different concentrations of spent engine oil on anatomical features of *G. arborea* grown on soil polluted with different concentrations of SEO showed that *G. arborea* was able to withstand high levels of applied SEO stress as seen in the stomata length and width (T1 to T3), which are not different from the control. his agreed with the report of Zhang *et al.* (2019) who similarly reported reduced stomata length in maize grown in crude oil-contaminated soil. Previous work has shown that stomata in plants were not only influenced by air pollution of automobile emissions, but also by soil or water contamination of environmental stress (Ahmad *et al.,* 2005; Rai *et al.,* 2005). Therefore, plants respond to pollution differently but *G. arborea* has ability to retain chlorophyll content, stomata size and improve its photosynthesis under stressful conditions.

Conclusion

The results of this study imply that *G arborea* has ability to germinate and survive in SEO polluted soil and accumulate heavy metals in higher proportions which could be due to phyto-remediating potential of the plant.

Based on this result, we can conclude that *Gmelina arborea* can absorb pollutants and clean an environment from pollution effects. This should therefore be the concern of everybody to guard against spent engine oil pollution on arable land in order to maintain a sustainable agricultural productivity. Therefore, it is important that further studies be carried out over a longer period for a better understanding of the impact on humans and climate change.

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