GROWTH OF *Saccharum spontaneum* (L.) IN SOIL CONTAMINATED WITH SPENT ENGINE OIL

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CERTIFICATION

This is to certify that this thesis was written by Elizabeth Nsidom Usen in partial fulfillment of the requirement for the award of M.Sc. degree in Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city, Nigeria.

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DEDICATION

This thesis is dedicated to my parents; Mrs. E. N. Usen and Mr. N.A. Usen.

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I am eternally grateful to God Almighty, my source of spiritual strength and counsel, for bringing me this far.

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ABSTRACT

Contamination of soils due to spent engine oil is an important environmental issue affecting soil characteristics and plant growth. This study was aimed at evaluating the effects of spent engine oil on growth parameters of *Saccharum spontaneum* L. for potential use in phytoremediation of oil polluted environments. Spent engine oil from a motor service garage was prepared in five concentrations of 4, 8, 16, 32 and 64ml/Kg. Tillers of *S. spontaneum* were planted in top garden soil and allowed to stabilize for four weeks before treatments were added. A control experiment in four replicates was also set up. The control had a significantly higher number of tillers compared to the different concentration treatment groups. The higher concentrations of 16, 32 and 64ml/Kg of spent engine oil contaminated soil did not produce any tillers at all. Total aliphatic hydrocarbon content was lowest in the control and highest in the 64ml/Kg treatment. Total polynuclear aromatic hydrocarbon was highest in the 32ml/Kg treatment. Soil chemical parameters did not show a dose dependent response to increase in treatment with spent engine oil. This report demonstrated that lower concentrations of spent engine oil enhanced the number of leaves, plant height and leaf area of *S. spontaneum*. Results showed that *S. spontaneum* was tolerant to, and thrived at low concentrations of contamination with spent engine oil but declined in growth and development at high concentrations and for extended periods.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND OF THE STUDY:

Anthropogenic activities release contaminants into the environment which can adversely affect wildlife and impact human health (Greipsson, 2011). These contaminants even at low levels pose a risk to biological organisms due to potential accumulation at higher trophic levels through the process of biomagnification. As a result of the steady growth in environmental pollution and anthropological disturbances to ecosystems, the study of abiotic stress responses in plants has become necessary (Alkio *et al*., 2005).

1.2 AUTOMOBILE SPENT ENGINE OIL

Crude oil, a naturally occurring hydrocarbon compound is utilized as fuel for vehicles, heating of homes, cooking gas and other fractions in the manufacture of synthetic products (Edema *et al*., 2009). The multipurpose nature of crude oil has made its availability a source of pollution to the environment. The introduction of petroleum and its products into the environment are of specific concern in pollution studies as a result of their structural complexity, slow biodegradability and bio-magnification potential (Kathi and Khan, 2011).

Motor oil (engine oil) is a refined product of crude oil and is applied as a lubricant in internal combustion engines for car, motor cycles, buses and commercial vehicles (Iqbal *et al*., 2016). Used motor oil that is collected from mechanical automobile workshops garages and industrial source like hydraulics oil, turbine oil, process oil and metal working fluids is referred to as spent engine oil (Osuagwu and Iwuoha, 2015). Spent engine oil disposal into gutters, watercourses, open vacant plots and farmland are a common practice among auto-mechanic operators (Nwoko *et al*., 2007).

Soil pollution resulting from motor oil contamination poses an important challenge in different parts of the world. Pollution due to motor oil is an important environmental issue affecting soil characteristics and plant growth. Iqbal *et al*. (2016) suggested that motor oil accumulation in the soil can permanently damage the soil characteristics and make it unfavorable for the survival and development of plant. Pollution due to oil inhibits normal oxygen exchange between soil and atmosphere due to its hydrophobic properties as well as inhibits seed germination, plant growth and also leads to premature death of plant (Njoku *et al*., 2009; Udom *et al*., 2012). These effects could also be as a result of the reduction in the level of available plant nutrients or a rise in the amounts of certain elements such as iron and zinc to toxic levels.

1.3 SACCHARUM SPONTANEUM L.

Saccharum spontaneum L. is a member of the grass family (Poaceae) and genus *Saccharum*. It is an erect perennial grass with fibrous roots and sometimes creeping rhizomes. In recent years, *S. spontaneum* has attracted serious attention for its potential in ecological restoration (Pandey *et al*., 2015a). It is popularly known as 'Wildcane' is a tall, perennial C_4 wasteland weed, with deep roots and rhizomes, growing up to 3 – 4 m in height.

1.5.1 Scientific Classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Monocots

(Unranked): Commelinids

Order: Poales

Family: Poaceae

Genus: *Saccharum*

Species: *S. spontaneum* L.

1.3.2 Description

S. spontaneum is a tall (100 – 600 cm) perennial grass with a creeping, tufted and rhizomatous rootstock. The stem is erect, polished, robust; internodes solid; node 5 – 10, waxy. Leaves linear-lanceolate, involute, with long hairs at base, base rounded; ligule 2 – 8 mm long, ovate, brown, membranous, ciliolate; leaf blade is glabrous, apex accuminate, base simple or tapering to the white midrib, scabrid to serrate along margins; sheath longer than internode. Inflorescence plumose panicles; peduncle hirsute above; panicle 15 – 60 cm long, silky white, axis silky pilose or hirsute, open, ovate, dense; racemes 3 – 17 cm long; rachis internodes filiform; spikelets homomorphic, 2.5 – 5.0 (-7.0) mm long, lanceolate, reddish-brown, paired (one sessile and the other pedicelled), pilose with long silky hairs. Fertile spikelets sessile, 0.35 – 0.70 cm long, lanceolate, dorsally compressed, two in the cluster, subequal; pedicels filiform, ciliate. Glumes similar, membranous above, chartaceous below; lower glume 3 – 4 × 1 mm, ovate lanceolate to elliptic, subcoriaceous to coriaceous, acuminate at apex, ciliate along margins, much thinner above, 2-keeled; upper glume 3 – 4 × 1 mm, ovate lanceolate, coriaceous, acute at apex, ciliate along margins, mucronate, much thinner above, without keels. Florets basal sterile and upper fertile; sterile florets barren, without significant palea; lemma 1 – 2 × 1 mm, lanceolate, hyaline, 0-veined, without mid vein, without lateral veins, acute at apex; fertile floret bisexual, first lemma 1–2 × 1 mm, linear, hyaline; second lemma 2 – 2.5 mm long, linear-lanceolate, hyaline; Palea absent or minute. Flower lodicules, cuneate, ciliate. Stamens three; anthers yellow or reddish, 1.5 – 2.0 mm. Ovary oblong; stigma white. Flowering and fruiting occurs from June to September. Flowers emerge just before rains and takes 1 – 2 months to produce seeds (Pandey *et al*., 2015a).

1.3.3 Distribution

This species is distributed across Africa and Italy through temperate Asia and tropical Asia (USDA, 2016).

1.3.4 Cultivation

It is propagated through seeds as well as vegetatively by creeping rhizomes and stem cuttings (Pandey *et al*., 2015a). Stem shows good regeneration potential even after 6 days of drying (Graham *et al*., 2014).

1.3.6 Significance of *S. spontaneum* in Phytoremediation

As a species with multiplicity of applications it has been utilized as biomass for ethanol and biogas production (Chandel *et al*., 2009). Due to its bad reputation as weeds and its ability to quickly colonize croplands it was neglected. However, *S. spontaneum* increasingly gained application for its potential in ecological restoration and stabilization of various waste dumps like fly ash dumps, acid mine dumps and sewage sludge (Pandey *et al*., 2015b; Pandey and Singh 2014; Kumar *et al*., 2015). Apuan *et al*. (2018) reported that *S. spontaneum* is potentially useful as a phytostabilization strategy for chromium contaminated soil.

1.4 STATEMENT OF THE PROBLEM

Pollution due to petroleum hydrocarbon is a growing concern all over the world due to its devastating effects on the ecosystem. Spent engine oil which is a refined product of crude oil, is a predominant culprit in this pollution menace because it is more often than not, improperly disposed of.

1.5 JUSTIFICATION FOR THE STUDY

Humans as well as animals rely on plants for sustenance. Plants require good soil for growth and development. It has become imperative that aside from cutting down on the rate of environmental degradation due to spent engine oil, measures aimed at cleaning up or remediating the affected soil be explored. This study is geared towards evaluating the effects of different concentrations of spent engine oil on the growth parameters of *Saccharum spontaneum*.

1.6 AIM OF THE STUDY

The aim of the study is to evaluate the effects of spent engine oil on growth parameters of *Saccharum spontaneum* L, for potential use in phytoremediation of oil polluted environments.

1.7 OBJECTIVES OF THE STUDY

The objectives of this study are to:-

1. To evaluate the effects of spent engine oil on the growth of *Saccharum spontaneum*
2. Assess the hydrocarbon content of soil contaminated with spent engine oil.
3. Evaluate the chemical properties of soil contaminated with spent engine oil.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 STUDY SITE

The experiment was conducted at the botanical garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Top soil (0 – 6 cm) was obtained from the botanical garden and properly homogenized by mixing. The homogenized soil was then put into 25 cm by 25 cm perforated bowls to a weight of 3 kg to increase aeration and avoid water logging. The soil was watered and allowed to settle.

2.2 PLANT MATERIAL

Tillers of *Saccharum spontaneum* were obtained from a domesticated population in Benin.

2.3 SPENT ENGINE OIL

Spent automobile engine oil was obtained from a motor service workshop located in Uselu Lagos Road, Benin City.

2.4 TREATMENT APPLICATION AND EXPERIMENTAL DESIGN

Saccharum spontaneum tillers were planted in the perforated bowls containing top garden soil without spent engine oil treatment for four weeks to enable them stabilize. Thereafter, six concentrations of spent engine oil - 0, 4, 8, 16, 32, 64 ml/Kg - were prepared and applied to the soil with the *S. spontaneum* tillers. The experiment was laid out in a randomized complete block design (RCBD) in four replications and lasted a total of 8 weeks.

2.5 DATA COLLECTION AND ANALYSES

The plants were analyzed weekly for eight weeks and the following growth parameters were recorded – plant height, number of leaves, number of tillers and leaf area. At the end of the experiment, the soil samples were analyzed for total hydrocarbon content (TPH), polyaliphatic and polyaromatic hydrocarbons, Fe, Pb, Cu, Zn content, pH, electrical conductivity (EC), total nitrogen and total organic carbon.

Data collected were subjected to analysis of variance (ANOVA) test for significance at 5% level of probability. Significantly different means were separated using the least significant difference (LSD) analysis.

Plant growth measurement

The height of each plant was measured at a regular intervals of seven days from the soil level to the terminal bud using a simple meter rule. The leaves and tillers produced were counted every 7 days (Odjegba and Sadiq, 2002).

The leaf area was determined using direct measurements and using the weighted regression equation:

$$A = b \times l \times w$$

Where b = the leaf shape coefficient

l= the length of the leaf

w= the width of the leaf at its widest point

A= the leaf area

Determination of Total Organic Carbon Content

Air dried soil was passed through a 2 mm sieve in order to remove large particles, roots, organic debris and ensure consistency. These soil samples were used for both carbon and nitrogen analyses. A weighed amount (1.0 g) of prepared soil sample was dispensed into a 250 ml conical flask. 10 ml of Normal potassium dichromate was added to the flask followed by the addition of 20 ml concentrated H_2SO_4. The flask was shaken for 1 min and allowed to cool. 10 ml of phosphoric acid was added to the solution followed by the pipetting of 1 ml of 1% diphenyl amine solution (indicator). Titration with 0.5 ferrous ammonia sulphate solution was done until there was colour change from dark violet to green. A blank determination was done for each soil sample (Onyeonwu, 2000).

Calculation

$$\frac{\text{Blank} - \text{sample} \times \text{Normality of Ferrous Ammonium sulphate } 0.03 \times 1.3 \times 100}{\text{Weight of sample}}$$

Determination of Total Nitrogen

The total nitrogen content of the soil sample were determined using micro Kjedahl digestion and colorimetric method (Bremmer and Mulvaney, 1982). 10 g soil sample was placed into 30 ml Kjedahl digestion flask. One tablet of a catalyst (Kjedahl) and 10 ml concentrated H_2SO_4 was added, and the mixture was hand shaken to ensure mixing. At completion of digestion, the mixture was clear and upon removal from the digestion chamber, it was allowed to cool. Then, 10 ml distilled water was added and the solution was decanted through a Whatman filter paper No 42 into a 100 ml volumetric flask.

The Kjedahl flasks were washed into 3 small aliquots of distilled water and all the washings were added into the volumetric flask via the filter paper and made up to volume. The nitrogen content of the filtrate was then determined colorimetrically. For colorimetric analysis, a standard nitrogen stock solution was prepared using dry ammonium sulphate and from the resultant 100 ppm nitrogen stock solution, 5, 10, 15, 20 and 25 ppm standards were prepared and in each standard, 4 ml concentrated H2SO4, and 0.95 g anhydrous sodium sulphate was added. A blank solution containing no nitrogen standard but having the same quantity of acid and anhydrous sodium sulphate was also prepared. Then, 5 ml of the digested filtrate was pipetted into a 25 ml glass flask, and 2.5 ml alkaline phenol, 1 ml sodium potassium tartrate and 2.5 ml of sodium hypochlorite were added. The mixture was hand shaken and made to 25 ml mark with distilled water. The solution was read colorimetrically at 630 nm, using a spectrophotometer.

Determination of Total Hydrocarbon Content (TPH)

2.5 g of air dried soil sample was dissolved in 10 ml of hexane and shaken after 10 minutes using a mechanical hand shaker. The solution was filtered using a Whatman filter paper No 42. The absorbance of this solution was read at 460 nm with a spectrophotometer using n-hexane as blank. The TPH (mg/kg) of the sample was calculated with reading obtained from the spectrophotometer (Akpoveta *et al*., 2011), using the formula below.

$$\text{TPH (mg/kg)} = \frac{\text{OD reading} \times \text{volume of solvent used}}{\text{Weight of the soil sample (Kg)}}$$

Determination of pH

20 g of air dried soil was weighed and passed through a 2 mm sieve, into a 50 ml beaker. 20 ml of distilled water was added, allowed to stand for 30 minutes and stirred occasionally with a glass rod. The electrode of the pH meter was inserted into the partly settled suspension and the pH was measured.

CHAPTER THREE

3.0 RESULTS

3.1 GROWTH OF SACCHARUM SPONTANEUM IN SOIL POLLUTED WITH SPENT ENGINE-OIL

The growth of *S. spontaneum* exposed to different concentrations of spent engine oil-contaminated soil was evaluated weekly for a total of 8 weeks. The parameters recorded were: number of leaves, plant height, number of tillers and leaf area.

3.1.1 Number of Leaves

Mean number of leaves per plant of *S. spontaneum* at week 1 after treatment was significantly different (P<0.05) across the plants subjected to the various levels of spent engine oil treatment (Table 3.1). The numbers of leaves of *S. spontaneum* grown on spent engine oil contaminated soil were all significantly higher than the control (0 ml/Kg) except that of the 32 ml/Kg concentration which was significantly lower. The plant grown on the 16 ml/Kg had the highest number of leaves.

The mean number of leaves per plant of *S. spontaneum* in the different concentrations of spent engine oil continued to increase weekly until after the 8^{th} week, where there was a reduction in the number of leaves from the previous week. However, this was not so for the control (0 ml/Kg) as the number of leaves remained constant from the 7^{th} week.

After the 8^{th} week, the mean number of leaves per plant of *S. spontaneum* grown in the 0 and 4 ml/Kg of spent engine oil contaminated soil exhibited a non-significant difference (P>0.05) from each other. They were however significantly higher than those grown in the soils contaminated with higher concentrations of spent engine oil (Fig 3.1).

Table 3.1: Number of leaves of *S. spontaneum* grown in soil contaminated with spent engine oil at different weeks after treatment.

Weeks after Treatment	Concentration of spent engine oil (ml/Kg)					
	0	4	8	16	32	64
1	6.00±0.22^{c}	6.75±0.30^{b}	6.75±0.34^{b}	7.25±0.36^{a}	5.75±0.20^{d}	6.75±0.30^{b}
2	7.50±0.32^{b}	7.50±0.31^{b}	8.00±0.36^{a}	6.25±0.22^{e}	6.75±0.26^{d}	7.25±0.38^{c}
3	8.50±0.36^{c}	8.50±0.36^{c}	8.00±0.39^{b}	9.00±0.36^{a}	8.25±0.31^{d}	7.75±0.36^{de}
4	9.00±0.34^{a}	9.00±0.32^{a}	8.50±0.33^{c}	8.75±0.39^{b}	8.25±0.32^{d}	7.75±0.32^{e}
5	9.00±0.33^{b}	8.75±0.40^{c}	8.50±0.31^{d}	9.25±0.41^{a}	8.75±0.39^{c}	7.75±0.32^{e}
6	9.00±0.36^{d}	9.50±0.39^{b}	9.25±0.40^{c}	10.50±0.49^{a}	9.25±0.36^{c}	8.75±0.39^{e}
7	9.75±0.41^{b}	10.50±0.45^{a}	10.50±0.44^{a}	9.50±0.42^{c}	9.50±0.40^{c}	9.25±0.43^{d}
8	9.75±0.39^{a}	10.00±0.47^{a}	9.00±0.51^{d}	9.25±0.43^{c}	8. 50±0.40^{d}	9.00±0.47^{d}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly (P<0.05).

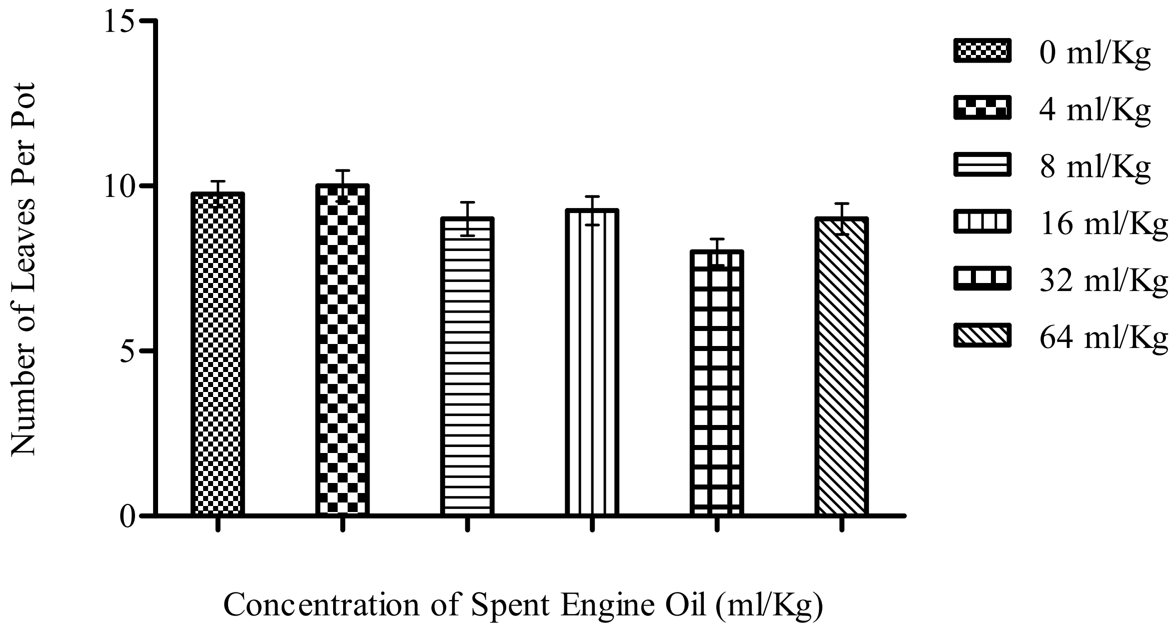

Fig 3.1: Mean number of leaves of *S. spontaneum* grown in soil contaminated with spent engine oil soil eight weeks after treatment.

3.1.2 Plant Height

Mean heights of the *S. spontaneum* plants grown in soil with different concentrations of spent engine oil after the 1st week as shown in Table 3.2 indicated that the plant grown in the 8 and 32 ml/Kg concentrations possessed the lowest plant height. This was significantly different ($P<0.05$) from the control as well as from the other different concentrations. However the other different treatments did not show any significant difference from the control.

The mean plant height continued to increase weekly up to the 8th week. After the 8th week of treatment, the control and the 4 ml/Kg treatments possessed the highest plant height compared to the higher range of treatment concentrations (Fig. 3.2).

Table 3.2: Plant height (cm) of *S. spontaneum* grown in soil contaminated with spent engine oil at different weeks after treatment.

Weeks after Treatment	Concentration of spent engine oil (ml/Kg)					
	0	4	8	16	32	64
1	7.95±1.31^{a}	7.98±2.01^{a}	7.75±1.56^{b}	8.03±2.31^{a}	4.48±1.27^{c}	8.98±2.56^{a}
2	11.88±5.68^{a}	13.43±4.25^{a}	10.70±3.32^{b}	11.83±3.25^{a}	8.15±3.11^{c}	8.98±2.17^{c}
3	15.13±4.65^{a}	14.73±3.56^{a}	12.65±3.89^{b}	15.10±4.23^{a}	10.10±4.01^{c}	8.33±3.38^{d}
4	16.15±3.25^{b}	19.40±5.68^{a}	15.70±5.61^{b}	18.35±5.21^{a}	16.55±5.22^{b}	15.15±4.65^{c}
5	20.58±8.12^{a}	21.60±8.71^{a}	17.38±7.65^{b}	20.98±5.88^{a}	18.53±5.89^{b}	17.45±6.56^{b}
6	24.13±4.23^{a}	24.83±8.54^{a}	20.60±7.98^{b}	24.93±6.78^{a}	21.48±7.63^{b}	20.15±7.11^{b}
7	34.58±9.56^{a}	36.65±9.76^{a}	27.60±8.45^{b}	33.23±8.78^{a}	27.73±8.77^{b}	26.4±7.86^{b}
8	38.6±5.97^{a}	42.75±6.89^{a}	31.33±5.99^{c}	36.08±5.75^{b}	32.83±6.11^{c}	31.45±5.99^{c}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly ($P<0.05$).

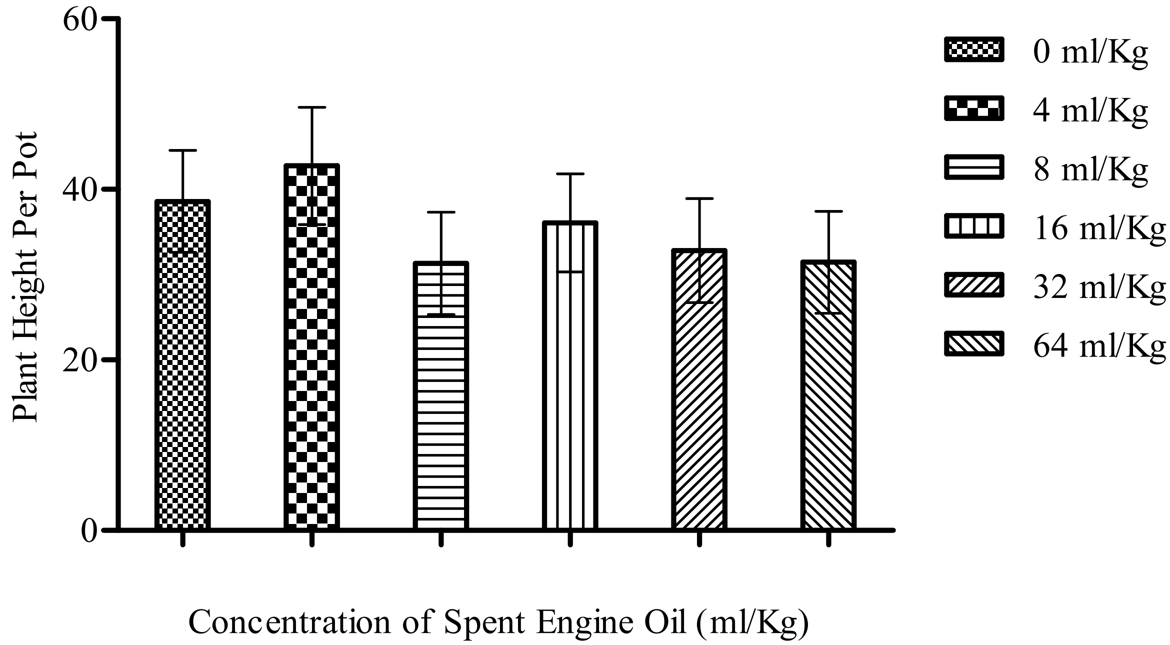

Fig 3.2: Mean plant height of *S. spontaneum* grown in soil contaminated with spent engine oil eight weeks after treatment.

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3.1.3 Number of Tillers

Tiller number differed significantly ($P<0.05$) among *S. spontaneum* plants treated with the different concentrations of spent engine oil (Table 3.3). Plants grown in soil contaminated with 16, 32 and 64 ml/Kg spent engine oil had no tillers.

After the 1st week of treatment, the tiller number of the control and 4 ml/Kg concentration did not show any significant difference ($P>0.05$) from each other. However, after the 2nd week their tiller numbers exhibited a significant difference onwards.

The number of tillers continued to decrease across the different concentrations of spent engine oil treatment towards the 8th week. At the 8th week, the control (0 ml/Kg) had the highest mean tiller number, which was significantly different from the higher treatment concentrations (Fig. 3.3).

Table 3.3: Mean number of tiller of *S. spontaneum* grown in soil contaminated with spent engine oil at different weeks after treatment.

Weeks after Treatment	Concentration of spent engine oil (ml/Kg)					
	0	4	8	16	32	64
1	2.00±0.063[a]	2.00±0.063[a]	1.00±0.041[b]	0	0	0
2	1.75±0.049[a]	1.00±0.036[c]	1.25±0.035[b]	0	0	0
3	2.00±0.064[a]	1.00±0.033[b]	1.00±0.036[b]	0	0	0
4	2.00±0.68[a]	1.00±0.039[c]	1.25±0.041[b]	0	0	0
5	2.00±0.61[a]	1.00±0.034[c]	1.25±0.039[b]	0	0	0
6	1.75±0.054[a]	1.00±0.039[c]	1.25±0.037[b]	0	0	0
7	1.75±0.051[a]	0.25±0.007[c]	1.25±0.044[b]	0	0	0
8	1. 50±0.044[a]	0.75±0.009[c]	1.25±0.042[b]	0	0	0

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly ($P<0.05$).

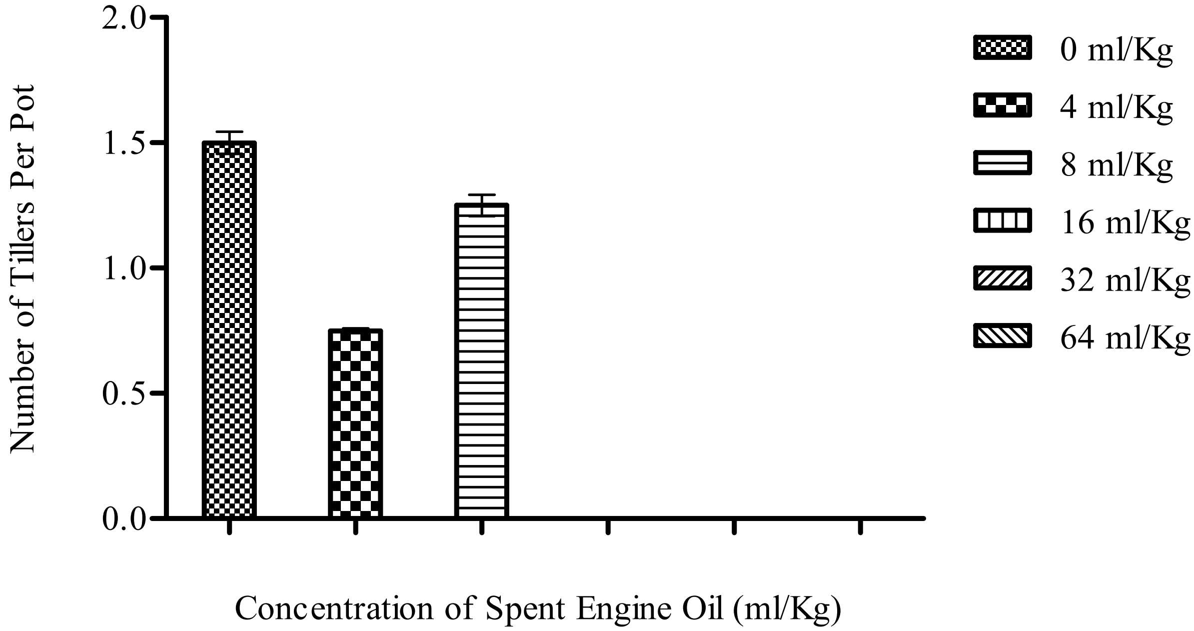

Fig 3.3: Mean number of tillers of *S. spontaneum* grown in soil contaminated with spent engine oil eight weeks after treatment.

3.1.4 Leaf Area (cm^2)

Mean area of leaf per plant differed significantly ($P<0.05$) after the 8th week among *S. spontaneum* plants treated with the different concentrations of spent engine oil (Table 3.4 and Fig 3.4) The untreated plants (control) and those grown in soil treated with 4 and 16 ml/Kg spent engine oil had significantly larger leaf area per plant (ranging from 97.54±8.96 cm^2 to 110.54±12.32 cm^2). Plants grown in soil contaminated with 8, 32 and 64 ml/Kg spent engine oil had significantly reduced mean leaf area.

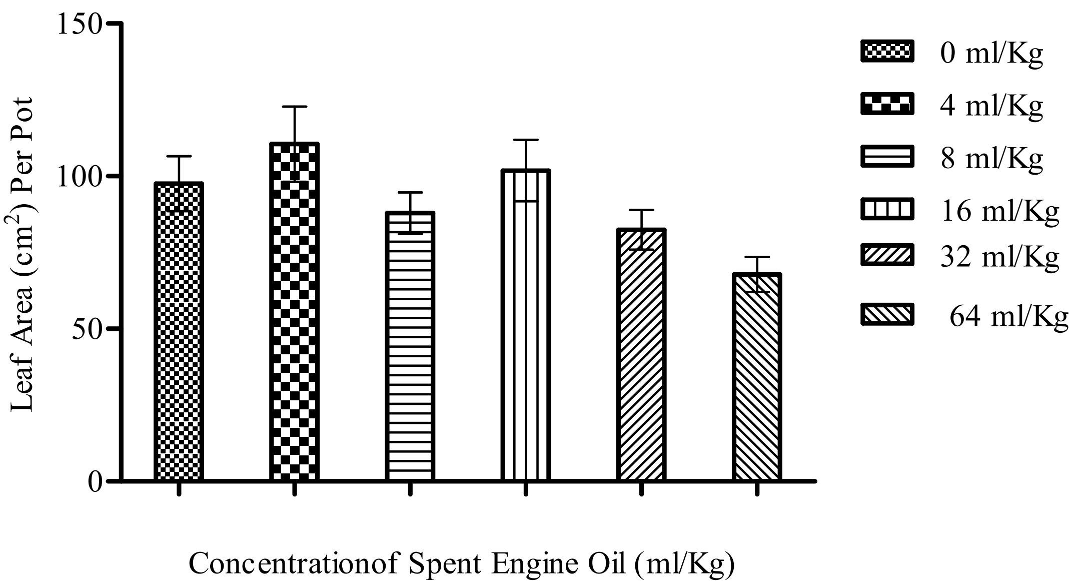

Fig 3.4: Mean leaf area of *S. spontaneum* grown in soil contaminated with spent engine oil eight weeks after treatment.

3.2 ANALYSIS OF SOIL CONTAMINATED WITH SPENT ENGINE-OIL

3.2.1 Hydrocarbon Content

The soil contaminated with spent engine oil in which *S. spontaneum* was grown, after the 8th week of treatment showed increase in total aliphatic hydrocarbon content in accordance with the concentration of the spent engine oil applied to the soil. This increase was significantly different ($P<0.05$) from each other, with the control (0 ml/Kg) having the lowest (0.312±0.001 mg/kg) total aliphatic hydrocarbon content and the highest treatment of 64 ml/Kg (2.569±0.005 mg/kg) having the highest concentration (Table 3.2).

Table 3.4: Total Aliphatic Hydrocarbon Content of contaminated soil (mg/kg).

SOIL COMPONENT	CONCENTRATION OF SPENT ENGINE OIL (ml/Kg)					
	0	4	8	16	32	64
Total (mg/kg) nC9 - nC44	0.312±0.001^{f}	0.427±4^{e}	1.128±0.005^{d}	1.264±0.002^{c}	1.502±0.001^{b}	2.569±0.005^{a}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly (P<0.05).

From Table 3.4, the polynuclear aromatic hydrocarbon content shows that acenaphthalene, benzo(k)fluoranthrene, indeno(1,2,3) perylene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were absent in the control as well as in the different concentrations of spent engine oil contaminated soil. However, naphthalene was present in the 0 ml/Kg, 32 ml/Kg and 64 ml/Kg spent engine oil contaminated soil but absent in the 4 ml/Kg, 8 ml/Kg and 16 ml/Kg concentrations. The naphthalene content was significantly higher ($P<0.05$) in the 32 ml/Kg spent engine oil contaminated soil than the 64 ml/Kg, which was also significantly higher than the 0 ml/Kg (control) concentration.

Acenaphthene was present in the 0 ml/Kg, 4 ml/Kg, 32 ml/Kg and 64 ml/Kg. Acenaphthene in the 64 ml/Kg contaminated soil was the highest and it was significantly different ($P<0.05$) from the others. This was followed by the 32 ml/Kg, concentration, which was significantly different from the 0 ml/Kg and 4 ml/Kg concentrations. It was however absent in the 8 ml/Kg and 16 ml/Kg concentrations.

Florene and pyrene were only present in the 32 ml/Kg and 64 ml/Kg concentrations, with the 64 ml/Kg concentration significantly higher ($P<0.05$) than the 32 ml/Kg treatment.

Phenathrene was found present only in the 16 ml/Kg and 32 ml/Kg treated soils, with the 32 ml/Kg contaminated soil significantly higher ($P<0.05$).

Anthracene and fluoranthene were absent in the 0 ml/Kg and 4 ml/Kg contaminated soil but present in the other concentrations. The 64 ml/Kg contaminated soil possessed the highest anthracene and fluoranthene contents which was also significantly different ($P<0.05$) from the other concentrations.

Benzo(a)anthracene was found only in the 8 ml/Kg, 32 ml/Kg and 64 ml/Kg spent engine oil contaminated soil. The content of benzo(a)anthracene in the 64 ml/Kg spent engine oil contaminated soil was the highest which was significantly different (P<0.05) from the other treatment concentrations.

Crysene was present only in the 0 ml/Kg, 32 ml/Kg and 64 ml/Kg spent engine oil contaminated soil. The crysene content of the 0 ml/Kg concentration was significantly lower (P<0.05) than the treatment concentrations.

From Table 3.4, benzo(a)pyrene was found only in the 0 ml/Kg contaminated soil, whereas benzo(k)fluoranthrene was found only in the 64 ml/Kg contaminated soil.

The total polynuclear aromatic hydrocarbon (PAH) content of the different concentrations of spent engine oil contaminated soil was found to be significantly different (P<0.05) from each other. The 32 ml/Kg concentration at $95.7 \pm 0.35 \times 10^{-2}$ mg/kg was the highest.

The total hydrocarbon content (TPH) content as shown in Table 3.4 indicates that the content increased as the concentration of the contaminated soil increased. This difference was significant (P < 0.05), with the highest concentration having the highest total hydrocarbon content.

Table 3.5: Polynuclear Aromatic Hydrocarbon Content of contaminated soil ($\times10^{-2}$ mg/kg).

	CONCENTRATION OF SPENT ENGINE OIL (ml/Kg)					
SOIL COMPONENT	0	4	8	16	32	64
Naphthalene	0.5 ± 0.12^{c}	-	-	-	11.7 ± 0.06^{a}	8.5 ± 0.03^{b}
Acenaphthalene	-	-	-	-	-	-
Acenaphthene	1.4 ± 0.09^{c}	1.3 ± 0.12^{c}	-	-	7.6 ± 0.07^{b}	12.1 ± 0.13^{a}
Florene	-	-	-	-	5.8 ± 0.12^{b}	12.2 ± 0.06^{a}
Phenathrene	-	-	-	1.6 ± 0.05^{b}	2.5 ± 0.08^{a}	-
Anthracene	-	-	1.2 ± 0.14^{c}	2.80 ± 0.13^{b}	2.5 ± 0.07^{b}	5.0 ± 0.06^{a}
Fluoranthene	-	-	1.2 ± 0.06^{c}	1.0 ± 0.15^{c}	2.2 ± 0.11^{b}	5.1 ± 0.08^{a}
Pyrene	-	-	-	-	20.4 ± 0.01^{b}	6.2 ± 0.02^{a}
Benzo(a)anthracene	-	-	0.4 ± 0.12^{c}	-	7.3 ± 0.17^{b}	5.9 ± 0.03^{a}
Crysene	0.5 ± 0.04^{b}	-	-	-	6.4 ± 0.12^{a}	6.0 ± 0.15^{a}
Benzo(a)pyrene	3.0 ± 0.02^{a}	-	-	-	-	-
Benzo(k)fluoranthrene	-	-	-	-	-	8.9 ± 0.09^{a}
Indeno(1,2,3) perylene	-	-	-	-	-	-
Dibenzo(a,h)anthracene	-	-	-	-	-	-
Benzo(g,h,i) perylene	-	-	-	-	-	-
Total PAH (mg/kg)	2.6 ± 0.06^{f}	1.3 ± 0.12^{e}	2.9 ± 0.17^{d}	5.4 ± 0.23^{c}	95.7 ± 0.35^{a}	80.1 ± 0.06^{b}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly ($P<0.05$).

Table 3.6: Summary of Soil Analysis Result

SOIL COMPONENT	CONCENTRATION OF SPENT ENGINE OIL(ml/Kg)					
	0	4	8	16	32	64
Aliphatics	0.312 ± 0.001^{f}	0.427 ± 4^{e}	1.128 ± 0.005^{d}	1.264 ± 0.002^{c}	1.502 ± 0.001^{b}	2.569 ± 0.005^{a}
PAH	0.026 ± 0.06^{f}	0.013 ± 0.12^{e}	0.029 ± 0.17^{d}	0.054 ± 0.23^{c}	0.957 ± 0.35^{a}	0.801 ± 0.06^{b}
Total TPH (mg/kg)	0.338 ± 0.061^{f}	0.443 ± 0.124^{e}	1.157 ± 0.175^{d}	1.318 ± 0.232^{c}	2.459 ± 0.351^{b}	3.370 ± 0.065^{a}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly ($P<0.05$).

3.2.2 Soil Chemical Parameters

The chemical analyses results as shown in Table 3.7 indicate that the pH value of the 8 ml/Kg and 16 ml/Kg contaminated soils were significantly higher than the control and other treatment concentrations.

Electrical conductivity of the control, 16 ml/Kg and 64 ml/Kg spent engine oil contaminated soil was significantly higher ($P<0.05$) than the other concentrations. They were however not significantly different from each other.

The total organic carbon (TOC) in the 32 ml/Kg and 64 ml/Kg concentrations was significantly higher ($P<0.05$) than the control and other concentrations of spent engine oil contaminated soil. The 8 ml/Kg had the lowest value of TOC which was significantly different from the other concentrations.

Total nitrogen content of the 8 ml/Kg, 16 ml/Kg and 64 ml/Kg concentrations was significantly higher ($P<0.05$) than the control and other concentrations of spent engine oil contaminated soil.

The Iron (Fe) content of the different concentrations of spent engine oil contaminated soil were all significantly different from each other. The 32 ml/Kg concentration had the highest iron content. The iron content in the control was only higher than the 8 ml/Kg concentration.

Lead (Pb) present in the different concentrations of spent engine oil contaminated soil was significantly different from each other. The 16 ml/Kg concentration had the highest lead content while the lead content in the control was higher than the other concentrations.

The copper (Cu) content of the different concentrations of spent engine oil contaminated soil was significantly different from each other. The 16 ml/Kg concentration possessed the highest copper content. The copper content in the control was only higher than the 4 ml/Kg concentration.

The zinc (Zn) content of the different concentrations of spent engine oil contaminated soil were significantly different from each other. The control had the highest zinc content.

Table 3.7: Chemical parameters of soil contaminated with spent engine oil.

SOIL COMPONENT	CONCENTRATION OF SPENT ENGINE OIL (ml/Kg)					
	0	4	8	16	32	64
pH	5.80±0.13^{b}	5.70±0.09^{b}	6.00±0.32^{a}	6.10±0.71^{a}	5.80±0.22^{b}	5.80±.21^{b}
Electrical Conductivity (µS/cm)	80.5±2.34^{a}	76.6±2.11^{b}	69.6±1.58^{c}	80.7±2.11^{a}	37.5±0.34^{d}	80.5±2.82^{a}
TOC (%)	0.18±0.08^{c}	0.17±0.09^{c}	0.15±0.05^{d}	0.20±0.03^{b}	0.24±0.03^{a}	0.22±0.02^{a}
Total Nitrogen (%)	0.063±0.003^{b}	0.067±0.001^{b}	0.070±0.005^{a}	0.077±0.006^{a}	0.062±0.004^{b}	0.069±0.002^{a}
Fe (mg/kg)	4575.09±13.24^{d}	4625.50±15.42^{c}	4024.73±52.47^{e}	1947.25±12.87^{f}	5847.00±56.23^{a}	4899.63±68.91^{b}
Pb (mg/kg)	6.12±0.94^{b}	4.91±0.45^{e}	5.19±0.51^{d}	6.58±0.74^{a}	5.74±0.69^{c}	4.73±0.49^{f}
Cu (mg/kg)	18.60±1.34^{e}	17.95±2.31^{f}	23.13±5.12^{b}	27.48±1.23^{a}	18.88±1.45^{d}	20.73±2.89^{c}
Zn (mg/kg)	95.48±4.32^{a}	19.50±2.10^{f}	46.49±3.51^{d}	37.62±2.89^{e}	60.91±3.98^{c}	67.38±4.25^{b}

Values are presented as Mean ± SEM. Values on the same row with different superscript differ significantly ($P<0.05$).

Key

TOC = Total Organic Carbon; Fe = Iron; Pb = Lead; Cu = Copper and Zn = Zinc.

CHAPTER FOUR

4.0 DISCUSSION

The presence of oil in the soil had deleterious effects on plant growth and development as it affected the biological, chemical and physical properties of the soil depending on the dose, type of the soil and other factors (Agbogidi and Egbochuna, 2010). This study evaluated the effects of different concentrations of spent engine oil on the growth parameters of *Saccharum spontaneum*. It was demonstrated that contamination of soil with spent engine oil significantly ($P<0.05$) affected the number of leaves (Table 3.1) after the 8th week of treatment. At the lowest concentration of 4 ml/Kg, the number of leaves was higher than the control, but this observation was not significantly different. The numbers of leaves grown in the higher concentrations of spent engine oil contaminated soil were all significantly lower than the control. This indicates that spent engine oil reduces the number of leaves of *S. spontaneum* at higher concentrations as well as in the long run. This is further supported by the reduction of number of leaves across the spent engine oil contaminated groups at the 8th week after treatment as compared to the preceding week. This report gives credence to the work by Iqbal *et al*. (2016) using *Parkinsonia aculeate,* where he observed that spent engine oil significantly affected the number of leaves. The reduction in the number of leaves is due to the increase in hydrocarbon content beyond the threshold for optimum leaf development.

The plant height of *S. spontaneum* was also significantly affected by the spent engine oil contamination. At concentrations higher than 4 ml/Kg, the plant heights were all significantly lower than the control group. However, as observed in the evaluation of number of leaves, the plant height of the control was not significantly different from that of the 4 ml/Kg spent engine oil contaminated soil; although at this concentration the plant height was higher. Iqbal *et al*. (2016) observed a significant reduction of plant height when *Parkinsonia aculeate* was grown in spent engine oil contaminated soil. Agbogidi and Illondu (2013) also reported a significant reduction in plant height grown in spent engine oil contaminated soil.

The development of tillers was significantly affected by contamination of the soil with spent engine oil. The control had a significantly higher number of tillers compared to the different treatment groups throughout the course of the study. The higher concentrations of 16, 32 and 64 ml/Kg of spent engine oil contaminated soil did not produce tillers at all. This is an indication that the spent engine oil had deleterious effects on the biochemical processes that result in tiller development. The presence of spent engine oil at high concentrations induced physiological stress on the plant occasioned by change in the chemical properties of the soil. Spent engine oil therefore prevents the spread and invasion of the contaminated soil by *S. spontaneum*.

The mean leaf area was also impacted by contamination with spent engine oil. The effect of spent engine oil was found to be significantly different ($P<0.05$) when compared with the control, however it was observed to not be concentration dependent. The control and those grown in soil treated with 4 and 16 ml/Kg spent engine oil had significantly larger leaf areas per plant compared to the plants grown in soil contaminated with 8, 32 and 64 ml/Kg spent engine oil which had significantly reduced mean leaf areas. The highest treatment concentration had the least leaf area. This report supports the findings of Olayinka and Arinde (2012), where it was observed that plants grown in soils with high concentrations of spent engine oil had the least leaf mean area development. Adu *et al*. (2015) reported that a negative relationship existed between the oil levels in the soil and the growth parameters (plant height, number of leaves and leaf area) of *Vigna unguiculata*. The spent engine oil resulted in the reduction of nutrient uptake which is necessary for leaf area expansion (Ogbuehi and Ezeibekwe, 2010). Ogbuehi *et al*. (2010) reported that oil contamination results in nutrient immobilization by microbes, which leads to low soil fertility as well as difficulty in nutrient uptake.

Spent engine oil contains aliphatic and polynuclear aromatic hydrocarbon contents which are responsible for the observed reduction in the number of leaves, plant height, number of tillers and leaf area of *S. spontaneum*. From the study, it was observed that the total aliphatic hydrocarbon content significantly increased with increasing concentration of spent engine oil contamination of the soil. The control had the least content, while the highest treatment of 64 ml/Kg had the highest total aliphatic hydrocarbon content. This supports the observation recorded above, that the higher concentrations had the least growth values at the 8th week after treatment. Hence, increase in total aliphatic hydrocarbon may have been responsible for the significant reduction in growth parameters across the treatment groups. The total polynuclear aromatic hydrocarbon content did not exhibit a dose dependent increase as the treatment concentration increased. This is suspected to be responsible for the absence of a dose dependent effect of spent engine oil contamination on the growth parameters of *S. spontaneum.* The control had a significantly lower total polynuclear aromatic hydrocarbon than the different concentrations of spent engine oil followed by the 4 ml/Kg treatment, whereas the 32 ml/Kg had the highest content. The increase in number of leaves, plant height and leaf area of *S. spontaneum* grown in 4 ml/Kg contaminated soil when compared with the control, although not significantly different, was due to the presence of small amounts of hydrocarbon contents. As reported by Agbogidi and Bamidele (2007), small amounts of hydrocarbon in the substrate can result in the enhancement of growth media and indirectly growth parameters. Ismail *et al.* (2014) also reported that pigeon pea (*Cajanus cajan*) and hyacinth bean (*Lablab purpureus*) grown on the lower concentration of 2.5 % of spent engine oil (SEO) contaminated soil had the longest shoots and leaf length when compared to the control after 3 months. As also reported by Vwioko and Fashemi (2005), the mean values of parameters like plant height, stem girth, leaf area, fresh and dry weights, and root length *Ricinus communis* L, were higher for 1% w/w of spent engine oil contaminated soil than control.

Result of soil chemical analyses show that the pH of the control is not significantly impacted by the contamination with different concentrations of spent engine oil except the 8 ml/Kg and 16 ml/Kg treatments where a significant increase in pH was recorded. The results show no relationship between pH and spent engine oil contamination. This finding is contrary to the report of Andrade *et al*. (2004), where he observed that pollution of soil with crude oil resulted in increased pH gradient. Generally, the soil chemical parameters did not show a dose dependent response to increase in treatment with spent engine oil except the total organic carbon (TOC) content result. The electrical conductivity of the soil in the control, 16 ml/Kg and 64 ml/Kg treatments groups were significantly higher than those of the 4 ml/Kg, 8 ml/Kg and 32 ml/Kg treatments. The control and 4 ml/Kg treatment had the lowest total organic carbon content. The total nitrogen of the control, 4 ml/Kg and 16 ml/Kg treatments were significantly lower than the other treatments. The 16 ml/Kg treated soil had the least iron content. The lead content of the control was significantly higher than the spent engine oil treated groups except the 16 ml/Kg treatment. Copper content of the control and 4 ml/Kg treated soil was significantly lower than the other treatment groups, with the 16 ml/Kg treated soil having the highest copper content. Zinc was significantly higher in the control than the spent engine oil treated groups. The 4 ml/Kg treated soil had the least zinc content. The absence of a dose dependent value of soil chemical parameters may be responsible for the absence of a dose dependent response in growth parameters of *S. spontaneum* grown in the different concentrations of spent engine oil contaminated soil up to the 7th week after treatment.

CONCLUSION

The presence of pollutants in the environment has adverse effects of the growth and development of plants. Spent engine oil deposited into the environment contaminates the soil, which may lead to reduction in plant growth parameters. However, as demonstrated in this report, lower concentrations of spent engine oil enhanced the number of leaves, plant height and leaf area of *S. spontaneum*. This observation shows that the plant is tolerant and thrives at contamination with very low concentrations of spent engine oil but declines in growth and development at high concentrations and extended periods.

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