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Anatomical and Physiological Effects of Spent-Engine Oil on Two Varieties of *Abelmoschus esculentus* (L.) Moench. from Malvaceae

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Abstract

The effect of different levels of spent engine oil application on germination, survival, growth, leaf anatomy, yield, nutrient content and heavy metals uptake of okra (*Abelmoschus esculentus* – 'Clemson' spineless variety and 'OLA 3 Local' variety) was assessed in the present study. Completely randomized design was used with five treatment levels of spent engine oil at 0 ml (control), 50 ml, 100 ml, 150 ml and 200 ml applied to 5 kg of soil. Data on germination, seedling survival, growth, chlorophyll nutrient and heavy metal content, as well as leaf epidermal features collected from the study were subjected to Statistical Package for Social Sciences (SPSS) analyses. Spent engine oil at 200 ml per pot significantly ($P < 0.05$) delayed seed germination for 4 days and reduced germination percentage by approximately 24% compared to the control. Plant height, number of leaves, leaf area, chlorophyll A, B and stomata area were reduced by 21.33-72.89%; number and dry weight of fruits were reduced by 67.4-13.58%. Number of stomata was increased on the adaxial surface by 57.73% and on the abaxial surface by 34.99%. Na, K, Cr, Cd and Fe contents increased by 0.0178-6.2698 mg/kg^{-1}. The present study has shown that plant constituents and anatomy can be influenced by spent oil contamination. Contamination of soil with spent engine oil therefore should be avoided in order to ensure sustainable crop plant productivity and to reduce the risk of heavy metals toxicity for human beings.

Keywords: germination; growth; heavy metal; nutrient level; yield

Introduction

Spent engine oils are mostly from the automobiles and frequently encountered at mechanic workshops (Anoliefo and Vwioko, 2001). The spent oil is disposed mainly on the vegetation areas nearer such mechanic shops. The methods and manners of disposal of the oil are of major concern to the environmentalists, scientists, agriculturists, health workers and other stake holders. In the recent time, many scientists have reported on the implications of the uncontrolled, improper and indiscriminate disposal of spent engine oils on the agricultural produces.

Many household, mechanic engineers inclusive deliberately cultivate crops in their neighbourhood for easy access to the crops when mature, or alternatively, the seeds of these crops are dispersed unknowingly to the surroundings and start germinating. The products of these actions somehow enter the food chain and thus constitute a serious health issues.

The situation described above is a common phenomenon in Nigeria where the automobile engineers are either unaware of the environmental hazards their actions are causing or due to the lack of proper orientation on how to dispose the used engine oils (Adesodun, 2004; Lauhanen *et al*., 2004). Onwuka *et al*. (2012) mentioned that the sources of pollution of the soils are mostly in the urban areas. The spent engine oils are said to be hazardous and unhealthy containing several pollutants including heavy metals (Meinz, 1999; Duffus, 2002). The danger in this is that most crops accumulate such metals in their tissues and they may not be affected physically, thereby producing juicy leaves and at times fruits which may be consumed.

Two varieties of *Abelmoschus esculentus* (L.) Moench. namely 'Clemson' spineless variety and 'OLA 3 Local' variety obtained in Nigeria were studied to assess the effects of the spent oils on their physiological and anatomical features. *Abelmoschus esculentus* of the family Malvaceae is cultivated for its fibrous fruits or edible green seed pods. The plant is a popular vegetable eaten in almost all part of the world (Duvauchelle, 2011). It contains high fiber, vitamin C, high folate content, high antioxidants, a good source of calcium and potassium.

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A perusal of literature reveals that there is little information on the effect of pollutants on the anatomy of the plant. The hereby research is therefore aimed at establishing the leaf architectural diversity of the two varieties of *Abelmuschus esculentus* under the influence of oil contaminated soil, as a pointer to environmental degradation. The objectives were to assess the effect of spent engine oil polluted soil on the germination, growth, survival patterns and fruiting, to determine the effect of spent oil on the anatomical adaptations of the plant species through their leaf epidermal diversities, to determine the effect of the pollutant on the composition of the plants species, to determine whether the plants are able to take up heavy metals from the polluted soil and at what rate if they are, as well as to determine whether there are variations in the response of the different species to the pollutants.

Materials and Methods

Description of the study area

The field experiment was conducted at the Botanical Garden of University of Ilorin, located in the southern Guinea savanna zone of Kwara State (8° 29" N, 4° 35" E), Nigeria, between November 2014 and February 2015. The site is characterized by bimodal rainfall distribution. The rainy season is from April to October with dry spells in late June and August, with the total annual rainfall ranging from 650 mm to 1,500 mm, the soil is sandy loam (Iwena, 2008). The temperature of the wet month's ranges between 24-27 °C, while in dry months it ranges between 29-35 °C (Klute, 1986).

Soil analysis

The procedures for physico-chemical properties analyses followed those earlier described by Walkley and Black (1934) and Anderson and Ingram (1993).

Experimental design

The experimental design was a randomized complete block replicated five times. Doses of 50 ml, 100 ml, 150 ml, 200 ml and 0 ml (control) of spent engine oil were used to prepare 5 kg of homogeneously mixed soil in polythene pots in which seeds were sown. The pots were shifted randomly twice during the period of experimentation to reduce the effect of sun and shade. The pots were kept free of weeds by hand weeding at intervals of three weeks after sowing. Measurements of the plant growth were noted for the following parameters: plant height, number of leaves, leaf length, and breadth and were done fortnightly. Leaf area was calculated as $LA = 0.75 \times L \times B$ (LA = Leaf Area, L = length and B = breadth (Kvet *et al.*, 1971).

Leaf segments of an area of 1cm^2 were macerated and the adaxial and abaxial membranes were separated from the mesophyll, stained in 1% safranin solution for about five minutes, mounted on glycerine on a microscope glass slide and observed using a binocular light microscope, using the field of view at x40. Stomatal complexes types (Dilcher, 1974; Metcalfe and Chalk, 1988), size (Franco, 1939) frequencies (Obiremi and Oladele, 2001), densities (Stace, 1965) and indexes were determined. Hand drawings of observations were taken. The frequency of each complex type was expressed as a percentage occurrence of such complex type based on all occurrences.

Stomatal index was calculated as: $SI = S\ S/(S+E) \times 100$.

Where: SI = Stomatal index, S = number of stomata per square millimeter; E = number of ordinary epidermal cell per square millimetre.

Shapes of trichome types were keenly observed and terminologies for identification and naming them followed those used by Dilcher (1974) and Metcalfe and Chalk (1988).

Plant parts (root, shoot, fruit and leaves) were oven-dried, ground in a Willey mill and digested with concentrated HNO_3 and concentrated HCL (3:1) at 150-175 °C for about 2 hours. Concentrations of plant nutrient elements Potassium (K) and Sodium (Na), as well as metal elements including Iron (Fe), Lead (Pb), Cadmium (Cd) and Chromium in the digested samples were determined using Atomic Absorption Spectrophotometer (AAS).

Data analysis

Data generated were analyzed using the Statistical Package for Social Sciences (SPSS) version 21. After testing the homogeneity of variance and normality of data, one way ANOVA was performed and significant differences were separated using Duncan's multiple range tests (DMRT). A *p*-value of < 0.05 was used. All data were expressed as mean ± standard error.

Results

Germination and number of surviving seedlings count

In *A. esculentus* 40 days local variety, the germination count of the control was significantly higher than all treatments at week 1 and 2 at $p < 0.05$ (Table 2). There was no germination in pots polluted with 100 ml and 150 ml for week 1, and the lowest germination count was recorded in

Table 1. Physico-chemical properties of experimental soil

Soil parameter	Values
Physical properties	
Textural class	Sandy-loamy
Particle size distribution	
Silt (%)	14.00
Clay (%)	5.04
Sand (%)	80.96
Chemical properties	
pH	8.60
Total acidity (cmolkg^{-1})	1.10
Organic carbon (%)	2.16
Organic matter (%)	3.74
Total Kjeldahl Nitrogen	0.03
Available phosphate	0.0219
Base saturation (%)	0.10
Cation exchange capacity	2.53
Ca (cmolkg^{-1})	0.09
Mg^{2+} (cmolkg^{-1})	1.07
K^+ (cmolkg^{-1})	0.03
Na^+ (cmolkg^{-1})	0.24

pots polluted with 150 ml of spent engine oil. For *A. esculentus* 'Clemson' spineless variety, the germination count of the control plants was significantly higher than all treatments at week 1 and week 3 at $p < 0.05$, all treatments were statistically the same at weeks 1 and 3. No germination was recorded for any treatment until week 2; the 150 ml plants did not germinate until the third week. Over all, at the end of 3 weeks, the highest germination was recorded in the control plants, while the 200 ml polluted plants had the lowest count.

For *A. esculentus* 40 days local variety, all control seedlings survived; the lowest seedling survival rates were recorded in the 150 ml tested plants. The survival rate of the control plants was significantly higher than 100 ml, 150 ml and 200 ml treatment plants, while the 150 ml treatment plants' survival ratio was significant lower than that of the control and other treatment plants at $p < 0.05$. For *A. esculentus* 'Clemson' spineless variety, the number of surviving seedlings of the control was observed to be significantly higher than all treatment plants; however, the different concentration treatment plants were found not to be statistically different from one another at $p < 0.05$. The control plants had the highest recorded survival rate, while the 200 ml plants had the lowest.

Growth parameters

Plant height

In *A. esculentus* 40 days local variety, the height of the control plants was significantly higher than those of 100 ml, 150 ml and 200 ml from week 3 to week 7 at $p < 0.05$. The lowest heights were recorded in the 150 ml pot plants throughout, although, they were not significantly different from plants in 100 ml and 200 ml pots at $p < 0.05$. The control plants had the highest recorded mean heights. For *A. esculentus* 'Clemson' spineless variety, similarly, the control plants had the highest heights for weeks 3, 5 and 7; they were also significantly higher than those in the 100 ml, 150 ml and 200 ml plants at $p < 0.05$. The 200 ml pot plants had the lowest stem heights throughout the weeks. The 50 ml plants were neither significantly different from the control plant nor were they from the 100 ml, 150 ml and 200 ml plants at $p < 0.05$ (Table 3).

Number of leaves

The mean number of leaves in *A. esculentus* 40 days variety revealed that the control and 50ml plants are not significantly different from each other; both were however significantly higher that plants in the 100 ml, 150 ml and 200 ml pots, the 100 ml, 150 ml and 200 ml plants were not

Table 2. Germination count and number of surviving seedlings as affected by different concentrations of spent engine oil

Treatment	1WAP	2WAP	3WAP
	Abelmuschus esculentus 40 days local variety		
Control	4.2000±1.78885^{a}	7.0000±0^{a}	7.0000±0^{a}
50 ml	6000±0.89443^{b}	4.4000±2.88097^{b}	5.4000±2.50998^{ab}
100 ml	0.0000±0^{b}	1.8000±1.78885^{cd}	2.2000±1.64317^{cd}
150 ml	0.0000±0^{b}	1.0000±1.22474^{d}	1.0000±1.22474^{d}
200 ml	1.4000±1.67332^{b}	3.6000±1.67332^{bc}	3.4000±1.94936^{bc}
	Abelmuschus esculentus 'Clemson' spineless variety		
Control	0.8000±1.09545^{a}	2.8000±1.48324^{a}	3.8000±1.48324^{a}
50 ml	0.0000±0^{b}	1.6000±1.14018^{ab}	1.6000±1.34164^{b}
100 ml	0.0000±0^{b}	0.4000±0.89443^{bc}	0.4000±0.89443^{b}
150 ml	0.0000±0^{b}	0.0000±0^{c}	0.6000±0.89443^{b}
200 ml	0.0000±0^{b}	0.8000±1.30384^{bc}	0.4000±0.54772^{b}

NB: values with the same letters are not significantly different from each other. ($p < 0.05$)
Key: 1WAP = One week after planting, 2WAP = Two weeks after planting, 3WAP = Three weeks after planting

Table 3. Plant heights as affected by different concentrations of spent engine oil

Treatment	3WAP (cm)	5WAP (cm)	7WAP (cm)
	Abelmuschus esculentus 40 days local variety		
Control	13.0000±0.70711^{a}	19.8000±1.64317^{a}	31.2000±3.49285^{a}
50 ml	10.0000±1.58114^{a}	16.6000±1.51658^{ab}	24.2000±3.49285^{ab}
100 ml	6.0000±4.00000^{b}	10.4000±6.65582^{bc}	16.4000±10.33441^{bc}
150 ml	4.2000±4.26615^{b}	8.0000±7.96869^{c}	11.4000±11.69615^{c}
200 ml	5.6000±2.07364^{b}	10.0000±3.67423^{bc}	15.6000±5.50454^{bc}
	Abelmuschus esculentus 'Clemson' spineless variety		
Control	11.6000±1.14018^{a}	19.4000±1.81659^{a}	27.6000±4.77493^{a}
50 ml	7.4000±4.21900^{ab}	12.2000±6.97854^{ab}	17.0000±9.74679^{ab}
100 ml	3.2000±4.38178^{b}	6.4000±8.79204^{b}	9.8000±13.42386^{b}
150 ml	2.6000±3.71484^{b}	4.6000±6.54217^{b}	7.2000±10.47378^{b}
200 ml	2.4000±3.57771^{b}	4.4000±6.65582^{b}	6.8000±10.54514^{b}

NB: values with the same letters are not significantly different from each other
Key: 3WAP = Three weeks after planting, 5WAP = Five weeks after planting, 7WAP = Seven weeks after planting

significantly different from each other at $p < 0.05$. The 150 ml plants had the lowest recorded leaf number while the control plants had the highest recorded leaves count (Table 4). For *A. esculentus* 'Clemson' spineless variety, the leaf numbers of the plants in the control pots were significantly higher than those of all the treatment concentrations for weeks 3, 5 and 7. The 50 ml plants were significantly higher than those of 100 ml, 150 ml and 200 ml at week 3; at weeks 5 and 7 however 100 ml plants were not significant different from the 50 ml, 150 ml and 200 ml plants, although 50 ml plants were significantly higher than those of 150 ml plants all at $p < 0.05$. The lowest numbers of leaves were recorded in the 200 ml plants and the highest were recorded in the control plants (Table 4).

Leaf area

For *A. esculentus* 40 days local variety, the leaf area parameters of the control and all treatment concentrations were observed to increase continuously from week 3 to week 7. There was a significant difference between the control and all the treatment plants from weeks 3 to 7, the 50 ml plants were also significantly higher in their leaf areas than all other treatment plants at week 3 and higher than 150 ml and 200 ml plants at week 5 and 7 at $p < 0.05$. The leaf areas were also observed to decrease progressively as the concentration of pollutant increased from the control to the 200 ml plants, with the control ones having the highest leaf area and the 200 ml plants the lowest (Table 5). Leaf area parameters for *A. esculentus* 'Clemson' spineless variety followed the same trend, as the parameters were observed to increase progressively with time and decrease as the concentration of pollutant increased; however, in the 100 ml, 150 ml and 200 ml plants', the leaf areas had no significant difference at weeks 3, 5 and 7. The control and 50 ml plants were however significantly different from each other and from the other treatments at $p < 0.05$. The control plants had the highest leaf areas, while the 200 ml plants had the lowest recorded leaf areas (Table 5).

Chlorophyll content

For *A. esculentus* 40 days variety, both chlorophyll A and B contents were observed to be reduced progressively, as pollutant concentration increased, from control to the 200 ml plants. The 200 ml plants were significantly lower in chlorophyll A contents than control and other treatment plants, but shared statistical identity with the 150 ml plants in chlorophyll B contents. Data for *A. esculentus* 'Clemson' spineless variety followed the same trend; however, the 100 ml and 150 ml plants were statistically identical with means values statistically distinct from neither the 50 ml nor the 200 ml plants, which were significantly different from each other statistically at $p < 0.05$ (Table 6).

Table 4. Number of leaves of plants as affected by different concentrations of spent engine oil

Treatment	3WAP	5WAP	7WAP
	Abelmuschus esculentus 40 days local variety		
Control	6.6000±0.89443^{a}	14.6000±0.89443^{a}	16.2000±1.30384^{a}
50 ml	5.8000±0.83666^{a}	12.0000±1.58114^{a}	13.6000±1.14018^{a}
100 ml	3.2000±2.04939^{b}	6.8000±3.96232^{b}	7.0000±4.00000^{b}
150 ml	2.0000±1.87083^{b}	4.6000±4.33590^{b}	5.2000±4.81664^{b}
200 ml	2.8000±1.30384^{b}	4.6000±2.40832^{b}	6.8000±2.28035^{b}
	A.esculentus 'Clemson' spineless variety		
Control	5.0000±0.70711^{a}	11.2000±1.30384^{a}	12.8000±0.83666^{a}
50 ml	3.0000±1.73205^{b}	5.2000±3.11448^{b}	6.4000±3.84708^{b}
100 ml	1.2000±1.78885^{c}	2.4000±3.28634^{bc}	3.0000±4.12311^{bc}
150 ml	0.8000±1.09545^{c}	1.2000±1.78885^{c}	1.4000±1.94936^{c}
200 ml	0.8000±1.09545^{c}	1.0000±1.41421^{c}	1.2000±1.78885^{c}

NB: values with the same letters are not significantly different from each other
Key: 3WAP = Three weeks after planting, 5WAP = Five weeks after planting, 7WAP = Seven weeks after planting

Table 5. Leaf area of plants as affected by different concentrations of spent engine oil

Treatment	3WAP (cm^2)	5WAP (cm^2)	7WAP (cm^2)
	Abelmuschus esculentus 40 days local variety		
Control	7.4140±0.68189^{a}	25.4880±2.92685^{a}	51.6680±8.88353^{a}
50 ml	5.3140±1.61246^{b}	13.7300±3.17013^{b}	34.9080±5.84401^{b}
100 ml	3.0500±1.84766^{c}	9.4300±5.39482^{bc}	22.6340±12.86607^{bc}
150 ml	1.6520±1.51571^{c}	6.7940±6.28988^{c}	12.4120±12.06083^{c}
200 ml	2.7320±0.37904^{c}	10.5640±1.61448^{bc}	20.3740±3.97404^{c}
	Abelmuschus esculentus 'Clemson' spineless variety		
Control	3.5140±0.28005^{a}	19.3420±2.06028^{a}	39.5160±6.87190^{a}
50 ml	2.1700±1.24980^{b}	10.1280±6.08132^{b}	22.7460±12.85597^{b}
100 ml	0.8880±1.22259^{c}	4.0480±5.61636^{c}	8.0800±11.06403^{c}
150 ml	0.4980±0.68397^{c}	1.1380±1.56164^{c}	3.0760±4.26522^{c}
200 ml	0.2740±0.38895^{c}	1.2660±1.76445^{c}	3.3040±4.68665^{c}

NB: values with the same letters are not significantly different from each other
Key: 3WAP = Three weeks after planting, 5WAP = Five weeks after planting, 7WAP = Seven weeks after planting

Stomatal parameters

The stomatal complex type observed in the two varieties was paracytic (Figs. 1a and b). Although there were variations in the epidermal cell shape and wall morphology, basic stomatal morphology was maintained and the leaves were observed to be amphistomatic. Table 7 presents the findings on number of stomata and epidermal cells on both adaxial and abaxial leaf surfaces, as well as the corresponding stomatal indices. Table 8 presents the sizes of the stomata present on both surfaces for the control and treatment plants.

The number of stomata and epidermal cells tend to increase with increase in pollutant concentration, while the stomatal index tend to reduce gradually as spent oil concentration increases, with variations between abaxial and adaxial surfaces, as the counts on the abaxial leaf surfaces were always significantly higher than those of the adaxial surfaces for all treatments and varieties studied at $p < 0.05$. For all crops, the control plants were always significantly lower than the 100 ml, 150 ml and 200 ml treatment plants and the 200 ml plants were consistently significantly higher than the control and 50 ml plants.

Trichome morphology

The trichome types observed were simple unicellular, branched unicellular and multicellular trichomes (Fig. 2; Tables 9 and 10). Length and breadth were variable, usually from 1,200 µm – 17,000 µm in length and 25-37 µm broad at base. Foot on top of epidermial cell or planted in the epidermal cell. Walls were observed to be thick and smooth;

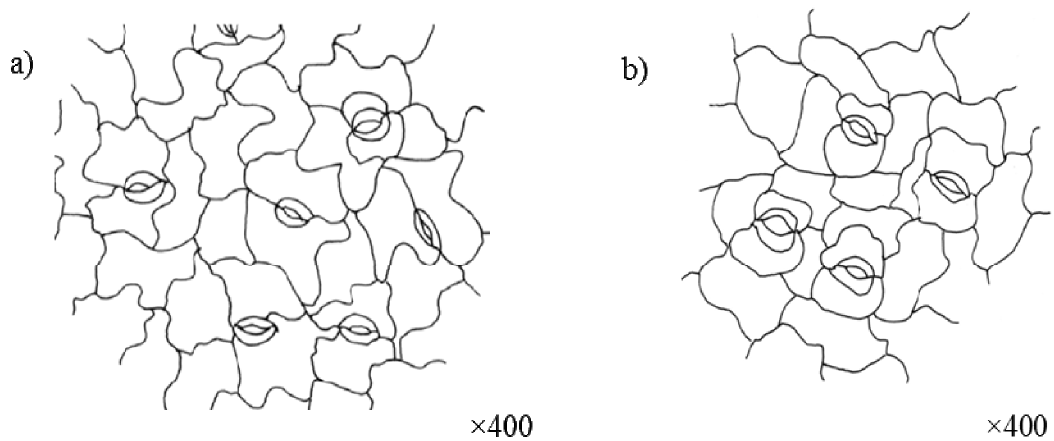

Fig. 1. Abaxial surface of *Abelmuschus esculentus* 'Clemson' spineless variety (a) and *Abelmuschus esculentus* 40 days local variety showing paracytic stomata; ×400

Table 6. Chlorophyll A and chlorophyll B content in plants as affected by different concentrations of spent engine oil

Treatment	Chlorophyll A (mg g^{-1})	Chlorophyll B (mg g^{-1})
	Abelmuschus esculentus 40 days local variety	
Control	0.6710±0.9735[a]	0.9054±0.08431[a]
50 ml	0.6018±0.17880[ab]	0.8253±0.07601[a]
100 ml	0.5862±0.13218[ab]	0.5788±0.04858[b]
150 ml	0.4296±0.12654[bc]	0.4486±0.04546[c]
200 ml	0.3820±0.08051[c]	0.4412±0.04024[c]
	Abelmuschus esculentus 'Clemson' spineless variety	
Control	1.0979±0.45645[a]	1.0507±0.28178[a]
50 ml	0.7750±0.06434[b]	0.5912±0.05352[b]
100 ml	0.5840±0.04274[bc]	0.4518±0.03045[bc]
150 ml	0.5265±0.02294[bc]	0.4522±0.04324[bc]
200 ml	0.4400±0.04690[c]	0.3452±0.02417[c]

NB: values with the same letters are not significantly different from each other ($p < 0.05$)

Table 7. Number of stomata and epidermal cells as influenced by different concentrations of spent engine oil

Treatment	Stomatal number	Epid. cell number	Stomatal index (%)
	Abelmuschus esculentus 40 days local variety		
Control adaxial	48.0000±3.39116[c]	122.6000±7.92465[e]	28.1360
Control abaxial	59.0000±4.30116[b]	154.6000±15.77340[cd]	27.6217
50 ml adaxial	50.2000±6.14003[c]	136.0000±5.70088[de]	26.9603
50 ml abaxial	61.8000±3.34664[b]	172.2000±7.59605[c]	26.4103
100 ml adaxial	51.8000±2.28035[c]	180.8000±9.49737[bc]	22.2700
100 ml abaxial	60.4000±4.03733[b]	200.6000±9.76217[b]	22.9890
150 ml adaxial	63.4000±3.20936[b]	250.4000±11.63185[a]	19.8249
150 ml abaxial	74.0000±4.52769[a]	256.4000±6.46529[a]	22.3971
200 ml adaxial	63.2000±1.92354[b]	242.2000±15.84929[a]	20.6942
200 ml abaxial	73.6000±4.56070[a]	233.0000±57.82733[a]	24.0052
	Abelmuschus esculentus 'Clemson' spineless		
Control adaxial	35.4000±4.82701[d]	115.0000±11.15796[e]	23.5372
Control abaxial	45.8000±4.86826[c]	131.8000±10.89495[e]	25.7888
50 ml adaxial	38.6000±4.03733[d]	136.0000±5.70088[e]	22.1077
50 ml abaxial	54.2000±4.43847[b]	174.4000±8.61974[d]	42.1462
100 ml adaxial	47.2000±4.65833[c]	184.2000±14.46375[cd]	35.9208
100 ml abaxial	56.0000±6.67083[b]	202.4000±10.45466[c]	21.6718
150 ml adaxial	56.4000±3.71484[b]	229.8000±18.82020[b]	24.5431
150 ml abaxial	69.8000±5.93296[a]	249.2000±15.75436[ab]	21.8809
200 ml adaxial	60.6000±2.30217[b]	245.4000±10.57355[b]	19.8039
200 ml abaxial	70.2000±6.05805[a]	270.2000±37.88403[a]	20.6228

NB: values with the same letters are not significantly different from each other. ($p < 0.05$)

Table 8. Stomatal size as influenced by different concentrations of spent engine oil contamination

Treatment	Stomata length (μm)	Stomata breadth (μm)	Stomatal area (μm^2)
	Abelmuschus esculentus 40 days local		
Control adaxial	27.20	18.30	390.86
Control abaxial	28.1	18.8	414.83
50 ml adaxial	27.10	16.5	351.12
50 ml abaxial	27.8	16.9	368.92
100 ml adaxial	21.3	13.7	229.14
100 ml abaxial	25.9	17.2	349.81
150 ml adaxial	19.5	14.1	215.90
150 ml abaxial	21.9	15.6	268.27
200 ml adaxial	21.8	16.2	277.31
200 ml abaxial	22.8	16.9	302.57
	Abelmuschus esculentus 'Clemson' spineless variety		
Control adaxial	25.60	16.70	335.71
Control abaxial	24.4	19.8	379.36
50 ml adaxial	20.60	13.5	218.37
50 ml abaxial	22.3	14.9	260.91
100 ml adaxial	19.3	13.7	207.62
100 ml abaxial	21.9	12.2	209.80
150 ml adaxial	6.2	5.1	24.83
150 ml abaxial	9.1	5.6	40.02
200 ml adaxial	6.3	4.2	20.78
200 ml abaxial	8.9	4.9	34.24

The trichomes were pointed at the distal end and were present on grooves of petiole, midrib, stem and on the adaxial surface of leaves. The unicellular trichomess were observed to be of the highest frequency, density and index, followed by the branched unicellular trichome. The multicellular trichome had the least frequency percentage and density in the control and treatment plants.

Yield parameters

For *A. esculentus* 40 days local variety, the parameters were observed to reduce progressively as pollutant concentration was reduced, with the following exceptions: fruit length and number of the 200 ml plants were higher than those of the 150 ml plants, fresh weight at 150 ml, dry weight at 150 ml and 200 ml were higher than values at 100

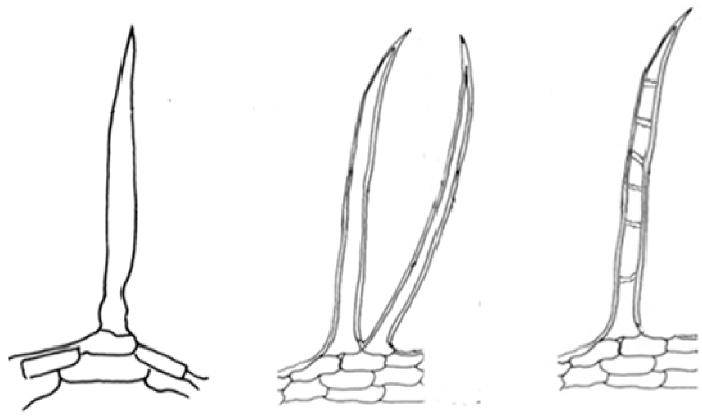

Fig. 2. Trichome types observed in the study; a- simple unicellular trichome; b- branched unicellular trichome; c- multicellular trichome X400

Table 9. Trichome parameters of *A. esculentus* 'Clemson' spineless variety, as affected by different concentrations of spent oil pollution

Treatment	Trichome type	Trichome frequency (%)	Trichome density (mm^2)	Trichome index (%)
Control adaxial	Unicellular	60.00	6	5.22
	Branched unicellular	30.00	3	2.61
	Multicellular	10.00	1	0.86
Control abaxial	Unicellular	66.67	6	4.58
	Branched unicellular	22.22	2	1.53
	Multicellular	11.11	1	0.76
50 ml adaxial	Unicellular	62.50	5	3.68
	Branched unicellular	37.50	3	2.21
	Multicellular	-	-	-
50 ml abaxial	Unicellular	58.33	7	4.02
	Branched unicellular	16.67	2	1.15
	Multicellular	25.00	3	1.72
100 ml adaxial	Unicellular	66.67	6	3.26
	Branched unicellular	22.22	2	1.09
	Multicellular	11.11	1	0.54
100 ml abaxial	Unicellular	57.14	8	3.81
	Branched unicellular	28.57	4	1.94
	Multicellular	14.29	2	0.98
150 ml adaxial	Unicellular	50.00	4	1.75
	Branched unicellular	12.50	1	0.45
	Multicellular	37.50	3	1.31
150 ml abaxial	Unicellular	58.33	7	2.81
	Branched unicellular	8.33	1	0.40
	Multicellular	33.33	4	1.61
200 ml adaxial	Unicellular	62.50	5	2.04
	Branched unicellular	25.00	2	0.82
	Multicellular	12.50	1	0.41
200 ml abaxial	Unicellular	60	6	2.22
	Branched unicellular	20	2	0.74
	Multicellular	20	2	0.74

Table 10. Trichome parameters of *A. esculentus* 40 days local variety, as affected by different concentrations of spent oil pollution

Treatment	Trichome type	Trichome frequency (%)	Trichome density (mm^2)	Trichome index (%)
Control adaxial	Unicellular	58.33	7	5.43
	Branched unicellular	33.33	3.17	3.17
	Multicellular	8.33	1	0.81
Control abaxial	Unicellular	57.14	8	4.88
	Branched unicellular	21.43	3	1.91
	Multicellular	21.43	3	1.91
50 ml adaxial	Unicellular	56.25	9	6.21
	Branched unicellular	26.67	4	2.86
	Multicellular	13.33	2	1.44
50 ml abaxial	Unicellular	58.33	7	3.91
	Branched unicellular	25.00	3	1.71
	Multicellular	16.67	2	1.15
100 ml adaxial	Unicellular	61.58	8	4.25
	Branched unicellular	23.08	3	1.64
	Multicellular	15.38	2	1.10
100 ml abaxial	Unicellular	60.00	9	4.31
	Branched unicellular	26.67	4	1.96
	Multicellular	13.33	2	0.99
150 ml adaxial	Unicellular	54.54	6	2.34
	Branched unicellular	27.27	3	1.19
	Multicellular	18.18	2	0.79
150 ml abaxial	Unicellular	50.00	7	2.66
	Branched unicellular	21.42	3	1.16
	Multicellular	28.57	4	1.54
200 ml adaxial	Unicellular	63.63	7	2.81
	Branched unicellular	18.18	2	0.82
	Multicellular	18.18	2	0.82
200 ml abaxial	Unicellular	56.25	9	3.72
	Branched unicellular	25.00	4	1.69
	Multicellular	18.75	3	1.27

ml. For fruit length and fresh weights, control plants had the second highest recorded data, after the 50 ml plants, the control plants also had the highest number of fruits. The 150 ml plants however had the highest dry weight. There was no significant difference in the length and dry weight of fruits among control and all treatment plants at $p < 0.05$.There were no fruit parameter data for 100 ml, 150 ml and 200 ml treatments of *A. esculentus* 'Clemson' spineless as the plants did not fruit. For all fruit parameter, the control plants' data were significantly higher than those of the 50 ml plants at $p < 0.05$ (Table 11).

Nutrient contents

For *A. esculentus* 40 days local variety, the Potassium and Sodium contents of the plants tend to increase with increasing pollutant concentration. The values for 200 ml plants were significantly higher than the control and other treatments at $p < 0.05$, as they had the highest recorded value in this regard. For *A. esculentus* 'Clemson' spineless variety, the data followed a similar trend; however, the 150 ml plants were significantly higher in Potassium and Sodium contents than all other treatments and the control plants at $p < 0.05$. The control and 50 ml plants were statistically the same in Potassium content; all others showed statistical distinction from one another for both nutrients at $p < 0.05$.The 100 ml and the 150 ml plants had the highest Potassium and Sodium concentrations respectively, while the control plants had the lowest Sodium and Potassium concentration (Table 12).

Heavy metal contents

For *A. esculentus* 40 days variety, Chromium contents of the control of and 50 ml plants were statistically identical at $p < 0.05$. The 150 ml and 200 ml plants also share statistical identity, the 100 ml plants were however not statistically different from both groups. The control plants had the least recorded Chromium content, while the 200 ml plants had the highest. For Cadmium concentration, the 100 ml plants were statistically higher than the 50 ml and control plants, but the 150 ml and 200 ml plants had no significant statistical difference from both the 100 ml and 50 ml plants at $p < 0.05$. The 100 ml plants had the highest Cadmium concentration, the 50 ml plants had the lowest Cadmium concentration and the control plants had no Cadmium content. The 200 ml plants were significantly higher in Iron content than other treatments and control at $p < 0.05$. The control and the 150 ml plants were statistically the same, but were significantly higher than the 50 ml and 100 ml plants which were statistically the same at $p < 0.05$ (Table 13).

Discussion

Germination and number of surviving seedlings count

The control seeds of all experimental plants used for this study germinated within 4 to 10 days after planting, whereas the seeds in the contaminated soil started germinating slowly and intermittently from the 6^{th} day and continued until about 3 weeks after planting.

Table 11. Yield and fruit parameters as affected by different concentrations of spent engine oil

Treatment	Fruit length (cm)	Fruit number	Fruit FW (g)	Fruit DW (g)
		Abelmuschus esculentus 40 days local variety		
Control	3.9000±0.36742[a]	17.8000±2.38747[a]	4.2800±1.31034[ab]	0.4638±0.13078[a]
50 ml	3.9800±0.25884[a]	11.8000±3.03315[b]	5.5800±1.81163[a]	0.2010±0.15421[a]
100 ml	2.5400±1.52578[a]	8.8000±6.05805[b]	2.2200±1.52381[b]	0.2946±0.36423[a]
150 ml	2.4400±2.60154[a]	3.8000±3.76829[c]	2.7600±3.19734[b]	0.5600±0.34662[a]
200 ml	2.9200±0.92304[a]	5.8000±2.38747[a]	1.8400±0.48270[b]	0.4008±0.17925[a]
		Abelmuschus esculentus 'Clemson' spineless variety		
Control	5.7600±1.34276[a]	14.8000±2.77489[a]	6.1200±1.93054[a]	0.5454±0.19389[a]
50 ml	0.7800±1.07331[b]	0.6000±0.89443[b]	0.6000±0.82462[b]	0.0805±0.12900[b]
100 ml	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]
150 ml	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]
200 ml	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]	0.0000±0[b]

NB: values with the same letters are not significantly different from each other ($p < 0.05$)
Key:Fruit FW = Fruit Fresh weight, Fruit DW = Fruit Dry Weight

Table 12. Nutrient contents of control and treatment plants as affected by different concentrations of spent engine oil

Treatment	Potassium (mg kg^{-1})	Sodium (mg kg^{-1})
	Abelmuschus esculentus 40 days local variety	
Control	1.3579±0.07022[c]	1.0379±0.02883[c]
50 ml	1.2897±0.01916[c]	1.1259±0.12564[bc]
100 ml	2.2661±0.25102[b]	1.1468±0.03854[bc]
150 ml	1.9858±0.06410[bc]	1.1709±0.01635[b]
200 ml	4.6688±0.88311[a]	1.5700±0.01545[a]
	Abelmuschus esculentus 'Clemson' spineless variety	
Control	0.9822±0.5716[d]	1.0054±0.00621[e]
50 ml	1.1803±0.10203[d]	1.2514±0.03010[c]
100 ml	2.5664±0.12109[c]	1.1634±0.02850[d]
150 ml	8.5933±0.23861[a]	1.5542±0.04372[a]
200 ml	7.2520±0.60036[b]	1.4453±0.03228[b]

NB: values with the same letters are not significantly different from each other ($p < 0.05$)

Table 13. Heavy metal contents of plants as influenced by the different concentrations of spent engine oil

Treatment	Chromium (mgkg^{-1})	Lead (mg kg^{-1})	Cadmium (mg kg^{-1})	Iron (mg kg^{-1})
		Abelmuschus esculentus 40 days variety		
Control	0.0033±0.00577[b]	0.0000	0.0000±0[c]	3.1800±0.04359[b]
50ml	0.0067±0.00577[b]	0.0000	0.0133±0.00577[b]	2.5833±0.05859[c]
100ml	0.0133±0.00577[ab]	0.0000	0.0300±.01000[a]	2.1067±0.01528[c]
150ml	0.0200±0.01000[a]	0.0000	0.0200±0.01000[ab]	3.4900±0.33645[b]
200ml	0.0233±0.00577[a]	0.0000	0.0267±0.00577[ab]	4.3200±0.54083[a]
		Abelmuschus esculentus 'Clemson' spineless variety		
50ml	0.0100±0.10[b]	0.0000	0.0100±0[a]	2.5267±0.41199[b]
100ml	0.0067±0.1155[b]	0.0000	0.0167±0.00577[a]	2.1600±0.54009[b]
150ml	0.0133±0.00577[b]	0.0000	0.0133±0.00577[a]	5.4767±0.12583[a]
200ml	0.0333±0.00577[a]	0.0000	0.0133±0.00577[a]	3.8867±1.54053[ab]
Control	0.0067±0.00577[b]	0.0000	0.0000±0[b]	3.4600±1.22650[b]

NB: values with the same letters are not significantly different from each other ($p < 0.05$)

The delay in germination of the treatment plants might be attributed to the presence of spent engine oil in the soil. Germination was recorded for every treatment concentration, but the percentage germination was observed to be reduced as concentration of pollutant increased. Agbogidi and Ilondu (2013) also observed that *Moringa oleifera* seeds planted in spent oil contaminated soil took up to six more days to sprout when compared with seeds planted in uncontaminated soils. They also reported that the number of germinated seeds was significantly reduced as the level of oil in soil increased. Chauhan and Goyal (1980) reported that the coating of seeds by oil may completely inhibit germination. Similarly, germination was delayed or inhibited as a result of application of spent oils in *Abelmuschus esculentus* (Osawuru and Abioye, 2012), pepper and tomato (Anoliefo and Vwioko, 1995), *Arachis hypogaea* (Agbogidi and Enujeke, 2012) and *Chromolaena odorata* (Anoliefo and Vwioko, 2001).

Plant height

Growth parameters such as plant height, number of leaves and leaf area which were examined in the course of the present work were observed to be significantly affected by spent engine oil contamination. The control plants averaged higher in these parameters than the treatment plants at different concentrations with the figures decreasing with an increase in spent oil contamination. This finding supports the reports of Osawuru and Abioye (2012) on their work on *Abelmuschus esculentus* where the control plants grew better than plants sowed in soils treated with spent oil. Udo and Fayemi (1975), Amakiri and Onofeghara (1984) also affirmed that most plant species suffer serious depression in growth as a result of poor soil condition, dehydration and impaired nutrient uptake by the roots resulting from high concentration of oil in soil.

Agbogidi and Eruotor (2012) reported that spent engine oil contamination on soil significantly affected the height, number of leaves, collar diameter and leaf area of *Jatropha curcas* seedlings with reductions in these parameters being concentration dependent. Vwioko and Fashemi (2005) among many other authors have reported that reduction in plant height is a visible effect of substances that are toxic, and thus, inhibit plant growth. Furthermore, dehydration occurs as a result of the presence of pollutants like spent engine oil in the soil and plants tend to reduce transpiration by shedding their leaves in order to maximize the little available water (Amadi *et al*., 1993). Leaf fall was observed in plants on the treated soils especially *A. esculentus* 'Clemson' spineless variety; this may be a cause for the observed reduction in leaf number and area (Agbogidi and Eruotor, 2012). The reduction in the number of leaves of the plants as pollutant concentration increased may be an adaptation to the drought created by the presence of the spent engine oil. The concentration-dependent reduction in leaf area and number of leaves agrees with the reports of Sharifi *et al*. (2007) on six plant species, Vwioko and Fashemi (2005) on *Ricinus communis* and Agbodigi *et al*. (2005) on *Leucaena leucocephala*.

Chlorophyll content

The leaves of the control plants were greener and darker than the treated plants with their paleness increasing with increasing spent engine oil concentration. Agbogidi and Ejemete (2005) and Agbogidi and Eshegbeyi (2006) reported that hydrocarbon from oil contaminated soils accumulate the chloroplast of leaves, thereby reducing their photosynthetic ability. Agbogidi and Ilondu (2013) and Agbogidi and Eruotor (2012) also reported spent engine oil concentration dependent chlorosis (yellowness of leaves) in *Moringa oleifera* and *Jatropha curcas* respectively. The chlorosis could be as a result of nutrient immobilization because Agbogidi and Ejemete (2005), Benka-Coker and Ekundayo (1995) and Benka-Coker and Ekundayo (1997) reported oil pollution as a cause for the unavailability of some essential nutrient elements, while the toxic ones may be more readily available.

Baruah *et al*. (2014) have shown that there is a great impact of crude oil contamination on the chlorophyll content of the leaves of *Cyperus brevifolius*, whereby chlorophyll a, chlorophyll b and total chlorophyll contents of leaves of plants grown in different concentrations of crude oil treated soils was found to be lower than those of the control plants. Their results also demonstrated that chlorophyll content was the lowest in the treatment that received maximum dose of crude oil. It also showed that chlorophyll content decreased with increased concentration of crude oil - all these findings are similar with the findings of the current research.

Stomatal parameters

The importance of microscopic features of the leaf epidermis has been realized lately by taxonomists so that recent taxonomic monographs are now considered incomplete without them (Esseitt *et al*., 2012). The stomatal complex and trichome types observed were the same for the 2 varieties and treatments did not change under the influence of spent engine oil pollution the architectural design.

Insignificant variation in leaf epidermal features in response to varied conditions implies that such features are stable; their expressions are under strong genetic control and may play a significant role in taxonomy (Alege *et al*., 2013). However, significant variations were observed in stomatal size, density accessory and ordinary epidermal cell sizes, which were observed to reduce with increase in pollutant concentration. Alege *et al.* (2013) reported that the increase in organic or inorganic nutrient through the application of fertilizer, especially chicken dung increased the size of the stomata and the guard cells of *Sesamum indicum*; this supports the findings of the present research in that since spent engine oil has been established to reduce nutrient availability to plant use, the stomata cell may reduce in size or shrink.

The work of Abdulrahaman and Oladele (2008) confirmed that plants with higher frequency of stomata with subsidiary cells such as cyclic, paracytic and diacytic, lower index and less heterogeneous composition of stomatal complex types and probably occurrence of trichome may be more suitable to adaptation in dry locations. This features where observed to be prominent in the studied species, especially in the oil treated plants. Since the reduction in the amount of water available by the presence of spent oil in the soil was inevitable, it is likely that this features were elaborated to cope with the drought.

Yield parameters

Yield parameters such as length, dry weight, fresh weight and number of fruits produced by the control and spent oil treatment plants were compared and a concentration dependent reduction in these parameters were observed in all treatments except the 200 ml spent oil treated plants of *A. esculentus* 40 days local variety, as they had higher fruit number than the 150 ml treated plants and were higher in dry weight than the 100 ml treated plants. *A. esculentus* 'Clemson' spineless variety plants treated with 100 ml, 150 ml and 200 ml of spent engine oil did not fruit at all. These variations may be an effect of spent oil polluted soil on the plant (Agbogidi and Ofuoku, 2005).

Agbogidi and Eshegbeyi (2006) reported that oil pollution exert the greatest influence on growth and yield of plants. Since according to Kathirvelan and Kalaiselvan (2007) the leaf surface area determines in large part the amount of carbon gained through photosynthesis and the amount of water lost through transpiration and ultimately the crop yield, the loss of leaves and the reduction of the leaf area as was observed in the current study implies that there would be low a photosynthetic efficiency of the plants, as much of the solar energy emitted by the sun would not be absorbed for photosynthesis, this ultimately leading to low yield of the plant (Walker *et al.*, 2001). Agbogidi and Ilondu (2013), Okon and Mbong (2013) and Agbogidi and Eruotor (2012) all reported reduction or absence of yield in plants grown in oil polluted soils.

Nutrient contents

The concentration of Sodium and Potassium in the studied plants variably increased respectively as the concentration of spent engine oil increased. Control plants of *A. esculentus* 40 days variety were also higher in Potassium than the 50 ml plants, while the 100 ml plants were higher in potassium contents than the 150 ml plants. The presence of oil in the soil significantly reduced the available forms of phosphorus and potassium to plants. Giovacchino *et al.* (2001) reported increased nitrogen, phosphorus, potassium, calcium, magnesium and sodium content in all parts of the maize crop planted with olive oil processing effluent. Mallika (2001) reported similar findings.

Heavy metal contents

Lead was not detected in all treatment and control plant parts, but Iron, Cadmium and Copper were detected in the two plant varieties. This shows that the plants were able to take up heavy metals from the soil and transport them to the aerial parts (Peralta-videa *et al.*, 2002, 2009; Osawuru and Ajiboye, 2012). This finding support the reports of Hall (2002), Osubor and Anoliefo (2003), Okafor and Nwanfei (2003), Abdua and Muazu (2007), Agbogidi *et al.* (2007a, b), Damisa *et al.* (2008), Agbogidi and Egbuchua (2010) and Agbogidi (2010) that heavy metals are abundant in soils contaminated with petroleum hydrocarbons, even though the concentration in plants were below the accepted limits (FAO, 198; FEPA, 2002). Cadmium was not detected in control plants, but was present in all treatment plants.

According to Achuba and Peretiemo-Clarke (2008) excess uptake of metals by crop plants may lead to toxicity in human nutrition, e.g. Cadmium and Zinc can lead to acute gastrointestinal and respiratory damages, acute heart, brain and kidney damages. Chromium, Cadmium and Iron concentration increased with increased spent engine oil concentration (Osawuru and Ajiboye, 2012). Iron had of the highest concentration in all the plants. High concentrations of heavy metals in soil can negatively affect plant growth and yield as these metals interfere with metabolic functions in plants including physiological and biochemical processes, inhibition of photosynthesis and respiration (Osubor and Anoliefo, 2003). This may be the reason for the stunted growth observed in the treatment plants. The findings of the present study show that soil contamination with spent oil can lead to a gradual accumulation of heavy metals, which may have direct impacts on plant life and, by extension, on human life (Agbogidi and Eruotor, 2012).

Conclusions

The results of the hereby study have demonstrated that soil contamination with spent engine oil can alter the physico-chemical properties of the soil and degrade its capacity to provide suitable habitat for plants' growth in such soil. *A. esculentus* seed germination was delayed or inhibited due to the presence of spent oil in the soil. Growth, chlorophyll content in leaves, Potassium and Sodium content, reproduction and leaf anatomical configuration, were also affected. The research has also shown that *A. esculentus* is able to accumulate heavy metals from the soil as an indication of environmental degradation. Although the amount of metals observed in the present study were not above the tolerable limits, these crops should however not be cultivated in areas polluted with spent engine oil and its associated products without initial soil amendment, as the metals which may be accumulated, with time, may rise in concentration to lethal levels, and thus are dangerous to human health.

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