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EFFECTS OF SPENT ENGINE OIL POLLUTION ON GERMINATION AND EARLY SEEDLING ESTABLISHMENT OF SIX PLANT SPECIES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The performance of six plant species subjected to various concentration of spent engine oil was the principal aim of this study. The experiment was arranged in a completely randomized design with three replicates each. Germination percentage, germination vigour, germination index, radicle and plumule lengths were assessed. The plant species showed differential response to concentration of spent engine oil which was dose-dependent. Significant correlation was observed among final germination percentage, shoot and root length and shoot and root weights respectively. The correlation between FGP and GI; FGP and SVI were significantly negative (r = -0.134; -0.371 (P < 0.001) indicating that the germination index and seedling vigor index were delayed with decrease in germination percentage. Indiscriminate disposal of spent engine oil should be checked because of its adverse effects on the germination and early seedling establishment in the tropics.

Keywords: Phytotoxic; germination; seedling; engine oil; growth.

1. INTRODUCTION

Petroleum and its allied product is the lynchpin of Nigerian economy. Spent engine oil is one of the petro-chemicals reported to be a major source of soil contaminant in Nigeria [1]. Agbogidi and Enujeke [2] defined engine oil as a pollutant in the environment that causes damage to the ecosystem as well as health hazard to human beings. It is commonly obtained after servicing and subsequent draining of used oil from automobiles engines [3]. It contains potentially toxic polycyclic aromatic hydrocarbons and heavy metals. As engine oil is used in automobile, it picks up a number of additional compounds from engine wear. This is made up of a mixture of different chemicals including petroleum hydrocarbons, chlorinated biphenyls, chlorodibenzofurans, additives, decomposition products and heavy metals that come from engine parts as they wear away [4].

Over the years there has been an increase in the amount of waste generated across the globe partly as a result of urbanization and population growth. Studies have shown that the activities of artisans in the automobile industry (which include mechanics, welders and sprayers) generate varieties of waste such as used engine oil [5]. Pollution incidence emanating fromspent engine lubricating oil has been reported to bemore widespread and prevalent than that of crude oil [6]. In Nigeria, as in many other countries, petroleum hydrocarbon contamination is widespread. Pollution arising from the disposal of used engine oil

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is one of the environmental problems in Nigeria and is more widespread than crude oil pollution [7]. The prevalent mode of indiscriminate disposal of these spent engine oils in the environment calls for urgent attention [6]. Contamination results from mishandling, deliberate disposal, spilling and leakage of petroleum products, such as gasoline, lubricating oils, diesel fuel, heating oils, used or spent engine oils [8]. Some heavy metals are required by plants at low concentrations (essential micronutrients), but at high than normal concentrations they may cause metabolic disorders and subsequently become growth inhibitors for most of the plant species [9]. Anoliefo and Vwioko [10] reported that the contamination of soil with spent engine oil caused growth retardation in plants, with the effect more adverse for tomato (*Lycopersicom esculentum*) than pepper (*Capsicum annum* L.).

Spent engine oil is indiscriminately disposed into gutters, water drains, open vacant plots and farms by auto technicians and allied artisans with workshops on the road sides and open places [11]. The consequences are the environmental pollution, contamination of water bodies, contamination of ground water and toxicity to animal and plants [12]. The release of contaminants into the environment by human activities has increased enormously over the past several decades. When these contaminants are deposited in soils, they cause higher risk of toxicity and soil infertility. Thus this posed a serious threat to the soil environment and properties [13].

The environmental impact of spent engine oil on growth and performance of plants have been reported by many researches. Germination of *Amaranthus hybridus* seeds were significantly affected in spent engine oil polluted soil [14]. Agbogidi et al. [15] showed that spent engine oil application to soil significantly reduced crop growth and yield in okra and five cultivars of soy beans, respectively. Anoliefo and Vwioko [16] reported a significant higher means plant height, leaf area and dry weight of *Commelina bengalensis* (day flower) at 0 mg g^{-1} oil pollution than at 50 mg oil g^{-1} pollution level. Further still, [17] reported that vegetative cutting of *Paspalum* conjugation (sour grass) grew well in absence of oil and salinity and that 75% of the test plant survived in low oiling but heavy oiling resulted in mortality.

Petroleum hydrocarbon contamination may adversely affect plants by retarding seed germination and reducing height, stem density, photosynthetic rate and biomass or resulting in complete mortality [18,19]. Identifying plants with the potential to phytoremediate specific mixtures of contaminants in soils is a foundational step to providing insights in the area of phytoremediation of contaminated soils. In light of the above, this study was carried out to assess the phytotoxic effect of spent engine oil on germination and early seedling growth of six plant species for use in phytoremediation of polluted sites.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out at the laboratory of the Department of Biology, Federal University of technology, Owerri, Southeast Nigeria.

2.2 Seed Collection and Experimental Design

Seeds used in this study were obtained from the gene bank of the International Institute of Tropical Agriculture, Ibadan. The accessions are TVu-10343, TVu-10366, TVu-13606, TVu-13630, TGm-504, TGm-570 and TGm-547. Seed viability test was carried out following the method of [18]. Ten (10) seeds of each accession were placed in 26mm Petri dishes lined with cotton wool and moistened with the measured concentration of spent engine oil. The concentrations were 3%, 6%, 9% and 0% which served as control, which was obtained by mixing appropriate volumes of SEO with distilled water. Each concentration served as a treatment. The experiment was laid out in a completely randomized design and replicated thrice. Prior to planting, seeds were aerated in water until the first sign of germination to ensure uniform germination of the seeds. The growth chamber was set to 22°C and 16:8 hours of light: dark, with a relative humidity of approximately 58.9%. It is difficult to provide optimum growth conditions (photoperiod, day-night temperature and relative humidity) for each plant species. Most plant species have been found to grow actively between a minimum of 12-hour photoperiod, an average relative humidity of 50%[19] and an average daily temperatures of 5-35°C, with the general assumption being that to achieve optimal growth that temperatures at night should be less than day temperatures by 3-10°C [20] but positive effects of lower night temperature has been found to be negligible or detrimental to plant growth [21], Thus, the selected growth chamber conditions for this study is optimal for plant growth. The Petri dishes were wetted with each level of contaminant and monitored for 14 days and the number of germinated seeds counted and recorded every morning at 9am. After 14 days, the root and shoot lengths of plants were measured and the final germination percentage calculated. Seeds were considered as germinated when roots were 2 mm long. The following parameters were calculated from the data obtained:

$$\textbf{Final germination percentage (GP)} = \frac{\text{Number of germinated seeds at final count}}{\text{Total number of seeds sets for bioassay}} \times \frac{100}{1}$$

$$\textbf{Germination index (GI)} = \text{GI} = \Sigma \frac{{}^{G}T}{Tt} = \frac{\text{[Number of germinated seeds]}}{\text{[Days of first count]}} + \cdots + \frac{\text{[Number of germinated seeds[22]}}{\text{[Days of last or final count]}}$$

$$\textbf{Seedling vigour index (SVI) SVI} = \frac{\text{Seedling length (mm) X Germination percent[23]}}{100}$$

2.3 Root and Shoot Length (cm)

TheRadicle and Plumule lengths were measured on a daily basis with the aid of a meter rule. Mean values were calculated and expressed in cm.

2.4 Whole Plant Fresh and Dry Weight (g)

Whole plant fresh weight was measured with the of analytical balance. Plant samples were air dried for 48hrs then oven dried at 80^0C to a constant weight and dry weights were measured in grams.

A scoring system was used to rank the accessions of each in-vitro screening test for their tolerance to spent engine oil at various concentrations. The aggregated score was computed as it was recommended by Fawzi [24] using the formula:

Score = $G_{0\cdot0}$ x $L_{0\cdot0}$ + 2 (G_{50} x L_{50}) + 3(G_{100} x L_{100}) + 4(G_{200} x L_{200}),

Where G = is the number of germinated seeds,
L = is the average length of the shoots of the germinated seeds, and
i= the concentration levels of spent engine oil.

According to this formula the highest score obtained was ranked as number 1 (the most SEO tolerant); the second highest score was ranked as number 2 (the 2nd most SEO tolerant), and so on.

Data were statistically analyzed using the one-way analysis of variance (ANOVA), and comparisons of means were carried out using the Duncan's test. Pearson's Correlation analysis was employed in order to study the extent of associations of one parameter on the other.

3. RESULTS AND DISCUSSION

The final germination percentage of the six plant species at various concentrations of spent engine oil under laboratory condition is presented in Fig. 1. The figure revealed a decrease in germination percentage with increased level of contaminant applied. The control (0%) had the highest percentage germination in all the varieties with maximum germination percentage in (100%) observed v3 and minimum (90.9%) observed in v4. The percentage germination of the seeds treated with spent engine oil were significantly different from each other when compared with the control ($p<0.05$). In the 3% contaminant level, the percentage germination of varieties V6, V5 and V3 where observed to be significantly lower than the control ($p<0.05$). Similar trend was also observed in 6% treated seeds in relation to their control counterparts. The 9% contaminant level soil had delayed emergence till the 14^{th} day after planting. Significant reductions were observed in the germination percentage of seeds sown in high spent oil level of contaminant level when compared with seeds grown in the control. There was no significant difference between V1 and V2 and V4 at 3% and 6% level of contaminant respectively. Nonetheless, varieties V1, V2, V3, V4, and V6 showed higher germination percentage when compared to V1 and V5. Generally, the results showed a decrease in germination percentage with increase in contaminant level.

Germination percentage may not completely interpret the possible delayed seed germination caused by phytotoxic plant contaminant or substances. Germination index parameters provide information on germination levels as well as temporal aspects of germination of seedlings. The germination index as affected by spent engine at various concentrations is depicted Fig. 2. The highest germination index was obtained from the control treatment in all the varieties and this was statistically ($p \leq 0.05$) different from that obtained in treatment with 3, 6 and 9% level of contaminant respectively. Significant marked reduction in germination index was also observed in 9% level of contaminant when compared to other treatments. In all the varieties assayed, germination index decreased with increase in contaminant concentration.

There was a significant decline in seedling vigor index of all the varieties as shown in Fig. 3. The varieties showed a considerable difference when compared with the control. The maximum and minimum values of vigor index as observed in the control are 49.3 in V2 and 37.6 in V6. The values of vigor index were found to be decreased with increased contaminant level.

Mean root and shoot length decreased significantly with increase an in contaminant level. However, the control (0%) treatment had the highest root and shoot length across the varieties than 3%, 6% and 9% level of contaminant (Figs. 3 and 4). Both parameters (root and shoot length) were observed to be shortest in 9% treatment. The result further showed a marked significant difference from each other ($p<0.05$). Also the seedlings of V4, V5 and V6 were shorter in length than in other accessions with similar level of contaminant ($p<0.05$). Results obtained showed that spent engine oil had a significant effect on the root and shoot length of the seedlings.

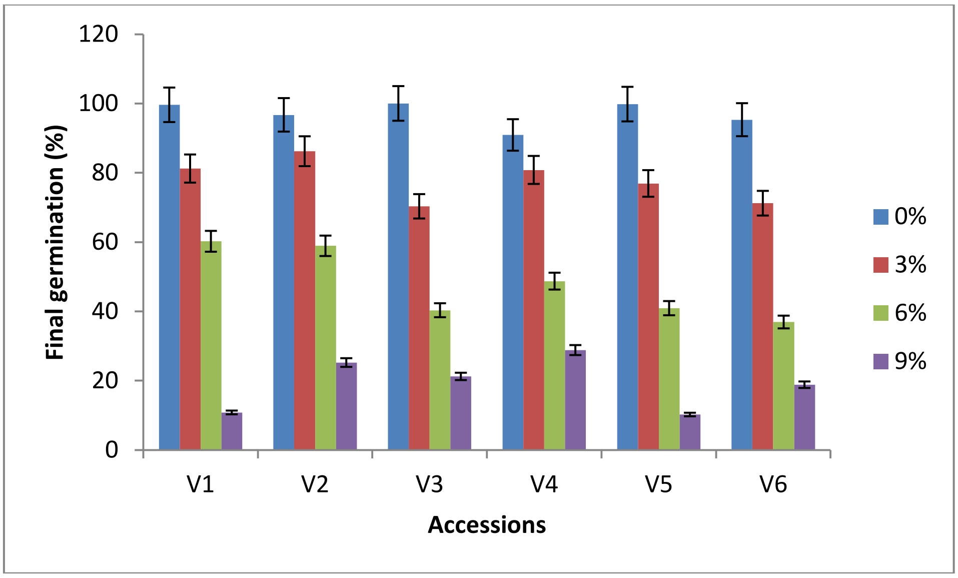

Fig. 1. Effects of SEO on germination percentage of six plant species
Values are mean ± S.E of three replicates. V1=TVu-10343, V2=TVu-10366, V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

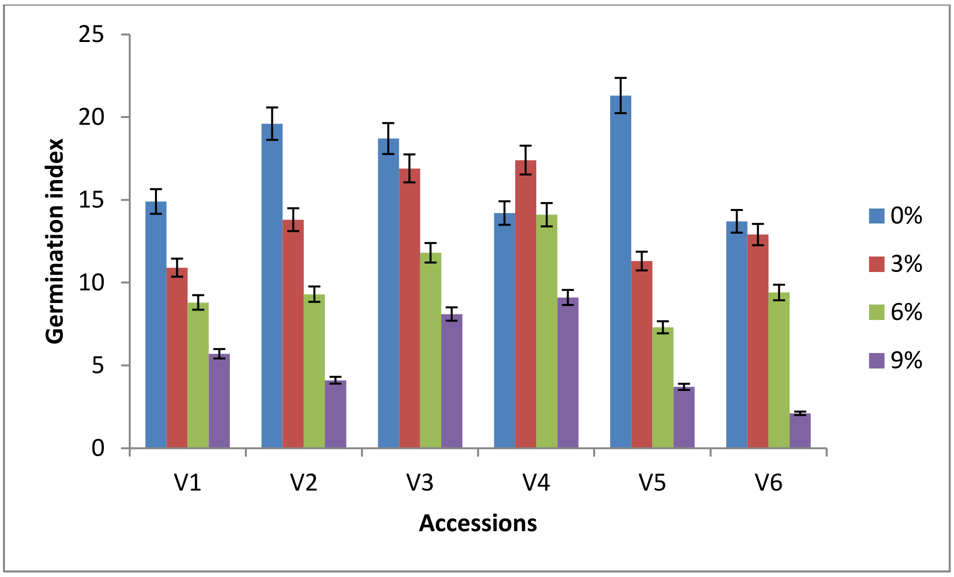

Fig. 2. Effects of SEO on germination index of six plant species as affected by difference concentration of SEO
Values are mean ± S.E of three replicates.V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

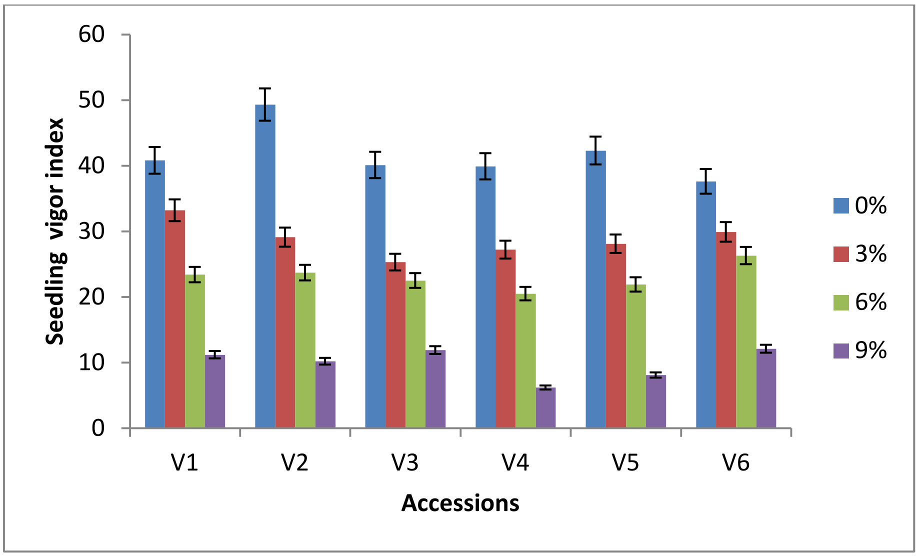

Fig. 3. Effects of SEO on seedling vigor index of six plant species
Values are mean ± S.E of three replicates.V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

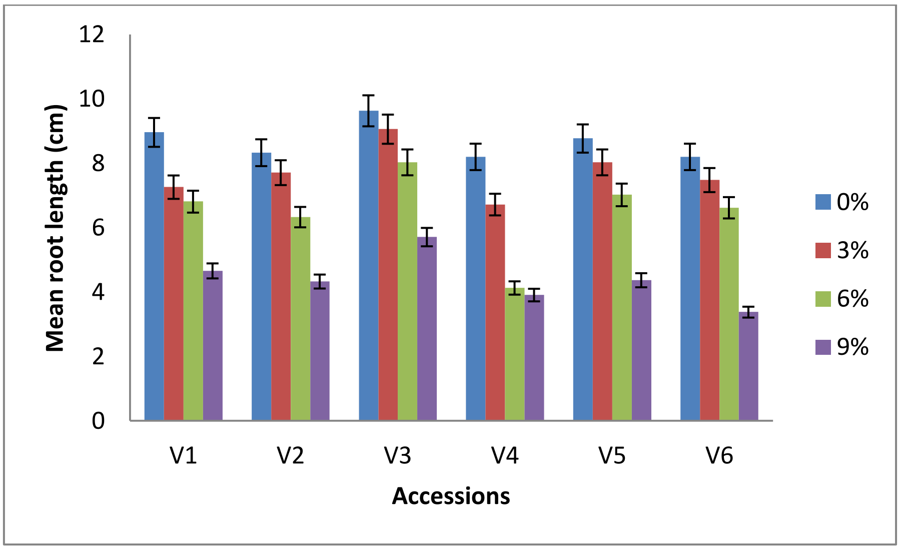

Fig. 4. Effects of SEO on seedling vigor index of six plant species
Values are mean ± S.E of three replicates, V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

Table 1. Pearson's correlation coefficient for varietal parameters over the study period

	FGP	GI	SVI	RL	SL	FW	DR
FGP	1.00						
GI	-0.134	1.00					
SVI	-0.371	0.331	1.00				
RL	0.381	0.221	-0.031	1.00			
SL	0.908**	0.331	0.521*	0.721**	1.00		
FW	0.781**	-0.291	0.838**	0.821**	-0.135	1.00	
DR	-0.071	-0.271	0.721**	0.583*	0.421*	0.921**	1.00

Where FGP = final germination percentage, GI = germination index, SVI = seedling vigour index, RL = root length, SL = shoot length, FW = fresh weight, DR = dry weight,
**= correlation significant at 0.05%, ** = correlation significant at 0.01%.*

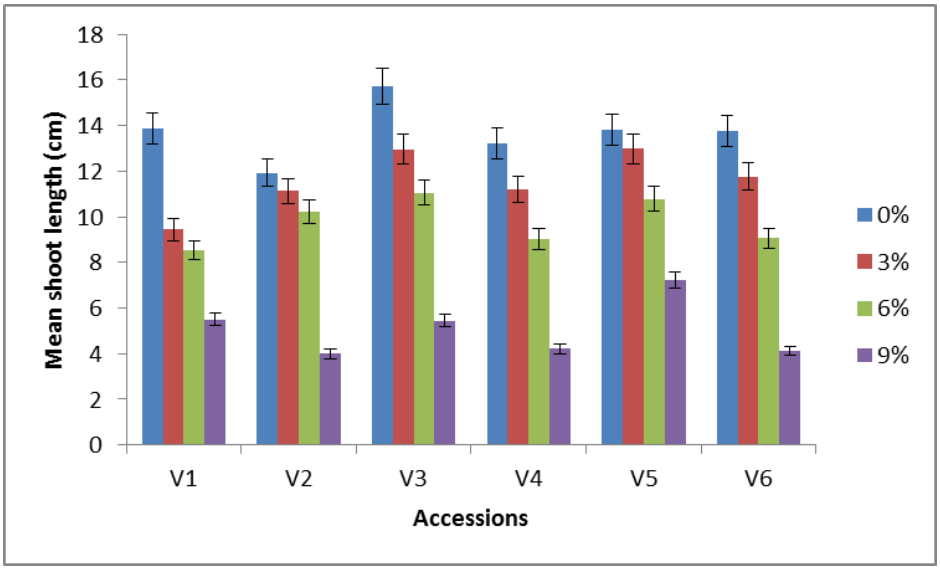

Fig. 5. Effects of SEO on seedling vigor index of six plant species
Values are mean ± S.E of three replicates.V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

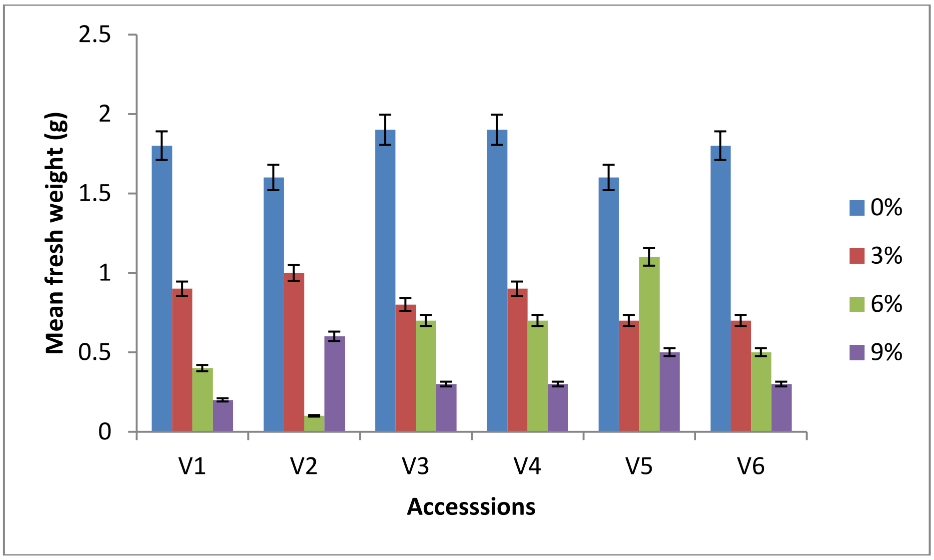

Fig. 6. Effects of SEO on fresh weight of six plant species
Values are mean ± S.E of three replicates readings.V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

Mean fresh and dry weights (g) of the seedlings treated with different levels of spent engine oil are presented in Figs. 4-5. Both parameters were constantly recorded to decreasing with increase in the level of contaminant. The results showed that fresh and dry weights of the accessions at 0% level were not significantly different but 3%, 6%, and 9% level of treatment differed significantly among the accessions and this was followed a marked reduction in 9% treatment. The effect was less pronounced in 3% level of treatment.

3.1 Correlation Analysis

Results of correlation analysis of the varietal parameters over the study period are presented in Table 1. It was observed that final germination percentage (FGP) showed positive and significant correlation with RL, SL, FW, and DR ($p < 0.001$). The correlation between FGP and GI; FGP and SVI were significantly negative ($r = -0.134$; -0.371 ($P < 0.001$) respectively indicating that the germination index and seedling vigor index were delayed with

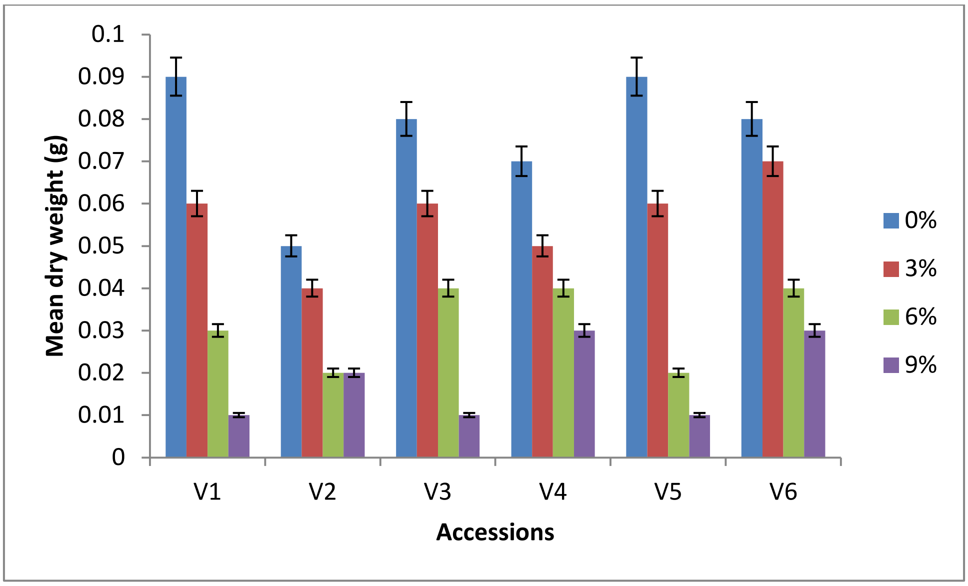

Fig. 7. Effects of SEO on dry weight of six plant species

Values are mean ± S.E of three replicates.V1=TVu-10343, V2=TVu-10366,V3= TVu-13606, V4=TVu-13630, V5=TGm-504, V6= TGm-570 and TGm-547

decrease in germination percentage. Seedling vigour index also showed a positive and significant correlation with FW(0.838) and DR(0.721) at $p < 0.001$.

3.2 Performance Ranking

Performance ranking of the plant species subjected to different concentration of spent engine oil is presented in Table 2. This was done to select the most tolerant accessions for further trial in the green house. The scores were calculated based on the number of germinated seeds and the average seedling shoot length following the method of [24]. Ranking of the accessions based on tolerance to contaminant level were: TVu-10366>TVu-10343>TGM-504>TVu-13605>TGM-570>TGM547 respectively.

Seed germination, radicle and plumule elongation assay are the simplest type of toxicity test used to determine preliminary effects of toxicity of contaminants on plants and can give a fair idea of plant tolerance to a specific level or type of contamination. Total germination percent (GP) is a commonly used index to measure the effects of phytotoxic substances [24,23]. It is the maximum percentage of germination that mainly depends on final measurements. However, this index cannot interpret the possible delayed germination caused by phytotoxic contaminant or substances. Therefore, GP is considered to be suitable for ecological studies rather than physiological process like germination [25,26]. A number of indices over GP have been proposed by many researchers to study the inhibitory activity of phytotoxic substances on germination process [27,28]. The use of plants to study toxicity of contaminants has been reported by previous authors [24,26,29]. In this study, the observed reduction in the measured parameters could be attributed to the numerous hydrocarbons, related compounds and chemical additives present in spent engine oil which are toxic to soil environment. The negative effect observed on the accessions may be due to the blockage of the cotyledon by the spent engine oil which inhibited the early germination and growth of the seedlings [30]. This is in agreement with the studies of [31].

Table 2. Performance ranking of the plant species subjected to different concentration of spent engine oil

Accessions	Scores	Ranking in order of tolerance
TVu-10366	76.06	1
TVu-10343	72.28	2
TGM-504	71.71	3
TVu-13606	69.24	4
TGm-570	68.14	5
TGm-547	65.22	6
Mean	70.44	
SD	3.757	
CV	5.3%	

The phytotoxic properties of spent engineoil to organisms have been reported by Banks et al. [29].

Due to the toxic nature of spentengine oil, the embryo of fluted pumpkin seeds could have been injured or killed as it gets in contact withthe oil and this finding is in line with reports presented by Omosun et al. [32]. Equally, these findings were in line with the result of the research conducted by Tanee and Akonye [33] that contamination of soil with spent oil consistently inhibited germination of hot pepper and tomatoes. The inhibitory of spent engine oil at 6 and 9% agrees with the findings of [31] that the level of oil seemed to exert significant influences on plant species and agricultural lands. The different accessions showed tolerance and sensitivity to spent engine oil. This can be observed in root and shoot length development of the seedlings. Banks et al. [29] reported similar difference in sensitivity of corn and red bean exposed to crude oil Rajan and Blackman [22] reported interspecific differences in the sensitivity of different accessions of *G. max* to crude oil. This could be attributed to the genetic make-up of the plants or due to differences in systemic uptake of oil compounds, nutrient availability and cell wall structural differences [31].

The observed increase in the plants grown in the control soils could be seen as the unadulterated nature of the soil structure which allowed normal metabolic activities of the test plant [34]. The reduction of the plant growth as observed in this study could be due to reduction of mineral element with increasing contaminant level as reported by Kirk et al. [30]. Reduction in growth might be interpreted as being due to general effects of the spent oil which may have shown up either in the distortion or reduction in the number of stomata per unit area of the leaf [29]. This might have affected photosynthetic process and subsequently, the amount of photosynthetic products synthesized. Such growth reductions due to soil oil pollution have been reported by Ogbo [31].

Interference with the soil-water-relation as well as, nutrient immobilization and the presence of heavy metals could also be responsible for the observed reduction in plant parameters as seen in this study. The significant percentage differences observed in the fresh and dry weight accumulation of the seedlings could be attributed to the high oil level in soil and hence uptake of ions (water and salts) is carried out by the roots [35]. The plants in control soils with their roots undisturbed could have absorbed enough nutrients when compared to the seedlings exposed to higher spent engine oil treatment.

Kirk et al. [30] reported that a reduction in shoot growth is a direct resultant effect of engine oil pollution in the root growths as roots are input organs for the absorption and translocation of water and mineral nutrients. Reduced dry mass accumulation following spent oil application on garden soil had been reported by Agbogidi and Ilondu [36-38] where they noted that hydrocarbons from oil contaminated soils accumulate in the chloroplast of plant leaves. This makes the photosynthetic ability of the leaves become reduced, thereby affecting translocation in affected plants which might be due to obstruction of the xylem and phloem vessels consequently causing reduction in photosynthetic products and dry matter content of the entire plants.

4. CONCLUSION

Six plant species were assessed for their tolerance to spent engine oil polluted soil at various concentrations. Results showed a dose dependent sensitivity and resistance to spent engine oil pollution in all the parameters measured. This study has demonstrated that spent engine oil has a significant effect on germination and growth parameters of the six plant species screened. It should therefore be the concern of all and sundry to guard against spent oil pollution of our arable agricultural land in order to maintain our quest for sustainable agricultural productivity. Further greenhouse studies are in progress to evaluate the growth and the development of the three plant species in successive advanced stages by observing the effect of spent engine oil on their growth, yield, plant biomass and dry weight, in addition to their phytoremediation ability to decrease the spent engine oil level in soils. The Pearson's correlation analysis revealed positive and significant association among final germination percentage, shoot and root length and shoot and root weight respectively. Ranking of the accessions based on tolerance to contaminant level were: TVu-10366>TVu-10343>TGM-504>TVu-13605>TGM-570>TGM547. Varieties TVu-10366, TVu-10343 and TGM-504 are recommended to be used in the reclamation of spent engine polluted sites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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