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Phytotoxicity of diesel soil contamination on the germination of *Lactuca sativa* and *Ipomoea batatas*

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Abstract

Phytotoxic effect of diesel contaminated soil on germination rate of *Lactuca sativa* and *Ipomoea batatas*, at two concentrations ranges (0-6ml and 0-30ml), were investigated and compared. Diesel soil contamination was simulated and soil samples were taken from contaminated soil at 1, 5, 10, 15, 25, 50, 75 and 100 days should be after planting. The result showed that in both plant species, diesel inhibited germination in a concentration dependent manner, Also, the influence of diesel contamination diminished with increased time duration; suggesting possible reduction in diesel toxicity over time. However, germination of lettuce was significant and negatively correlated (r^2= -0.941) with diesel contamination as compared to sweet potato (r^2= -0.638).Critical concentration of diesel in relation to seed germination of *L. sativa* was lower than vegetative germination of *I. batatas,* indicating that germination *of I. batatas* was less sensitive to diesel contamination as compared to *L. sativa*.

Key words

Diesel contamination, Germination rate, *Ipomoea batatas, Lactuca sativa,*Phytotoxicity

Introduction

Oil spill is one of the most serious environmental problems confronting the world (ITOPF, 2011). Pollution through oil spill has largely been associated with large spillages from oil rigs and tanker ships in sea and coastal land. However, little attention has been paid to 'minor' spillages of petroleum products like diesel on inland environments such as agricultural land. Inland pollution from petroleum products are far more complicated, unpredictable and constitute vast majority of oil spills in many countries (Fingas, 2001). Diesel spills are by far the largest of all products from crude oil (SAPIA, 2007) as it is used in many sectors, which include transport and non-transport/commercial uses. Pressure to feed the ever growing world population has increased use of machinery on farms. Increased use of machinery is expected to lead to more spillage of diesel on farms and associated effects on cultivated crops.

Seed germination is one of the most reliable method used to test phytotoxicity of hydrocarbons contaminated soil (Jyoti and Smita, 2012) as seed germination is the most sensitive stage of plant growth to environmental pollution (Millioli *et al.,* 2009). Hence, seed germination is often used as an indicator of plant's ability to survive hydrocarbon pollution (Njoku, 2009). Several reports are available on the adverse effects of diesel on seed germination of peanut, corn, cowpea and sorghum (Ogbo, 2009), safflower and corn (Asli and Houshmandfar, 2011). These reports reveal that reduction in seed germination is dependent on the level of diesel in soil and crop species grown.

Lactuca sativa, commonly known as lettuce, has been recommended by USEPA and FDA as a bio-indicator of soil pollution (Fletcher, 1991; Da Silva Júnior, 2013). However, despite recommendation of lettuce for acute toxicity test by USEPA and FDA and fact that diesel is highly toxic and most likely among petroleum products to be spilt on agricultural land; study on the effect of diesel soil contamination on germination of lettuce is meagre. Also few studies have been carried out to compare the effects of diesel contamination between seed germination and vegetative propagated crops. In light of the above, the present

study was carried out to compare the effect of diesel contaminated soil and duration of contamination on seed germination of lettuce and vegetative by propagated sweet potato.

Materials and Methods

Study area and diesel concentrations/contamination Treatments : The present study was carried out in germination chamber of the Department of Botany, University of Zululand in Kwa-Zulu Natal province(KZN) of South Africa. The conditions in the germination chamber were as follows: average temperature 22.65°C; humidity 73.25 %. The soil moisture was maintained at field capacity.

Agriculturally productive soil was collected from University's farm, air dried, homogenized using a spade and sieved through a grid wire mesh to remove stones. Diesel used for contamination was purchased from Zulu Fuel at Empangeni, KZN. Simulation of diesel contamination was done by spiking soil with diesel in a 20 l pot. Two sets of incremental diesel concentrations were tested at two different concentration ranges: 0-30 ml diesel concentration range which included 5, 10, 15, 20, 25 and 30 ml diesel kg^{-1} soil, while 0-6ml diesel concentration range consisted of 1, 2, 3, 4, 5 and 6 ml diesel kg^{-1}soil, respectively.

Soil and diesel analyses : A composite soil sample was taken for chemical analyses from potted soil before and after diesel treatments from each of the treatment replicates. Hydrometer method (Bouyoucos, 1962) was used to determine relative amount of soil separates (sand, silt and clay) and soil textural class was determined, based on USDA-FAO soil textural triangle (FAO, 1990). The pH of soil samples were determined following the method of Bates (1954). Total carbon and nitrogen content were analysed by Automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation USA, Michigan, USA; Matejovic, 1996) and vanadium pentoxide as catalyst. Phosphorus, potassium, zinc, copper and manganese were extracted using Ambic-2 extracting solution (0.25M ammonium carbonate (NH4CO3)+0.01M disodium ethylene diamine tetra acetate (NaEDTA)+0.01M ammonium fluoride (NH_4F) + 0.05g l^{-1}Superfloc). Potassium, zinc, copper and manganese were determined by atomic absorption spectrophotometer and phosphorus was determined from the extract by modified method of Murphey and Riley (1962) molybdenum blue procedure (Hunter, 1974). Acidity, calcium and magnesium were extracted with potassium chloride solution. Calcium and magnesium ions were determined by atomic absorption spectrophotometer. Hydrocarbon contents in diesel was analysed by Gas chromatography and heavy metals were determined by atomic absorption spectrophotometer.

Experimental set up : 30 g and 350 g of soil were drawn from both concentration ranges at 1, 5, 10, 15, 25, 50, 75, and 100 days after diesel contamination and placed in petri and Styrofoam dishes. Soil was then moistened with distilled water. Germination of lettuce was done by placing 10 sterilized seeds which had passed viability test done using floatation method (Bell *et al.,* 1993) on the contaminated soil. Vegetative propagation of sweet potato was done by planting five cuttings each with three buds of sweet potato in Styrofoam dishes. Cuttings were planted in such a way that two buds were buried in soil and one bud was above soil. Both petri and Styrofoam dishes were incubated in seed germination chamber for 14 days at 28 °C in light (Serrano, *et al.,* 2007). Sprouting of lettuce seeds and any of the buds on sweet potato cuttings was considered as germination. Seeds/cuttings that failed to sprout after 14 days were regarded as dead. The effect of diesel contamination and duration on germination of both plant species were investigated at 100 (D100), 75 (D75), 50 (D50), 25 (D25), 15 (D15), 10 (D10), 5 (D5) and 1 (D1) days after soil contamination.

Statistical analysis : The data collected were first subjected to ANOVA using GenStat Release 12.1 (PC/Windows Vista) (VSN International Ltd., 2009). Mean of treatments were separated for least significant difference at 5% level ($LSD_{0.05}$). Mathematical functions expressing correlation and regression relationship between diesel concentrations and germination/plant height of lettuce and sweet potato were obtained using curve fitting programme of Table Curve 2D v5.01.01 (Systat Software Inc., San Jose, CA, USA, 2002). Critical diesel concentration and toxicity index for each crop was interpolated from regression relationship.

Results and Discussion

In the present study, it was observed that all the 30 seeds of lettuce and15 cuttings of sweet potato planted in control soil germinated. Hence, reduction in germination rate of lettuce and sweet potato planted in diesel contaminated soil are attributable to the toxic effects of diesel. In diesel contaminated soil, germination of both plant species was significantly and negatively correlated with diesel level in soil at both 0-30 ml (lettuce: r^2= -0.975, sweet potato: r^2= -0.973) and 0-6 ml (lettuce: r^2= -0.984, sweet potato: r^2= -0.914) concentration ranges (Fig. 1 and 2).

At 5 ml diesel kg^{-1} soil which was the least diesel treatment investigated at 0-30ml kg^{-1} range, diesel reduced the mean germination of lettuce seeds to 48.33%. It must however be noted that a significant reduction in germination was not achieved until 15 day of contamination when germination of lettuce was reduced to 36.67% and that at after 50 days diesel had no effect on germination of lettuce seed as 100% germination was achieved (Table 1). In contrast, diesel showed no significant effect on germination/sprouting of sweet potato cuttings mean germination was 99.58% at 5 ml diesel kg^{-1} (Table 2).

Significant effect of diesel soil contamination on germination of sweet potato cutting was first observed at 15ml kg^{-1}

soil treatment. At that concentration diesel reduced germination of sweet potato cuttings by 20% at 1D (80% germination was achieved). Again, diesel had no effect on germination of sweet potato 25 days onwards (Table 2). In comparison to lettuce seed, diesel soil contamination on germination of lettuce seed was significant even at 75D as lettuce seed germination was reduced to 83.33%. Further, lettuce germination decreased by 10% highest duration *i.e.*, 100 D of diesel contamination in the present study.

At 30 ml diesel kg^{-1} soil, which was the highest diesel soil treatment at 0-30ml kg^{-1} range, the effect of diesel on germination of lettuce was severe, with no germination recorded till 15D of contamination. At 30 ml diesel kg^{-1} soil treatment germination of lettuce, reduced by 70% at 100 day. In contrast, at 30ml kg^{-1} soil treatment, effect of diesel on germination of sweet potato was no longer significant after 50 days however, germination of sweet potato was restored to 100% by 75th day (Table 2).

At 0-6ml kg^{-1} diesel concentration range, diesel effect on germination of both species was far less than that of 0-30 ml kg^{-1} soil treatment. Although diesel had significant effect on germination of lettuce at 1ml kg^{-1} pollution on 1st day on 5th day no significant effect was observed and by 10th day germination of lettuce seed was restored to 100%. At 6ml kg^{-1}, which was the maximum diesel concentration tested at 0-6ml kg^{-1} concentration range; diesel affected germination of lettuce up to 15 days, but afterwards (D≥ 25days), no significant effect of diesel on germination of lettuce seed was observed as germination was restored to 100%. Generally, at 0-6ml concentration range,the reducing effect of diesel on germination of both plant species was not significantly different from control. Diesel soil contamination

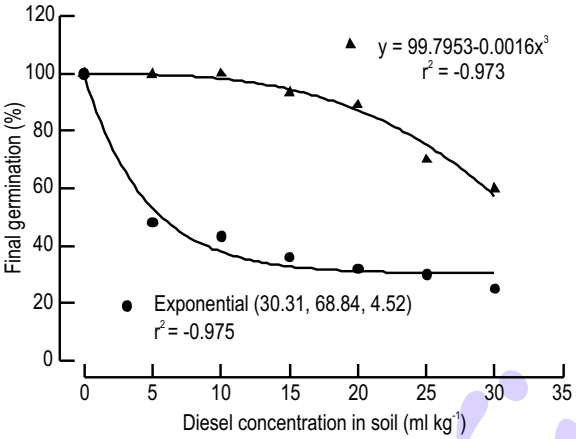

Fig. 1 : Effect of diesel contamination in soil at 0-30 ml kg^{-1} concentration range on germination of lettuce (●) and sweet potato (▲)

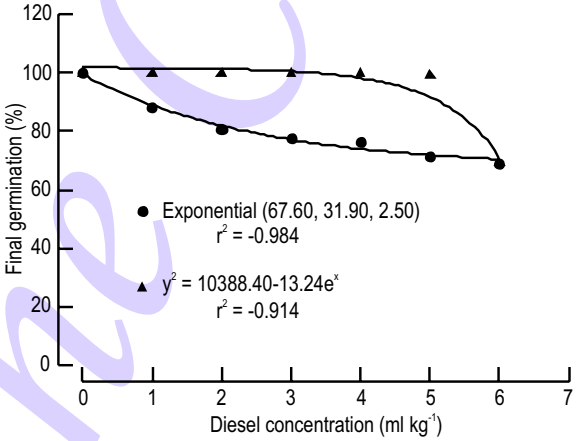

Fig. 2 : Germination response of lettuce (●) and sweet potato (▲) to diesel contamination in soil at 0-6 ml kg^{-1} soil contamination concentrations range

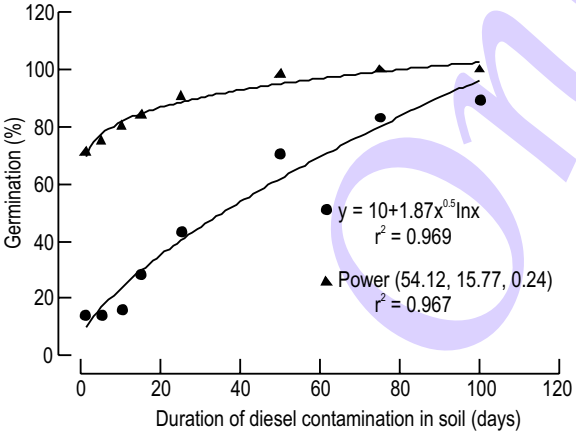

Fig. 3 : Effect of duration on germination of lettuce (●) and sweet potato (▲) at 0-30 ml diesel kg^{-1} soil contamination range

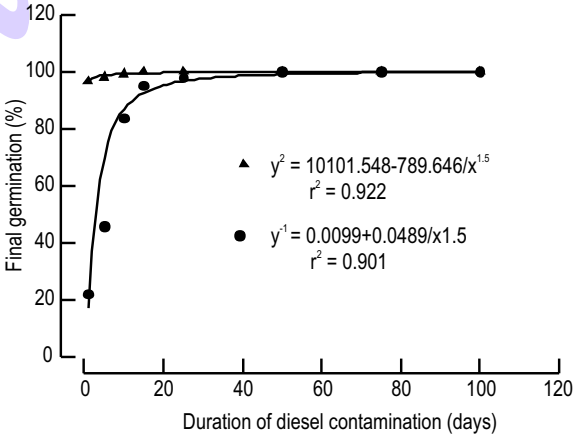

Fig. 4 : Germination responses of Lettuce (●) and sweet potato (▲) to duration at 0-6 ml kg^{-1} soil concentration range

Table 1 : Interactions of diesel concentration and age/duration of contamination on the germination of lettuce seeds (0-30 ml kg^{-1} range)

Duration (D) (days after contamination)	Diesel concentration in soil (ml diesel kg^{-1} soil)							Lsd (5%)	[y]Mean Germination (%)
	0	5	10	15	20	25	30		
1	100.00[a]	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	-	14.29
5	100.00[a]	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	-	14.29
10	100.00[a]	3.33[a]*	10.0[b]*	0.00[a]*	0.00[a]*	0.00[a]*	0.00[a]*	7.64	16.19
15	100.00[a]	36.67[b]*	20.0[c]*	16.67[b]*	13.33[b]*	10.0[b]*	3.33[a]*	13.78	28.57
25	100.00[a]	46.67[c]*	40.0[d]*	36.67[c]*	30.00[c]*	30.0[c]*	20.0[b]*	12.67	43.33
50	100.00[a]	100.00[d]	83.33[e]	63.33[d]*	50.00[d]*	46.6[d]*	50.0[c]*	18.72	70.48
75	100.00[a]	100.00[d]	96.67[f]	83.33[e]*	76.67[e]*	70.0[e]*	56.6[d]*	15.29	83.33
100	100.00[a]	100.00[d]	96.67[f]	90.00[f]	86.67[f]	83.3[f]*	70.0[e]*	14.80	89.52
[x]Mean germination (%)	100.00	48.33	43.33	36.25	32.08	30.00	25.00	-	45.00

* Means along the same row (D) are significantly different from the control ($p<0.05$). Means along the same column with same letters are not significantly different ($p<0.05$). [x]Main effect of diesel concentrations. [y]Main effect of age (D) of contamination

Table 2 : Interactions of diesel concentration and age/duration of contamination on the vegetative germination of sweet potato cuttings (0-30 ml kg^{-1} range)

Duration (D) (days after contamination)	Diesel concentration in soil (ml diesel kg^{-1} soil)							LSD (5%)	[y]Mean Germination (%)
	0	5	10	15	20	25	30		
1	100.0[a]	100.0[a]	100.00[a]	80.00[a]*	*66.67[a]	33.3[a]*	20.00[a]*	17.09	89.52
5	100.0[a]	100.0[a]	100.00[a]	80.00[a]*	*73.33[a]	46.6[b]*	26.67[a]*	13.24	75.24
10	100.0[a]	100.0[a]	100.00[a]	93.33[b]	86.67[b]	53.3[bc]*	26.67[a]*	15.29	80.00
15	100.0[a]	96.67[a]	100.00[a]	93.33[b]	86.67[b]	60.0[c]*	53.33[b]*	13.78	84.29
25	100.0[a]	100.0[a]	100.00[a]	100.00[b]	100.00[c]	73.3[d]*	60.00[b]*	76.43	90.48
50	100.0[a]	100.0[a]	100.00[a]	100.00[b]	100.00[c]	93.33[e]	93.33[c]	11.46	98.10
75	100.0[a]	100.0[a]	100.00[a]	100.00[b]	100.00[c]	100.0[e]	100.0[c]	-	100.00
100	100.0[a]	100.0[a]	100.00[a]	100.00[b]	100.00[c]	100.0[e]	100.0[c]	-	100.00
[x]Mean germination (%)	100.00	99.58	100.00	93.33	89.17	70.00	60.00	-	87.44

* Means along the same row (D) are significantly different from the control ($p<0.05$). Means along the same column with same letters are not significantly different ($p<0.05$). [x]Main effect of diesel concentrations. [y]Main effect of age (D) of contamination

reduced germination of lettuce by 19.40 % (Table 3); whereas, germination of sweet potato was only reduced by 0.77 % (Table not shown), i.e. mean germination of lettuce and sweet potato were 80.60 and 99.23 respectively. Hence, the adverse effect of diesel on germination rate was stronger in lettuce seeds than sweet potato cuttings. A negative correlation between diesel contamination in soil and germination of some crops like *Vigna unguiculata* (Njoku *et al*., 2009) and *S. terebinthifolius* (Bona *et al*., 2011) have been reported.

Duration of diesel contamination also affected germination in both plant species leading to strong positive correlation (@ 0-30ml: lettuce r^2= 0.984, sweet potato r^2= 0.914 @0-6ml: lettuce r^2=0.901, sweet potato r^2= 0.922) between duration and germination of lettuce at both 0-30 (Fig. 3) and 0-6 ml diesel kg^{-1} soil (Fig. 4). However, at 0-30 ml diesel kg^{-1} soil treatment, marked differences were observed in lettuce and sweet potato due to duration of diesel pollution. The difference observed was prominentat at early stage of diesel contamination and diminished with time. Lettuce was more sensitive to duration as compared to sweet potato. At 0-30 ml kg^{-1} diesel concentration, the mean germination of lettuce varied within a large range from 14.29% in 1-day-old diesel exposure to 89.52% in 100-day-old exposure (Table 1). In contrast, sweet potato varied within a narrower range from 89.52% in 1-day-old exposure to 100% in 100-day-old exposure (Table 2). At 0-6 ml kg^{-1}concentration range, some difference was noticed in the manner in which germination of lettuce and sweet potato responded to duration of diesel contamination. At1D treatment, mean seed germination of lettuce was 21.9% and it required 50 days for the mean seed germination of lettuce to be restored to 100 % (Table 3). In contrast, vegetative germination of sweet potato was slightly depressed (3.33%) (Table not shown), as compared to seed germination of lettuce which was depressed by 78.1% at 1D pollution. Further, germination of sweet potato was restored to 100% in 15 days of diesel exposure as compared to 50 days for lettuce. It is clear from the study that germination of lettuce and sweet potato was dependent on diesel concentration, duration of contamination and plant species.

A strong positive correlation between duration of diesel contamination and germination of lettuce seeds and sweet potato cuttings in the present study clearly indicated that longer the time between contamination and planting, higher was germination in

Table 3 : Interactions of diesel concentration and age/duration of contamination on the germination of lettuce seeds (0-6 ml kg^{-1} range)

Duration (D) (days after contamination)	Diesel concentration in soil (ml diesel kg^{-1} soil)							LSD (5%)	[y]Mean germination (%)
	0	1	2	3	4	5	6		
1	100.00[a]	26.67[a]*	26.67[a]*	0.00[a]*	0.00[a]*	0.00a*	0.00[a]*	11.46	21.90
5	100.00[a]	80.00[b]	46.67[b]*	46.67[b]*	33.33[b]*	13.33[b]*	0.00[a]*	7.64	45.71
10	100.00[a]	100.00[c]	73.33[c]	73.33[c]	80.00[c]	86.67[c]	73.33[b]*	7.64	83.81
15	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	86.67[c]*	80.00[c]*	-	95.24
25	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	86.67[c]*	100.0[d]	-	98.10
50	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	100.00
75	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	100.00
100	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	100.00
[x]Mean germination (%)	100.00	88.33	80.83	77.50	76.67	71.67	95.83	-	80.60

*Means along the same row (D) are significantly different from the control ($p<0.05$). Means along the same column with same letters are not significantly different ($p<0.05$). [x]Main effect of diesel concentrations. [y]Main effect of age (D) of contamination

both species. Improved germination with increase in duration of diesel contamination might be done to loss of hydrocarbons from the soil. The loss may be due to evaporation and microbial degradation. Increased germination has also been reported in *S. terebinthifolius*, cowpea and maize with increase in duration of diesel contamination (Ogbo, 2009; Bona, 2011).

Critical concentration is the amount of diesel in soil required to reduce germination by 10% were interpolated from regression relationships of pooled data at both concentration ranges (Fig. 5). It was determined from interpolation of regression relationship that critical diesel concentration for toxicity in relation to seed germination of lettuce was 1.32 ml as compared to 15.5 ml for vegetative propagation of sweet potato. Critical concentration (Table 4) for each diesel treatment level as interpolated from combined regression of 0-6ml and 0-30ml showed that critical concentration of diesel increased with duration of diesel contamination in soil. Also, the critical concentrations of diesel for toxicity at all duration of diesel contamination for sweet potato were higher than that of lettuce (Table 4). In lettuce, critical concentration increased from 0.16ml (1D) to 17.74 (100D) at the end of the study. In case of sweet potato, critical concentration of diesel increased from 10.83 at 1st day to 21.6 at 25th day. As duration of diesel pollution increased after 50 days, diesel contamination did not reduce vegetative propagation of sweet potato by 10%, hence no critical concentration of diesel for toxicity was reported.

A marked difference observed in lettuce and sweet potato in response to diesel contamination in soil is an indication of difference in sensitivity of germination of these two plant species to diesel soil contamination. Difference in sensitivity of different crops to diesel contamination has been reported between *Cucumis sativus*, *Brassica oleracea* and *Barbarea verna* (Cruz *et al*., 2013); safflower and corn (Asli and Houshmandfar, 2011). Reasons for more sensitivity of lettuce when compared with sweet potato may be attributed to the fact that sweet potato was propagated from cuttings. Cuttings are matured plants which are already hardened by forces of nature. In contrast, lettuce was germinated from seeds. Seed germination is known to be vulnerable to inhibitory substances (Taiz and Zeiger, 2010). Inhibitory substances such as diesel can alter or prevent the initiation of metabolic processes in seeds which may ultimately affect cell growth and seed germination. Lettuce seeds are small and are easily covered with diesel sheen. This covering of lettuce seed acts as a barrier for entry of water and oxygen into the seeds. In sweet potato, only part cuttings in soil are covered with diesel while other parts above soil have access to oxygen. Water and oxygen are essential for germination (Taiz and Zeiger, 2010). Also, penetration of diesel kills the embryo of lettuce seeds. In contrast, sweet potato cuttings do not germinate through embryo and propagate via buds present on the cuttings. Hence, sweet potato may not be as vulnerable as lettuce to diesel soil contamination.

The cause of diesel toxicity to plants is usually considered due to presence of heavy metals in diesel (Millioli *et al.,* 2009) and deficiency of N, Ca, K and P (Wyszokowski and Ziolkowska, 2008; Njoku *et al*., 2009). In the present study, diesel and soil chemical analysis were done to determine the cause of diesel toxicity on seed germination i.e., whether the toxicity was due to heavy metal, nutrient deficiencies or direct effect of hydrocarbons. The results indicated that the concentration of heavy metals in diesel was generally low, ranging from 7.18 ppm for V to 2.33 ppm for Fe (Table 5). Also in diesel polluted soil, concentration of heavy metals in soil did not follow a definite pattern Furthermore, none of the heavy metals analysed were in high concentrations that could induce toxic effects in test plants studied. Insignificant changes in the level of heavy metals may be due to presence of low metals in diesel used in the present study (Table 5). Diesel soil contamination induced an increase in organic carbon content of soil. Increase in organic carbon of soil may be attributed to the presence of carbon in diesel. It has been reported that diesel has high content of organic carbon

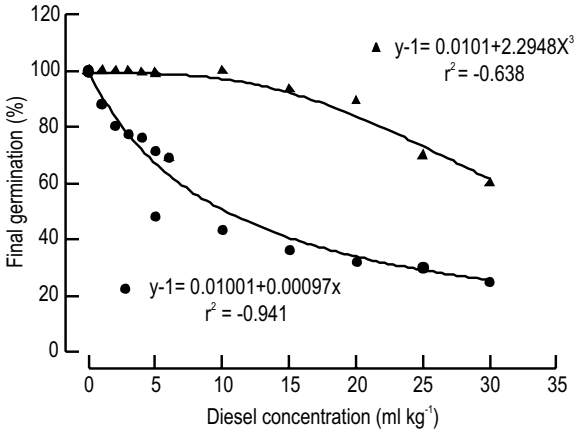

Fig. 5 : Effect of diesel contamination concentration in soil on the germination of lettuce (●) and sweet potato (▲) for the pooled data in the 0-6 and 0-30 ml kg^{-1} diesel concentration ranges

Table 4 : Critical Concentrations of diesel for toxicity in relation to germination of lettuce and sweet potato

Duration (D) (days after contamination)	Critical diesel concentrations associated with 10 % reduction in germination (ml diesel kg^{-1} soil)	
	Lettuce	Sweet potato
1	0.16	10.83
5	0.37	12.05
10	1.49	14.07
15	2.80	16.73
25	4.09	21.6
50	8.87	-
75	12.96	-
100	17.74	-

Table 5 : Concentrations of some heavy metals in diesel used

Heavy Metals	Concentration (ppm)
Fe	2.3318
Zn	1.9371
Cr	0.3637
Pb	2.6427
Cu	0.5211
Cd	0.0013
Hg	0.0003
V	7.1834
Ni	2.1526
Ba	0.4266

Joergensen *et al*. (1995) reported that a gram of diesel contained 0.71g C. The sandy loam soil (18 % clay, 8 % silt and 74 % coarse silt and sand) used in the present study was slightly acidic and although the changes caused by diesel contamination on pH could be attributed to the presence of diesel hydrocarbons, the changes were insignificant as pH fell within the optimum range required for growth of both lettuce and sweet potato. The increase observed in macronutrients may be due to the presence of N and K in diesel used (Pena *et al*., 2007). However, decrease in macro nutrients (Ca and Mg) may be due to temporal immobilization of nutrients by diesel hydrocarbons (Bayram *et al*., 2009). Although diesel soil contamination altered the concentrations of N, P, K, Mg and Ca in soil used in the present study. Alterations of all these nutrients did not follow a definite pattern. The concentration of nutrients as altered by diesel contamination were further compared with the critical concentrations and sufficiency range established by various authors for both lettuce and sweet potato (Table 6). It was established that alteration in soil nutrients and pH caused by diesel contamination in both crops were within the optimum range required for growth of lettuce and sweet potato over the entire range of diesel concentration tested in the present study (Table 6). At 0-6 ml diesel range soil contamination caused far less insignificant changes than 0-30 ml diesel range, hence no table is presented in this report.

Non-involvement of both soil nutrients and heavy metals in inducing toxic effect of diesel on germination of test plants indirectly confirms that toxic effect of diesel soil contamination was due to presence of hydrocarbons in diesel. In the present study, polynuclear aromatic hydrocarbon and BTEX represented 0.37% and 0.0021% of the total hydrocarbon present in diesel. PAH varied widely, ranging from 1775.53 ppm phenanthrene to 1.05 ppm dibenzo (a, h) anthracene (Table 7). BTEX in diesel was mainly xylene isomers representing about 58.29% of the total BTEX present diesel in (Table 7). Improved germination of crops with increased duration also corroborates this hypothesis, since toxic hydrocarbons are lost from soil through volatilization or degradation (Onuoha *et al.,* 2011). If diesel toxicity on germination was due to heavy metal pollution, duration of diesel pollution would have no influence on germination. Similarly, no improvement in seed germination would have occurred with duration of pollution if toxic effect of diesel was due to nutrient deficiencies. Thus, it was inferred that diesel affected germination of lettuce seeds and sweet potato cuttings due to the presence of hydrocarbon compounds (PAH, BTEX and aliphatic hydrocarbons). The exact mechanism by which hydrocarbons affected seed germination of lettuce and vegetative propagation of sweet potato could not be ruled out from this study. Whatever the mechanism be, vegetative germination of sweet potato was less sensitive to hydrocarbons. The practical implication of this study is that, since sweet potato exhibited better germination at higher diesel concentration, it may be useful if further evaluated for phytoremediation of diesel contaminated soil. Whereas, sensitive species like lettuce can be used to monitor the level of diesel in soil. The present study highlights that vegetative germination was also adversely affected by diesel contamination, but was less sensitive than seed germination. Germination of lettuce seeds and sweet potato cuttings were concentration dependent and phytotoxic effect of diesel on germination of lettuce and sweet potato decreased with increase in duration of diesel contamination. Lastly, the critical concentration of diesel for

Table 6 : Soil chemical analyses after diesel treatment at concentration range 0-30 ml

ml diesel kg^{-1} soil	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	pH	Zn ppm	Mn ppm	Cu ppm	OC %	N %
0	350.00	143.33	1646.00	261.33	6.26	87.03	3.00	15.80	3.37	0.24
5	333.33	221.67	1670.33	255.00	6.40	79.80	2.33	13.33	2.87	0.22
10	330.00	236.67	1586.33	248.00	6.19	86.47	2.67	14.53	3.97	0.27
15	330.00	233.00	1549.33	258.67	6.08	87.77	2.67	14.93	3.83	0.29
20	353.33	236.67	1506.33	241.67	6.17	90.70	3.00	15.53	4.20	0.29
25	353.33	274.00	1499.00	245.67	6.15	94.37	3.33	15.87	5.60	0.40
30	396.67	249.67	1531.67	236.67	6.20	83.03	4.00	16.00	5.27	0.41
mean	349.52	227.86	1569.86	249.57	6.21	87.02	3.00	15.14	4.16	0.30
r^2	0.969	0.894	0.996	0.728	0.743	0.630	0.710	0.520	0.950	0.903
LSD	40.95	39.30	129.40	30.90	0.20	8.76	0.76	2.35	0.91	0.08
CC/SR	110-365	120-500	800-1800	219-438	5.5 - 7.0	30-150	20-30	20-30	e"0.20	e"0.20

CC/SR = Critical concentration/sufficiency range, OC = organic carbon. Soil properties with similar superscripts along the same column are not significantly different (p<0.5).ppm = parts per million = mg kg^{-1} = μg g^{-1}; 10 000 ppm = 1 percent

Table 7 : Hydrocarbon composition of the diesel

Hydrocarbon	Concentration (ppm)
PAH	
Naphthalene	1438.9156
Acenaphthylene	52.9216
Acenaphthene	77.1736
Fluorene	243.5930
Phenanthrene	1775.5283
Anthracene	24.4193
Fluoranthene	13.6358
Pyrene	15.9533
Benzo(a) anthracene	12.6010
Chrysene	30.4386
Benzo(b)fluoranthene	3.0563
Benzo(k)fluoranthene	2.0229
Benzo(a) pyrene	2.6208
Indeno{1,2,3-cd}pyrene	3.8619
Dibenzo(a,h)anthracene	1.0537
Benzo(g,h,i)perylene	3.0448
BTEX	
Benzene	2.8691
Toluene	3.4002
Ethyl Benzene	2.2181
P-Xylene(C8H10)	2.0107
M-Xylene(C8H10)	5.979s6
0-Xylene(C8H10)	3.8686

toxicity on germination of sweet potato was higher than that of lettuce. Hence, it could be inferred that germination of lettuce seed was more sensitive to diesel contamination in soil than vegetative propagation of sweet potato cuttings.

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