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Assessment of Root Growth Characteristics of *Glycine max* linn. (Soybean) in Diesel Oil Polluted Sandy Loam Soil of Owerri, Imo State, Nigeria

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ABSTRACT

This study assessed the root growth characteristics of *Glycine max* linn. in diesel oil-polluted sandy-loam soil of Owerri, Imo State, Nigeria. The experiment was conducted in an open field in the Teaching and Research Farm of Imo State University, using randomized complete block design. The treatments were 0litre (control), 1.0litre, 1.5litres, 2.0litres of diesel oil respectively replicated five times. Data were obtained at two week intervals. Results obtained showed that numbers of root/plant, root length/plant, and root dry weights/plant were reduced by the 2.0 litres pollution level and it was significantly different ($p < 0.05$) compared to the control treatment. Percentage emergence was found to be significantly reduced by the 2.0 litres pollution level when compared to the control 1.0 litre, and 1.5 litres respectively. Also Nitrogen, Phosphorous and Potassium content of root of soybean were significantly reduced by 2.0 litres of diesel oil pollution level at all the growth stages compared to the control. It was observed that 1.0litre pollution level showed a stimulatory role in all parameters measured compared to 1.5litres and 2.0litres respectively. The result showed that diesel oil pollution significantly reduced the growth and development of soybean roots, thus consequently could lead to poor yield and productivity of soybean plants

Keywords: Assessment, *Glycine max*, Root growth, Diesel fuel, Pollution

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INTRODUCTION

The advancing industrialization of the world economy has led to a large increase in the consumption of petro-chemical compounds in most countries including Nigeria; the dynamically growing number of motor vehicles led to increased demand for fuel, which in turn leads to more production and processing more crude oil. It also stimulates fuel transport, storage and distribution. As a result, the tendency of petroleum substances permeating into environment has increased (Budny *et al.*, 2002). Pollution of the soil with petroleum derivatives is often observed in municipal soils, around industrial plants and in areas where petroleum and natural gas are obtained (Adam *et al.*, 2002; Clark, 2003). Processing and distribution of petroleum hydrocarbon as well as the use of petroleum product is the main cause of soil contamination (Ayotammo *et al.*, 2006). Changes in soil properties due to contamination with petroleum derived substances can lead to water and oxygen deficit as well as shortage to available forms of nitrogen and phosphorous (Wyszkowska and Kucharski, 2000).

Contamination of the soil environment can also limit its protective functions, upset metabolic activity, unfavourably affects chemical characteristics, reduce fertility and negatively influence plant production (Gong *et al.*, 1996 and Wyszokowski *et al.*, 2004). This situation threatened human health and that of the organism that are dependent on soil (Aboribo, 2001). Soil pollution through diesel oil pollution poses a serious environmental threat as it generally reduce the growth of plants found within the radius of pollution (Nwaogu *et al.*, 2008).

Diesel oil can enter into environment through leakage from storage containers, refueling of vehicles, wrecks of oil tankers and warships carrying diesel oil, through improper disposal by mechanics when cleaning diesel tankers. Soil pollution through such many small and common sources of these products poses large environmental threat (Wyszokowski and Ziokowska, 2008).

Trapp *et al.* (2001) reported that the treatments of petroleum and its derivatives delayed the period of germination, reduced percentage germination, plant height, leaf production and biomass of *Vigna unguiculata*. Also Ogbo (2009), reported that diesel oil contamination of the soil caused reduction in the length of the radicles of *Arachis hypogea*, *Vigna unguiculata*, *sorghum bicolor*, and *Zea mays*.

The findings of Anoliefo *et al.* (2001), show that the soil contaminated with crude oil derivatives depressed and inhibited the growth of *Arachis hypogea*. Treatment of soils with crude oil derivatives significantly delayed the period of germination, reduced percentage germination, plant height and leaf production as well as biomass of *Vigna unguiculata* (Adedokun and Ataga, 2007).

Soybean is an important source of high quality but inexpensive protein and oil. It has an average protein content of 40% and oil content of 20%. Soybean has a superior amino acid profile. Its protein has great potential as a major source of dietary protein. The oil

produced from soybean is highly digestible and contains no cholesterol. A by-product from the oil production (soybean cake) is used as a high-protein animal feed in many countries.

Soybean also improves soil fertility by adding nitrogen from the atmosphere. This is a major benefit in African farming systems, where soil have become exhausted by the increasing need to produce more food for the increasing human and natural populations in the face hardly available and expensive fertilizers for farmers. This study is aimed at highlighting the effect of diesel oil on root growth characteristics of *Glycine max linn.* Considering the fact that good root growth will lead to healthy plant and consequently good yield and sufficient food production.

MATERIALS AND METHODS

Field experiments to Assess of root growth characteristics of Soybean (*Glycine max L.*) under diesel oil pollution were conducted during 2010 planting season at the Teaching and Research Farm of Imo State University. Owerri lies between the latitudes $5^{\circ}10^1\text{N}$ and $6^{\circ}0^1\text{N}$ and longitudes $6^{\circ}35^1\text{E}$ and $7^{\circ}0^1\text{E}$ within the Southeast rainforest agricultural zone of Nigeria.

Certified seeds of soybean were purchased from Imo State Agricultural Development Programme (IMOADP). The diesel oil was obtained from Texaco Fueling Station, Okigwe Road, Owerri, Imo State.

The land was ploughed and soil was raised to beds of 1m x 1m x 0.75m. Different levels (0.0, 1.0, 1.5 and 2.0litres respectively) of diesel oil contaminant were poured on the beds and incorporated by pulverizing the soil using a spade to achieve even distribution of the diesel oil in the soil. Treated soils were left for 7 days before planting in order to enable the volatile substances contained in the diesel oil which are toxic to plant, escape into the atmosphere.

The soybean seeds were sown at a depth of 2cm with spacing of 10cm x 10cm at the rate of 2 seeds per hole with total of 10 stands per bed which was thinned down to five seedlings per bed at 14 days after germination to obtain seedlings of equal vigour. The field layout was arranged in a randomized complete block design, with four treatments and five replications. Data were collected on the following parameters; percentage emergence, number of roots/plant, root length/plant, root dryweight/plant, root nitrogen, potassium and phosphorous content

The percentage emergence: percentage emergence were calculated using the formular below

$$\frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seed Planted}} \times \frac{100}{1}$$

Number of root

Plant roots were gently washed in distilled water to remove soil particles. Hand lens was used to count number of roots.

Root length

Root length was measured by measuring the longest root (cm) using ruler.

Root dry weight

The root dry weight of the plants were determined as described by Merk *et al.*,(2004) by oven drying at 60⁰C for 24hours after which its weight was taken through analytical weighing balance (Metler balance P 163).

Nitrogen content

The root Nitrogen content on dry weight basis at different stages (2,4,6,8 and 10 WAP) was estimated by modified kjeldahl's method. (Osborne and Vogt, 1978)

Phosphorous content

Phosphorous was estimated by vanadomolybdo-phosphoric yellow colour method (Jackson, 1967).

Potassium content

Potassium was determined by photometer method (Jackson, 1967)

Data were subjected to Analysis of Variance using spss x 14 for windows. Analysis of variance conducted at $\alpha = 0.05$ to determine treatment significance.

RESULTS

The effect of diesel oil pollution on percentage emergence, number of root, root length, root dry weight Nitrogen content, phosphorus content and potassium content of *Glycine max linn* were presented in table 1-7 respectively.

Generally the various responses of parameters to diesel oil contamination appear to be dose dependent. The ability of *Glycine max* to germinate in diesel oil contaminated soil and control soil varied greatly depending on the pollution level. 2.0 litres treatment level of diesel oil showed a statistically significant decrease in percentage emergence (26%) ($p < 0.05$) compared to the control (50%). Also 1.0 litre treatment significantly increased percentage emergence (32%) compared to the 1.5 litre (28%), as shown in table 1.

Table 2, showed that number of roots per plant statistically decreased significantly by 2.0 litres diesel oil treatment at 2,4,6 8 and 10 WAP compared to the control treatment. On the other hand, 1.0 litre stimulated increase in the number of roots as shown in table 2, which was significantly different ($p < 0.05$) compared to 1.5 litres treatment at 2,4,6, 8 and 10WAP respectively.

Root length showed an interesting growth response in at 2 WAP in 1.5 litres (10.660cm) which was significantly different ($p < 0.05$) compared to control (7.320cm). Similarly, 2.0 litres of diesel oil treatment also showed the same trend (8.344cm) which was significantly different ($p < 0.05$) compared to the control (7.320cm). whereas at 4, 6, 8 and 10WAP, 2.0 litres diesel oil treatment levels statistically decreased the root length of *Glycine max linn* which was significantly different compared to the highest root length (18.920cm, 22.600cm, 18.580cm and 22.080cm respectively) recorded in control at 4, 6, 8 and 10WAP.

Mean root dry weight of *Glycine max* were consistently higher in control treatment than in diesel oil polluted soils. At every growth stages of development (2,4,6, 8 and 10WAP) 2.0 litres diesel oil recorded decreased root dry weights, (0.003g, 0.016g, 0.082g, 0.062g and 0.028g respectively) which was significantly different ($p < 0.05$) compared to the increased root dry weights (0.026g, 0.073g, 0.306g, 0.352g and 0.216g respectively) recorded in control plots.

The root Nitrogen content (%) as measured (Table 4), demonstrated significantly increased root Nitrogen content in 1.0litre treatment level compared with 2.0 litres diesel oil treatment level. Thereafter, control (0 litre diesel oil) significantly ($p < 0.05$) recorded higher Nitrogen content at 2, 4, 6, 8 and 10WAP. (1.444%, 1.454%, 1.890%, 1.386% and 1.934% respectively) than the lower Nitrogen content (0.320%, 0.692%, 1.180%, 0.420% and 0.058% respectively) recorded in 2.0 litres treatment level. However, 1.5litres diesel oil treatment level at 4,6,8 and 10WAP, recorded increase in Nitrogen content (1.212%, 1.604%, 0.978% and 0.345% respectively) which was significantly difference ($p < 0.05$) compared to the values (1.158%, 1.504%, 0.963% and 0.117% respectively) recorded in 1.0litre treatment level.

Root phosphorus content in table 6, demonstrated the same trend as root Nitrogen content were 2.0litres diesel oil recorded significantly lowest phosphorus content compared to the highest phosphorus content recorded in control at 2WAP, 4,6,8 and 10WAP respectively. Similarly, root phosphorus was significantly higher in 1.5litres treatment level at 6,8 and 10WAP compare to the lower phosphorus content recorded in 1.0litre treatment level as indicated in table 6.

Root potassium content was found to be significantly increased in control (0litre) treatment level compared to the decreased potassium content recorded in 2.0litres treatment at 2WAP, potassium content recorded in 2.0litres treatment at 2WAP 4, 6, 8 and 10WAP respectively. However, at 4WAP and 10WAP treatment 1.5 litres gave significantly higher potassium content (0.237% and 0.052% respectively) than the lower potassium content (0.213% and 0.035% respectively) recorded in 1.0litre treatment level. It was

observed that there was gradual increase in the value of parameters recorded from 2WAP to 8WAP after which there was drastic decrease at 10WAP.

Table 1: Percentage Emergence

Treatments	Percentage (%)
0litre	50 ^a
1litre	32.00 ^b
1.5litres	28.00 ^c
2.0litres	26.00 ^d
LSD _{0.05}	0.0502

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 2: Number of Roots

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	10.680 ^a	48.00 ^a	70.00 ^a	76.200 ^a	63.400 ^a
1litre	5.000 ^b	36.00 ^b	61.400 ^b	57.400 ^b	30.000 ^b
1.5litres	5.800 ^c	30.600 ^c	30.000 ^c	22.400 ^c	19.600 ^c
2.0litres	5.600 ^d	20.200 ^d	20.00 ^d	19.600 ^d	10.000 ^d
LSD _{0.05}	0.064	0.090	1.429	0.100	0.091

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 3: Root Length (cm)

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	7.320 ^d	18.920 ^c	22.600 ^a	18.580 ^a	22.080 ^a
1litre	7.380 ^L	23.120 ^a	21.480 ^b	14.980 ^b	9.920 ^b
1.5litres	10.660 ^a	22.653 ^b	19.700 ^c	9.800 ^d	5.920 ^c
2.0litres	8.344 ^b	13.740 ^d	13.260 ^d	11.500 ^c	3.560 ^d
LSD _{0.05}	0.076	0.299	0.075	0.082	0.081

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 4: Root Dry Weight (g)

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	0.026 ^a	0.073 ^a	0.306 ^a	0.352 ^a	0.216 ^b
1litre	0.009 ^b	0.054 ^b	0.250 ^b	0.142 ^b	0.520 ^a
1.5litres	0.006 ^c	0.042 ^c	0.138 ^c	0.152 ^c	0.126 ^c
2.0litres	0.003 ^d	0.016 ^d	0.082 ^d	0.062 ^d	0.028 ^d
LSD _{0.05}	0.001	0.028	0.008	0.007	0.066

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 5: Root Nitrogen Content (%)

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	1.444 ^a	1.454 ^a	1.890 ^a	1.386 ^a	1.934 ^a
1litre	1.222 ^b	1.158 ^b	1.504 ^b	0.963 ^b	0.147 ^c
1.5litres	0.974 ^c	1.212 ^c	1.604 ^c	0.978 ^c	0.345 ^b
2.0litres	0.320 ^d	0.692 ^d	1.180 ^d	0.420 ^d	0.058 ^d
LSD _{0.05}	0.008	0.005	0.008	0.006	0.005

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 6: Root Phosphorus Content (%)

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	0.170 ^b	0.166 ^a	0.331 ^a	0.082 ^a	0.198 ^a
1litre	0.274 ^a	0.133 ^b	0.130 ^c	0.053 ^c	0.032 ^c
1.5litres	0.127 ^c	0.118 ^c	0.152 ^b	0.071 ^b	0.056 ^b
2.0litres	0.054 ^d	0.071 ^d	0.149 ^d	0.039 ^d	0.006 ^d
LSD _{0.05}	0.006	0.050	0.006	0.001	0.007

Means followed by the same letter in the same column do not differ significantly (p<0.05).

Table 7: Root Potassium Content (%)

Treatments Levels	2WAP	4WAP	6WAP	8WAP	10WAP
0litre	0.331 ^a	0.333 ^a	0.608 ^a	0.464 ^a	0.255 ^a
1litre	0.283 ^b	0.213 ^c	0.304 ^b	0.330 ^b	0.035 ^c
1.5litres	0.208 ^c	0.237 ^b	0.066 ^c	0.315 ^c	0.052 ^b
2.0litres	0.089 ^d	0.155 ^d	0.055 ^d	0.112 ^d	0.029 ^a
LSD _{0.05}	0.005	0.001	0.050	0.007	0.006

Means followed by the same letter in the same column do not differ significantly (p<0.05).

DISCUSSION

The effect of diesel oil concentration in the soil was found to have significantly affected the percentage emergence of soybean. Increased concentration of diesel oil (2.0 litres) reduced significantly percentage emergence in soybean compared to the control (0 litre) which recorded very high percentage emergence. This reduction in percentage emergence could be attributed to decrease in oxygen availability and toxicity of diesel oil to germinating seeds, and consequently, increased competition for oxygen between the germinating seeds and microorganism thereby affecting physiological function within the seed. This finding is to observation of Vwioko and Fashemi (2005) who reported, exposing plant species to contaminated soil with various petroleum products causes reduction in germination rate which resulted from coating of oil on seed surface, thereby affecting physiological functions within the seed.

A similar effect was also observed by Adam and Duncan (2002), who reported reduction in germination rate in several plant species mainly in commercial crop caused by petroleum contamination. However the level of contamination determines the extent of damage and also inhibition. At high levels of contamination (1.5litres and 2.0 litres) although there was germination in all the soybean plants, there was a reduction in the seed germination of soybean. On the other hand Wang *et al.* (2001), and Adam and Ducan (2002) suggested that reduction in germination of seeds could be attributed to the formation of polar compounds dissolved in water which could penetrate the seed coat, exerting polar narcosis.

Application of different levels of diesel oil pollution significantly affected production of roots in the Soybean. , Under field conditions, control treatment gave the highest number of roots compared to diesel oil polluted plots. In this investigation under field conditions, high level of diesel oil pollution (2.0litre) drastically reduced the number of roots produced at early growth stage (2WAP) and maturity stage (10WAP). This could be due to shrinking of root size, suffocation due to lack of oxygen and anatomical changes in cells of roots in the

region of apical meristem caused by the presence of pollutant. This observation agreed with the work of Omosun *et al.* (2008) who reported reduction in parenchyma cell and thickness of cell wall of root and stems in *Amaranthus* hybrides in diesel oil polluted soil.

The ability of plant roots to grow in the soil was hampered by the diesel oil pollution level (2.0 litres). This could be due to stress imposed by high diesel oil dose, which limit nutrient availability, water uptake and lack of oxygen for root growth. It was also observed that diesel oil pollution altered soil physiochemical properties which in turn affected the establishment of crop stand. Wyszowska and Kucharaski (2000), reported that changes in the soil properties due to contamination with petroleum derived substances can lead to water and oxygen deficits as well shortage of available forms of Nitrogen and phosphorus.

The moderate diesel oil pollution (1.0litre) level gave comparatively high root length which was significantly different ($P<0.05$) from severe polluted soil (2.0litre) as observed. This showed that diesel oil pollution stimulate growth in plant at low pollution level. This was attributed to enhance nutrients availability due to high microbial activities, resulting in high organic matter content. This assertion is in conformity with work of Ekpo and Ebeagwu(2009) who reported increase is attributed to the fact that at lower level of pollution, diesel oil acts rather as a source of nutrient to the organisms which when degraded enhance the fertility of the soil.

Root development in respect to root dry weight result revealed that diesel oil pollution levels significantly affected the root dry weight of soybean. The root dry weight was significantly reduced in 2.0 litres pollution level than observed in control treatment and 1.0 litre pollution level. This could be due to reduction in the amount of assimilates translocated from leaves to root region due to blockage of xylem by diesel oil hydrocarbon which limit absorption of nutrients (N,P, and K) necessary for synthesis of organic compounds. In addition, the reduction could be attributed to interference in the root metabolic pathway in phloem and xylem structure due to anatomical and morphological aberration caused by diesel oil pollution in the soil, which is similar to findings of Omosun *et al.* (2008).

The level of diesel oil concentration significantly influenced the root Nitrogen content of the soybean crop. The control treatment (no diesel pollution,) was found to contain high root Nitrogen content in comparison with the low root Nitrogen recorded in 1.0litre, 1.5litres and 2.0litres treatments at every growth stage. The reduction of Nitrogen content in the treated plants could be due to decrease in available Nitrogen form due to inhibitory effect of diesel oil pollution, the acidity of the soil and reduced microbial activities. This assertion is in conformity with findings of Achuba and Peretiemo-Clark (2007) who reported, that such increase in soil acidity can affect the microbial distribution in the soil reducing their activities in the rhizosphere. Since petroleum products are known to reduce Nitrogen availability (Agbogidi *et al.*, 2007), that could be the cause of reduction in Nitrogen content of root leading to reduced growth in all the growth parameters measured. Since uptake of water and nutrients is carried out by the root system, untreated plants with sound, healthy roots grow normally while treated plants could suffer from anatomical aberration thereby reducing the ability of plants to absorbed nutrients and water from the

soil. The adverse effects could be due to disruption of absorption and uptake of nutrients by petroleum products (Njoku *et al.*, 2009). According to Baran *et al.* (2002), the degrading effect of petroleum – derived compounds on soil leads to severe Nitrogen and Phosphorus depletion, depletion of water balance and biological equilibrium

The data on phosphorous content followed the similar trend with that on Nitrogen content, where root phosphorus content was higher in the control than in 1.0litre, 1.5litres and 2.0litres treatments. It implies that absorption of Nitrogen enhanced the absorption of phosphorus and cations. Other researchers also believe that one of the impacts of Nitrogen increase is the increase of cations absorption which is due to the increase of the plant metabolic activity, acceleration in most of processes and increases of the plant absorption power (Adeairan and Bongorko, 1995, Staal *et al.*, 1991). The root phosphorus was lower in the 2.0 litres diesel oil treatments than in the control treatment at all the stages of soybean growth. Wyzkowski *et al.*, 2004.and Xu and Johnson 1997 explained that reduction of phosphorus content could be due to bacteria and fungi in the soil consume available phosphorus and other macro-elements, restricting the uptake of these elements by plant (Wyzkowski *et al.*, 2004, Xu and Johnson, 1997). It can be inferred in this study that plants take up essential and non-essential elements from soil in response to concentration gradients induced by selective uptake of ions by roots or by diffusion of elements in the diesel oil contaminated soil

Root potassium uptake or absorption by the roots of the Soybean showed, that increase in level of diesel oil concentration in the soil decrease potassium content of plant roots. This reduction could be attributed to the fact that the diesel oil formed a hydrophobic layer of over the root, which limited the absorption of macro-elements and water. This agrees with the work of Quinones – Aquilar *et al.* (2003). The high rate of K⁺ content absorbed in 1.0 litre of diesel oil treatment as shown in the test crop is due to low level of diesel oil contamination. Anoliefo and Edegba (2001), reported that low level oil pollution could be easily be degraded by natural rehabilitation in soils, increase organic matter in soil and improve the fertility, physical and chemical properties of the soil.

It could be concluded that the deleterious effect of diesel oil on the root growth characteristics of soybean was relative to its concentrations and the higher the concentration, the greater the effects of the diesel oil. The tendency of the soybean to still thrive at 1.0 and 1.5 litres pollution level indicates that it can survive in an environment slightly contaminated with diesel oil while high pollution (2.0 litres) hinders plant germination and growth. The study has demonstrated that soil contamination with diesel oil has a highly significant effect of reducing macro-element composition of soybean. The impairment obtained on the biochemical biomarker (number of root, root length, root dry weight, Nitrogen, Phosphorous and Potassium content) must have been due to the inhibitory effects of the high concentration of diesel oil which shall consequently affect the quantity and quality of food materials produced by this crop specie.

Recommended are increased public awareness of the prospective environmental plight in oil operations and pre-emptive remedies amongst the oil industries as well as more research projects and funding of reclamation of polluted agricultural soils.



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