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Performance of *Glycine max* (L.) Merr. in disel oil contaminated soil

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SUMMARY

A pot culture experiment was carried out to study the influence of *Glycine max* L. Merr. (Soybeans) in a soil contaminated with diesel oil. The soil was artificially contaminated with different concentrations of diesel oil varying from 0 to 20 ml. The result showed that the seed germination of *Glycine max* recorded on 7 and 14 DAS (days after sowing) was maximum in control T_1 and that in T_5 had poor germination percentage. The biometric parameters like shoot and root length, number of leaves per plant, fresh and dry weight of the plant analysed on 30, 60 and 90 DAS and the number of flowers and root nodules recorded on 60 DAS were found to be higher in uncontaminated control T_1 and lower in T_5. The biochemical parameters like protein and carbohydrate content observed on 30, 60 and 90 DAS was found to be maximum in T_5 and minimum in control T_1. The chlorophyll content of the control T_1 was more on 30 DAS and 60 DAS, and decreased in the 90 DAS in all the treatments. There was a gradual decrease in the chlorophyll content as the concentration of diesel oil increased. There was a decrease in the leghaemoglobin content as the concentration of diesel oil was increased. In yield parameters, number of pods per plant, number of seeds per pod and the weight of a pod were maximum in T_1 and minimum in T_5 on 90 DAS.The initial and final concentration of petroleum hydrocarbons in all treatments were analysed and it was noticed that after the harvest period (90 DAS), there was a decline in the concentration of petroleum hydrocarbons in the soil. The different soil parameters like pH, EC, nitrogen, phosphorus, potassium, iron, copper, manganese and zinc showed a gradual increase in contaminated soil than control at the onset of the investigation. But at the end of the investigation, the micronutrients and macronutrients showed a decrease in their amounts in polluted treatments than control. On the other hand pH and EC were further increased in the diesel oil treated soil than control.

Key words : Soybean, Diesel oil, Biometric, Biochemical, Yield and soil parameters

Soil pollution with petroleum and its derivatives is one of the causes of degradation of natural environment. Toxicity of refinery products depends on their physical and chemical properties. The traditional methods used to clean up petroleum-contaminated soils were land filling and incineration, which are labor intensive and costly. Hence, there is an urgent need to tackle the most hazardous pollutants like heavy metals, pesticides and other xenobiotics in soil environment by bioremediation and phytoremediation techniques (Riis *et al*., 1995 and Olson *et al*., 1999).

Biodegradation of refinery product is one of the methods of combating pollution caused by petroleum-derived compounds (Margesin and Schinner, 1997). An alternative method is phytoremediation, the use of plants and microbes associated with roots to remove, contain or render harmless environmental contaminants. It is cost-effective and low maintenance method of remediating oil-contaminated soils on site (Cunningham *et al*., 1996; Kirkpatrick *et al*., 2006).

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Legumes are thought to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen in soils, which are deficient in oil-contaminated soils. Legumes do not have to compete with microorganisms and other plants for limited supplies of available soil nitrogen at oil contaminated sites (Gudin and Syratt, 1975).

The present study deals with utilization of a leguminous crop, soybean (*Glycine max* (L.) Merr.) that has the potential to phytoremediate petroleum hydrocarbons.

MATERIALS AND METHODS

The study was laid out in a completely randomized design, consisting of five treatments. All the treatments were replicated three times. The soil was cleaned absolutely by removing stones and other unwanted materials and was homogenized properly. Then each pot was filled with 2 kg of soil in 1: 1 ratio of red sandy loam soil: sandy soil and treated with diesel fuel to achieve concentration of 0ml, 5 ml, 10 ml, 15 ml and 20 ml, respectively. Viable seeds were selected and about 10 – 15 seeds were sown in each pot.

Germination percentage:

On 7 and 14 days after sowing (DAS) the number of seedlings germinated was counted and the germination percentage was calculated using the following formula (ISTA, 1993).

$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Number of seeds sown}} \times 100$$

Collection of plant samples on 30, 60 and 90 days:

The plant samples were collected carefully on 30, 60 and 90 days after sowing and various biometric and yield parameters were recorded. The samples were also analysed for total protein, carbohydrates, chlorophyll and leghaemoglobin content.

– Estimation of protein (Lowry *et al*., 1951)

– Estimation of chlorophyll (Arnon, 1949)

– Estimation of carbohydrate (Anthrone Method – Hedge and Hofreiter, 1962)

– Estimation of leghaemoglobin (Appleby and Bergersen, 1980)

Pre and post harvest soil sample analysis:

The soil sample before and after harvesting the experimental plants were collected and soil characteristics were noted.

RESULTS AND DISCUSSION

The germination percentage recorded on 7, 14 days after sowing are presented in Table 1. The highest germination percentage was recorded in control (T_1) on 7 DAS (64 per cent) and 14 DAS (98 per cent). The lowest germination percentage was recorded in (T_5) on 7 DAS (51 per cent) and 14 DAS (64 per cent). Thus the germination percentage was higher in control and decreased as the concentration of diesel oil increased. The results obtained from the current experiment were similar to those obtained by Anoliefo and Vwioko (1995), who reported that the germination percentage of chillies and tomatoes decreased as the spent oil concentration was increased.

Table 1 : Influence of diesel oil contaminated soil on germination percentage age of *Glycine max* (L.) Merr.

Treatments	Germination percentage (%)	
	7 DAS	14 DAS
T_1	64	98
T_2	60	87
T_3	56	82
T_4	53	73
T_5	51	64

The results of the biometric parameters analyzed on 30, 60 and 90 days after sowing of soya bean are depicted in Table 2, 3 and 4. The root length was higher in the treatment without fuel oil (T_1) on 30 DAS (29 cm), on 60 DAS (31 cm) and on 90 DAS (40 cm). The root length was minimum in (T_5) on 30 DAS (12 cm), on 60 DAS (21 cm) and 90 DAS (25 cm). The longest shoot length observed in the pot without contamination of fuel (T_1) on 30 DAS (25 cm), on 60 DAS (39 cm) and on 90 DAS (47 cm). The shoot length was least in (T_5) on 30 DAS (20 cm), on 60 DAS (31 cm) and on 90 DAS (32 cm). In this study we have noted a remarkable decrease was noted in plant growth in contaminated soil than the control. This result was consistent with the works of previous authors (Kinghorn, 1983; DeSong, 1980) who reported the same in cereals.

More number of leaves was observed in control (T_1) on 30 DAS (14), on 60 DAS (16) and on 90 DAS (17). The least number of leaves was obtained in (T_5) on 30 DAS (11), on 60 DAS (12) and on 90 DAS (14). The results were in conformity with the findings of Wiltse *et al*. (1998) who reported that the plant leaves of alfalfa uptake hydrocarbons from the contaminated soil and transfer them to the atmosphere through the leaves.

On the 30th day it was noted that plants of all treatments did not bear any flowers. On the 60th day the number of flowers decreased from T_1 to T_5 as the concentration of diesel oil was increased. The T_1 treatment had more number of flowers (16) than T_5, which showed least number of flowers (8). The number of flowers in spent lubricant oil treated soil decreased than control as reported by Vwioko and Fashemi (2005) in *Ricinus communis*.

The number of nodules recorded in the control was highest and significantly different from plants grown in diesel oil contaminated soil. There was a progressive decrease in nodulation as the concentration of diesel oil increased. The mean value of the nodule was higher in control T_1 (14) and least in T_5 (3). Nwoko *et al*. (2007) reported that leguminous plants are capable of fixing nitrogen and have advantage in the remediation of oil contaminated soil and provide their own nitrogen fertilizer through nitrogen fixation to correct imbalance in C: N ratio. The results were similar to the findings of Adam and Duncan (2003) in *Vicia sativa* who reported that the mean nodules was significantly reduced in contaminated plant compared to control plants but nodules on contaminated plants were more developed than the nodules of control plants.

The fresh weight was found to be maximum in control (T_1) on 30 DAS (3.5 g), on 60 DAS (3.6 g) and on 90

Table 2 : Influence of diesel oil polluted soil on root length (cm) and shoot (cm) on 30, 60 and 90 DAS of *Glycine max* (L). Merr.

Treatments	Root length (cm)			Shoot length (cm)		
	30	60	90	30	60	90
T_1	29	31	40	25	39	47
T_2	28	31	38	24	35	40
T_3	27	30	34	22	34	36
T_4	26	30	33	21	32	34
T_5	12	21	25	20	31	32
S.E.$\pm$	4.1958	2.3542	2.4595	1.7360	3.6799	1.7374
C.D. (P=0.05)	9.3488*	5.2455**	5.4802**	3.8681^{NS}	8.1993^{NS}	3.8713**
C.D. (P=0.01)	13.2989*	7.4615**	7.7953**	5.5022^{NS}	11.6631^{NS}	5.5067**

(Data represents the mean of three replications), SEd- Standard error deviation, CD- Critical difference, DAS- Days after sowing
* and ** indicate significant of value at P=0.05 and0.01, respectively , N.S.- Non significant
T_1 – Control, T_2 - 5 ml of diesel, T_3 - 10 ml of diesel, T_4 - 15 ml of diesel, T_5 - 20ml of diesel

Table 3 : Influence of diesel oil polluted soil on number of leaves, flowers and root nodules of *Glycine max* (L). Merr.

Treatments	Number of leaves			Number of flowers	Number of nodules
	30	60	90	60	60
T_1	14	16	17	16	14
T_2	13	14	16	15	9
T_3	13	14	15	11	8
T_4	12	13	15	9	7
T_5	11	12	14	8	3
S.E. $\pm$	0.6667	1.1926	0.8692	2.2010	1.2699
C.D. (P=0.05)	14.854**	2.6572^{NS}	1.9368**	4.9042*	2.8184**
C.D. (P=0.01)	2.1130**	3.7798^{NS}	2.7550**	6.9760*	4.0091**

Table 4 : Influence of diesel oil polluted soil on fresh weight (g) and dry weight (g) on 30, 60 and 90 DAS of *Glycine max* (L). Merr.

Treatments	Root length (cm)			Shoot length (cm)		
	30	60	90	30	60	90
T_1	3.5	3.6	4.6	0.62	0.68	0.86
T_2	3.0	3.4	3.5	0.54	0.56	0.66
T_3	2.8	3.0	3.3	0.52	0.55	0.60
T_4	2.7	2.8	2.9	0.49	0.50	0.56
T_5	1.6	2.2	2.3	0.33	0.34	0.38
S.E.$\pm$	0.1765	0.1680	0.3746	0.0662	0.5530	0.0217
C.D. (P=0.05)	0.3933**	0.3743**	0.8347**	0.1474*	0.1232**	0.0484**
C.D. (P=0.01)	0.5595**	0.5324**	1.1874**	0.2097*	0.1752**	0.0689**

(Data represents the mean of three replications), SEd- Standard error deviation, CD- Critical difference, DAS- Days after sowing
* and ** indicate significant of value at P=0.05 and0.01, respectively , NS Non-significant
T_1 – Control, T_2 - 5 ml of diesel, T_3 - 10 ml of diesel, T_4 - 15 ml of diesel, T_5 - 20ml of diesel

DAS (4.6 g). The least fresh weight was obtained in (T_5) on 30 DAS (1.6 g), on 60 DAS (2.2 g) and on 90 DAS (2.3 g). The maximum dry weight was obtained in (T_1) on 30 DAS (0.62 g), on 60 DAS (0.68 g) and on 90 DAS (0.86 g). The lowest dry weight was obtained in (T_5) on 30 DAS (0.33 g), on 60 DAS (0.34 g) and on 90 DAS (0.38 g). The results of the current experiment were similar to the findings of Vwioko and Fashemi (2005) who reported a decreasing fresh and dry weight of *Ricinus communis* as the concentration of spent lubricant oil increased. It was due to the adverse effect of environmental pollution on plant growth, morphological aberrations and reduction in biomass.

On 30 DAS the highest protein content was obtained in T_5 (69 mg / g) and lowest in T_1 (56 mg / g). The protein content was maximum in T_5 (82 mg/ g) and minimum in T_1 (61 mg /g) on 60 DAS. Protein content on 90 DAS was more in T_5 (307 mg / g) and less in T_1 (74 mg / g)

(Table 5).The results were similar to the findings on Nwoko *et al*. (2007) who reported that plants grown in spent oil contaminated soil showed an increase in protein content due to the ability of plants to detoxify oxides of nitrogen in leaves by synthesis of protein and amino acids, this indicates plants grow under SO_2 stress (Murray *et al*., 1992).

The higher amount of carbohydrates was found in T_5 (85 mg / g, 114 mg / g and 309 mg / g) on 30, 60 and 90 DAS, respectively. The least amount was noted in T_1 (56 mg / g, 97 mg / g and 107 mg / g) on 30, 60 and 90 DAS, respectively (Table 5) .The carbohydrate content was higher in plants under stress than control due to the changes in the amyloplast membrane and electrolyte leakage. The results are in agreement with Hayashi *et al*. (1992).

The leghaemoglobin content was maximum in the root nodules of control T_1 (0.500) at 60 DAS. It was lower in T_5 (0.202). There was a progressive decrease in the nodulation as the concentration of the diesel oil increased (Table 6). Leghaemoglobin, an efficient oxygen scavenger helps in nitrogen fixation by providing sufficient oxygen to nitrogen fixing bacteria (Verma, 2005). The leghaemoglobin content decreased with the increased concentration of diesel oil due to the reduction in acetylene content in soya bean as reported by Rosa *et al*. (2005).

The chlorophyll content of the control was highest on 30 DAS, 60 DAS and decreased on 90 DAS. The results were tabulated in Table 7. There was a gradual decrease in the chlorophyll content as the concentration of diesel oil increased. Chlorophyll content is an indication of the level of physiological condition of the plant species. The results obtained were similar to the findings of Agrawal (1992) who reported that the reduction in chlorophyll content was due to the fact that the test crops grow under stress.

The results of number of pods / plant, number of seeds / plant and weight of a pod are presented in Table 8. The yield components were significantly different in the entire sampling regimes. The number of pods / plant was greater in control T_1 (8) and lesser in T_5 (2). The number of seeds / plants was recorded maximum in control T_1 (25) and minimum in T_5 (4). The weight of a pod / plant was higher in control T_1 (0.547 g) and lower in T_5 (0.173 g). From the results it was clear that the control recorded the highest yield values compared to those contaminated with diesel oil, (*i.e.*) increase in concentration decreased the yield values. The results are in conformity with Achuba (2006) who reported that the low pod yield and yield components recorded in the plants that received treatment than control was due to the presence of oxides of sulphur and nitrogen in the spent oil.

The results of the residual petroleum hydrocarbons in soil after the harvest of soybean are tabulated in Table 9. The results revealed that the leguminous crop, *Glycine*

Table 5 : Influence of diesel oil polluted soil on protein (mg/g FW) and carbohydrate (mg/g FW) on 30, 60 and 90 DAS of *Glycine max* (L). Merr.

Treatments	Protein (mg / g FW)			Carbohydrate (mg / g FW)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	56	61	74	56	97	107
T_2	60	65	197	63	103	144
T_3	63	68	204	68	105	163
T_4	67	76	272	81	109	204
T_5	69	82	307	85	114	309
S.E. ±	2.1705	1.8012	4.9889	5.2999	1.6465	22.0000
C.D. (P=0.05)	4.84**	4.0134**	11.1160**	11.8090**	3.6687**	49.0193**
C.D. (P=0.01)	6.88**	5.7089**	15.8119**	16.7977**	5.2186**	69.7275**

Table 6 : Influence of diesel oil polluted soil on leghaemoglobin content of *Glycine max* (L). Merr. on 60 DAS

Treatments	Leghaemoglobin content
T_1	0.500
T_2	0.416
T_3	0.343
T_4	0.297
T_5	0.202
S.E. ±	0.0044
C.D. (P=0.05)	0.0098**
C.D. (P=0.01)	0.0140**

(Data represents the mean of three replications),
SEd- Standard error deviation, CD- Critical difference,
DAS- Days After Sowing
* and ** indicate significant of value at P=0.05 and0.01, respectively , NS Non-significant
T_1 – Control, T_2 - 5 ml of diesel, T_3 - 10 ml of diesel, T_4 - 15 ml of diesel, T_5 - 20ml of diesel

Table 7 : Influence of diesel oil polluted soil onchlorophyll content (mg/g FW) of *Glycine max* (L). Merr. on 30, 60 and 90 DAS

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Total chlorophyll		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	0.006	0.006	0.004	0.005	0.007	0.004	0.010	0.013	0.008
T_2	0.004	0.005	0.003	0.004	0.005	0.003	0.008	0.010	0.006
T_3	0.003	0.005	0.003	0.004	0.005	0.003	0.007	0.008	0.006
T_4	0.002	0.004	0.002	0.002	0.003	0.001	0.003	0.007	0.003
T_5	0.002	0.002	0.001	0.001	0.002	0.001	0.002	0.004	0.003
S.E.U	0.0009	0.0006	0.0020	0.0009	0.0007	0.0013	0.0017	0.0019	0.0036
C.D. (P=0.05)	0.0020**	0.0012**	0.0044 NS	0.0019**	0.0016**	0.0029NS	0.0037**	0.0043*	0.0080NS
C.D. (P=0.01)	0.0029**	0.0018**	0.0062NS	0.0028**	0.0022**	0.0041NS	0.0053**	0.0061*	0.0114NS

DAS=Day after sowing

Table 8 : Influence of diesel oil polluted soil on yield of *Glycine max* (L). Merr. on 90 DAS

Treatments	Number of pods / plant	Number of seeds / plant	Weight of a pod (g)
T_1	8	25	0.547
T_2	7	13	0.450
T_3	6	11	0.384
T_4	4	8	0.263
T_5	2	4	0.173
S.E. $\pm$	0.5164	1.1353	0.0128
C.D. (P=0.05)	1.1506**	2.5296**	0.0285**
C.D. (P=0.01)	1.6367**	3.5982**	0.0406**

(Data represents the mean of three replications),
SEd- Standard error deviation, CD- Critical difference,
DAS- Days After Sowing
* and ** indicate significant of value at P=0.05 and0.01, respectively , NS Non-significant
T_1 – Control, T_2 - 5 ml of diesel, T_3 - 10 ml of diesel, T_4 - 15 ml of diesel, T_5 - 20ml of diesel

Table 9 : Petroleum hydrocarbon content (%) of pre and post harvest soil

Treatments	Before harvest (%)	After harvest (%)
T_1	0.00	0.00
T_2	0.67	0.60
T_3	0.84	0.79
T_4	1.01	0.95
T_5	1.18	0.99

max can tolerate oil polluteds oil and a good candidate for phytoremediation. Initial diesel oil concentration was 0.67 per cent, 0.84 per cent, 1.01 per cent and 1.18 per cent, respectively. The greatest decline in diesel oil concentration which occurred after the harvest period (90 DAS) was 0.60 per cent, 0.79 per cent, 0.95 per cent and 0.99 per cent, respectively. The plant roots have stimulated rhizodegradation of diesel oil by stimulating the degradative activity of petroleum hydrocarbons degrading organisms which in turn increased the desorption of diesel oil to degrading organisms (Singer *et al*, 2003). Liste and Felgentreu (2006) reported the removal of petroleum hydrocarbons from oil by mustard and vetch. They proved that after 95 days, 68.7 per cent and 59 per cent of initial total petroleum hydrocarbon content was reduced to 15.6 per cent and 12 per cent by mustard and vetch.

At the onset of the investigation the pH of the hydrocarbon-contaminated soil was generally higher than the pH of the control soil. The electrical conductivity was elevated compared to normal soils as a result of contamination. The total nitrogen, potassium and phosphorus in the hydrocarbon-contaminated soil decreased as the concentration of diesel oil was increased than in the control soil. The micronutrients namely iron, copper, zinc and manganese were higher in diesel-contaminated soil than the control. The results were similar to the findings of Obire and Nwaubeta (2002) reported the same in 5% diesel oil hydrocarbon contaminated soil. Stephanie *et al*. (1999) also reported the electrical conductivity of hydrocarbon contaminated soil to be 4dS/m compared to normal soils which was <I dS/m.

At the end of the investigation the pH was higher than at the initial stage. The Electrical conductivity was elevated in the polluted soils than control after the harvest period. The values of nitrogen, phosphorus and potassium decreased in hydrocarbon treated soil after the harvest period. The micronutrients also started to decline after the harvest period in the polluted treatments which may be due to the utilization of nutrients like copper and manganese for photosynthesis and that of iron and zinc for enzyme production by the test crop (Table 10). The increase in pH was due to the release of by products during hydrocarbon degradation. (Rahman *et al*., 2003). The decline in soil nitrogen and phosphorus could be due to the fact that phosphorus and nitrogen is essential for biodegradation (Atlas *et al*., 1978). Thus as the hydrocarbon degradation proceeds the phosphorus and

Table 10 : Physico-chemical characteristics of soil before and after harvest

Treatments	Pre harvested soil									Post harvested soil								
	pH	EC	N	P	K	Fe	Mn	Zn	Cu	pH	EC	N	P	K	Fe	Mn	Zn	Cu
T_1	7.3	0.19	98	8.5	190	5.3	0.7	1.7	1.1	8.0	0.20	90	6.8	128	3.8	0.4	1.1	0.7
T_2	7.5	0.20	92	8.0	190	5.3	0.7	1.8	1.2	8.1	0.21	84	6.5	122	4.1	0.4	1.2	0.8
T_3	7.5	0.23	87	8.0	180	5.5	0.7	1.9	1.2	8.1	0.24	73	6.5	120	4.2	0.5	1.4	0.8
T_4	7.6	0.24	78	8.0	170	5.8	0.7	1.9	1.2	8.1	0.25	70	6.3	115	4.2	0.6	1.6	0.8
T_5	7.8	0.24	70	7.5	165	6.1	0.7	2.0	1.2	8.1	0.25	65	6.2	110	4.6	0.6	1.8	0.9

All the values of N, P and K are expressed in kg/acre and EC in dS/m
All the values of Fe, Mn, Zn and Cu are expressed in ppm
T_1 – Control, T_2 - 5 ml of diesel, T_3 - 10 ml of diesel , T_4 - 15 ml of diesel , T_5 - 20ml of diesel

nitrogen was being exhausted. The results were similar to the findings of Odu *et al*. (1985) who reported that the decreased value of micronutrients was good enough to maintain the soil fertility.

Conclusion:

Diesel oil contamination represents a significant stress for soil biological community. The carbon: nitrogen (C: N) ratio of the soil is altered when petroleum hydrocarbons contaminate the soil. The added carbon stimulates microbial numbers but causes an imbalance in C: N ratio, which results in immobilization of soil nitrogen by the microbial biomass, making it unfavourable for plant growth. Thus the plants of leguminosae prove useful in this case because, leguminous plants fix atmospheric nitrogen and produce their own nitrogen for growth. Hence proves more successful at growing on petroleum hydrocarbon contaminated sites.

From the present investigation it is concluded that the selected leguminous crop, soybean accelerates the removal of diesel fuel in soil and serves as a viable, low cost remedial technology for diesel contaminated soil. The petroleum hydrocarbons may not accumulate in plant tissue to a great extent. The potential for magnification in food chain may exist if the consumer organism cannot biodegrade, detoxify or eliminate the contaminants they acquire from eating plants. The plants that have accumulated waste material have to be harvested, disposed or incinerated which results in formation of ash with high metal content and hence reduces the environmental impact of this technology.

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