Evaluation of Phytoremediation Potential of some Leguminous Crops on Crude Oil Polluted Soil in the Niger Delta Area

ABSTRACT

Since petroleum hydrocarbon contamination is a major pollution problem in the Niger Delta environment, the relative tolerance of some leguminous species to crude oil contamination and their potential in the phytoremediation of crude oil polluted soil was assessed in a greenhouse. Seeds of cowpea (*Vigna unguiculata L.* Walp), lablab (*Dolichos lablab*), mucuna (*Mucuna untilis*) and Soybean (*Glycine max*) were planted in soil polluted with 2 and 4% (v/w) crude oil and no pollution, 7 days after oil treatment in a 3x4 factorial arrangement, replicated thrice. Parameters assessed included germination percentage, plant height, leaf area, plant top and root biomass, .nodule number and plant nutrient N uptake. Whereas, at 4% pollution, germination of cowpea, lablab and soybean was depressed by 35, 40 and 60%, respectively, germination in mucuna, though delayed, was depressed by only 10%. Oil treatment significantly reduced ($P<0.05$) plant height, leaf area and biomass dry matter (DM) for all leguminous species except mucuna. In both oil polluted and unpolluted soils, mucuna formed more nodules and took up more soil N. At 4% pollution, mucuna produced more than 6, 4 and 3 times the biomass produced by soybean, lablab and cowpea, respectively. Therefore, at 4% pollution, if 1 ton dry matter of mucuna is turned-in as green manure, it may add 18kg/ha N to the soil compared to 11kg N for soybean. This study revealed the potentials of the legumes in the phytoremediation of crude oil contaminated soil and tolerance to oil pollution rating them in the order: mucuna>cowpea>lablab>soybean

Key words: Phytoremediation, Mucuna, Cowpea, Lablab, Soybea, Oil pollution

Introduction

Petroleum hydrocarbon contamination caused by crude oil spill is major problem in the Niger Delta environment ((Oyedeji and Immanuel, 2023)) due to the widespread occurrences of oil spills and the so much risks is posed to human life and the environment (Nemati *et al.*, 2024; Ayibanoa Ibaba and Dickson, 2024). This create several environmental problems including its adverse effect on soil condition (Nemati et al., 2024; Ossai *et al.*, 2020; Hentati *et al.*, 2013; Macci *et al.*, 2013), microorganisms and plants. As part of efforts to remedy the situation, investigators have examined the feasibility of some post-oil spill rehabilitation measures (Ossai *et al.,* 2020; Amadi *et al.*, 1993) on the rate of soil recovery and crop improvement. Three methods commonly used in treating petroleum hydrocarbon contamination are chemical, Physical, and biological remediation, out of which phytoremediation, a biological remediation method, is becoming increasingly of interest, owing to its distinctive advantages as a green, safe technology with low cost and wide variety of potential applications (Xiao, 2015). But in most cases of oil pollution of soils, N and P alone have been observed to be most limiting to biodegradation and crop growth (Chijioke *et al.,* 2014; and Amadi *et al.,* 1993).

Among the fertilizer nutrients, nitrogen deficiencies have been observed to be most widespread in Nigeria (Kamara *et al*, 2005). And in the soils from the Coastal plain sand deposits of Southern Nigeria, Ijah *et al* (2021) reported that N is more limiting to crop production than P. The successes recorded by Amadi *et al* (1993), when they used some N rich inorganic and organic nutrient supplements to reclaim an oil polluted soil are therefore understandable. Today, there is some level of awareness created among the people in the oil producing areas that application of N rich materials to polluted soils could accelerate soil recovery. But the fertilizer supplies from the National Fertilizer Company of Nigeria (NAFCON) cannot even meet the fertilizer requirements of the nation's farmers not to talk of using same for the reclamation of the several polluted sites in the country. Since fertilizer is not readily available, the alternative could have been livestock, wastes but people show much aparthy to their use because of their offensive odour and bulk. Phytoremediation and bioremediation are reported as cost-effective and environmentally friendly technologies (Hussien *et al.,* 2022). Phytoremediation, an emerging technology, uses plants to clean up contaminants in the environment (Ibaba and Dickson, 2024) which is natural, environment friendly and more cost effective (Xiao, 2015). Several studies have consistently demonstrated that some plant species especially grasses and legumes have the potential to degrade petroleum hydrocarbons in soil. (Godwin and Peter, 2014; Akpokodje *et al.* 2019, Ibaba and Dickson, 2024). Since organic agriculture is taking the centre stage, there is need to source for alternative sources of N-rich materials that can be used for the rehabilitation of polluted sites. Using leguminous crops as green manure is worth trying because of their ability to fix N and enrich soils that are deficient in the nutrient. This study seeks to provide information on the relative tolerance of four leguminous crop species, cowpea (*Vigna unguiculata L.* Walp). Lablab (*Dolichos lablab*), Mucuna (*Mucuna utilis*) and Soybean (*Glycine max*), to oil pollution, and their potentials as green manure crops for reclaiming oil polluted sites in the country.

Materials and Methods

Experimentation:

The Nigerian Bonny light crude with a specific gravity of 0.8343 was used for this greenhouse studies. Surface soil (0 to 15cm depth) was collected from a one year fallow patch of land by the Acada village of the Rivers State University of Science and Technology, Port Harcourt, bulked, air dried and passed through a 2-mm sieve. Two and half kilograms of the composite sample was put into plastic planting buckets. The experiment was a 3 x 4 factorial fitted into a completely randomized design (CRD), replicated thrice. The factors were oil (0, 2 and 4% (v/w) and the leguminous crops species (cowpea, lablab, mucuna and soybean). Details of the oil treatment levels are as shown in Table 1. Listed in Table 2 is the chemical characteristics of the sandy loam soil used for the studies.

All pots were watered consecutively for one week with tap water before the respective pots were polluted with the three levels of oil. One week after pollution, ten (10) seeds each of the leguminous species were planted separately to the respective pots thinned to 2 seedlings 2 weeks after planting (2WAP). The pots were watered at intervals of 2 days to about field capacity throughout the period of growth. Weeding was by hand picking and the hand-picked weeds deposited back in the respective pots to decompose. Growth parameters were monitored weekly and plants harvested 8WAP.

Germination counts were taken per pot per treatment combination and percentage germination calculated using the formula:

$$\frac{\text{No of Seedlings (emerged)}}{\text{No. of Seedlings (planted)}} \times 100$$

Plant height was measured using a meter rule. Leaf area (cm^2) was assessed by measuring the total length and breadth (at the broadest point) of the leaves on a plant and multiplying the factor. At harvest, nodulation nodule/ numbers were taken after washing the roots in a sieve to prevent loss of the nodules. After washing and drying of plant tops and roots in an oven at 70^{o}C for 3 days, plant top and root biomas weights were taken.

Table 1: Treatment levels showing the volume of oil applied per 2.5kg soil and the leguminous species planted.

Treatment	Rate of Application	Vol. of Oil
Oil	0%	0ml
	2%	50ml
	4%	100ml
Leguminous Species	Cowpea	
	Lablab	
	Mucuna	
	Soybean	

Soil Analyses

The soil samples were subjected to chemical analyses at the beginning of the experiment and after harvest of the crops. Standard methods were used in the analyses of the soils as described in Udo et al (2009) Estefan et al. (2013).

Table 2: Chemical Characteristics of the Experimental Soil before Treatment Application

Texture	Sandy loam
pH	4.77
Ca^2+	2.80 cmol/kg
Mg^2+	1.40 cmol/kg
K+	0.14 cmol/kg
PO_4^{3-}	82.63 mg/kg
Org.C	1.59%
Total N	0.08%
C/N ratio	20

Plant Tissue Analysis)

Oven dried plant samples were ground in a hammer mill. Subsamples of the milled samples were digested using acid mixture (Perchloric acid – sulphuric acid – silicic acid mixture). After digestion, the digests were put in 100ml volumetric flasks and made to mark. Appropriate portions of the aliquots were then taken for nutrient concentration determination using standard methods.

Data Analysis

Simple linear regression analysis was carried out on the effect of oil treatment on germination percentage, plant height, leaf area, plant top biomass, plant root biomass, nodule number yield as well as soil properties and plant nutrient uptake.

Results and Discussion

Effect of Oil Treatment on Seed Germination and Plant Growth Parameters

Seed germination:

Germination counts (%) of the leguminous species as affected by oil contamination are illustrated in Figure 1. Crude oil pollution at the 2% level delayed and decreased the germination of the leguminous species. For example, maximum germination occurred in all species for the unpolluted soil at 5DAP. But for the 2% oil level, it occurred at 6DAP and 8DAP for cowpea and mucuna, respectively. For lablab and soybean under the same level of pollution, germination stopped at 4DAP and 5DAP, respectively. At the 4% level also, germination of mucuna seeds were delayed to 8DAP while the germination of cowpea and lablab stopped at 5DAP. Germination of the soybean seeds stopped at 4DAP only. Comparing the rate of germination of the legume seeds, oil contamination reduced germination by 10, 35, 40 and 60% for mucuna, cowpea, lablab and soybean, respectively. Though germination in mucuna was delayed by oil pollution, 75%

germination was recorded at both levels of pollution which is only 10% below the unpopulated condition (Figure 1). A similar trend was reported by Lale *et al.* (2014). They recorded reduced plant height, laminar leaf area, number of leaves and shoot dry weight of cowpea plant cultivated in a soil contaminated by spent lubricating oil. Thus, mucuna is more tolerant to the adverse effects of oil on germination relative to the other leguminous species. It might be possible that mucuna seed is of higher quality since germinability of seeds is a mere reflection of the quality of seed than environmental influence, with the environment exerting only a marginal influence on seedling establishment (Amadi *et al*, 1993). In this case, mucuna, whose seed sizes were relatively larger, probably had more stored energy for use by the developing seedlings. This might have enabled mucuna to withstand the adverse effect of oil on germination. Our observation on the germination of these crops supports and re-enforced the findings of Uzoho *et al.*, (2004); Ekpo et al., (2012) and Amadi *et al* (1993) on the effect of oil on seed germination. These authors variously observed that increasing the concentration of oil beyond 3% in soil reduced germination of maize and okra seeds. In this study, germination of cowpea, lablab and soybean seeds was decreased drastically by 2% oil in the soil. Hussien *et al.* (2022), similarly, reported progressive decline in seed germination percentage with increase in crude oil percentage for *V. rosea* seeds in the soil. However, Adeyemi and Adeyemi (2020) attributed the deleterious effects of crude oil on plant germination to cytotoxicity of crude oil constituents. The poor germination of the crops in this study might be attributed to the poor wettability and aeration in the soil, in addition to the toxic effects of the oil (Kekere *et al.*, 2011). Also, oil coatings on seed surfaces affected the physiological functioning within the seed (Amadi et al, 1993), leading to the poor germination.

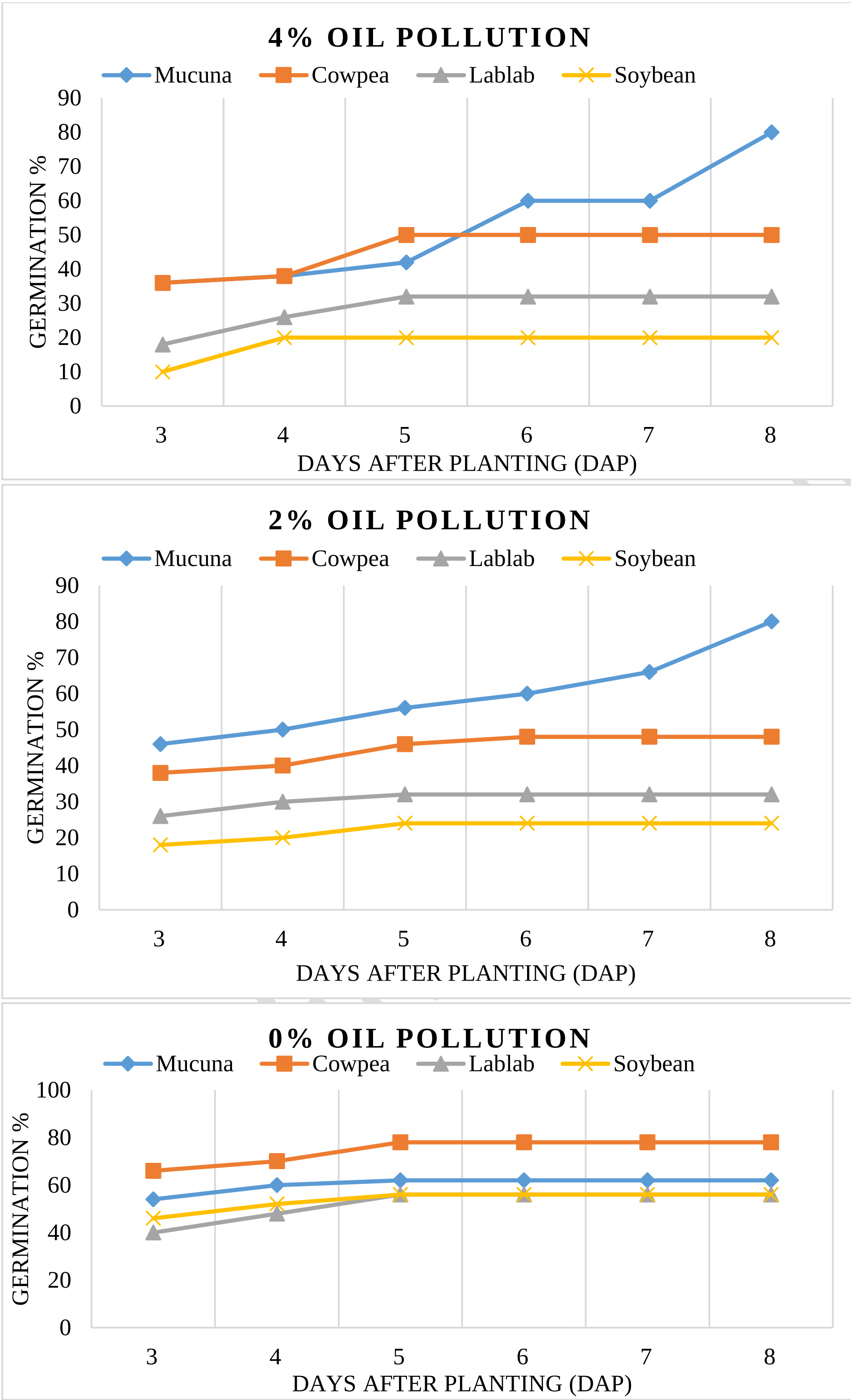

Fig. 1: Daily Percentage Germination of the Leguminous Species at the Different pollution Levels

TABLE 3: **The relationship between oil treatment and percentage germination (8DAP), plant height (8WAP), leaf area (8WAP), nodule number, plant top and root biomass production at harvest as expressed by correlation coefficient (r value) and regression equation.**

Correlation Factor	**Significance (r) level**	**Regression Equation**
Oil: Germination	-0.655 ***	Y = 51.26-0.05X
Oil: Plant Height	-0.353*	Y = 64.13-0.01 X
Oil: Leaf Area	-0.457**	Y = 42.02-0.05 X
Oil: Plant Top Biomass	-0.608*	Y = 7.49 – 0.22X
Oil: Plant Root Biomass	-0.172ns	Y = 3.18 – 0.11 X
Oil: Nodule Number	-0.740**	Y = 32.45- 0.06 X

Plant Growth:

At 8WAP, oil pollution exerted significantly negative impact on all growth parameters except plant root biomass (Table 3). There was increase in height of all the legumes with time but increasing oil levels depressed heights of all species except mucuna and that of cowpea at 8WAP (Figure 2). The height of mucuna at 8WAP varied from 130 cm at the 2% oil level to 140 cm at the 4% oil i.e. about 62% of the height of mucuna grown in unpolluted soil. Similarly, the heights of cowpea planted in soils polluted with 2% and 4% oil levels were 58% and 65% respectively, relative to that in the unpolluted soil. The heights of lablab and soybean on the other hand decreased with increase in oil concentration. Adeyemi and Adeyemi (2020) reported decreasing plant height of *Phaseolus vulgaris L* with increasing oil level which was attributed to decreased availability of plant nutrients and oxidative stress.

The depressing effect of increasing oil concentration on the growth of these legumes was also evident in the leaf area recorded for the crops except mucuna (Figure 3). Like plant height, leaf area increased with time. The leaf area recorded for mucuna varied from 53cm^2 in the 2% oil level to 59cm^2 in the 4% pollution level. These values correspond to 80% and 89% relative to that in the unpolluted soil. Using these two growth parameters to assess the crops, mucuna appears to be more tolerant to oil pollution. But Odeyemi and Immanuel (2023) reported reduction in leaf area and nodulation in the instance of crude oil in soil, and ascribed it to impaired metabolic processes in plants growing in the polluted soil. The results in this study might be as a result of the ability of mucuna to form more nodules even in polluted condition (Figure 4). Whereas mucuna formed 82, 48 and 23 nodules at 0, 2 and 4% oil levels while that of lablab was only 37, 12 and 13, respectively. It is possible that the greater number of nodules in mucuna enhanced more N uptake which encouraged more luxuriant growth of the crop both in polluted and unpolluted conditions. Moreover, mucuna seems to be better in withstanding oxidative stress and impaired metabolic processes in oil polluted soils.

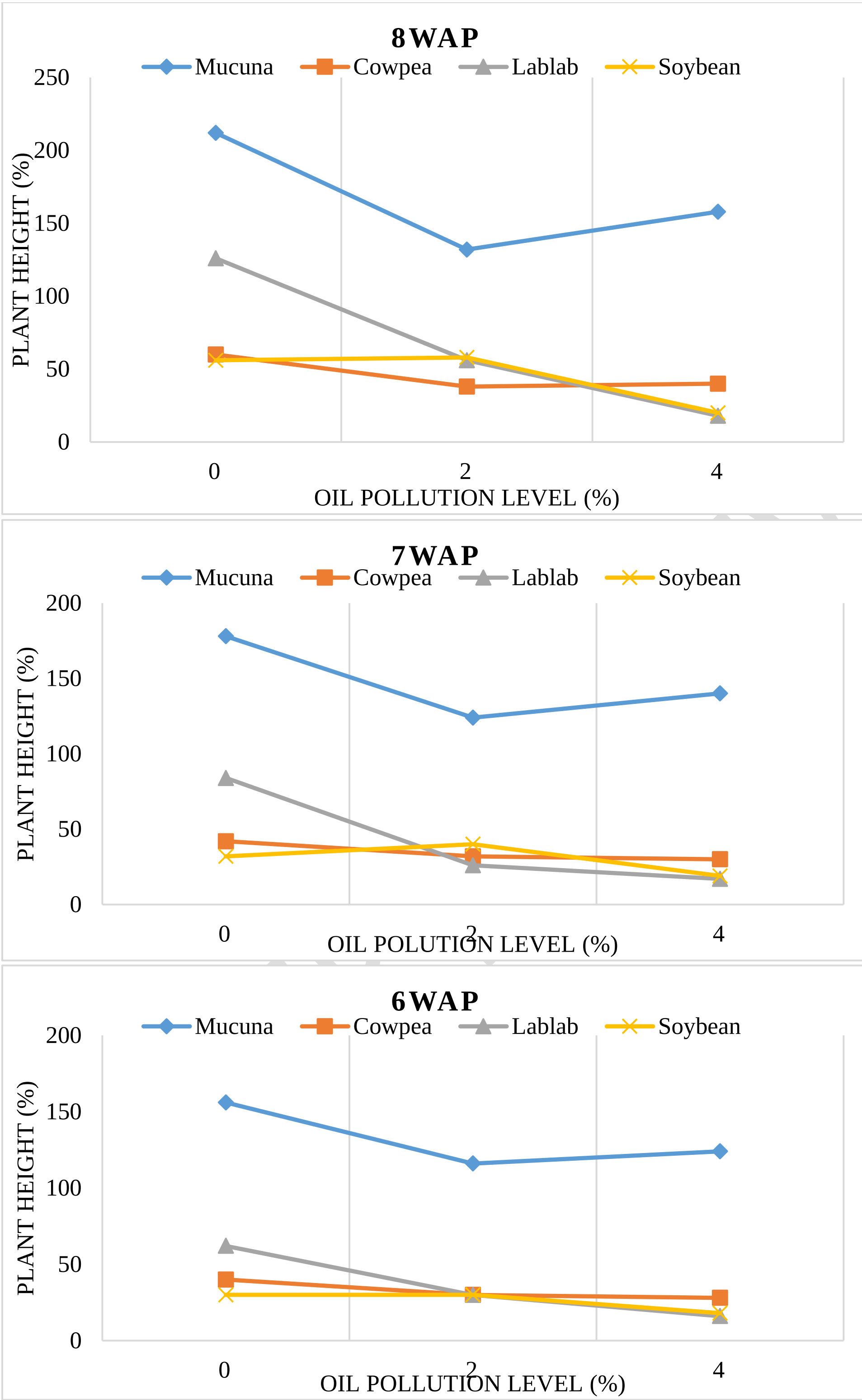

Figure 2: Mean Plant Height (Weekly) of the Leguminous Species at the Three Oil Levels

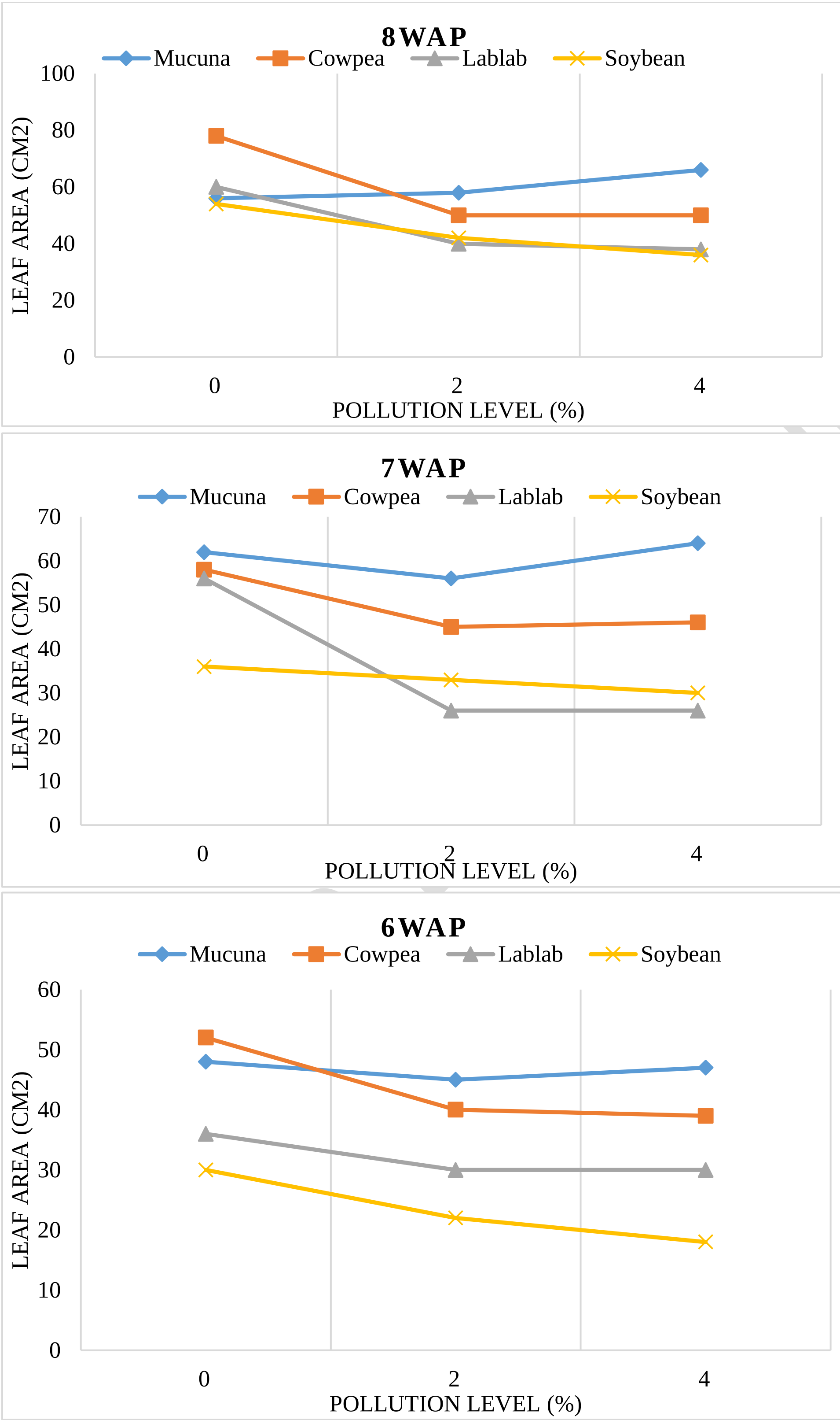

Figure 3: Mean Leaf (Weekly) of the Leguminous Species at the Three Oil Levels

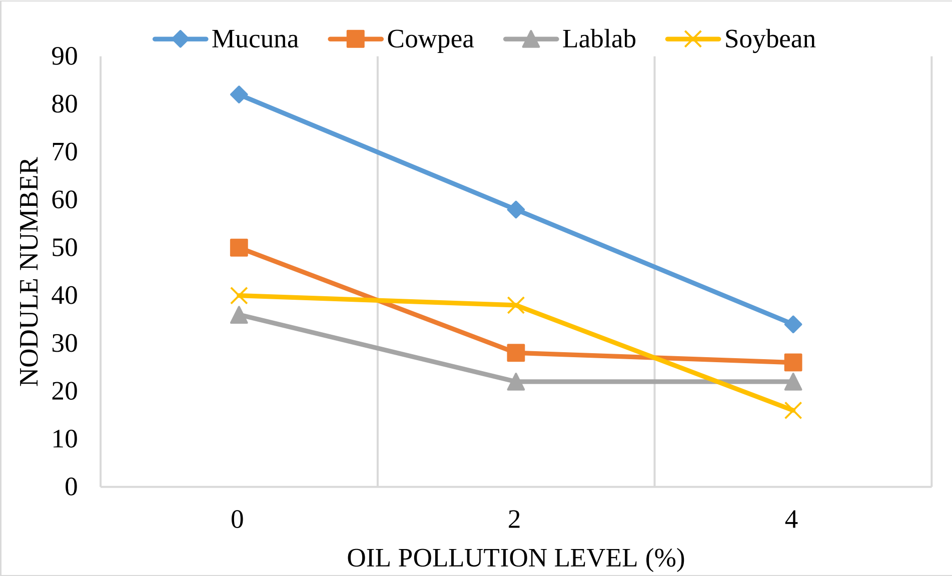

Figure 4: Effect of Oil Pollution on the nnmber of Nodules formed in each Leguminous Species

Effect of Oil Treatment on Yield
Dry Matter Yield:

As already recorded for the growth parameters, whereas, increasing concentration of oil depressed plant top and root biomass of cowpea, lablab and soybean, that of mucuna decreased from the unpolluted condition to the 2% oil level and increased when the oil level increased to 4% (Figure 5). The result tend to corroborate the results of growth parameters that mucuna is more tolerant to oil pollution among the 4 leguminous species. This perhaps, is due to its ability to nodulate well in polluted soils. At 4% oil level, mucuna was able to produce 56% and 86% plant top and root biomass relative to that in unpolluted soil. Lablab on the other hand produced only 24% and 32% of the respective biomass relative to that in polluted condition. Pre-planting oil pollution, perhaps, exerted deleterious effect on the performance of these legumes by altering soil physical, chemical and biological properties (Nemati et al., 2024; Amadi *et al*, 1993). These in turn affected the rate of synthesis and translocation of vital mineral nutrients in the plants. Mucuna probably have greater ability to withstand the deleterious effects.

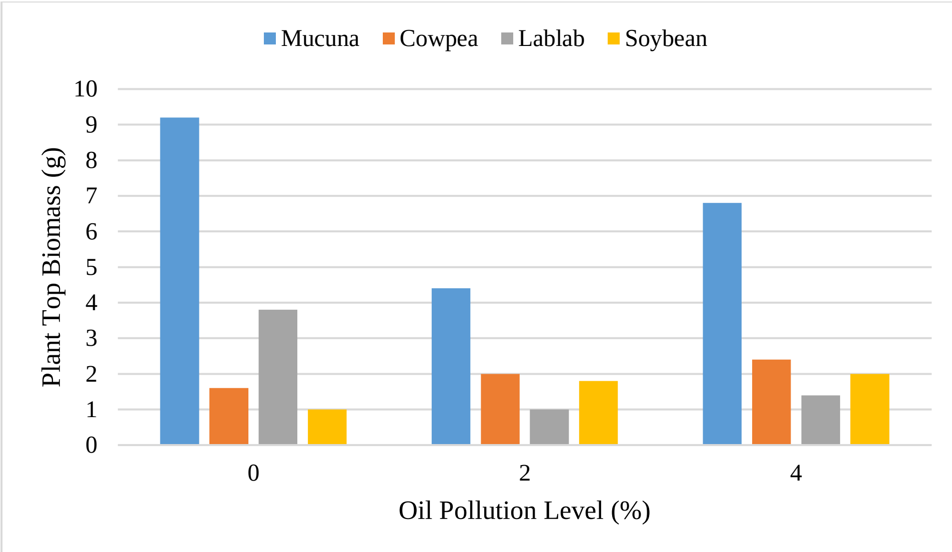

Figure 5: Effect of Crude Oil Pollution (%) on Biomass Production of the Legumes

Effect of Oil Treatment on Soil Properties
Soil Properties:

The results of the effect of oil pollution and the leguminous species on soil chemical properties are shown on Table 4. Soil pH values increased slightly with increasing levels of oil. These results agreed with the findings of Macci *et al.* (2013) and Uzoho *et al.* (2004) who variously reported that oil buffered acid soil pHs toward neutral values. This shift in pH is understandable because the sandy loam soil used is low in organic matter and therefore low in buffering characteristics. Unlike pH, exchangeable cations were not affected appreciably. Some decrease in soil P due to oil pollution was however noticed. Organic C increased with increase in oil level; the increase is more marked than pH. This increase might be due to addition of carbon from the degradation of the hydrocarbons to the organic C pool (Macci *et al.*, 2013). On the other hand, N did not vary significantly. Rather, the correlation between oil concentration and soil N was positive (Table 5). But Amadi et al (1993) working on a similar soil reported large-scale reduction in soil N due to oil treatment which they attributed to N immobilization by microbial biomass and plant tissues. Since crude oil is generally low in N that is accessible to microbes (Wolicka and Borkowski, 2012) and hydrocarbon degrading organisms naturally, are present in any soil whose population size increases rapidly in response to input of oil into the environment (Vincent *et al.*, 2011), there must have been an increase in the population of microorganisms, especially the hydrocarbon degrading forms in the oil polluted pots. Ordinarily, these organisms require N and other metabolic feed stocks to degrade the oil which results in the reduction of N and P in oil polluted soils (Kekere *et al.*, 2011 and Ekpo et al., 2012). But the results in Table 6 indicated no reduction in N of the polluted soils over the unpolluted. This may mean that the N-fixing organisms associated with the legumes helped to fix and increase the N level of the polluted soils. Ekpo *et al.* (2012) and Uzoho *et al.* (2004) had earlier reported slight increases in soil N due to increases in oil doses which they attributed to increased activity of N-fixing organisms in the oil-contaminated soils. These results emphasized the role played by leguminous species in fixing and increasing N level even in oil polluted soils.

TABLE 4: Effect of oil treatment on soil chemical properties planted to the different leguminous species

Oil Treatment %	Leguminous Species	pH (H_2O) 1:25	Org. C %	Total N %	Ca++	Mg++	K+	Avail P mg/kg	C/N ratio
0	Cowpea	4.66	1.79	0.08	2.40	0.62	0.12	110.52	22
	Lablab	4.69	1.76	0.07	2.00	0.29	0.16	105.26	25
	Mucuna	4.83	1.40	0.06	2.20	0.81	0.15	92.98	23
	Soybean	4.78	1.68	0.07	2.60	0.41	0.10	66.66	24
2	Cowpea	4.85	4.95	0.09	2.40	0.40	0.17	52.63	55
	Lablab	4.88	3.35	0.11	2.40	0.31	0.18	98.24	30
	Mucuna	4.68	4.68	0.06	2.20	0.60	0.14	78.94	78
	Soybean	4.76	4.74	0.07	2.60	0.22	0.13	43.86	68
4	Cowpea	5.06	4.55	0.08	3.00	0.28	0.18	43.86	57
	Lablab	4.99	5.88	0.11	2.80	0.61	0.17	87.72	53
	Mucuna	4.92	4.99	0.07	2.20	0.80	0.15	75.44	71
	Soybean	4.89	5.54	0.09	2.40	0.41	0.14	43.86	62

The results on Table 5 show that oil pollution had significant positive correlations with soil pH, organic C and C/N ratio. This positive relationship is an indication that their levels in the soil were increased by oil treatment.

TABLE 5: The relationship between oil treatment and soil properties after harvest as expressed by correlation coefficient (r value) and regression equation.

Correlation Factor	Significance (r) level	Regression Equation
Oil Treatment Vs		
pH	+0.769*	Y = -16.23 + 10.53 X
Ca2+	+0.456ns	Y = -3.11 + 2.77 X
Mg2+	+0.016ns	Y = 0.74 – 0.13 X
K+	+0.475ns	Y = 65.61 + 32.88 X
Org. C	+0.910***	Y = 1.94 + 0.92 X
N	+0.438ns	Y =- 87.42 + 43.75 X
P	-0.538ns	Y = -75.08 – 0.04 X
C/N ratio	+0.752**	Y = 47.21 + 0.06 X

Effect of Oil Treatment on Plant Nutrient Uptake
Nutrient Uptake:
The concentration of nutrients in the leguminous crops at the different oil levels after harvest are as presented in Table 6. And in Table 7 is shown the correlation between oil treatment and the nutrients in the plants. Oil treatment had a significantly negative correlation with P in the crops (r=-0.769*). The negative correlation between oil treatment and plant nutrient concentration is an indication that their uptake by these crops was adversely affected. Apart from Mg and K, all the others gave negative correlation with oil treatment.

TABLE 6: Effect of oil treatment on nutrient concentration in the tissues of the legumes.

Oil Treatment	Leguminous Species	%				
		N	P	K	Ca	Mg
0	Cowpea	1.43	0.53	4.00	1.74	2.67
	Lablab	1.82	0.26	2.75	1.80	4.96
	Mucuna	1.46	0.39	2.50	1.46	2.11
	Soybean	1.32	0.25	2.88	1.82	2.86
2	Cowpea	1.39	0.08	4.88	1.43	2.95
	Lablab	1.78	0.05	4.00	1.26	3.47
	Mucuna	1.76	0.05	2.75	1.35	4.47
	Soybean	1.02	0.06	3.75	1.35	3.02
4	Cowpea	1.32	0.05	4.38	1.28	3.22
	Lablab	1.53	0.06	3.50	1.58	5.76
	Mucuna	1.76	0.04	2.63	1.88	4.08
	Soybean	1.11	0.05	2.75	1.20	2.82

TABLE 7 The relationship between oil treatment and nutrient concentration in the leguminous species as expressed by correlation coefficient (r) value and regression equation.

Correlation Factor	**Significance (r) level**	**Regression Equation**
Oil Treatment N	-0.044ns	Y = 2.04 – 0.28 X
P	0.798**	Y = 17.82 – 8.83 X
K	+0.152ns	Y = 2.74 + 0.33X
Ca	-0.387ns	Y = 6.95 – 2.72 X
Mg	+0.327ns	Y = 2.50 + 0.52 X

From the results in Table 6, the highest concentration of N in plants harvested from soils polluted with 2% oil was lablab (1.78%) followed by mucuna (1.76%) while the least was in soybean (1.02%). At 4% pollution level, mucuna with 1.76% concentration was highest while soybean was least (1.11%). Thus, 1 ton of mucuna dry matter from 4% oil polluted field, turned-in as green manure could add about 18kg of N to the soil while soybean under similar condition will return only 11kg N. The total biomass production (plant top and root biomass) at the 4% oil pollution level for mucuna was 17.3g against 2.8g for soybean (Figure 5). These results revealed that mucuna produced more than 6, 4 and 3 times the biomass produced by soybean, lablab and cowpea, respectively. Combining the biomass production and nutrient uptake qualities for these leguminous species, the crops maybe rated in the order mucuna>cowpea>lablab>soybean.

Conclusion

And whereas increasing oil concentration increased pH and organic matter levels in the soil, it had no noticeable effect on soil N. This is because the N-fixing organisms and their associated legumes helped to fix and improve N level even in oil polluted soil condition. The results showed that whereas oil treatment decreased germination in cowpea, lablab and soybean, germination in mucuna was only delayed and not decreased even at the 4% oil level. The same trend was observed in plant height, leaf area and biomass production. Mucuna in polluted soils formed more nodules than others and mobilized more soil N into its tissues. These findings point to the fact that among the legumes studied, mucuna has the greatest potential for use as green manure and phytoremediation of crude oil polluted soils. It is however recommended that further study should be carried out with higher oil concentrations supplemented with organic manures.

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2.

3.

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