International Journal of Agriculture Innovations and Research
Volume 6, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 09/03/2018 | Accepted on: 19/03/2018 | Published: 05/04/2018

Revegetation Potentials of Cowpea (*Vigna Unguiculata* l. Walp) on a Crude oil Polluted Soil Amended with Cow Dung

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Abstract **– The field study was conducted to investigate the effect of the application of cow dung to crude oil polluted soils on the growth of cowpea. Four quantities of crude oil (0, 100, 200 and 300mL) and four concentrations of cow dung (0, 20, 40 and 60g) were used respectively. It comprised of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) plots with each plots measuring 1m x 1m. The experiment was laid out in a randomized complete block design. Some plant growth parameters such as plant height, number of leaves and leaf area were recorded at 7DAP, 2, 4, 6, 8 and 10 weeks after planting. The plant dry matter yield was also determined. Results obtained at the end of the field experiment showed that plant height increased from 4.2cm to 37.0cm at the rate of 300ml crude oil and cow dung application of 40g. There was a continuous increase in percentage germination, number of leaves, leaf area and dry matter yield. Results of soil analysis during the experiment revealed a general negative correlation coefficient indicating enhanced remediation during the trial periods.**

Keywords **– Cow Dung, Crude Oil, Cowpea, Growth, Revegetation.**

I. INTRODUCTION

Consistent oil exploration, refining and transportation activities have increased the frequency of oil pollution in our environment. Environmental pollution is recognized globally as a threat to human, plants and other animal population that interact with the environment (Biney, *et al.,* 1987). Contamination of the environment by petroleum derivatives, whether as a consequence of acute or chronic events, constitutes an additional source of stress on both plants and microorganisms. Crude oil spillage on soil generally reduces aeration by blocking airspaces between soil particles hence create condition of anaerobiosis (Rowell, 1977) and causes root stress in plant which also reduces leaf growth (Smith *et al*., 1989). Contamination of the soil environment can also limit its protective function, upset metabolic activity, negatively affect its chemical characteristics, reduce soil fertility and negatively influence plant production (Wyszkowski *et al*., 2004). This threatens human health and that of the organisms that are dependent on the soil.

Bioremediation is defined as "the application of biological treatment to cleanup hazardous chemicals" It has also been described as any process that utilizes microorganisms (bacteria, fungi, cyanobacteria and actinomycetes) and green plants (phytoremediation) or their products to return the polluted environment to its original condition. Bioremediation is an option that offers the possibilities to destroy or render harmless various contaminants using natural biological activity.

In Nigeria, most of the terrestrial ecosystem and shore-lines in oil producing communities are important agricultural land under continuous cultivation. The adverse effects of crude oil pollution on these arable agricultural lands have given rise to various soil treatment options such as the use of surfactants, alternate carbon substrates, organic and inorganic manures and bioremediation plants as bioremediation strategies (Raskin *et al*., 1997).

Organic manures such as cattle dung as well as plants have over time been used to improve soil fertility. Cow dung is widely available at almost cost free in the environment. Cow dung also known as cow manure is the undigested plant matter (waste product) of bovine animal species which has passed through the animal guts. The resultant fecal matter usually combined with soil bedding and urine is rich in minerals and often used as agricultural manure, providing food for a wide range of animals and fungus species. The use of cow dung on crude oil contaminated soils will also protect the soil structures, provide utilizable nutrients (Ogboghodo *et al.,* 2005).

Cowpea (*Vigna unguiculata*) is an annual grain legume and it is a main source of dietary protein which complements staple low-protein cereal and tuber crops. Cow-pea can be used to control erosion and restore fertility in poor soil. It is also used as a cover crop to suppress weeds. Fodder obtained from cowpea plays a particularly vital role in feeding animals during the dry season.

The objectives of this study therefore were to access the performance of cowpea grown on crude oil polluted soils and to evaluate the effect of cattle's dung on the performance of cowpea grown on crude oil populated soil.

II. MATERIALS AND METHODS

The field experiments were conducted at the Faculty of Agriculture Teaching and Research Farm; University of Benin. The experiment comprising of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) plots.

Treatments and Experimental Design

The experiments were laid out in 4 x 4 factorial arrangement fitted into a Randomized Complete Block Design (RCBD) and replicated three times. Each replication was made up of sixteen beds each carrying a treatment, After the preparation of beds the soils was left for two

weeks and treated with four rates (0, 100, 200 and 300 ml) of crude oil (bonny light blend). The crude oil was spilled on the surface of the soil in simulating what generally occurs in case of oil spills. Two weeks after crude oil treatment, four rates (0, 20, 40 and 60 gm) of air- dried, ground cow dung manure was applied to polluted soils. The cow dung manure was thoroughly mixed with the soil using hand trowel to ensure uniform distribution within the soil. Each quantity of crude oil served as a treatment with the 0ml treatment serving as the control.

Treated soils were left for about two weeks before planting. Three cowpea seeds were planted per hole and later thinned to two seedlings per hole after seven days of planting.

Growth Parameters Measured

Parameters measured include plant height, number of leaves, leaf area (leaf length and width), fresh weight of plant, dry matter yield and number of pod. Crop emergence (germination percentage) was taken as a percentage of the ratio of seedlings at 7 days after planting to the actual number of seeds planted. Plant height, number of leaves and leaf area were measured at two weeks intervals while dry matter yield, fresh weight of plant and number of pod were measured after harvesting plant i.e. at the end of the experiment.

Determination of total Petroleum Hydrocarbon and Pah in Crude Oil Samples

Procedure

The samples were cold-extracted in a conical flask for two hours in each case using 100% dichloromethane according to the method of (Shahunthala *et al*., 2004). The solvent from the resultant solution was removed by means of a rotary evaporator under vacuum (pressure not greater than 200mbar) and finally by a flow nitrogen at not more than 30^{o}C to yield the extracted organic matter (EOM).

The extracted organic matter (EOM) was analysed by capillary gas chromatography. TPH was analysed with the GC-FID (Gas Chromatography – Flame Ionization Detector) while the PAH was analysed with the GC-MS (Gas Chromatography - Mass Spectrometry) Clarus - 500 Perkin Elmer according to the method of (Ashraf, 2014). The GC-FID system consist of a HP5890 SERIES II, Hewlett-Packard, Waldbrown, Germany GC equipped with flame ionization detector and ATLAS software data processor (USA). The gas chromatographic column used was Ultra-1932530, a non-polar, fused-silica capillary column (30m × 250µm inner diameter × 0.20µm film thickness) (USA). Helium gas was used as the carrier gas at a low flow rate of 1ml/min at a pressure of 75kpa. The injector temperature was set at 250^{o}C, and detector temperature at 310 ^{o}C. The temperature program used was; 2 minutes hold time at 250, a ramp to 13^{O}C at 3^{O}C/min followed by 3 min hold time, a ramp to 240 ^{O}C at 7 ^{O}C /min and a final ramp to 285 ^{O}C at 12 ^{O}C with an 8 minute hold time.

Table 1. Concentration of PAH's in crude oil

PAH (ml/l)	Nigerian crude oil
Acenaphthene	1.072
Acenaphthylene	1.046
Anthracene	0.522
Benzo (a) pyrene	0.076
Benzo (b) flouranzthene	0.023
1, 12-Benzoperylene	0.007
1, 2, 5, 6 Dibenzanthracene	0.002
Fluoranthene	0.450
Fluorene	0.284
Indeno (1, 2, 3) pyrene	0.002
Naphthalene	0.163
Phenanthrene	0.143
Pyrene	0.621
Benzo(k)fluorathene	BDL

Table 2. Concentration of TPH's in crude oil

TPHs	Bonny light crude oil (mg/kg)
C_{10}	0.2002
C_{11}	0.0432
C_{12}	0.0421
C_{13}	0.0523
C_{14}	0.4934
C15	0.0060
C16	BDL
C17	0.1480
Pristane	0.1218
C18	0.3200
Phytane	BDL
C19	1.7480
C20	1.6860
C21	1.7100
C22	1.5910
C23	1.3930
C24	1.1830
C25	0.0330
C26	0.8110
C27	0.7070
C28	1.120
C29	0.7450
C30	0.2260
C31	0.7570
C32	0.4300

III. Result

Germination Percentage, GP (%)

The result for germination percentage is presented in Table 6. Germination was 100% in Treatment (300ml of crude oil, 20g of cow dung) and had a low germination rate of 83.3% in Treatments (100ml of crude oil, 20g of cow dung, 100ml of crude oil, 40g of cow dung, 300ml of crude oil, 40g of cow dung) respectively. Statistical analysis for percentage germination from the control and the treatment groups indicate no significant difference (P>0.05).

Plant Height

Result of plant height shows a continuous increase during the experiment. The result had a higher value of 37.0 in Treatments (100ml of crude oil, 40g of cow dung and 300ml

of crude oil, 40g of cow dung) and low value of 4.2 in treatment (300ml of crude oil, 60g of cow dung).

Statistical analysis of plant height for 7 days after planting (7DAP) indicate no significant difference (P>0.05) while the comparison of a forth night result up till 10 weeks indicate statistically significant increase (P<0.05) in plant height (Table 3).

Number of Leaves

Leaf production in cowpea had a higher value of 96 in Treatments (300ml of crude oil, 20g of cow dung) and a lower value of 82.6 in treatment (300ml of crude oil, NA of cow dung) at the end of the experiment i.e 10 weeks after planting (WAP).

Statistical analysis for number of leaves from all the treatment groups indicate no significant difference (P>0.05) these shows that crude oil and cow dung introduced at different concentration did not significantly affect the number of leaves when compared with the control (Table 4).

Leaf Area

The result of leaf area revealed a higher value of 64.5cm^2 in treatments (200ml of crude oil, 40g of cow dung) and low value of 8.6cm^2 in treatment (100ml of crude oil, NA of cow dung) and ranged from 58.0cm^2 to 64.5cm^2 at the end of the experiment i.e. 10 WAP.

Statistical analysis for leaf area from all the treatment groups indicate no significant difference (P>0.05) these shows that crude oil and cow dung introduced at different concentration did not significantly affect the leaf area when compared with the control.

Dry Matter Yield

The dry matter yield of cowpea at the end of the field experiment ranged from 6.5g to 12.7g. The dry matter weight increased from 8.6g (0ml of crude oil, NA of cow dung) in the control to 12.6g (100ml of crude oil, NA of cow dung) at the lowest crude oil application level, this was followed by an unstable increase and decreases with increasing level of crude oil application and that of increase in the rate of cow dung amendment.

Statistical analysis for dry matter yield obtained from all the treatment groups indicate no significant difference (P>0.05) these shows that crude oil and cow dung introduced at different concentration did not significantly affect dry matter yield when compared with the control.

Fresh Weight of Plant

The fresh weight of plant at the end of the experiment ranged from 46.6g to 98.2g. The fresh weight of plant decreased from 59.7g in the control (0ml of crude oil, NA of cow dung) and increased at the lowest crude oil application 85.5g (100ml of crude oil, NA of cow dung).

Statistical analysis for fresh weight of plant obtained from all the treatment groups indicate no significant difference (P>0.05) these shows that crude oil and cow dung introduced at different concentration did not significantly affect fresh weight of plant when compared with the control (Table 6).

Number of POD

Pod production in cowpea was higher in Treatments (200ml of crude oil, 60g of cow dung) with a value of 16.2 and low in treatment (100ml of crude oil, 40g of cow dung) with a value of 5.3. Statistical analysis for result of number of pod obtained after harvesting indicates there were no significant differences (P>0.05) between the control and treatment groups.

Table 3. Effect of Application of Cow Dung to Crude Oil Polluted Soil on Plant Height

TREATMENTS	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	10 WEEKS
0(NA)	7aC	17.9bC	42.2aB	66.8aA	85.1aA
0(20)	7.1aC	18.2bC	53.2aB	70.9aAB	89.1aA
0(40)	7.9aD	18.2bD	41.7aC	62.9aB	86.7aA
0(60)	8.0aD	18.5bD	47.1aC	70.8aB	89.1aA
100(NA)	6.6aC	17.8aBC	33.1aB	69.9aA	89.6aA
100(20)	6.9aC	19.4abBC	39.5aB	72.6aA	93.5aA
100(40)	8.1aD	17.8abD	58.8aC	77.4aB	94.7aA
100(60)	6.9aC	19.4abC	44.8aB	65.7aAB	88.4aA
200(NA)	7.9aD	21.3aD	42.0aC	63.3aB	80.0aA
200(20)	7.1aD	20.8aCD	44.1aBC	68.4aAB	86.1aA
200(40)	8.2aC	19.6aC	51.6aB	75.3aA	85.5aA
200(60)	7.5aD	21.6aCD	49.4aBC	68.7aAB	83.9aA
300(NA)	6.3aC	18.5bBC	40.8aB	64.2aA	82.6aA
300(20)	6.5aE	18.1bD	58.2aC	79.2aB	96.0aA
300(40)	6.7aC	18.2bC	50.0aB	73.0aAB	90.0aA
300(60)	6.8aC	17.7bC	46.7aB	68.9aAB	87.6aA

[a] Different letters in the same column indicate significant difference (P<0.05)
[A-E] Different letters in the same row indicate significant difference (P<0.05)

Table 4. Effect of Application of Cow Dung to Crude Oil Polluted Soil on the Number of Leaves.

TREATMENTS	7 DAP	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	10 WEEKS
0(NA)	4.8aD	9.9aD	20.8aC	24.8aBC	28.9aB	34.7aA
0(20)	4.7aD	9.9aD	18.9aC	21.2aBC	26.5aB	32.5aA
0(40)	4.9aD	11.5aD	21.7aC	25.6aBC	29.5aAB	35.0aA
0(60)	4.8aE	10.3aD	21.5aC	24.6aC	29.4aB	36.9aA
100(NA)	4.5aD	9.3aCD	18.3aBC	20.9aABC	27.5aAB	33.5aA
100(20)	4.7aE	9.9aD	19.1aC	19.3aC	25.4aB	31.3aA
100(40)	5.1aE	9.7aDE	18.5aCD	22.9aBC	30.8aAB	37.0aA
100(60)	4.7aD	9.7aCD	18.8aBC	22.1aB	30.0aAB	36.3aA
200(NA)	4.9aE	10.3aD	20.2aC	21.8aC	26.7aB	32.9aA
200(20)	4.3aE	9.4aD	20.8aC	23.6aC	29.3aB	36.7aA
200(40)	4.7aD	10.7aD	21.4aC	24.5aBC	29.5aAB	35.4aA
200(60)	4.5aD	9.7aCD	20.7aBC	24.5aAB	29.5aAB	35.2aA
300(NA)	4.7aD	9.1aD	20.6aC	24.4aBC	30.6aAB	35.3aA
300(20)	4.3aE	9.7aD	19.3aC	23.3aC	27.8aB	32.3aA
300(40)	4.7aD	9.3aD	21.2aC	25.7aBC	32.1aAB	38.0aA
300(60)	4.2aC	9.3aC	19.0aB	20.5aB	25.7aAB	31.8aA

[a-b] Different letters in the same column indicate significant difference (P<0.05)
[A-E] Different letters in the same row indicate significant difference (P<0.05)

Table 5. Effect of Application of Cow Dung to Crude Oil Polluted Soil on Leaf Area (Cm^2)

TREATMENTS	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	10 WEEKS
0(NA)	9.7aE	19.5aD	34.9aC	49.3aB	58.3aA
0(20)	10.0aE	18.9aD	34.7aC	49.5aB	61.1aA
0(40)	9.1aE	18.7aD	33.6aC	50.9aB	60.8aA
0(60)	11.2aD	23.1aC	36.6aB	52.6aA	59.2aA
100(NA)	8.6aE	18.4aD	29.5aC	46.5aB	61.9aA
100(20)	10.6aD	19.4aD	31.3aC	48.5aB	59.3aA
100(40)	12.6aD	22.7aC	35.4aB	51.4aA	58.0aA
100(60)	10.0aE	17.8aD	31.1aC	48.3aB	60.1aA
200(NA)	9.2aD	18.0aD	32.7aC	48.8aB	60.9aA
200(20)	9.0aE	17.8aD	32.4aC	49.5aB	59.1aA
200(40)	9.7aE	20.0aD	34.3aC	54.2aB	64.5aA
200(60)	10.3aE	21.4aD	33.6aC	49.5aB	62.3aA
300(NA)	9.2aE	19.7aD	33.3aC	49.1aB	59.4aA
300(20)	9.1aE	19.2aD	31.9aC	49.3aB	59.6aA
300(40)	9.3aE	19.0aD	34.9aC	50.3aB	60.1aA
300(60)	9.7aE	18.4aD	32.6aC	48.9aB	60.1aA

[a] Different letters in the same column indicate significant difference (P<0.05)
[A-E] Different letters in the same row indicate significant difference (P<0.05)

Table 6. Effect of Cow Dung Application to Crude Oil Polluted Soil on Germination Percentage, Dry Matter Yield of Cowpea, Fresh Weight of Plant of Cowpea and Number of Cowpea POD

TREATMENTS	GERMINATION %	DRY MATTER YIELD	FRESH WEIGHT OF PLANT	NUMBER OF POD
0(NA)	97.7a	8.6a	59.7a	15.3ab
0(20)	97.7a	11.4a	56.6a	5.5ab
0(40)	97.7a	10.0a	46.6a	14.8ab
0(60)	97.7a	12.6a	80.7a	15.2ab
100(NA)	97.7a	12.6a	85.5a	10.2b
100(20)	83.3a	6.5a	64.3a	10.3b
100(40)	83.3a	12.3a	76.3a	5.3b
100(60)	97.7a	8.5a	60.5a	5.7b
200(NA)	90.7a	10.7a	80.0a	11ab
200(20)	90.7a	8.6a	65.2a	10.5ab
200(40)	95.3a	9.5a	98.2a	10.3ab
200(60)	93.0a	11.4a	93.6a	16.2ab
300(NA)	95.3a	9.4a	75.9a	14.2a
300(20)	100.0a	12.4a	76.5a	14.3a
300(40)	83.3a	13.7a	96.6a	12.7a
300(60)	93.0a	12.4a	78.1a	12.0a

[a] Different letters in the same column indicate significant difference (P<0.05)
[a-b] Different letters in the same column indicate significant difference (P<0.05)

KEY:
NA = NO AMENDMENT.
20 = 20g of cattle dung.
40 = 40g of cattle dung.
60 = 60g of cattle dung.
0 = No crude oil.
100 = 100ml crude oil in a 1sqm or 1x1m.
200 = 200ml crude oil in a 1sqm or 1x1m.
300 = 300ml crude oil in a 1sqm or 1x1m.

IV. Discussion

There was a continuous increase of plant height during the experiment. Plants have been reported by to grow better with adequate soil nutrients even in the face of crude oil pollution Onuh *et al.*, (2008) which was also observed in this experiment.

A 100% plant emergence was attained with the treatment combination of 300ml of crude oil and 20g of cow dung. The increased percentage germination associated with the high rates of cow dung amendment as pollution level increased may be attributed to nutrient addition and stimulation of the microbial activities.

The production of pods as well as flowering was not negatively affected. Results from production of pods on each plant indicates there were statistically significant differences ($P<0.05$). The production of pods and flowering occurred at about the same period. The pods were produced after six weeks of planting. A normal *Vigna unguiculata* planted in an unadulterated soil produces its pods after 50 days. There were significant difference ($P<0.05$) in the production of pods and flowering between the control and the treatment groups, it shows that there is an element of growth enhancing factor in the crude oil. Agbogidi *et al.,* 2007 observed that small amount of hydrocarbon in substrates can enhance growth media and indirectly growth characteristics.

Results from the number of leaves on each plants indicates no statistically significant difference ($P>0.05$) between the control and the treatment groups, Indicating no effect of crude oil or amendment on the growth of leaves. Pair wise comparison of the number of leaves as the week progressed indicates statistically significant differences ($P<0.05$) between week 2, 4, 6, 8 and 10 thus indicating steady growth within the period of the experiment. This was in conformity with the work of (Agbogidi*et al.,* 2007) who observed that small amount of hydrocarbon in substrates can enhance growth media and growth characteristics.

The addition of cow dung to soils contaminated with crude oil led to increase of dry matter Content of cowpea grown in the soils. The results from the dry matter content indicate no statistically significant difference ($P>0.05$) between the control and the treatment groups. This is similar to the findings of Merkl *et al*., (2005a) who did not observe any significant difference on the influence of fertilizers on the shoot biomass of tropical pasture grass (*Brachiaria brizantha*). The increase in the dry matter content observed in this study could be attributed to continuous growth of the plant.

The addition of cow dung led to increase in the leaf areas. The noticed increase in the leaf area could be due to the general better growth of the plants grown in contaminated soils that had cow dung added to it. This may be as a result of the addition of soil amendment which improves the soil condition

The results of plant biomass from the experiment indicate no statistically significant difference ($P>0.05$) between the control and the treatment groups. These reports are in line with those of (Zahir *et al.,* 2010), who determined significant increase in plant biomass with different crude oil concentrations. The increase in plant weight might be induced owing to the positive influence of cow dung on the cellular division, during the development process.

V. Conclusion

The study has shown that *Vigna unguiculata* could be used to remediate crude oil polluted site due to its potential to tolerate some levels of crude oil. Waste utilization is currently receiving great research attention globally, and the findings of this research work identified the usefulness of cow dung in bioremediation of crude oil polluted agricultural soils. It can be concluded that crude oil contaminated soil may result in low soil fertility. But this can be remedied by the addition of organic nutrient supplements especially cow dung as the quantity of supplement added may have significant effect on there mediation process. Moreover, it can be deduced from this research work that plants can perform relatively better in crude oil polluted agricultural soil if such soil is amended with reasonable quantity of animal waste especially Cow dung. The addition of cow dung provides nutrients essential for plant growth. Since *Vigna unguiculata is* widely distributed and have proved successful in bioremediation of crude oil contaminated soils, it can be beneficial for many other tropical countries facing the problem of crude oil spillage.

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