PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL: THE EFFECT OF GROWTH OF *Glycine max* ON THE PHYSICO-CHEMISTRY AND CRUDE OIL CONTENTS OF SOIL

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Abstract
The remediation of oil contaminated soils has been a major problem in oil producing countries and recently use of plants to clean such soils has been on investigation. In order to identify plants that can enhance the remediation of crude oil contaminated soil, the effect of the growth of *G. max* on the physico-chemistry and crude oil content of soil contaminated with different concentrations of crude oil was investigated in this study. The results revealed that the pH, moisture and organic matter contents of soils contaminated with crude oil were significantly affected by the growth of *G. max* at differently levels of significance ($P<0.001$, $P<0.01$ and $P< 0.05$). Crude oil loss was enhanced in soil with 25g crude oil in the presence of *G. max*. Although the growth of the *G. max* did not significantly affect the crude oil level in the 50g and 75g treatments, the soils became more favourable for plant growth as weeds sprouted from the contaminated soil vegetated with *G. max*. The implication of the findings of this study is that within 110 days, growth of *G. max* can lead to cleanup of crude oil contaminated soil and the reduction in toxicity of crude oil in soil. The ability of *G. max* to reduce the level of crude oil in oil polluted soil can help to restore polluted soils back for agricultural use. The high acceptability of *G. max* due to its high nutritional value, high adaptability and ease of propagation will make it an easy tool for remediation of soil contaminated with crude oil. [Nature and Science. 2009;7(12):22-30]. (ISSN: 1545-0740)

Keywords: Contamination, Crude oil, *Glycine max*, Cleanup, pH, Moisture, Organic matter

Introduction

Since commercial exploration of petroleum started in Nigeria in 1958 (Okoh, 2003), petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways. The agricultural lands have become less productive (Dabbs, 1996) and the creeks and the fishing waters have become more or less dead (Okpokwasili and Odokuma; 1990; Odokuma and Ibo, 2002). Several civil unrests due to environmental degradation due oil exploration have also been witnessed in the Niger Delta region of Nigeria (Inoni *et al*., 2006).

The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites (Frick *et al*., 1999). These techniques however have some adverse effects on the environment and are also expensive (Frick *et al*., 1999; Lundstedt, 2003). Recently, biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum. Phytoremediation is the use of plants and/or associated microorganisms to remove, contain or render harmful material harmless (Cunningham *et al*., 1996; Schwab and Banks, 1999; Merkl, 2005). It has been shown to be effective for different kinds of pollutants (contaminants) like heavy metals, radionuclides and broad range of organic pollutants (Schroder *et al*., 2002; Schnoor, 2002) According to Pivetz (2001), plants for phytoremediation should be appropriate for the climatic and soil conditions of the contaminated sites. Such plants should also have the ability to tolerate conditions of stress (Siciliano and Germida, 1998a). Njoku, *et al.,* (2008a) demonstrated that *G. max* germinates and grows in crude oil polluted soil. Also Frick *et al.* (1999) included *G. max* in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. However, no record has shown that *G. max* can remediate crude oil polluted soil. It is therefore important to study the ability of *G. max* to affect the physico-chemistry (pH, moisture and organic matter contents) and the crude oil content of soil polluted with crude oil. The overall goal of this investigation was to evaluate the suitability of *G. max* for use in remediation of crude oil polluted soil.

The study is significant for some reasons. Firstly, phytoremediation has mostly involved the use of weeds (Aprill and Sims, 1990; Lee and Banks, 1993; Schwab and Banks, 1994; Qui *et al*., 1997; Banks *et al*., 2000). The use of food crops will improve the economic value of the technique (Van de Lelie *et al*., 2001). Secondly, although the conditions in the tropics favour phytoremediation, few researches have been carried on this technique in the tropics (Gallegos Martinez *et al*., 2000; Merkl *et al*, 2005a). There is the need therefore to evaluate the potentials of phytoremdiation in the tropics especially in Nigeria where pollution due to oil activities is high. In addition, the high nutritional value of *G. max* makes it acceptable by many and Njoku *et al*., (2008b) reported that *G. max* has the potential of growing in sandy loam soil, a soil type found in Niger Delta region of Nigeria.

Materials and methods

This study was carried out in the Biological garden of the University of Lagos, Akoka Lagos, Nigeria. The crude oil (Wellhead medium) was obtained from the SPDC Port Harcourt while the *G. max* was obtained from the Gene Bank Section of IITA Ibadan, Nigeria. The soil used is sandy loam soil and the treatments included 25g, 50g, and 75g crude oil mixed with 4000g of the soil filled in plastic containers. For each treatment, the control had no *G. max* grown on it. Both the treatments and the control were replicated thrice. Seven seeds of *G. max* were sown into each of the containers at 2cm depth and the containers were moderately watered regularly to keep the soils moist.

Soil samples were collected at the surface and 15cm depth from each container every 21 days (3 weeks) for 105 days (15 weeks). The collected soil samples were used to investigate the effect of *G. max* on the pH, moisture and organic matter contents of crude oil polluted soil. The soils from the surface and 15cm depths were usually mixed together and the mixture used for the study of the above physico-chemical features. The soil samples used in the study of the effect of G. *max* on the crude oil content of the soil were collected on the 110th day of sowing of the seeds of *G. max* in the soils.

The pH of the homogenized soils was determined following the protocols outlined by Eckerts and Sims (1995). The soils were air-dried and sieved to remove large particles and debris. 5g of the sieved soils were mixed with 5mls of distilled water and stirred very well after which mixture was allowed to stand for 30 minutes. The electrode of a pH meter was put into slurry of the soil-water mixture and the pH of the soil was read off. The moisture content of the soil samples was determined according to the method of Schneekloth *et al.* (2002). The procedure of Schulte (1995) was used to determine the organic matter content of the soil samples.

The amount of crude oil the soil samples was determined using air-dried soils that were sieved through 1mm mesh. The crude oil in the soil was first extracted with n-hexane by shaking with a mechanical shaker for 30 minutes as was described by Okolo, Amadi and Odu (2005). The soil-crude oil-n-hexane mixture was filtered into a beaker of known weight through a Whatmann No.1 filter paper. The crude oil content of the filtrate was determined after heating the beaker at 40^{o}C to a constant weight (Merkl, Schutze-Kraft and Infante, 2005b). The amount of crude oil lost from the soil was determined as the amount of crude oil added to the soil minus that in the soil at the time of analysis.

The effect of *G. max* on the pH, moisture, organic matter and crude oil contents of the soils was determined by comparing each parameter in soil with *G. max* with that in soil with *G. max.* Statistical analyses of the data obtained were done using Graphpad Prism 5.0 package using a 2 way ANOVA followed by Bonferroni posttests at 5%, 1% and 0.1% significance levels. Correlation analyses were also carried out.

Result and Discussion

The growth of *G. max* generally reduced the acidity of the crude oil polluted soil. However on days 21 and 42, the growth of *G. max* led to increase in the acidity of crude oil polluted soil (Table 1). On days 21 and 63, the pH of the control differed significantly from those of soil with 50g crude oil and *G. max* ($t = 2.701$ for day 21 and $t = 3.696$ for day 63) and those of the soil with 75g crude oil and *G. max* ($t = 2.985$ for day 21 and $t = 3.838$ for day 63). Negative correlations exist between the pH of soils with *G. max* and soils without *G. max* for each concentration of crude oil ($p = 0.350$, 0.083 and 0.683 for 25g, 50g and 75g crude oil concentrations respectively). A perfect positive correlation exists between the pH of the soil with 50g crude oil and the soil with 75g crude oil (p = 0.017) while no correlation exists the soils with 25g crude oil and *G. max* and 75g crude oil and *G. max*.

The positive correlation between the pH of the soils and the amount of crude oil added to the soil may be an implication that crude oil pollution leads to increase in soil pH. This is similar to the findings of Andrade *et al*. (2004) and Ayotamuno *et al*. (2004) who observed increase in the pH of soils polluted with crude oil. In the opinion of Dibble and Bartha (1979), the higher pH of soils with *G. max* than in soils without *G. max* means that higher degradation of crude oil took place in soil with *G. max* than in soils without G. max. The trend of the pH over the period of studies was against the expectation going by the reports of Ayotamuno *et al*. (2004) and Merkl *et al*. (2005c). These researchers reported that the pH of soils decreased as a result of degradation of crude oil. This decrease in the pH of soil with degradation of crude oil could be due to accumulation of organic acids produced during degradation in the soil (Merkl *et al*., 2005a) or the production of acid radicals through nitrification (Tisdale and Nelson, 1975). However, since soil bacteria thrive better in neutral than in acidic soils (Song *et al*., 1986; Phung, 1988), the increase of the soil pH towards neutral condition means more favourable conditions for soil bacteria. Many researchers have reported that bacteria play good role in the degradation of crude oil (Atlas and Bartha, 1977; Amund and Igiri, 1990; Frick *et al*., 1999; Van Hamme, Singh and Ward, 2003). This means that as observed in this study, growth of *G. max* can enhance the bacteria population in crude oil polluted soil and thereby lead to higher degradation of crude oil in the soil. The continual increase in the soil pH as the period of the study increased means that there was continual increase in favourable conditions soil bacteria and for biodegradation (Dibble and Bartha, 1979).

Table 1: The effect of *G. max* on the pH of crude oil polluted soil. Values are means ± standard error of three replicates

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	4.73±0.233	5.30± 0.115	5.37±0.067	5.87±0.186c	5.37±0.067a	5.50±0.153b	5.60±0.153a
42	5.03±0.176	5.30±0.173	5.23±0.067	5.57±0.145	5.37± 0.033	5.07±0.120	6.23±0.433□
63	5.07±0.088	5.17±0.067	5.33±0.333	5.37±0.233	5.77±0.176a	4.97±0.088	5.97±0.240a□
84	5.03±0.067	5.13±0.088	5.37± 0.067	5.37±0.203	5.80±0.231	4.97±0.133	5.97±0.186□
105	5.00±0.115	5.13±0.033	5.37±0.120	5.37±0.233	5.87±0.233	4.97±0.088	5.97± 0.203□

Note: a = significant difference between treatment and control at $p<0.05$ significant difference between treatment and control, b= significant difference between treatment and control at $p<0.01$, c = significant difference between treatment control at $p<0.001$, * = significant difference between soil with *G. max* and soil without *G. max* at $p<0.05$,+ = significant difference between soil *G. max* and soil without *G. max* at $p<0.01$, □= significant difference between soil *G. max* and soil without *G. max* at $p<0.001$

The growth of *G. max* in soils polluted with 25g crude oil led reduction of the moisture content of the soil. The reverse was the case for the soils with 75g crude oil. In the case of the soils with 50g crude oil, the growth of *G. max* led to reduction of the moisture in the first 42 days and afterwards the growth of *G. max* enhanced the moisture content of the soil (Table 2). The control has positive correlation with the treatments and there is a positive correlation among the treatments.

Crude oil pollution causes among other things low permeability and low infiltration of water into the soil (Hutchinson *et al*., 2001; Andrade *et al*., 2004). These conditions can lead accumulation of water on the soil surface and an artificial drought in the subsurface layer of soil. This can lead to difficulty for the roots to absorb water and nutrients which in the water as the roots usually grow deeper into the soil subsurface layers. The growth of plant root into soil help to create pores in the soil and thereby enhance water penetration and infiltration in soil polluted with crude oil. This increased water penetration and infiltration could be the cause of low moisture contents of soil contaminated with 25g crude oil and that had *G. max* grown on it as observed in this study. This can help to eliminate water logging of crude oil polluted soil and can lead to increased aeration of the soil. The increased aeration can lead to increase in the activities aerobic microbes in the soil and this can lead to increase in the degradation of oil.

Since the phytotoxic effect of crude oil increases with the concentration of the crude (Cullie and Blanchet, 1958), the higher moisture content of the soil with 75g crude oil and *G. max* than in the soil with 75g crude oil and no *G. max* could be due to inhibition of root growth by such amount of crude oil. The inhibition of root growth can lead to low penetration of water and higher accumulation of water on the soil surface. Reduction of transpiration is one of the phytotoxic effects of crude oil (Baker, 1970). The reduction in transpiration also affects the rate at which water absorption and uptake as these are controlled by transpiration pull (Taylor *et al*., 1997; Kent, 2000). Therefore higher moisture content in the soil with 75g crude oil and *G. max* than in soil with 75g crude oil and no *G. max* could be attributed to reduced loss of water due transpiration and subsequent reduction in the rate of water absorption in such soil. A possible cause of the difference between the trend of moisture content in the soil with 25g crude oil and soils with 50g and 75g crude oil is that because better growth of *G. max* in soil with 25g crude oil led to more absorption of water from the soil than from soils with 50g and 75g crude oil.

Table 2: The effect of *G. max* on the percentage moisture content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	13.04±0.211	13.50±1.381	12.24± 0.701	9.97± 0.573	10.31± 0.693	5.20±1.743	12.59± 0.763□
42	11.06±0.647	13.59±0.935	12.42± 0.739	10.05±0.427	11.12±1.832	3.34±0.975	13.32± 0.978□
63	13.16±0.230	14.03±0.420	13.06± 0.502	12.41±0.290	15.41±0.188	8.19±1.236	15.85± 0.593□
84	13.43±0.578	14.05±0.677	13.12± 0.430	12.74±0.133	15.52±0.133	8.16±1.196	15.97±0.477□
105	15.06±0.920	14.07±0.580	14.40± 0.534	13.66±1.420	16.45±0.423	8.86±0.700	14.84±0.629□

Note: a = significant difference between treatment and control at $p<0.05$, b= significant difference between treatment and control at $p<0.01$, c = significant difference between treatment control at $p<0.001$, * = significant difference between soil with *G. max* and soil without *G. max* at $p<0.05$,+ = significant difference between soil *G. max* and soil without *G. max* at $p<0.01$, □= significant difference between soil *G. max* and soil without *G. max* at $p<0.001$

The organic matter content of the soil was reduced by the growth of *G. max* in the first 42 days (Table 3). This might be due to the use of growth of *G. max* in the first 42 days might be as a result of the use of the organic matter by the *G. max* as it grew. Since the plants were in their early growth stages, they could possibly be absorbing nutrients from the soil and returning little or none to the soil. Such could have caused lesser accumulation of organic matter in the vegetated soil than in non-vegetated soil. Ayotamuno *et al*. (2004) reported similar observation of lower organic matter contents in vegetated soil.

From day 63, the growth of *G. max* enhanced the accumulation of organic matter in the soils. The observed higher organic matter accumulation in vegetated soil as from day 63 has some interpretations. Firstly, it is possible that *G. max* started shedding its leaves from after the first 42 days and the decomposition of such leaves increased the organic matter content of the vegetated soil more than that of the non-vegetated soil. The release of organic carbon to the soil due to degradation of crude oil possibly led to accumulation of more organic matter in the vegetated soil than in the non-vegetated soil. This is because organic carbon is a major component of organic matter (Okolo *et al*., 2005). Also the fixation activities in the root nodules of the plant also had a possible impact on the amount of organic matter accumulated in the vegetated soil.

The organic matter content of the soils has negative correlation (p = -0.237) with the days of sampling and positive correlation (p = 0.767) with the amounts of crude oil added to the soil. This means that while the organic matter contents of soil polluted with crude oil decreases with time, it increases with the quantity of crude oil added to soil. Apart from the 25g treatment, the soil organic matter was significantly affected by the addition of crude oil and growth of *G. max* at different levels of significance (P<0.001, P<0.01, P<0.05) for the different days of study. The growth of *G. max* however did not produce any significant effect on the organic matter content within each concentration of crude oil. There was negative correlation between the organic matter content of the control and the treatments. The 25g and 50g treatments have a perfect correlation (± 1) and same applies to the 25g and *G. max* and 50g and *G. max* treatments

Table 3: The effect of *G. max* on the percentage organic matter content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	0.89±0.118	1.95± 0.529	1.55±0.041	2.47±0.176	2.14 ±0.284	3.16±0.180	2.54± 0.170□
42	1.29±0.651	1.53± 0.073	1.15±0.367	2.22±0.115	1.65± 0.103	2.70±0.306c	2.21± 0.111□
63	0.90±0.096	0.96± 0.060	1.14±0.042	1.67±0.111	1.63±0 .071	2.05±0.140c	1.97± 0.119□
84	0.91± 0.096	0.99± 0.018	1.29±0.168	1.70±0.124	1.66 ±0.100	1.87±0.204c	1.98± 0.127□
105	0.91± 0.142	1.35±0.066	1.33±0.123	1.88±0.140	1.78 ±0.061	1.91±0.228c	1.95± 0.030□

Note: a = significant difference between treatment and control at p<0.05, b= significant difference between treatment and control at p<0.01, c = significant difference between treatment control at p<0.001, * = significant difference between soil with *G. max* and soil without *G. max* at p<0.05 ,+ = significant difference between soil *G. max* and soil without *G. max* at p<0.01, □= significant difference between soil *G. max* and soil without *G. max* at p<0.001.

The effect of crude oil the pH, moisture and organic matter content of soil observed in this study conforms with the reports of Njoku *et al*., (2008c) that these change with addition of crude oil to soil. Soil pH, soil moisture and soil organic matter contents have influence on the soil properties. The organic matter content of soil improves the binding processes in the soil. Such binding reduces water drainage and improves water retention ability of soil. Therefore the low organic matter in soil with 25g crude oil could be a cause of the low water accumulation in that soil. Excess binding of the soil particles together reduces root penetration and inhibit the absorption of materials. This can lead to malnourishment of plants even in the presence of abundant nutrients.

The amount of crude oil lost from the soil contaminated with 25g crude oil was enhanced by the growth of *G. max*. However in soils with 50g and 75g crude oil, more crude oil was lost from soils without *G. max* than in soils with *G. max* (figure 1). It is however worthy to note that in this study weeds were observed to have sprouted out from the contaminated soils with *G. max* and none of such was observed in the non-vegetated soil. This shows that even though the growth of *G. max* did not produce any significant effect on the percentage of crude oil lost from the soils the plant can reduce the quantity and toxicity of crude oil in soils. This is shown by the lesser amount of crude oil left in soil with 25g crude and *G. max* than in soil with same amount of crude oil and no *G. max* and the sprouting of weeds from the soils with 50g and 75g crude oil and *G. max*. The sprouting of the weeds indicates that the toxicity of crude oil in the vegetated soils reduced to the extent of allowing for the growth of weeds in such soils. This confirms the findings of Siciliano and Germida (1998a) that plants may not reduce the concentration of contaminants and yet can reduce the toxicity of such contaminants. For example, Siciliano and Gemida (1998a), observed a reduced toxicity of 2,3-dichlorobenzoic acid and 3-chlorobenzoic acid without reduction in the contaminant concentration in vegetated soil. The reduction is also a mechanism of phyoteremediation going by the definition of phytoremediation as a technique of rendering harmful materials harmless using plants and their associated microbes (Cunningham *et al*. 1996; Pivetz, 2001). Conversely, the absence of such weeds from soils without *G. max* indicates that the soil has not reach the level that will enable plants to grow.

The effect of *G. max* on the removal of crude oil from the soil polluted with 25g crude oil is similar to the findings of Aprill and Sims (1990), Lee and Bank (1993), Schwab and Banks, (1994) and Merkl *et al*. (2005b) who reported higher degradation of petroleum hydrocarbon in vegetated soils than in non-vegetated soil. The higher removal of crude oil observed in this study conforms with the reports of Frick *et al*. (1999) who listed *G. max* as one of the plants that can remediate petroleum hydrocarbon (anthracene) polluted soil. It also conforms with the suggestions of Njoku *et al*. (2008b) who suggested that *G. max* can be tried for its efficacy to remediate crude oil polluted soil. The removal of crude oil by *G. max* possibly occurred through one of the several mechanisms of phytoremediation. Such mechanisms include polymerization of the contaminants (Adler *et al*., 2004), interaction of the plant with fungi and bacteria (Siciliano and Germida, 1998) and production of root exudates and plant materials which serve as source of carbon, nitrogen and phosphorus for petroleum degrading microbes (Horvath, 1972; Rajaram and Sethunathan, 1975; Alexander, 1977; Smith, 1990; Burken and Schnoor, 1996). Nitrogen fixed in the soil by legumes reduces plant/microbes competition for nitrogen and thereby increase plant growth exudates production. This increases the ability of plants to increase the degradation of pollutants.

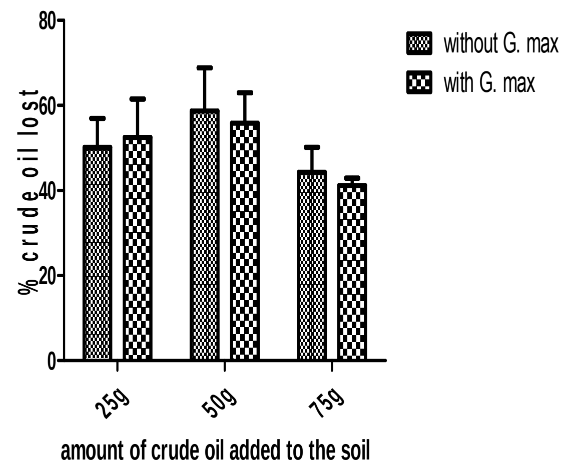

Figure 1: The effect of *G. max* on the removal of crude oil from polluted soil. Values are means ± standard error of three replicates.

Conclusion

The findings of this study indicate that growth of *G. max* in crude oil contaminated soils affects the physico-chemistry of the soil enhancing the degradation of crude oil. For instance, the significant effect that the growth of *G. max* produced on the pH and moisture content of the soil with 75g crude oil indicates that *G. max* affects the physico-chemistry of crude oil contaminated soil. It can also be inferred from the findings of this study that the growth of *G. max* in crude oil contaminated soils reduces the toxicity of crude oil in the soil. This is going by the sprouting of weeds in the soils with *G. max* and none of such soils without *G. max*. We suggest that to soil augments like cow dung should be added to crude oil contaminated soil to enhance the increase the efficacy of using *G. max* in remediating crude oil contaminated soils as Njoku *et al*. (2008a) have reported that addition of cow dung to crude oil contaminated soils enhances the growth of *G. max* in such soil.

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