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PHYTOREMEDIATION OF CRUDE OIL POLLUTED SOIL USING *GLYCINE MAX* AND *MEGATHYRSUS MAXIMUS*

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ABSTRACT

The potential of Glycine max (soybean) and Megathyrsus maximus (guinea grass) for the purpose of phytoremediation of crude oil polluted soil was investigated in this paper. This was done by examining three soil samples labelled A, B and C for a period of eight weeks. Sample A contained crude oil, Sample B contained crude oil, G.max and cow dung, and Sample C contained crude oil, M.maximus and cow dung. The pH of samples A, B and C increased from 5.44 to 6.27, 6.95 and 7.01 respectively. The total hydrocarbon content for samples A, B and C reduced by 14.33%, 45.89% and 60.80% respectively. The total nitrogen content of samples A increased from 0.156% to 0.169%, however, for samples B and C, it decreased to 0.136% and 0.128% respectively. The total organic carbon content for samples A, B and C decreased from 4.22% to 3.45%, 2.01% and 2.11% respectively. The potassium content for samples A, B and C increased from 0.30ppm to 0.36ppm, 0.79ppm and 0.84ppm respectively. The phosphorus content in samples A, B and C reduced by 41.87%, 58.45% and 59.65% respectively. These results show that G. max is more effective in remediating crude oil polluted soil compared to M. maximus.

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1. INTRODUCTION

Crude oil pollution occurs whenever unrefined crude oil spills into the environment where it is exploited, explored or handled, thereby decreasing the nitrogen and phosphorus contents of the soil (Oyibo, 2013; Obinna *et al.*, 2015). Crude oil provides the soil with excessive hydrocarbon which affects soil enzymatic activities due to the inability of soil microbes to degrade the excess hydrocarbons. Significant environmental pollution, obliteration of aquatic life and dilapidation of agricultural land are among the evils linked to crude oil exploration and exploitation (Njoku *et al.*, 2009).

Traditional physicochemical methods of cleanup such as aeration, excavation, transportation and inceneration are often expensive, difficult and inefficient (Njoku *et al.*, 2012; Oyibo, 2013). These may also be of high impact, hence, detrimental to soil structure and fertility (Norris *et al.*, 1994). Biological measures have recently been preferred compared to the chemical and physical techniques for remediation due to their low cost and ability to hinder accumulation of contaminants (El-Nawawy et al., 1987; Bonnier et al., 1980). Research has shown that some plants are able to remediate crude oil polluted soils through a method referred to as phytoremediation (Farrell *et al.*, 2000; Merkl *et al.*, 2005; Njoku *et al.*, 2009).

Phytoremediation involves the use of plants to remove, transfer, stabilize and/or degrade pollutant in soil, sediment and water (Odu *et al.*, 2006; Chibuike and Obiora, 2013; Anna and Anna, 2015). Phytoremediation is a passive low-cost method that has the potential to address organic and inorganic contaminants. The objective of this study is to evaluate and compare the potential of *G. max* and *M. maximus* for the phytoremediation of crude oil polluted soil.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

This study was carried out on the farm project site at the Faculty of Agriculture, University of Benin, Edo State, Nigeria. The crude oil (light sweet crude, 36.3 ^{o}API and 0.16 wt% sulphur) (Enekwe *et al.*, 2012) was obtained from Warri Refinery and Petrochemical Company (WRPC), Effurun, Delta State, Nigeria. *G. max* and *M. maximus* were obtained from the farm project at the Faculty of Agriculture, University of Benin. Previously uncontaminated loamy soil samples were collected from the Faculty of Agriculture, University of Benin. The soil samples were collected in a single day with the use of a hand trowel at a depth of 10 cm and then sun-dried for two days to remove moisture, sieved through a 2 mm mesh and then stored in a polyethylene bag at room temperature. All reagents used were of analytical grade.

2.2. Characterization of Soil

The following physicochemical properties of the soil were analysed before and after contamination in order to determine the effect of phytoremediation on the soil: pH, total hydrocarbon content, total organic carbon, total nitrogen content, phosphorus content and potassium content.

2.3. Phytoremediation Studies

An amount (75 g) of crude oil was mixed thoroughly with 4000 g of loamy soil in plastic containers. This contamination procedure was done in three plastic containers and labelled A, B and C. The soil samples were left for two days to allow for proper settling of the crude oil. Cow dung manure was added to soil samples B and C to enhance plant growth and thus facilitate the remediation process (Njoku *et al.*, 2009). Seven seeds of *G. max* were sown in sample B at 2 cm depth (Njoku *et al.*, 2009), *M. maximus* was transplanted to sample C, while sample A served as the control (with no *G. max* or *M. maximus* grown on it). The samples were kept in a greenhouse to prevent contamination from insects and they were moderately watered regularly to keep them moist (Njoku *et al*., 2009). The phytoremediation process was allowed to proceed for a total period of eight weeks.

2.4. Analytical Methods

Soil samples were collected from each container on a weekly basis to analyse for pH, organic carbon, potassium, nitrogen, phosphorus and total hydrocarbon content. The pH of the soil samples was determined using a glass electrode pH meter and the procedure was as described by McLean (1982). The nitrogen content of the sample was determined by the Kjeldahl method as described in Bremner, (1960). The organic carbon present in the sample was determined using the chromic acid wet oxidation method of Walkley and Black (1965). The available phosphorus in the sample was determined using the colorimetric Molybdenum blue procedure as reported in Bray and Kurtz, (1945). The amount of potassium was determined using a flame photomer (Toth *et al*., 1948). The total hydrocarbon content was determined spectrophotometrically at a wavelength 460 nm.

3. RESULTS AND DISCUSSION

3.1. Physico-Chemical Properties of the Soil used for the Study

Table 1 shows the physico-chemical properties of the soil used for the study before and after contamination with crude oil. It was observed that there was a decrease in pH from 5.84 to 5.44. This is due to the fact that Escravos crude is slightly acid (Ogboghodo *et al*., 2004). This result is consistent with earlier reports (Akubugwo et al., 2009; Nwaogu and Onyeze, 2010) The addition of the crude oil led to an increase in the amount of organic carbon from 2.05% to 4.22%. This is due to the high carbon content in the oil that may have been converted to soil organic carbon. This may also be attributed to changes in the metabolic processes of the soil microflora following crude contamination, thereby reducing its carbon mineralizing capacity (Osuji and Onojake, 2006).

Table 1: Physico- chemical properties of the loamy soil used for the study before and after contamination

Parameters	Before	After
pH	5.84	5.44
Total organic carbon (%)	2.05	4.22
Total nitrogen content (%)	0.189	0.156
Phosphorus content (mg/kg)	9.85	12.54
Potassium content (ppm)	0.34	0.30
Total hydrocarbon content (mg/kg)	0.053	86.45

Similar findings have been reported (Ellis and Adams, 1961; Benka-Coker and Ekundayo, 1995). It was also observed that there was a decrease in the nitrogen and potassium content from 0.189% to 0.156% and 0.34% to 0.30% respectively. Crude contamination may have also led to the inactivation of nitrogen fixing bacteria soil and other microbes responsible for organic decomposition in the soil thereby leading to a reduction in the soil nitrogen level. Reduction in potassium content may have been caused by the temporal immobilization of this nutrient by soil microbes as a result of crude contamination of the soil (Wang *et al*., 2013). Nutrient immobilization following oil pollution of soil has also been reported by De Jong (1980) and Jobson *et al*. (1974). A significant increase in the total hydrocarbon content was observed from 0.053 mg/kg to 86.45 mg/kg. This is expected as crude oil is essentially made up of hydrocarbons (Ogboghodo et al., 2004). This finding supports earlier reports from Agbogidi et al. (2007) who noted increase in hydrocarbon content in crude oil polluted soil in Southern Nigeria. The phosphorus content also increased from 9.8 ppm to 12.54 ppm. Maximal solubility of phosphorus occurs at a pH of 6.5, meaning that the nutrient increases in solubility up to a pH of 6.5(Riser-Roberts, 1998). Hence the reduction in soil pH that resulted after crude contamination may have led to an increase in available phosphorus in the soil.

3.2. pH

Figure 1 shows pH variation of the soil samples with time. The pH of the control sample showed a slight decrease in the course of the 8 weeks of treatment. This could be as a result of low biodegradation of hydrocarbon in the sample (Njoku et al., 2009). The pH of sample B and C increased from 5.44 to 6.95 and 7.01 respectively within the 8 weeks period of the experiment. The soil sample that had *M. maximus* generally had the highest pH values and the soil sample that had no treatment had the lowest pH values. The pH of the control sample showed a slight change from acidic to less acidic pH. This is as a result of low biodegradation of hydrocarbon in the sample (Njoku et al., 2009). The soil sample treated with *M. maximus* (sample C) had the highest final pH value and the soil sample that had no plant treatment (sample A) had the lowest final pH value. On contaminating the sample (i.e. week 0), all samples were acidic. Over time, an increasing trend was observed. At the end of the experiment, soil sample C was observed to be alkaline while soil sample B was observed to be less acidic compared to the control sample. This suggests that the treatment tended to decrease the acidity of the soil samples. This could have resulted from the biodegradation of crude oil by microorganisms under anaerobic conditions in the soil pores. A similar trend was also reported by Ebere *et al.* (2011), where pH increased averagely from 5.21 to 10.1 for the remediation of crude oil polluted soil. The pH values observed throughout the 8 weeks of bioremediation for all samples were within the optimum range for plant growth (5.5-7), as well as 6-9 as stipulated by the Federal Environmental Protection Agency (FEPA) (FEPA, 2002).

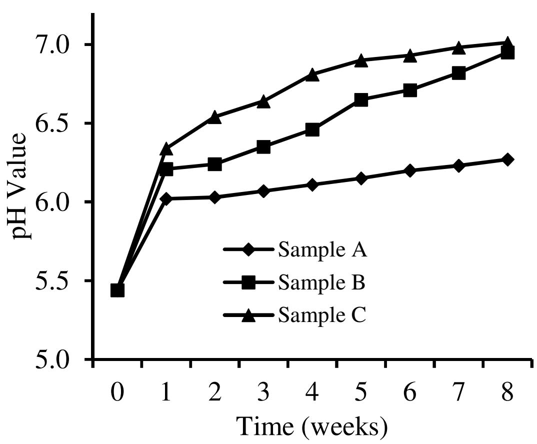

Figure 1: pH value variation with time

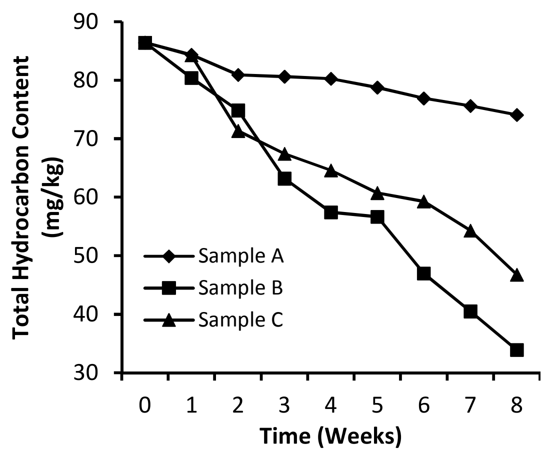

Figure 2: Variation of total hydrocarbon content with time

3.3. Total Hydrocarbon Content

Figure 2 shows the variation of the total hydrocarbon content in the soil samples with time. A considerable decrease in the total hydrocarbon concentration occurred in both treated samples compared to the control sample (sample A) after 8 weeks of phytoremediation. This conformed to the results obtained by Tanee and Albert (2011) who observed a decrease in total hydrocarbon content in a crude oil polluted soil. The control did not show significant reduction in the total hydrocarbon content. At the end of the 8 weeks treatment period, the reduction in hydrocarbon content of the polluted soil samples were 60.8% and 45.89% for sample B and C respectively, while that for sample A was 14.33%. Residual hydrocarbon reduction was significantly enhanced and was best in the soil sample treated with *G. max* (sample B).

3.4. Total Nitrogen Content

Figure 3 shows the variation of total nitrogen content in the soil samples with time. It was observed that there was a continuous decrease in the nitrogen content with time after an initial increase in the first week. The initial increase in nitrogen content may be attributed to the addition of the cow dung manure after the day of contamination of soil samples with crude oil (beginning of week 0). In addition, during this period, the microorganisms present in the soil samples tries to adapt to their new environment and no activity takes place. At the end of week 1, the microorganism in the soil samples and the roots of plants began to utilise the nitrogen present in the soil. The reduction in the values of nitrogen content in the polluted samples might be due to immobilization of nutrients and minerals by crude oil (Jobson *et al.*, 1974; De Jong, 1980) The soil remediated with *M. maximus* (sample C) had a greater reduction in nitrogen content. The rate of reduction of nitrogen content of sample B was lower than that of sample C because legumes such as *G. max* have the ability to fix nitrogen; i.e., legumes do not have to compete with microorganisms and other plants for limited supplies of available soil nitrogen at oil contaminated sites (Amusat *et al.*, 2016). Microorganisms require nitrogen to degrade organic soil contaminants, that is why the rate of depletion of the nitrogen content in sample B and C was higher than that of sample A since there are limited microorganisms present in the control sample to utilize the nitrogen present (White *et al.*, 2012).

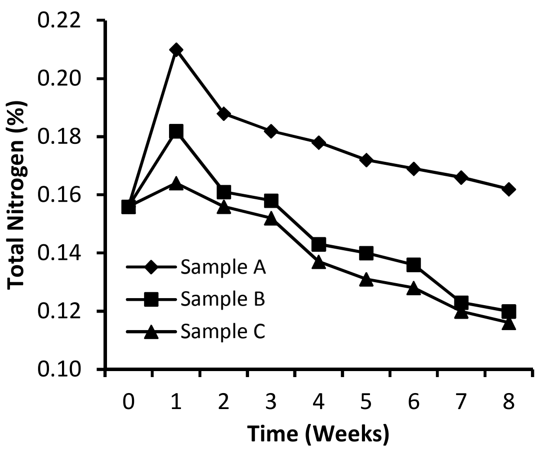

Figure 3: Variation of total nitrogen with time

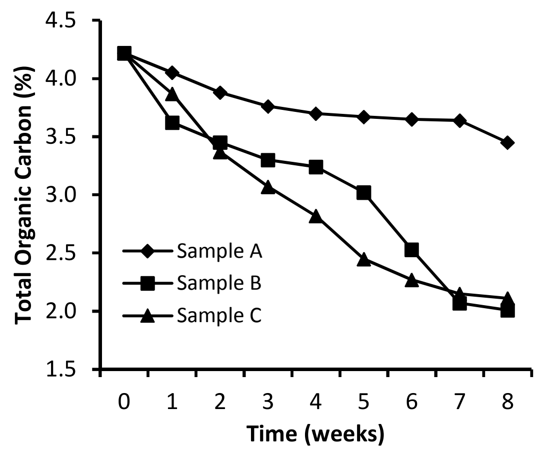

Figure 4: Variation of total organic carbon with time

3.5. Total Organic Carbon

Figure 4 shows the variation of total organic carbon in the soil samples with time. It can be seen from the figure that there was a decrease in total organic carbon in the treated samples after eight weeks of phytoremediation. At the end of the treatment period, the soil sample containing *M. maximus* had a higher total organic carbon compared to soil sample remediated with *G. max*. The results for all the samples after eight weeks of phytoremediation showed reductions from the initial value of 4.22% to 3.45%, 2.01%, and 2.11% for samples A, B and C respectively. 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 a similar observation of lower organic matter contents in vegetated soil. Organic carbon is a major component of organic matter (Okolo *et al.*, 2005).

3.6. Potassium Content

Figure 5 shows the variation of potassium content with time in the soil samples. It was observed that there was a general increase in potassium content in the soil samples and this may be attributed to the presence of cow dung used since it has inherent nutrients in it which actually improved the soil nutrient content. The potassium content increased from 0.30 ppm after contamination with crude oil to 0.36 ppm, 0.79 ppm and 0.84 ppm for sample A, B and C respectively. Sample C had the highest potassium content while sample A which had the least potassium content at the end of the eight weeks of the experiment.

3.7. Phosphorus Content

Figure 6 shows the variation of phosphorus content in the soil samples with time. It was observed that there was a reduction in phosphorus content of the soil samples. There was a decrease in the phosphorus content from the initial value of 12.54 mg/kg to 7.29 mg/kg, 5.21 mg/kg and 5.06 mg/kg for samples A, B and C respectively.

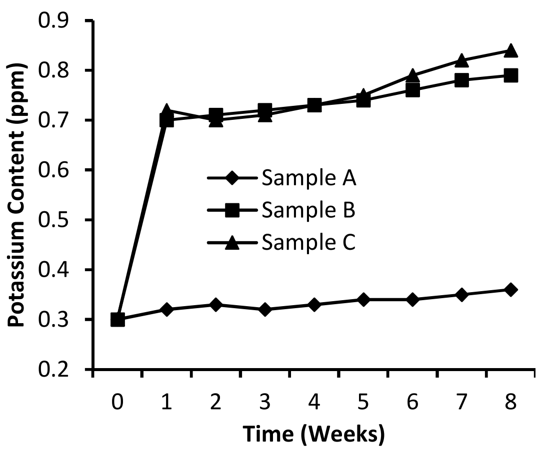

Figure 5: Variation of Potassium content with time

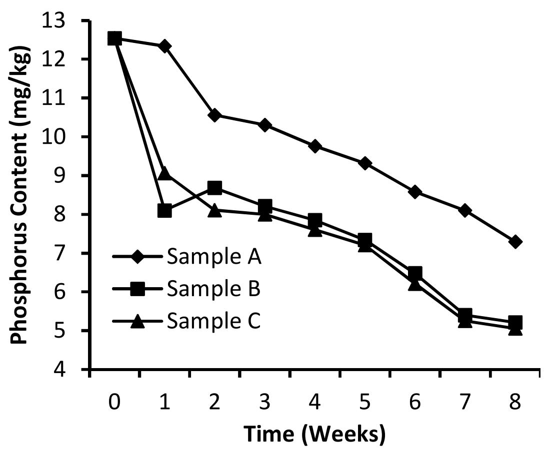

Figure 6: Variation of phosphorus content with time

The results of previous studies showed that oil contamination decreased available phosphorus concentration by various degrees (Wang et al., 2009; Eneje et al., 2012). In this study available phosphorus present at the end of the experiment was 7.29 mg/kg, 5.21 mg/kg and 5.06 mg/kg for sample A, B and C respectively. In the present study, lower available phosphorus concentrations may have been caused by two reasons. Firstly, microbes in soils which utilizes petroleum hydrocarbons as a carbon source, could utilize considerable amounts of available phosphorus when they degrade the hydrocarbons (Wang *et al.*, 2009). Secondly, phosphorus solubility is maximized at pH 6.5 (Wang *et* *al*., 2013), and consequently, the higher pH values in samples B and C could also lower the available phosphorus concentration compared with that in the control sample. From the experimental results, sample C had the highest pH value and as such it had the lowest phosphorus value at the end of the eight weeks of the experiment while sample A with the lowest value of pH had a higher amount of phosphorus at the end of the eight weeks of the experiment compared to sample B and C.

4. CONCLUSION

G. max better remediates the crude oil polluted soil compared to *M. maximus* since the rate at which it degrades total hydrocarbon content is higher than that of *M. maximus*. Thus legumes are preferable in the phytoremediation of crude oil polluted soil compared to grasses. The soil micro-nutrients were being used up during petroleum hydrocarbon degradation. This is shown by the reduction in nitrogen, phosphorus and organic carbon content of soil during the course of the experiment.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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