Bioremediation of a Crude Oil Contaminated Soil Using Water Lettuce (*Pistia stratiotes*)

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Abstract
Laboratory-scale studies were carried out using a nutrient formula produced from Pistia stratiotes plant to achieve bioremediation of crude oil impacted loamy soil. This study assessed the efficacy of powdered Pistia stratiotes (water lettuce) as potential bio stimulant in the remediation of crude oil contaminated soil using three test treatments (10ml, 50ml and 100ml) at three test concentrations (20, 40 and 60 g) of powdered water lettuce and control. The remediation process was monitored by assaying the total hydrocarbon content (THC) and soil pH before and after amendment with the powdered P. stratiotes for 180 days. The result showed increase in soil pH and THC in comparison with the control due to soil contamination by crude oil. However, there was a significant reduction ($p < 0.05$) in soil pH and THC with the introduction of powdered P. stratiotes at different concentrations. This study reveals the potentials of using P. stratiotes in the remediation of crude oil contaminated soil. Above all, this study also demonstrated that powdered P. stratiotes is a potentially strong bio stimulant.

Keywords: *Bioremediation; bio stimulant; crude oil; Pistia stratiotes; pH, total hydrocarbon content.*

INTRODUCTION

According to the Environmental Protection Agency in 2004 report, the United States had more than 40,000 contaminated sites. The Exxon Valdez oil spill in Prince William Sound, Alaska in 1989 and the BP Deep water Horizon spill in the Gulf of Mexico in 2010 are the two worst environmental disasters in US history with a total release of 0.75 and 210 million barrels of crude oil, respectively, which are still affecting some of the most productive and vulnerable marine ecosystems (Spier *et al*., 2013). Other, major incidents in the past few decades include the Minamata disease in Japan, the Union-Carbide Bhopal disaster, large-scale contamination of Rhine river, release of radioactive material in Chernobyl accident and progressive deterioration of aquatic habitats and conifer forests in the Northeastern US, Canada and parts of Europe. These have revealed the necessity to prevent the escape of effluents into the environment (Kumar *et al.,* 2011).

However, contamination of soil and aquatic ecosystems constitutes a major environmental problem in oil producing nations, especially Nigeria. It has been documented to affect seed germination and plant growth, leading to low agricultural yield and food shortage (Ogbo *et al.,* 2009). To overcome these drawbacks, a much better perspective is to completely destroy the pollutants or to transform them into some biodegradable substances. This approach can be achieved by using a technique known as bioremediation. This acts as an option to clean and safe environment and its resources by destroying various contaminants using natural biological activities. It is considered as safer, cleaner, cost effective and environment friendly technology which generally has a high public acceptance and can often be carried out at any site.

Crude oil when spilled on land affects the physicochemical properties of the soil such as temperature, structure, nutrients status and pH. Atuanya, (1987) reported that crude oil hamper proper soil aeration as oil film on the soil surface acts as physical barrier between air and soil thereby causing a breakdown of soil texture followed by soil dispersion. Since crude oil is a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds, the chemical compositions can have diverse effects on different micro-organisms within the same ecosystem. Crude oil destroys soil microorganisms causing reductions in biomass, the damaging effects are due to suffocation and toxicity of the crude oil (Odu, 1977). When crude oil is spilled on land, the light hydrocarbon fractions evaporate while the greasy fractions permeate slowly into the soil and are slowly biodegraded by microbes which naturally inhabit the soil. These inherent soil micro-organisms carry out the process of biodegrading the crude as time progress. Crude oil changes the soil's redox potential ratio and also increases the soil's pH. Thus, as crude oil pollution levels increases, soil pH also increases. Also, concentration and composition of hydrocarbons, nutrients, oxygen, moisture and temperature control the rate of degradation (Flowers, *et al.,* 1984). However, the process of biodegradation can be accelerated in the presence of nutrients such as nitrogen, potassium, phosphorus etc.

In regards to this frequent reports of oil spillages in Niger Delta, there is need to seek for a cost effective method for the remediation of crude oil contaminated soil. The process of bio-remediating using powdered water lettuce (*Pistia stratiotes*) as a nutrient source (bio-fertilizer), offers an alternative measure which would not only be effective in the regeneration of crude oil contaminated site but also affordable to encourage local participation in clean-up programs because it is environmentally friendly. This research work assessed the bio stimulation efficacy of water lettuce *(Pistia stratiotes)* as an effective agent in remediating a crude oil contaminated soil.

MATERIALS AND METHOD

2.1 Collection and Preparation of Materials

Water lettuce was harvested from Taylor Creek in Bayelsa State by hand picking the plant during the day time. Freshly harvested water lettuce (*pistia stratiotes)* was shredded in pieces using a standard table knife after washing with clean tap water following Collins (2009). The samples were oven dried at 80°C and ground into powder using a grinding mill.

The loamy soil samples were analyzed for its physico-chemical properties as follows: Total Nitrogen (TN), Phosphate (P), Total Organic Carbon (TOC), pH and Total petroleum Hydrocarbon (TPH).

The physico-chemical analyses of all the test samples were carried out using solvent extraction method which is in accordance with Standard Test Methods (ASTMD).

2.2 Experimental Design

The experimental design used for this research is a randomized complete block design.

Table 1.1: Bioremediation experimental design of the study

Experimental group	Sample point		
Set 1 (control)	Con 1	Con 2	Con 3
Set 2 (10ml)	10ml 20g WL	10ml 40g WL	10ml 60g WL
Set 3 (50ml)	50ml 20g WL	50ml 40g WL	50ml 60g WL
Set 4 (100ml)	100ml 20g WL	100ml 40g WL	100ml 60g WL

Samples were taken for laboratory analyses in two (2) months intervals at 0 hour, 60dpt, 120dpt and 180dpt.

2.3 Remediation Method

Baseline data of the soil was carried out prior to crude oil contamination, analyzing soil pH, total nitrogen, phosphate and total organic carbon. Crude oil contaminated soil samples were simulated in a site at Eagle Island using heavy crude oil. Soil was mixed with varying concentrations of crude oil (10, 50 and 100 ml) and was left undisturbed for 21 days to mimic a natural crude oil site, then different concentrations of the contaminated soil was amended with varying proportions of water lettuce nutrient powder (20, 40 and 60 g). The various combinations were contained in a shovel hole size (2.5 feet deep) respectively and each combination made up a sample point, thus making a total of twelve (12) sampling points. The content of each sample was thoroughly mixed with glove hands to ensure even distribution of crude oil and powdered water lettuce.

Sampling started immediately after the soils were amended with nutrients, this was called zero (0) day or zero-hour study. The pH and total hydrocarbon content of all the 12 cells were monitored every 60 days for a period of 6 months. During the 180-days experimental period, the air temperature ranged from 23 ºC to 32 ºC.

2.4 Statistical analysis

Data were analyzed using Statistical Package for Social Sciences version 21 (IBM) while a two – way Analysis of Variance (ANOVA) was used to assess the interactions between the treatment and control in relation to the pH, Total petroleum hydrocarbon and Total organic carbon over time, the statistical significance of the values obtained was based on a p-value of (0.05) at 95% level of confidence

RESULTS AND DISCUSSION

Table 1.2 shows the baseline data from the analysis of the soil characteristics and the physico-chemical properties of powdered water lettuce.

Table 1.2: Preliminary analysis of soil and Water Lettuce

Parameters	Soil	Water lettucce
pH	4.22	7.75

S/N	Sample	Treatment	0dpt	60dpt	120dpt	180dpt
1	20g WL	Control	4.55	5.71	5.43	6.13
2		10ml	4.82	5.43	4.70	6.11
3		50ml	3.48	5.16	4.74	5.77
4		100ml	3.85	5.45	4.26	5.80
5	40g WL	Control	4.60	5.64	5.53	6.20
6		10ml	5.04	5.50	4.89	5.86
7		50ml	4.24	5.05	4.91	5.78
8		100ml	3.82	5.12	4.84	5.90
9	60g WL	Control	4.91	5.56	5.63	6.26
10		10ml	4.89	5.51	4.86	5.80
11		50ml	3.83	4.82	4.74	5.80
12		100ml	3.52	5.18	4.69	5.82

Parameters	Soil	Water lettucce
Phosphate (mg/kg)	13.77	28.88
Total Nitrogen (%)	0.05	2.32
Total hydrocarbon content (mg/kg)	194.46	434.80

Effects of processed water lettuce (nutrient) on physio-chemical parameters of the soil

The results of the remediation effects of the different quantities of powdered water lettuce on the various concentrations of contaminated soil with respect to two (2) parameters viz: pH and Total Hydrocarbon (THC) at different stages of remediation are shown in Table 2.

Table 2: Values of water lettuce pH recorded during the period of study
N/B: DPT (days past treatment); **WL** (water lettuce)

Table 3: Values of water lettuce THC recorded during the period of study

S/N	Sample	Treatment	0dpt	60dpt	120dpt	180dpt
1	20g WL	Control	140.75	55.56	14.81	11.11
2		10ml	233.33	66.67	48.15	37.04
3		50ml	344.44	170.37	88.89	51.85
4		100ml	400.00	183.34	107.41	96.3
5	40g WL	Control	211.12	51.86	20.37	14.82
6		10ml	303.70	27.04	14.81	3.70
7		50ml	422.22	75.94	55.56	55.56
8		100ml	540.75	155.56	74.07	66.67
9	60g WL	control	100.00	48.15	25.93	18.52

10	10ml	225.93	70.37	33.33	11.11
11	50ml	391.05	155.56	70.37	66.67
12	100ml	444.45	129.63	85.17	81.48

DISCUSSION

The temperature range recorded in this study (23ºC -32ºC) is in agreement with that of Nunes *et al.,* (2020), they recorded an air temperature range of 20ºC to 37ºC during the 180-days experimental period.

Effect of crude oil contamination on soil pH during bioremediation

Soil pH value in Crude oil Contaminated Soil (CCS) was observed to be up to 6.11 and significantly higher than the control soil 4.22 ($p < 0.05$) Table 1.2. This is similar to the work of Felix *et al.,* (2020) who observed a soil pH value of 7.12 in polluted soil and significantly higher than the control 6.31. The results gotten from this study is in agreement with the work done by Ayotamuno *et al.,* (2006) who showed that increased organic matter has lowered the soil pH in a model crude oil contaminated soil. Crude oil contaminated soil showed lower pH over time. This suggests that *P. stratiotes* is a good bio-stimulant that can be used to decrease the pH of contaminated soils and improve soil properties.

Effect of crude oil contamination on soil THC during bioremediation

The removal effect of total hydrocarbon content which was used as one of the indicator parameters for remediation at various levels of crude oil contamination, were observed to reduce with time. This signifies that there was an improvement in the efficiency or degree of remediation offered by the bio-stimulant. The results from this research showed that total hydrocarbon content reduced from 540.75 mg/kg to 3.70 mg/kg, at 180 days past treatment, which signifies that water lettuce is a potent bio stimulant.

In addition, the findings from this research work is similar to that done by Shahsavarietal, *et al.,* (2013), whose report on the degradation of aliphatic hydrocarbons demonstrated that the addition of plant residues such as hay, pea, wheat and other types of plant residues to a contaminated soil led to statistically significant increases in the degradation of hydrocarbons in the soil compared with those obtained in the control soil. For example, 83% and 70% of total hydrocarbon degradation occurred in soil mixed with pea straw and mixed residues respectively over 90 day compared to only 57% degradation in control soils.

CONCLUSION

The results from this research study have shown that water lettuce is effective in the supply of limiting nutrients necessary for bioremediation of crude oil impacted media such as loamy soils. This cost-effective fertilizer can be harnessed into preserved powdery forms and be used for bioremediation by Oil and Gas Companies.

The results show that biological treatment using water lettuce (*Pistia stratiotes*) nutrients are effective in the supply of limiting nutrients necessary for bioremediation of crude oil contaminated soil. The effect of time played a more definite role in aiding the remediation of the crude oil from the soil in all cases studied. These inferences were reached after subjecting the data collected to a 2 – way Analysis of Variance.

This research has also demonstrated that powdered *P. Stratiotes* is likely a potent bio stimulant in the remediation of soils contaminated with crude oil. This was buttressed by the significant decrease in soil pH and total hydrocarbon content (THC). These environmental friendly remedial actions are geared towards sustainable development in the Niger Delta.

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