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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 as potential bio stimulant in the remediation of crude oil contaminated soil using three test treatments (20ml, 60ml and 100ml) at three test concentrations (20g, 40g and 60g) of nutrient and a control (0g). 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 90 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. Contaminated soil amended with 40g of P. stratiotes had the highest THC loss of (30%) on the final day of remediation. In a 90 day study, the control set-up had its total culturable heterotrophic bacterial population of the P. stratiotes recipe increase from 1.4×10^7 Cfu/g to 2.8×10^7 Cfu/g, while the total culturable heterotrophic fungal count in the P. stratiotes treatment ranged from 7.0×10^4 Cfu/g to 4.0×10^4 Cfu/g. Statistical analyses showed significant difference at $p \leq 0.05$ level for the two conditions (Pistia stratiotes nutrient treated soil and control). The total culturable hydrocarbon utilizing bacteria in P. stratiotes treated polluted soil increased from 1.0×10^6 Cfu/g to 6.8×10^5 Cfu/g, while the total culturable hydrocarbon utilising fungal counts increased significantly from 1.9×10^4 Cfu/g to 4.8×10^4 Cfu/g for P. stratiotes treatment. The results from this research demonstrated that powdered P. stratiotes is an effective agent that can be used to decrease the pH of contaminated soil and improve soil properties, hence aids in remediating a crude oil contaminated soil.

Keywords: Bioremediation; bio stimulant; crude oil; hydrocarbon utilizing bacteria; hydrocarbon utilizing fungi; *Pistia stratiotes*; total hydrocarbon content; total heterotrophic bacteria; total heterotrophic fungi.

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. Bioremediation acts as an alternative option to use for a clean and safe environment by degrading 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.

A widely practiced bioremediation technology that exploits the capability of microbes to degrade and/or detoxify petroleum pollutants in the soil is termed biostimulation. This procedure results in the stimulation of the growth and activity of the indigenous microorganisms present in the contaminated site through the addition of nutrients in order to accelerate the rate of natural biodegradation Nikolopoulou and Kalogerakis (2010). There exists extensive literature that have reported that high concentrations of petroleum hydrocarbon, containing around 80% carbon can lead to a rapid reduction in the concentration of inorganic nutrients present in the soil e.g. nitrogen and phosphorus, Alexander (1999). Nitrogen is an example of a nutrient that is found in terrestrial

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environments in many forms. It is an essential nutrient which supports soil microbial growth and activity, increasing the rate of microbial cell growth, reducing the lag phase of microbes, supporting a large microbial population and hence, increasing the rate of hydrocarbon degradation, Walworth *et. al.* (2007). Biostimulation often includes the addition of nutrients and electron acceptors such as (P, C, N and O_2) and represents an effective technology for restoring oil polluted and nutrient deficient sites, Li *et. al.* (2007). The main advantage of biostimulation is that enhanced biodegradation takes place by the native microbial communities which have already acclimatized to their environment. However care must be taken in the amount of nutrients added; for example the addition of excess quantities of nitrogen may result in inhibition of the soil microbial community, Chaillan *et. al.* (2006).

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

Soil Preparation and physicochemical analysis

Crude oil was collected from a flow station in Agbada 1 Aluu community and was analyzed for total hydrocarbon content (THC) and pH. Using a 10m soil auger, 30cm of loamy soil samples (sub-soil) were obtained directly from a farm land in Rumuekini community according to the procedures stated by the Food and Agricultural Organization of United Nations (FAO) (2007). The soil sample was analyzed for its total hydrocarbon content and pH.

Soil pH was measured using a pH meter (Model PHS-3C). The physico-chemical analyses was carried out using solvent extraction method which is in accordance with Standard Test Methods (ASTMD).

Plant preparation

Water lettuce was harvested from Taylor Creek in Bayelsa State by hand picking the plant during day time. Freshly harvested *P. stratiotes* were shredded in pieces using a standard table knife after washing with clean tap water following Ayotamuno *et al.* (2006). Next, the shredded *P. stratiotes* was oven-dried at 80°C and ground into powder form using a grinding mill. The presence of such minerals as carbon (C), nitrogen (N) and phosphorus (P) in the grounded water lettuce was determined to confirm the remediating properties of the water lettuce plant.

Soil contamination

Experimental design was used for this research where exactly 16 kg of loamy soil was air dried and sieved with a standard mesh. Equal quantities (1kg) of soil were contaminated with varying concentrations of crude oil (0, 20, 60 and 100ml) and was left undisturbed for 14 days to mimic a natural crude oil site. The various combinations were contained in a one (1) litre container and each combination was called a cell, thus making a total of sixteen (16) cells. These cells were amended with varying proportions of water lettuce powder form (0, 20, 40 and 60g). The content of each cell was thoroughly mixed to ensure even distribution of crude oil and the powdered plant. The experiment was allowed to commence and the containers were watered and mixed twice a week to provide sufficient oxygen and suitable environment for bacteria to grow, hence improving the soil aeration and thus providing sufficient oxygen required by the microbial community which in turn favor the growth of indigenous bacteria in the soil. The pH and total hydrocarbon content (THC) of all the 16 cells were monitored every four weeks for a period of 12 weeks. An ambient temperature range of 25^{o}C to 35^{o}C was maintained all through the period of experiment and this was achieved by covering all the entrances with nylon sheets.

Bioremediation analysis

Bioremediation assessment was done by comparing outputs of the baseline physicochemical analysis before and after crude oil soil contamination. Upon the introduction of the powdered *P. stratiotes* as amendments, soil pH and THC were determined at 0, 30, 60 and 90 days after contamination, THC percentage loss was also quantified.

Microbial analysis

Soil sample was collected into sterile re-sealable bags and transferred to the Microbiology Laboratory within 30 minutes for analyses (Prescott *et al.,* 2005). Distinct representative bacterial colonies were repeatedly transferred into freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 24 hours for purification of bacterial isolates. Similarly, distinct fungal colonies were sub cultured repeatedly on freshly prepared Potato Dextrose Agar plates for 72 hours for purification of fungal isolates (Prescott *et al.,* 2005).

Discrete colonies on the Nutrient Agar plates were aseptically transferred into 10% (v/v) glycerol suspension, well labeled and stored as stock cultures for maintenance of the bacterial isolates. While pure cultures of fungal isolates were sub cultured into Potato Dextrose Agar in bijou bottle for preservation of the fungal isolates (Prescott *et al.,* 2005).

RESULTS AND DISCUSSION

The results of investigations as shown in Table 1 indicate soil characteristics of both the control and crude oil contaminated soil using physical and biological treatments.

Table 1: Preliminary test

Parameters	soil	Crude oil	Water lettuce
pH	CS = 6.45 CCS = 7.70	4.05	7.75
THC (mg/kg)	CS = 197.32 CCS = 897.00	1399.32	434.80
Total Nitrogen (%)	0.16	0.22	2.32
Total organic Carbon (%)	4.99	99.9	6.96
Phosphorus (mg/kg)	49.1		28.9

CS = Control soil; **CCS =** Crude oil contaminated soil

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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: Results of Bioremediation of crude oil contaminated soil with water lettuce from week 0 to 12

Cells	Week 0		Week 4		Week 8		Week 12	
	pH	THC (mg/kg)	pH	THC (mg/kg)	pH	THC (mg/kg)	pH	THC (mg/kg)
1*	7.65	19.7	6.85	19.7	6.78	19.7	6.60	19.7
2	7.70	89.7	6.95	63.2	6.70	59.6	6.25	53.4
3	7.45	192.8	6.85	142.2	6.65	141.7	6.10	120.7
4	7.10	708.6	7.15	238.6	6.70	225.5	5.85	219.3
5*	7.05	19.7	6.45	19.7	6.35	19.7	5.60	19.7
6	7.05	107.6	7.00	77.1	6.80	50.2	6.75	49.7
7	7.20	376.7	7.15	172.6	7.05	151.1	6.90	137.2
8	7.40	358.8	7.30	295.5	7.15	249.8	7.05	205.8
9*	7.65	19.7	6.90	19.7	6.70	19.7	6.50	19.7
10	7.95	216.3	7.50	72.2	7.15	71.7	7.05	44.4
11	8.40	309.5	8.25	230.1	7.45	224.6	7.15	169.9
12	8.50	587.5	8.30	305.8	7.60	305.8	7.40	302.7
13*	7.80	19.7	7.65	19.7	7.05	19.7	7.20	-
14	8.70	237.7	8.65	72.9	8.45	53.8	7.65	38.5
15	9.55	245.3	8.70	224.3	8.35	200.4	8.05	171.3
16	9.55	364.2	8.60	358.8	8.55	295.8	8.10	291.5

Baseline studies

Results indicate that at week 0, the total culturable heterotrophic bacterial count (THB), total culturable heterotrophic fungal count (THF), total culturable hydrocarbon utilizing bacterial count (HUB) and total culturable heterotrophic fungal count (HUF) in the control set-up ranged from 1.0×10^4 cfu/g to 7.0×10^4 cfu/g, whereas the microbial count for natural attenuation (NA) ranged from 1.4×10^4 cfu/g to 7.0×10^4 cfu/g.

Table 3: Water lettuce week 0 (cfu/g)

S/N	Samples	THB	THF	HUB	HUF
1	Control	1.4×10^7	7.0×10^4	1.4×10^5	1.0×10^4
2	N/A	5.0×10^6	7.0×10^4	1.8×10^5	1.4×10^4
3	20g	1.4×10^8	1.5×10^5	3.7×10^5	1.9×10^4
4	40g	1.8×10^8	7.0×10^4	1.0×10^6	2.6×10^4
5	60g	2.3×10^8	2.6×10^5	1.0×10^6	3.0×10^4

Results indicate that week 12 microbial count for the control set-up ranged from 2.8 × 10^7 cfu/g to 7.8 × 10^5 cfu/g, while the microbial count for natural attenuation (NA) ranged from 1.8 × 10^5 cfu/g to 5.0 × 10^5 cfu/g.

Table 4: Water lettuce week 12 (cfu/g)

S/N	Samples	THB	THF	HUB	HUF
1	Control	2.8 ×10^7	4.0× 10^4	7.8 ×10^5	6.0 ×10^3
2	N/A	2.6× 10^6	1.8× 10^5	5.0 ×10^5	2.4 ×10^3
3	20g	4.2× 10^7	5.0× 10^4	6.8 ×10^5	3.0 ×10^3
4	40g	3.6× 10^8	9.0× 10^4	6.2 ×10^5	3.3 ×10^4
5	60g	6.6×10^8	3.3×10^5	6.4 ×10^5	4.8 ×10^4

The remediation of crude oil in the various cells was monitored with respect to the time at varying water lettuce concentration. This degradation was monitored by measuring the concentration of Total Hydrocarbon Content (THC) which was used as an indicator of remediation. It was observed that for 20, 60, 100ml crude oil contamination, the THC concentration fell with time at varying water lettuce concentration. The plots are shown in Fig. 1-3.

However, an attempt to understand the actual factor between time and water lettuce concentration that was responsible for remediation required that a 2-way Analysis of variance (ANOVA) be performed for the effect of time and the effect of water lettuce on the remediation process for the 20, 60, 100ml crude oil contamination. Using the excel data analysis tool and assuming a Null hypothesis (H_0) of no significant effect of remediation process and an Alternative hypothesis (H_1) of a significant effect on the remediation process. A significant effect is accepted when P-Value is less than 0.05 and a no significant value is accepted when P-Value is greater than 0.05.

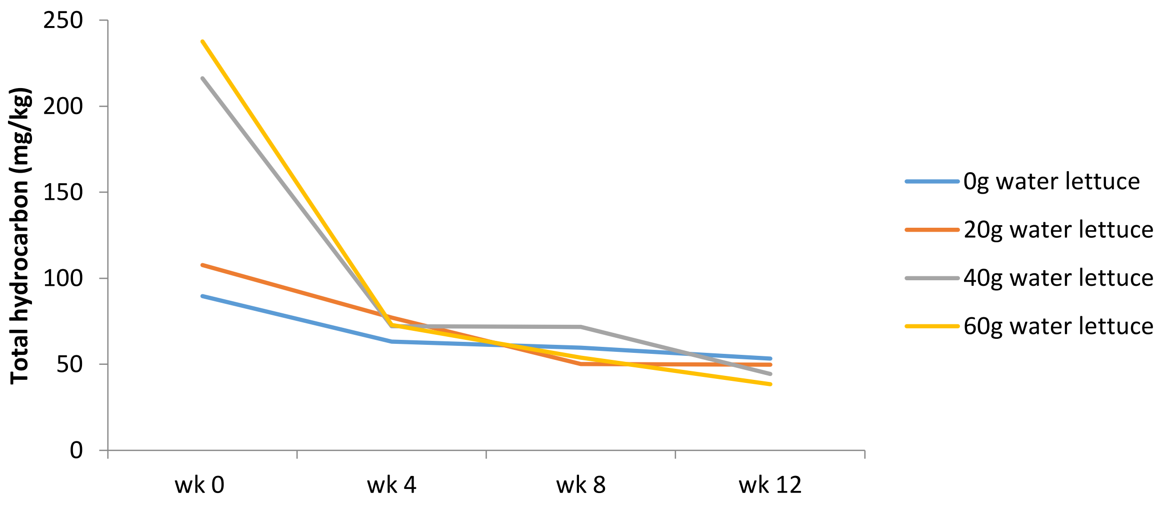

Fig. 1 Bioremediation of 20ml crude oil contaminated soil with varying amount of water lettuce

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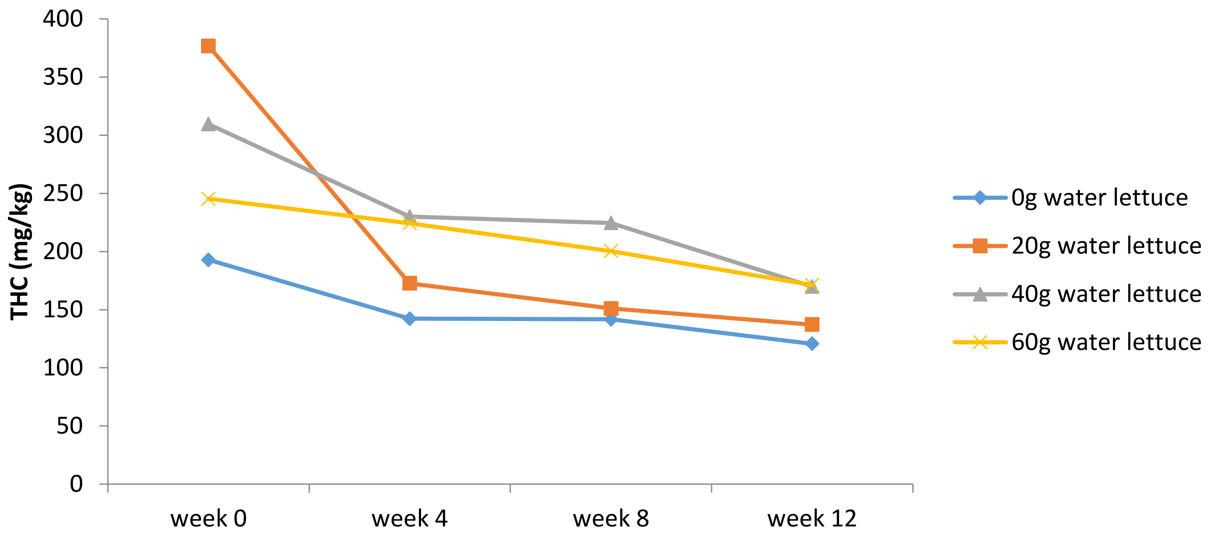

Fig. 2 Bioremediation of 60ml crude oil contaminated soil with varying amount of water lettuce

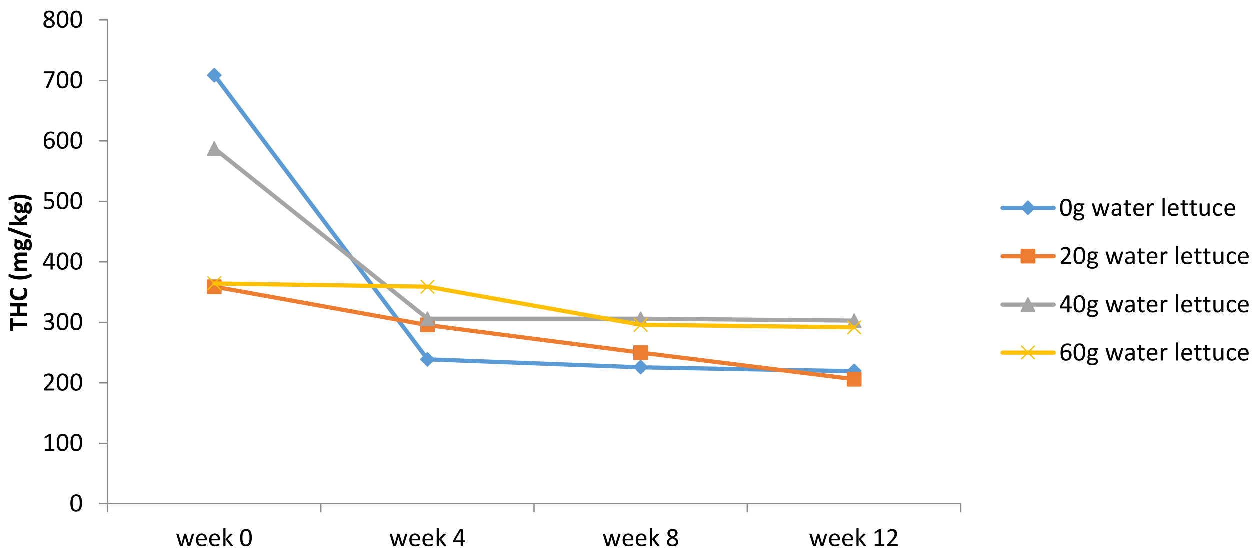

Fig. 3 Bioremediation of 100ml crude oil contaminated soil with varying amount of water lettuce

For 20ml crude oil contamination, it was observed that P-Value was less than 0.05 for the effects of time and greater than 0.05 for the effect of water lettuce. This implies that the time factor seem to play an important role in bioremediation than water lettuce contribution at 20ml crude oil contamination.

For 60ml crude oil contamination, P-Value was less than 0.05 for the effects of time and greater than 0.05 for the effect of water lettuce. This implies that the time factor also played an important role in bioremediation than water lettuce contribution at 60ml crude oil contamination.

For 100ml crude oil contamination, P-Value was less than 0.05 for the effects of time also less than 0.05 for the effect of water lettuce. This implies that both time factor and water lettuce played important role in bioremediation at 100ml crude oil contamination. Therefore, water lettuce helped in bio remediating soils contaminated with up to 100ml crude oil.

DISCUSSION

Microbial count

The higher counts of hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi recorded in all the *P. stratiotes* amended soil compared to the un-mended polluted soil (natural attenuation) might be due to the result of the presence of appreciable quantities of nitrogen (N) and phosphorus (P), especially high nitrogen content, which is a necessary nutrient for bacteria and fungi biodegradative activities, Adesodun and Mbagwu (2008). The results are in agreement with the finding of Abioye *et. al.* (2009), who recorded higher counts of HUB and HUF in used lubricating oil contaminated soil amended with brewery spent grain, banana skin and spent mushroom compost. The higher microbial population counts (Table 3 & 4) in crude oil contaminated soil amended with *P. stratiotes* is accompanied by significant crude oil biodegradation (Fig. 1-3), also indicating that the indigenous soil microbes utilized a portion of the carbon (C) supplied by the crude oil as a potential nutrient source.

The response of indigenous hydrocarbon utilizing bacteria to the bioremediation treatment was generally positive with higher microbial count occurring progressively as time elapsed (Table 3 & 4). The bacterial and fungal species exhibited ability to either degrade or utilize the different petroleum hydrocarbon. A study of a lubricant oil contaminated sandy loamy soil in Port Harcourt, documented the use of poultry manure to increase the number of indigenous hydrocarbon utilizing bacteria count significantly, Okolo *et. al*. (2005). This probably was due to the presence of additional nutrient in the organic matter amended soil which enhanced the indigenous microbe's degradation capabilities by stimulating the growth of microorganisms responsible for biodegradation of the pollutant.

Effect of crude oil contamination on soil pH during bioremediation

Soil pH value in crude oil contaminated soil was observed to be up to 7.70 and significantly higher than the control soil 6.45 ($p < 0.05$; Table 1). However, slight decrease in soil pH was witnessed in the control soil with increasing days after contamination. This suggests natural attenuation consistent with the work of Njoku *et. al.* (2009). Furthermore, *P. stratiotes* significantly decreased soil pH in crude oil contaminated soil with increasing days after contamination ($p< 0.05$). This is likely due to *P. stratiotes* production of low molecular weight organic acids that lower soil pH and improves soil fertility through enhanced nitrification and degradation of pollutants, Tang and Angela (2019b). The results from this work corroborates the work of Ayotamuno *et. al.* (2006) who showed that increased organic matter has lowered the soil pH in a model crude oil contaminated soil. This suggests that powdered *P. stratiotes* is an effective agent that can be used to decrease the pH of contaminated soil and improve soil properties.

Effect of crude oil contamination on soil THC during bioremediation

Total hydrocarbon content concentration in the crude oil contaminated soil for the three assayed treatment was significantly higher than the control ($p<0.05$). Total hydrocarbon content concentration reduced with increasing days after contamination. Upon soil amendment with *P. stratiotes*, THC

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significantly decreased with increasing days after contamination across all treatments. THC was lowest in soil treated with 60 g of powdered *P. stratiotes* at 90days after contamination (38.5), whereas it was highest in crude oil contaminated soil at first day of contamination (708.6). This result indicates that the use of *P. stratiotes* in the treatment of crude oil contaminated soil can aid in THC reduction. Total hydrocarbon content significantly decreased at 30, 60 and 90 days after contamination ($p < 0.05$; Table 2). Contaminated soils amended with 40 g of powdered *P. stratiotes* had the highest THC loss (30%), following this was contaminated soil amended with 60g (29%). Compared with other treatments, crude oil contaminated soil with no treatment showed the least total hydrocarbon content loss (20%). Although soil total hydrocarbon content can be reduced by natural attenuation, it takes a longer time comparison with the use of organic matter as stimulants. This result corroborates the findings of Akpe *et. al.* (2015), who demonstrated high total hydrocarbon content loss in hydrocarbon polluted soils within 56 days after biostimulation with plantain peels and guinea corn shaft. They suggested that the high THC loss recorded was due to the presence of nutrients from organic wastes.

CONCLUSION

This study will contribute to knowledge in the following ways: Knowledge on its use in supplying limiting nutrients necessary for bioremediation of crude oil impacted media such as loamy soils. Provide information that this cost effective manure can be harnessed into preserved powdery forms and be used for bioremediation by Oil and Gas Companies.

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 demonstrated that powdered *P. Stratiotes* is an effective agent in remediating a crude oil contaminated soil. This was buttressed by the significant decrease in soil pH and total hydrocarbon content (THC) over time. This environmental friendly remedial action geared towards sustainable development in the Niger Delta.

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