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Bioremediation of a crude oil contaminated soil using water hyacinth (*Eichhornia crassipes)*

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Abstract

Crude oil contamination is a growing environmental concern in most oil processing regions of the world. This study assessed the efficacy of powdered *Eichhornia crassipes* (water hyacinth) as potential bio stimulant in the remediation of crude oil contaminated soil using three test treatments (20ml, 60ml and 100ml) and a control. The remediation process was monitored by assaying the total hydrocarbon content (THC) and soil pH of the soil on 16 cells for 90 days. However, there was a significant reduction ($p < 0.05$) in soil pH and THC with the introduction of powdered *E. crassipes* at different concentrations. Contaminated soil amended with 40g of *Eichhornia crassipes* had the highest THC loss of (41%), following this was contaminated soil amended with 60g of *Eichhornia crassipes* (31%) on the final day of remediation. The total culturable hydrocarbon utilizing bacteria in *Eichhornia crassipes* treated polluted soil increased from 1.4×10^6 Cfu/g to 8.2×10^5 Cfu/g while the total culturable hydrocarbon utilizing fungal counts in the *Eichhornia crassipes* treatment increased from 1.2×10^4 to 4.5×10^4 Cfu/g from the zero hour to the 90th day of the study. The time effect of the remediation process had P-value less than 0.05 for 20, 60 and 100ml crude oil contamination signifying that the time factor played important role in the remediation process. The use of organic nutrient sources such as *Eichhornia crassipes* nutrient powder is of good use as source of limiting nutrient needed for bioremediation of crude oil impacted medium.

Keywords: Bioremediation; Crude oil; Hydrocarbon utilizing bacteria; *Eichhornia crassipes*; Hydrocarbon utilizing fungi; Total hydrocarbon content.

1. Introduction

Before the I960's, little attention was paid on the effects of crude oil spillages. However, three notable incidence sparked international attention namely: the Torrey Canyon wreck of 1967 (off the coast of England), the 1989 Exxon Valdez spill off the coast of Alaska and the 1991 massive release of crude oil during the Gulf war. These led to an increase in environmental research and the adoption of several national and international control practices such as the Oil Pollution Act of 1990. Despite these controls, environmental hazards and petroleum contamination remains a prevalent issue. Accidental large-scale oil spills present a significant volume of contaminants around the world. In addition to such catastrophic accidents, small spills from low-level continuous seeps, offshore exploration, tank washings and other related activities can also cause a variety of environmental problems because of the presence of toxic compounds [1]. The toxic compounds in crude oil consist of a wide range of hydrocarbons, nitrogen-oxygen compounds, sulfur compounds and heavy metals, which may cause acute and chronic effects on flora and fauna [2], thus remediation of these pollutants is vital. Also, contaminated soils could pose potential risks for human habitation and the establishment of agricultural steads due to increased exposure and the associated bio-accumulation of harmful hydrocarbon compounds from underground water and agricultural products [3]. Hence, this research is highly relevant in the Niger

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Delta region of Nigeria, where frequent oil-spills arising from crude-oil exploration and development activities have devastated farm lands and other agricultural settlements [4].

Remediation process like, land farming, soil washing, vapour extraction, thermal desorption, composting, incineration and the use of oil booms and solidification have been used for the clean-up of oil contaminated sites; however they are disruptive, labour intensive and relatively expensive processes [5]. Bioremediation which is the use of micro-organisms through the addition of fertilizers to improve their population or the direct addition of micro-organisms have been studied as means of remediating the harmful effect of crude oil pollution. 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 indigenous soil micro-organisms carry out the process of biodegrading of the crude with time. Existing bioremediation techniques are natural attenuation, bioaugmentation and biostimulation [6]. These techniques rely on the underlying functionality of increasing the soil organic matter contents and decreasing soil pH for improved outcomes. In some instances, organic matter is added directly to the contaminated soil or indirectly in compost form as a biostimulant [3]. Often in contaminated soils, nutrients, aside from carbon are depleted, therefore in order to increase the efficiency of bioremediation, the addition of nutrients such as nitrates and phosphates to enhance the growth of hydrocarbonoclastic microbes is crucial [7] and this is termed bio-stimulation. Despite the many positive impacts of petroleum hydrocarbons to human industrialization and activity, environmental contamination by petroleum-based substances represent a major cause of marine and terrestrial pollutions. Among the various remediation technologies, bioremediation is considered a clean, cost-effective and environmentally friendly approach, unlike other physical and chemical methods, it does not lead to secondary contamination, generally resulting in the complete mineralization of hydrocarbons.

This research work investigated on the possibility of using powdered water hyacinth (*Eichhornia crassipes*) as a source of nutrient in bioremediation process of soil contaminated with crude oil and the possibility of it serving as a soil conditioner.

2. Materials and methods

2.1. Collection and Preparation of Materials

Water hyacinth was harvested from Taylor Creek in Bayelsa State by hand picking the plant during the day time. Freshly harvested *E. crassipes* was shredded in pieces using a standard table knife after washing with clean tap water following Ayotamuno *et al.* [8]. The sample was oven dried at 80°C and ground into powder using a grinding mill. The presence of such minerals as carbon (C), nitrogen (N) and phosphorus (P) in the grounded water hyacinth was determined to confirm the remediating properties of the water hyacinth plant.

Bonny light crude oil was collected from a flow station in Aluu community and was analyzed for the following parameters: Total Organic Carbon (TOC), Volatile Matter, Total Hydrocarbon Content (THC), Total Kjeldahl Nitrogen (TKN), Ash Content and pH.

Loamy soil samples (subsoil) were collected from a farm land in Rumuekini community according to the procedures stated by the Food and Agricultural Organization of United Nations [9]. The soil samples were analyzed for its physico-chemical properties as follows: Total Kjeldahl Nitrogen (TKN), Total Organic Carbon (TOC), Volatile matter, Ash content, Phosphate, Potassium, Nitrate, Sodium, Calcium, Magnesium, Organic matter and pH.

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. Remediation Method

Crude oil contaminated soil samples were simulated in the Green house using suitable loamy soil and heavy crude oil. Equal quantities (1kg) of soil were mixed with varying concentrations of heavy crude oil (0, 20, 60 and 100ml) and was left undisturbed for 14 days to mimic natural crude oil site. After which different concentrations of the contaminated soil samples were amended with varying proportions of water hyacinth (0, 20, 40 and 60g). The various combinations were contained in a one (1) liter container and each combination was called a cell, thus making a total of sixteen (16) cells. The content of each cell was thoroughly mixed to ensure even distribution of crude oil and powdered *E. crassipes* 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. 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°C to 35°C was maintained all through the period of experiment.

2.3. Microbial analysis

Soil sample was collected into sterile re-sealable bags and transferred to the Microbiology Laboratory within 30 minutes for analyses [10]. 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 [10].

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 [10].

3. Results and discussion

Table 1 indicate the results from the analysis of the soil characteristics and the outcome of various treatments employed using physical and biological treatments. Also, the physico-chemical properties of the uncontaminated soil, crude oil and processed water hyacinth before contamination were equally indicated.

Table 1 Preliminary test

Parameters	Uncontaminated soil	Water hyacinth	Crude oil
pH	6.45 CCS = 7.70	7.00	4.05
Total organic carbon (TOC)%	4.99	9.87	99.90
Volatile matter %	5.99	92.35	100.0
Total Hydrocarbon Content (THC) mg/kg	197.32 CCS = 897.00	1242.35	1399320.00
Total Kjeldahl Nitrogen (TKN)%	0.161	2.87	0.22
Ash Content %	-	7.65	-
Organic matter %	5.98	-	-
Nitrate (NO_3) mg/kg	64.22	-	-
Phosphate (PO_4)mg/kg	49.13	95.37	-
Potassium (mg/kg)	39.98	-	-

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

The outcome of the remediation effects of different quantities of processed powdered water hyacinth on the various concentrations of crude oil contaminated soil (CCS) with respect to two (2) parameters using: 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 hyacinth 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*	9.45	19.7	8.35	19.7	7.20	19.7	7.20	19.7
2	8.55	107.6	8.30	82.5	6.80	49.7	6.60	44.4

3	8.50	210.8	8.15	229.3	6.75	215.2	6.65	124.6
4	8.45	516.1	7.95	373.6	6.60	340.8	6.20	247.5
5*	7.55	19.7	7.50	19.7	6.55	19.7	6.25	19.7
6	7.50	148.0	7.15	64.5	7.05	47.1	6.75	38.5
7	7.55	318.4	7.45	242.2	7.25	172.6	7.05	158.3
8	7.55	766.9	7.40	269.1	7.20	248.0	7.05	227.8
9*	7.75	19.7	7.65	19.7	6.85	19.7	6.65	19.7
10	8.30	215.3	7.55	103.6	7.05	99.1	6.65	92.8
11	7.55	529.5	7.40	252.1	7.30	202.3	6.90	200.4
12	7.55	560.6	7.30	512.1	7.30	396.4	7.20	290.6
13*	7.85	19.7	7.55	19.7	7.00	-	6.85	-
14	8.65	304.9	7.95	88.8	7.25	87.0	6.95	53.6
15	7.95	748.9	7.65	347.1	7.60	263.2	7.60	195.5
16	7.95	865.6	7.50	485.7	7.45	428.7	7.10	335.9

3.2. Baseline studies

Table 3 shows 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 were 1.6×10^7 cfu/g, 9.0×10^4 cfu/g, 1.7×10^5 cfu/g and 1.4×10^4 cfu/g respectively. Microbial count for natural attenuation (NA) were 7.0×10^6 cfu/g, 2.5×10^5 cfu/g, 2.0×10^5 cfu/g and 3.0×10^4 cfu/g respectively.

Table 3 Water hyacinth week 0 (cfu/g)

S/N	**Samples**	**THB**	**THF**	**HUB**	**HUF**
1	Control	1.6×10^7	9.0×10^4	1.7×10^5	1.4×10^4
2	N/A	7.0×10^6	2.5×10^5	2.0×10^5	3.0×10^4
3	20g	3.4×10^7	7.0×10^4	6.0×10^5	2.9×10^4
4	40g	2.8×10^8	1.1×10^5	1.4×10^6	3.2×10^4
5	60g	6.4×10^8	-	6.6×10^5	1.2×10^4

Table 4 Water hyacinth week 12 (cfu/g)

S/N	Samples	THB	THF	HUB	HUF
1	Control	3.6×10^7	5.0×10^4	9.4×10^5	8.0×10^3
2	N/A	3.0×10^6	1.0×10^5	7.1×10^5	9.0×10^3
3	20g	6.4×10^7	7.0×10^4	7.2×10^5	2.5×10^4
4	40g	4.8×10^8	1.6×10^5	5.8×10^5	4.5×10^4
5	60g	8.1×10^8	8.0×10^4	8.2×10^5	3.0×10^4

Table 4 shows week 12 had 3.6×10^7 cfu/g, 5.0×10^4 cfu/g, 9.4×10^5 cfu/g and 8.0×10^3 cfu/g respectively, while microbial count for natural attenuation (NA) were 3.0×10^6 cfu/g, 1.0×10^5 cfu/g, 7.1×10^5 cfu/g and 9.0×10^3 cfu/g respectively.

4. Discussion

The remediation of crude oil in the various cells was monitored by measuring the concentration of Total Hydrocarbon Content (THC) over time which was used as an indicator of remediation. It was discovered that at 20, 60 and 100ml crude oil contamination, the THC concentration decreased with time at varying water hyacinth concentration. The plots are shown in Figures 1-3.

A 2-way Analysis of variance (ANOVA) was used to evaluate the effect of time and the effect of water hyacinth on the remediation process for 20, 60 and 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. Tables 5-7 give summary results of the Excel 2 Factor ANOVA for the effect of both time and water hyacinth for 20, 60 and 100ml crude oil contamination. The time factor showed a significant effect while the water hyacinth did not show much effect.

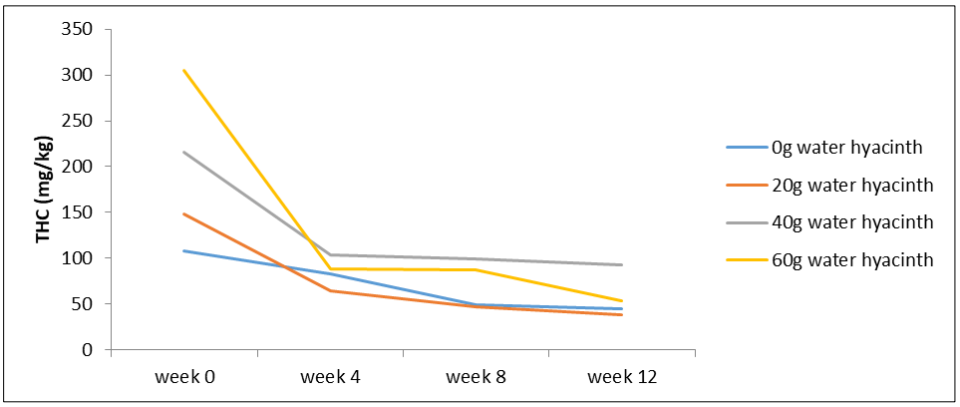

Figure 1 Weekly changes 20ml of total hydrocarbon content in contaminated soil amended with water hyacinth

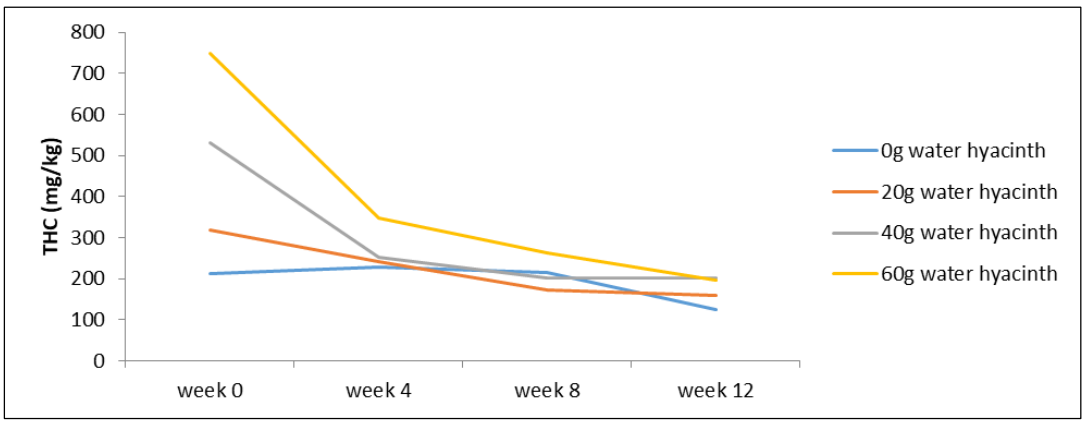

Figure 2 Weekly changes 60ml of total hydrocarbon content in contaminated soil amended with water hyacinth

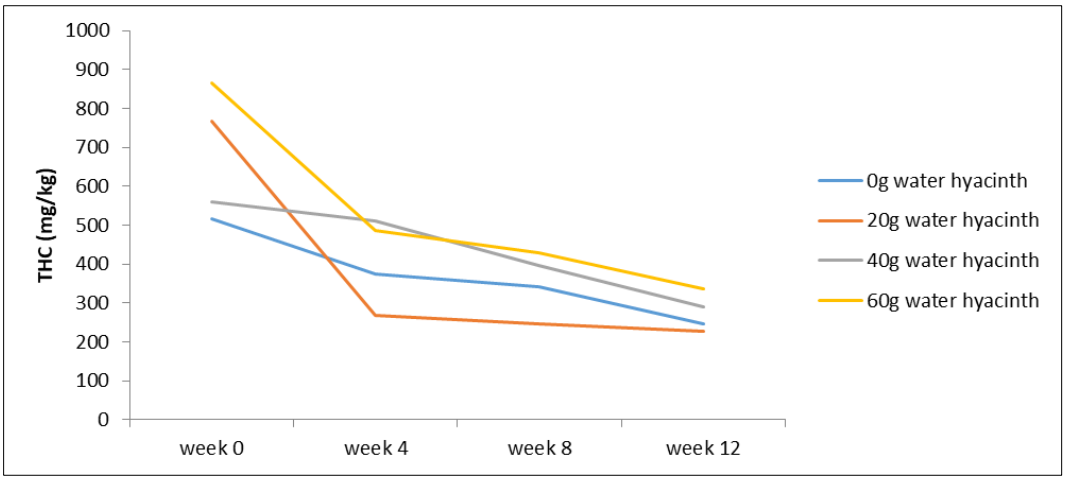

Figure 3 Weekly changes 100ml of total hydrocarbon content in contaminated soil amended with water hyacinth

4.1. Weekly variation at 20ml of Total hydrocarbon content

The THC in the *Eichhornia crassipes* amended crude oil contaminated soil was highest at 60g which ranged from 304.9 mg/kg at week 0 to 53.6 mg/kg on the final day of remediation. At week 12, the highest THC loss was at 40g with 41% while the least was at 20g with 17% loss.

4.2. Weekly variation at 60ml of Total hydrocarbon content

At 60g the THC in the *Eichhornia crassipes* amended crude oil contaminated soil reduced at the zero hour from 748.9 mg/kg to 195.5 mg/kg at the 90th day of study. The highest THC loss was at 40g while the least was at 0g, with 30% and 18% loss respectively on the 90th day of remediation.

4.3. Weekly variation at 100ml of Total hydrocarbon content

The THC in the *Eichhornia crassipes* amended crude oil contaminated soil decreased from 865.6 mg/kg to 335.9 mg/kg at 60g in the final day of remediation. At week 12, the highest THC loss was at 60g with 31% while the least was at 20g with 21% loss.

The results gotten from the weekly variation of Total hydrocarbon content in *Eichhornia crassipes* against the treatment shows that bioremediation occurred more at the highest level of nutrient supply across the three treatment levels, which in turn implies that the higher the nutrients in a media the more the degradation of pollutants.

Table 5 Anova for 20 ml crude oil contamination

Anova: Two-Factor without replication				
SUMMARY	***Count***	***Sum***	***Average***	***Variance***
0g	4	284.2	71.05	877.6833
20g	4	298.1	74.525	2516.336
40g	4	510.8	127.7	3430.18
60g	4	534.3	133.575	13307.43
week 0	4	775.8	193.95	7444.483
week 4	4	339.4	84.85	262.27
week 8	4	282.9	70.725	690.0692
week 12	4	229.3	57.325	597.9292
ANOVA				

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	13479.67	3	4493.224	2.994466	0.088049	3.862548
Week	46890.3	3	15630.1	10.41653	0.002764	3.862548
Error	13504.58	9	1500.509			
Total	73874.56	15				

Table 6 Anova for 60ml crude oil contamination

Anova: Two-Factor without replication				
SUMMARY	***Count***	***Sum***	***Average***	***Variance***
0g	4	779.9	194.975	2263.443
20g	4	891.5	222.875	5398.663
40g	4	1184.3	296.075	24789.5
60g	4	1554.7	388.675	61517.03
week 0	4	1807.6	451.9	56727.41
week 4	4	1070.7	267.675	2890.843
week 8	4	853.3	213.325	1423.703
week 12	4	678.8	169.7	1257.367

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
sample	89941.95	3	29980.65	2.782972	0.10215	3.862548
week	184949.9	3	61649.96	5.722695	0.017983	3.862548
Error	96956.01	9	10772.89			
Total	371847.8	15				

Table 7 Anova for 100ml crude oil contamination

Anova: Two-Factor without replication				
SUMMARY	*Count*	*Sum*	*Average*	*Variance*
0g	4	1478	369.5	12405.35
20g	4	1511.8	377.95	67520.82
40g	4	1759.7	439.925	14654.69
60g	4	2115.9	528.975	54174.05
week 0	4	2709.2	677.3	27696.46

week 4	4	1640.5	410.125	12444.2
week 8	4	1413.9	353.475	6262.329
week 12	4	1101.8	275.45	2311.817

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
sample	65042.71	3	21680.9	2.405968	0.034669	3.862548
week	365163	3	121721	13.50759	0.00111	3.862548
Error	81101.71	9	9011.301			
Total	511307.4	15				

The following conclusion can be deduced from the Tables 5-7

At 20ml crude oil contamination for water hyacinth, it was observed that the P-value was less than 0.05 for the effects of time 2.76×10^{-3} and P-value greater than 0.05 for the effect of water hyacinth was 0.088049. The Alternative hypothesis was accepted for the effect of time while the Null hypothesis was accepted for the effect of water hyacinth, respectively. This implies that the time factor seem to play an important role in bioremediation than water hyacinth contribution at 20ml crude oil contamination.

At 60ml crude oil contamination for water hyacinth, the P-value was less than 0.05 for the effects of time 0.017983 and P-value greater than 0.05 for the effect of water hyacinth was 0.10215. The Alternative hypothesis was accepted for the effect of time while the Null hypothesis was accepted for the effect of water hyacinth, respectively. This implies that the time factor seem to play an important role in bioremediation than water hyacinth contribution at 60ml crude oil contamination.

At 100ml crude oil contamination for water hyacinth, the P-value was less than 0.05 for the effects of time 1.11×10^{-3} and P-value less than 0.05 for the effect of water hyacinth was 0.034669. The Alternative hypothesis was accepted for the effect of time and water hyacinth. This implies that both time factor and water hyacinth played important role in bioremediation at 100ml crude oil contamination. Therefore, water hyacinth helped in bio remediating soils contaminated with up to 100ml crude oil. It helped in amending the soil by adding nutrients to the contaminated soil.

Managing water hyacinth which is a problematic plant species in the Nigerian water ways by putting it into effective use such as in bioremediation will definitely be a welcome development in Sub-Saharan Africa and other parts of the world where the plant posses' ecological problems and cheaper remediation technologies are regularly sought for.

The response of indigenous hydrocarbon utilizing bacteria to the bioremediation treatment was generally positive with higher population occurring progressively as time elapsed. Odokuma and Dickson [11] in a bioremediation study in a mangrove swamp in new Calabar River, Rivers State was able to scale-up the hydrocarbon-utilizing bacteria using NPK fertilizer. The NPK fertilizer increased the bio load of the polluted mangrove soil from 1.5×10^{3} to 1.5×10^{9} Cfu/g.

5. Conclusion

The long term aim of bioremediation design is to develop a cost effective and environmentally friendly approach. Water hyacinth powder apart from being cost effective is also environmentally friendly.

The results of this study have shown that biological treatment using water hyacinth (*Eichhornia crassipes*) nutrient are effective in the supply of limiting nutrients necessary for bioremediation of crude oil contaminated soil. This was buttressed by the significant decrease in soil pH and total hydrocarbon content (THC), in concert with high THC loss.

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. These environmental friendly remedial actions are geared towards sustainable development in the Niger Delta.

Compliance with ethical standards

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Disclosure of conflict of interest

Author declare that there are no conflicts of interest related to this research article.

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