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Bio-remediation of a crude oil contaminated soil using water hyacinth (*Eichhornia crassipes*)

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ABSTRACT

The effect of water hyacinth on the remediation of crude oil contaminated soil was studied ex-situ for 1.5, 3.0 and 4.5% crude oil contaminated soil (Heavy Crude) obtained from the Niger Delta region. The volume of the different percentages of crude oil contamination was equivalent to 3.17, 6.35 and 9.70l/m^2 of land area respectively. The remediation process was followed by monitoring the THC of the soil with time on 16 cells. After a period of 10 weeks, bean seeds were planted on the remediated soil to observe if these various cells would sustain plant growth for the first 15 days. Results obtained were analysed with a 2- Factor Analysis of Variance Excel tool for data analysis. The effect of water hyacinth on the remediation process had P-values greater than 0.05 at 1.5 and 3% which indicated that water hyacinth application may not be necessary for remediating crude oil contaminated soil at 1 and 3%. However, the P- value less than 0.05 for crude oil contaminated soil at 4.5% which indicated that the water hyacinth may be necessary for remediating crude oil contaminated soil above 3 - 4.5%. Also, the time effect of the remediation process had P-value less than 0.05 for 1.5, 3.0 and 4.5% crude oil contamination signifying that the time factor play important role in the remediation process.

INTRODUCTION

Increased petroleum exploration activities in the Niger Delta have resulted in an unprecedented release of crude oil, polluting the land and water sources. Also, illegal tampering of well heads, flow lines, pipelines, manifolds and flow stations have contributed to the total amount of crude oil entering the environment. With these frequent reports of oil spillages in Niger Delta, there is need to seek for a cost effective method for remediation of crude oil contaminated soil.

Crude oilwhen spilled on land affects the physicochemical properties of the soil such as temperature, structure, nutrient status and pH. [1]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 [2]. 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.

The process of crude oil clean up on land has been extensively researched upon [3]. Remediation processes like, Landfarming, Soil Washing, Vapour Extraction, Thermal Desorption, Composting and many others are either expensive or not environmentally sound. Bioremediation, the use of micro-organisms via 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 hydrocarbons 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. [4] [5] and [6] observed that soil naturally contain adequate diversity of microbes and the capacity to degrade saturates and light end aromatics (the mobile fractions).

Furthermore, concentration and composition of hydrocarbons, nutrients, oxygen, moisture and temperature control the rate of degradation [7][8][9][10] and [11].However, the process of biodegradation can be accelerated in the presence of nutrients such as nitrogen, potassium, phosphorus etc. In many cases these nutrients are supplied in chemicals via fertilizers which in many occasions are easily washed away after a heavy rainfall.

The process of bio-remediation using water hyacinth grass (Eichorniacrassipes) as a nutrient source (bio-fertilizer), offers an alternative measure which would not only be effective in the regeneration of the site and affordable but, in addition encourage local participation in clean-up programs because it is environmentally friendly. This research work investigated on the possibility of using water hyacinth as a source of nutrient in bioremediation process of soil contaminated with heavy crude oil and also the possibility of it serving as a soil conditioner.

MATERIALS AND METHODS

2.1 Collection and Preparation of Materials

Water hyacinth plant was harvested from a river in Bayelsa State. It was dried and ground into powder using a grinding mill. The presence of such minerals as carbon, nitrogen and phosphorus in the ground water hyacinth was determined to confirm the remediating properties of the water hyacinth grass.

Crude oil samples were collected from a flow station in Agbada 1 community and were analysed 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 Mgbuoba community. The soil samples were analysed for its physio-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: Treatment and Analysis

Crude oil contaminated soil samples were simulated in the laboratory using suitable loam soil and heavy crude oil. Because soil contaminated with more than 5% Total Petroleum Hydrocarbon by weight of crude oil do not readily degrade [12], thus remediation was only studied at 1.5, 3.0, and 4.5% crude oil contaminated soil.This heavy crude oil contamination was equivalent to 3.17, 6.35 and 9.70*litres/m*^{2} of land area, respectively.Also, because bioremediation is not effective below 150 – 250mm depth [12], the container for the bioremediation purpose contained crude oil contaminated soil not exceeding 150mm.

2.3: Remediation Method

Equal quantities (1kg) of soil were mixed with varying concentrations of heavy crude oil (0, 1.5, 3.0 and 4.5%). The different concentrations of the contaminated soil samples were mixed with varying proportion of water hyacinth (0, 20, 40 and 60g). The various combinations were contained in a 1.5 litre 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 water hyacinth.

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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 two weeks. An ambient temperature range of 25^{o}C to 35^{o}C was maintained all through the period of experiment.

RESULTS AND DISCUSSION

The results of investigations as shown in Table 1 indicate soil characteristics and the outcome of various treatments employed viz: physical and biological treatments. Also, the physio-chemical properties of the Soil, Crude Oil, and Water Hyacinth beforecontamination are equally indicated.

Table1: PRELIMINARY TEST (characteristics of uncontaminated Soil, processed Water Hyacinth and Raw Crude Oil)

Parameters	**Uncontaminated Soil**	**Water hyacinth**	**Crude Oil**
pH	7.94	8.75	9.82
Total organic carbon (TOC)	3.36	13.2	20.52
Volatile Matter %	3.60	69.90	99.50
Total Hydrocarbon content (THC)	1.00	14.00	31,800 mg/kg
Total Kjeldahl Nitrogen (TKN) %	0.007	0.77	0.14
Ash Content %	96.4	30.1	0.5
Organic Matter (%)	5.0		
Nitrate (NO_3) (mg/100g)	22.06		
Phosphate (PO_4) (mg/100g)	1.73	575 mg/kg	
Potassium (mg/100g)	106.5		
Sodium (mg/100g)	143.5		
Calcium (mg/100g)	462.0		
Magnesium (mg/100g)	238.0		

3.1: Effects of processed Water Hyacinth (Nutrient) on physio-chemicalparameters of the soil.

The results of the remediation effects of different quantities of processed Water Hyacinth on the various concentrations of contaminated Soil with respect to two parameters viz: pH and Total Hydrocarbon (THC) at different stages of remediation are shown in Tables2.

Table 2: Results of Bioremediation of crude oil polluted soil with waterhyacinth from week 0 toweek 10

Cells	**Week 0**		**Week 2**		**Week 4**		**Week 6**		**Week 8**		**Week 10**	
	pH	**THC (mg/kg)**	**pH**	**THC (mg/kg)**	**pH**	**THC (mg/kg)**	**pH**	**THC (mg/kg)**	**pH**	**THC (mg/kg)**	**pH**	**THC (mg/kg)**
1*	7.94	1.00	7.90	1.00	7.80	1.00	7.70	1.0	7.64	1.0	7.51	1.0
2	7.84	120	7.86	90	7.62	90	7.54	80	7.42	70	7.21	60
3	7.74	220	7.83	200	7.99	190	7.95	180	8.22	160	8.31	140
4	7.92	410	7.98	400	7.90	380	7.85	320	7.72	260	7.69	220
5*	7.83	1.0	8.44	1.00	8.48	1.00	8.46	1.00	8.40	Nil	8.56	Nil
6	7.82	130	8.53	120	8.36	100	8.32	80	7.50	60	8.53	30
7	7.99	230	8.56	220	8.28	160	8.24	140	7.98	120	7.73	100
8	7.95	420	8.52	410	8.36	300	8.30	260	7.92	240	8.00	180
9*	7.99	1.00	8.71	1.00	8.68	1.00	8.61	1.00	8.46	1.00	8.39	1.00
10	8.01	140	8.77	120	8.42	80	8.38	40	8.29	30	8.50	20
11	8.06	235	8.71	220	8.38	140	8.35	130	8.24	110	8.61	80
12	8.07	425	8.62	415	8.49	315	8.42	220	8.25	180	8.79	140
13*	8.10	1.00	8.84	1.00	8.85	1.00	8.78	1.00	8.30	Nil	9.09	Nil
14	8.05	145	8.84	140	8.62	60	8.58	40	8.74	10	9.28	Nil
15	8.02	240	8.89	220	8.63	180	8.60	160	8.77	108	8.93	60
16	8.02	430	8.82	420	8.58	320	8.54	240	8.15	220	8.70	140

DISCUSSION

The remediation of crude oil in the various cells was monitored with respect to the time at varying water hyacinth concentration. This degradation was monitored by measuring the concentration of Total Hydrocarbon Content (THC) which was used as an indicator of remediation. It was found out that for 1.5, 3.0 and 4.5% crude oil contamination, the THC concentration was observed to fall with time at varying water hyacinth concentration. The plots are shown in Figures 1 –3.

However, an attempt to understand the actual factor between time and water hyacinth 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 hyacinth on the remediation process for the 1.5, 3.0 and 4.5% 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 3 -5 give summary results of the Excel 2 factor ANOVA for the effect of both time and water hyacinth for 1.5, 3 and 4.5% crude oil contamination.The time factor showed a significant effect while the water hyacinth do not seem to show any effect

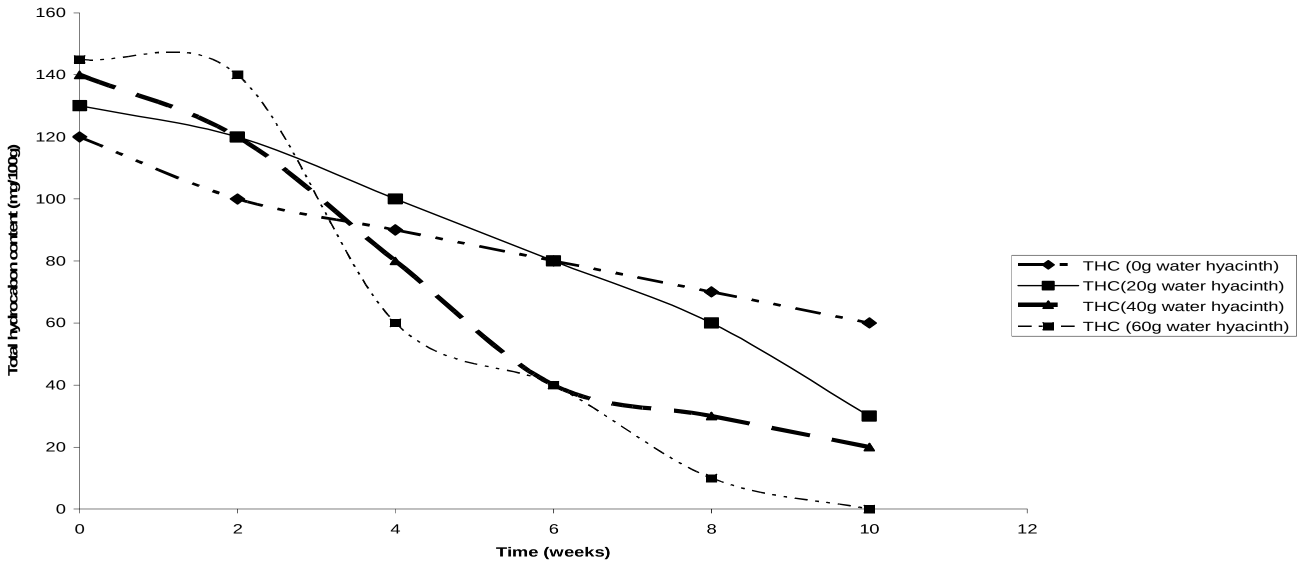

Fig. 1 BIOREMEDIATION OF 1.5% CRUDEOIL POLLUTED SOIL WITH VARYING AMOUNT OF WATER HYACINTH

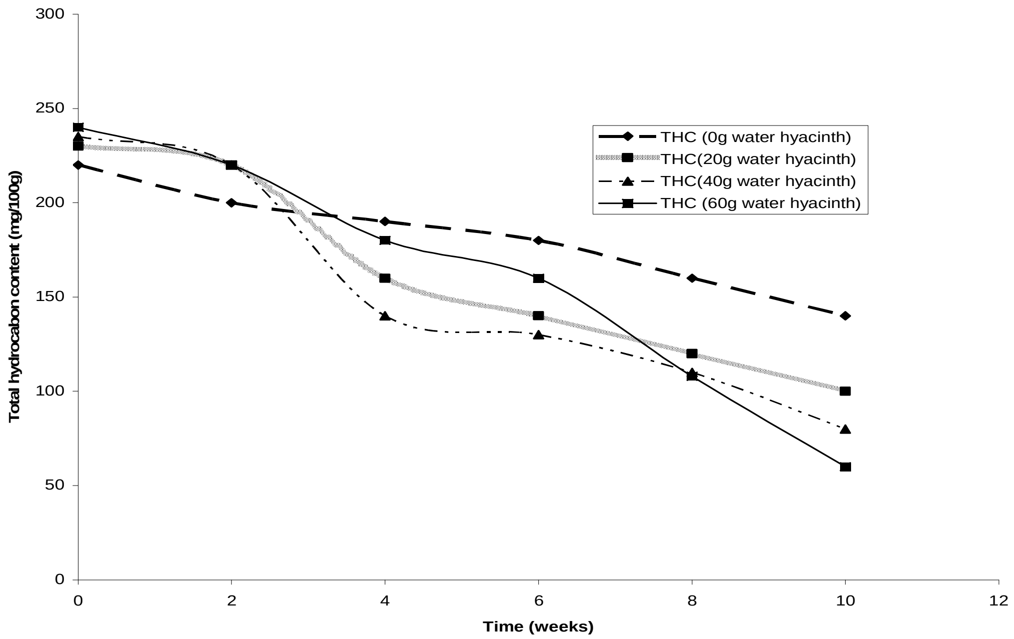

Fig 2 BIOREMEDIATION OF 3.0% CRUDEOIL POLLUTED SOIL WITH VARYING AMOUNT OF WATER HYACINTH

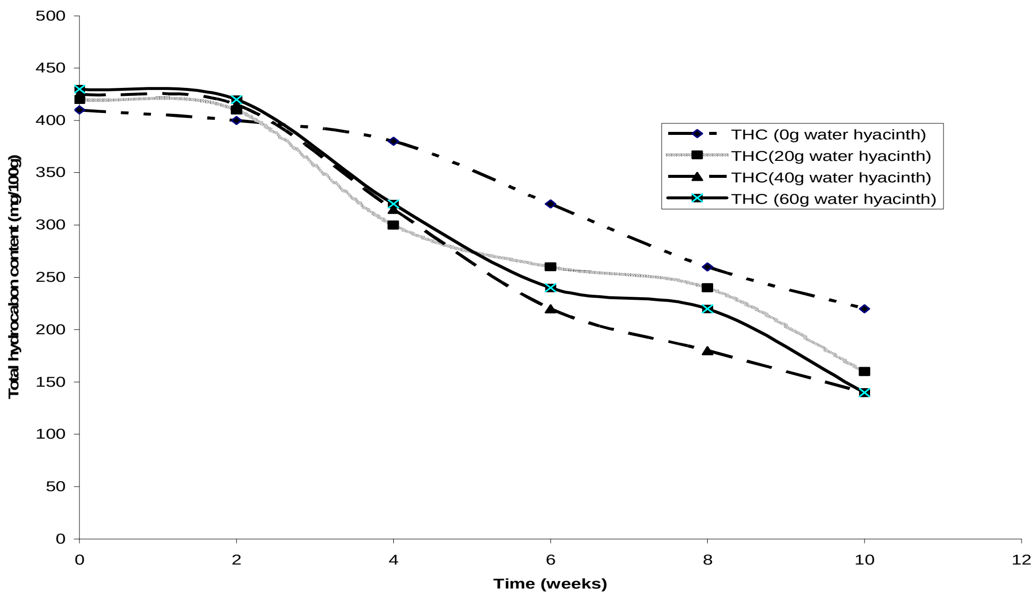

Fig. 3 BIOREMEDIATION OF 4.5% CRUDEOIL POLLUTED SOIL WITH VARYING AMOUNT OF WATER HYACINTH

Table 3: Anova for 1.5% crude oil contamination

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Week 0	4	535	133.75	122.9167
Week 2	4	480	120	266.6667
Week 4	4	330	82.5	291.6667
Week 6	4	240	60	533.3333
Week 8	4	170	42.5	758.3333
Week 10	4	110	27.5	625
0g water hyacinth	6	520	86.66667	466.6667
20g water hyacinth	6	520	86.66667	1426.667
40g water hyacinth	6	430	71.66667	2496.667
60g water hyacinth	6	395	65.83333	3984.167

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	***P-value***	*F crit*
Weeks (time)	36105.21	5	7221.042	18.78645	**5.58E-06**	2.901295
Water hyacinth	2028.125	3	676.0417	1.758808	**0.198119**	3.287383
Error	5765.625	15	384.375			
Total	43898.96	23				

Table 4: Anova for 3.0% crude oil contamination

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Week 0	4	925	231.25	72.91667
Week 2	4	860	215	100
Week 4	4	670	167.5	491.6667
Week 6	4	610	152.5	491.6667
Week 8	4	498	124.5	587.6667
Week 10	4	380	95	1166.667
0g water hyacinth	6	1090	181.6667	816.6667
20g water hyacinth	6	970	161.6667	2816.667
40g water hyacinth	6	915	152.5	3817.5
60g water hyacinth	6	968	161.3333	4618.667

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	***P-value***	*F crit*
Weeks (time)	54355.21	5	10871.04	27.21256	**5.08E-07**	2.901295
Water hyacinth	2739.458	3	913.1528	2.285819	**0.120458**	3.287383
Error	5992.292	15	399.4861			
Total	63086.96	23				

Table 5: Anova for 4.5% crude oil contamination
Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Week 0	4	1685	421.25	72.91667
Week 2	4	1645	411.25	72.91667
Week 4	4	1315	328.75	1239.583
Week 6	4	1040	260	1866.667
Week 8	4	900	225	1166.667
Week 10	4	660	165	1433.333
0g Water hyacinth	6	1990	331.6667	6176.667
20g Water hyacinth	6	1790	298.3333	10256.67
40g Water hyacinth	6	1695	282.5	14727.5
60g Water hyacinth	6	1770	295	13430

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	***P-value***	*F crit*
Weeks (time)	213334.4	5	42666.88	66.52983	**1.06E-09**	2.901295
Water hyacinth	7936.458	3	2645.486	4.125068	**0.025539**	3.287383
Error	9619.792	15	641.3194			
Total	230890.6	23				

The following conclusion can be deduced from the Tables 3-5.

For 1.5% crude oil contamination (3.17 *litres/*m^2 *of land area*), it was observed that the P-Value less than 0.05 for the effects of time is 5.58 x 10^{-6} and P-value greater than 0.05 for the effect of water hyacinth is 0.198119. 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 1.5% crude oil contamination. It therefore, follows that it might not be necessary to add water hyacinth for bioremediating soils contaminated with 1.5% crude oil. The micro-organisms and nutrients in the soil can naturally carry out the bioremediation in the soil without any addition of nutrient or amendment.

For 3.0% crude oil contamination (6.35 *litres/*m^2 *of land area)*, the P-value less than 0.05 for the effects of timeis 5.08 x 10^{-7} and P-value greater than 0.05 for the effect of water hyacinth is 0.120458. Thus, the Alternative hypothesis was also 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 3.0% crude oil contamination. It therefore, follows that it might not be necessary to add water hyacinth for bioremediating soils contaminated with 3.0% crude oil. This was because the micro-organisms and nutrients in the soil can naturally carry out the bioremediation in the soil without any addition of nutrient or amendment.

For 4.5% crude oil contamination *(9.70 litres/*m^2 *of land area),* the P-value less than 0.05 for the effects of time is 1.06 x 10^{-9} and P-value less than 0.05 for the effect of water hyacinth is 0.025539, 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 4.5% crude oil contamination. Therefore, water hyacinth helped in bioremediating soils contaminated with up to 4.5% crude oil. It helped in amending the soil by adding nutrients to the contaminated soil.

Also, a plot of the Total Hydrocarbon Content (THC) against Water Hyacinth at the end of the 10 weeks was depicted in Fig. 4. It shows that remediation was possible for 1.5, 3.0 and 4.5% crude oil contamination. However, the plot reveals that remediation occurs much faster in cells with 1.5% crude oil contamination followed by that of 3.0% and finally 4.5%.

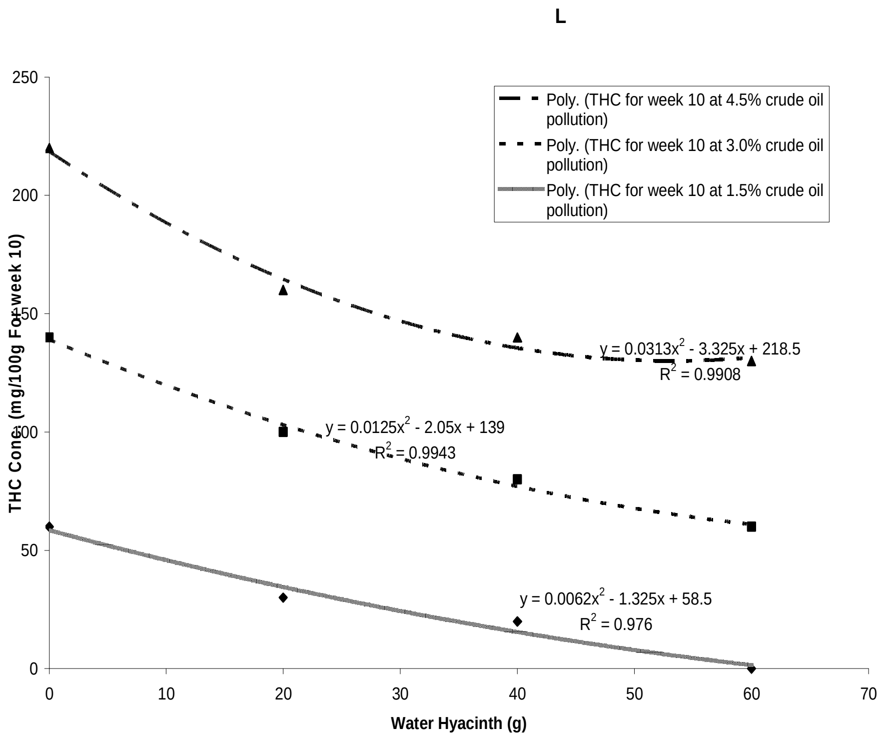

Fig 4: THC conc. against Water Hyacinth at 10 weeks.

CONCLUSION

This research work was intended to understand the effects of water hyacinth application on soil contaminated with crude oil concentrations at 1.5, 3.0 and 4.5%, being equivalent to the volume of crude oil of 3.17, 6.35 and 9.70*litres/m*^{2} of land area, respectively. The remediation process was followed by monitoring changes in the THC of the contaminated soil.

Remediating with varying amounts of water hyacinth for a period of ten weeks revealed that this particular soil type, which is the prevalent soil type of Niger Delta, is capable of remediating crude oil levels at 1.5 and 3.0% as time progressed with the water hyacinth application contributing less to the whole remediation exercise. While at crude oil concentration above 3.0 - 4.5%, water hyacinth application may play a role which aided in the whole remediation process. 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. It therefore, means that the Niger Delta soil is capable of self-remediating crude oil contamination of about 1- 3%. Thus, Natural Attenuation is possible at 1- 3% crude oil soil contamination while above 3.0 - 4.5%, the process of natural attenuation need to be enhanced by nutrient amendment in the form of fertilizers, for example, water hyacinth which can be seen as an organic fertilizer because of its high phosphate content.

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