Performance Evaluation of *Vernonia Galamensis* and *Vernonia Amygdalina* Species in Bioremediation of Petroleum Contaminated Sandy Soils in the Niger Delta.

Nmegbu, Chukwuma Godwin Jacob
Department of Petroleum Engineering,
Rivers State University, Port-Harcourt,
Nigeria
gnmegbu@gmail.com

Nnadikwe, Johnson
Department of Chemical Engineering,
Rivers State University, Port-Harcourt,
Nigeria
johnsonnwadikwe@yahoo.com

Abstract
The immense impact of polluted soils initiated by crude oil or petroleum products are devastating, causing land loss, property loss and impedes palatable agricultural environment. Quite a number of studies have been conducted in this area of remediation, yet more studies are required to determine more specifications for proposed remedial processes. This work therefore is focused on the evaluation of bitter leaf performance on hydrocarbon polluted soil using two species; Vernonia galamensis and Vernonia amygdalina. The micro-organism analysis states that three bacterial species (P. aeruginosa, S. aureus and E. coli) were present in the bitter leaf extracts. The leaf extracts were prepared by sun drying them, room drying them, using them wet and blended into the contaminated sandy-loam soil. Results from the analysis showed that the wet blended vernonia extracts performed best in the remediating action, remediating more than 50% of the initial values. A range of 10g-40g of bitter leaf was used in the contaminated soils for 40 days which showed a total reduction of the contaminants in the soil. Finally, models were developed to predict the remediating effects of hydrocarbon contents, lead, zinc and chromium as dependent variable while the mass of bitter leaf, the time of utilization and the pH of the soil are independent variables. The level of significance attained was less than 0.05 for the models and the R^2 was appreciable.

Keywords: *Bioremediation, Contaminated Soils, Venonia Amygdalina and Vernonia Galamensis*

I. Introduction

Bioremediation is a waste management method that involves the use of organisms for the removal or neutralization of pollutants from contaminated areas (Environmental Inquiry, 2016). Theo in the United States is EPA, bioremediation "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances". Technologies can generally be classified both on site and on land on-site bioremediation of contaminated material at the treatment site, while ex-situ involves the removal of contaminated material from the outside. Some technologies bioremedias example pitooremiatsia related, biointegratsia, biolehikingi, agricultural land, bioreactors, composting, bioagualizatsia, and rizopilpiratsia biotimitatsia. Bioremediation can occur on its own (natural

or intimate bioremiatsia adekvatsiis) or it can be effectively only fertilizers, oxygen, leaves and other means through which to promote pollution eating microbes in the growth medium (hyperzoid electrocoagulation) framework. The nitrogen status of the soil and nitrogenous organic chemicals can cause some of the biodegradation (Olson and Tsai, 1992) and the soil mass, which is the high capacity of the polluters adsorbulma soil to reduce emissions due to biodegradirebas biomegradirebis, chemicals, microbes (O'Loughlin e*t al.*, 2000), limited bioavailability. The success of the latter has approved microbial strains on average to be added to improve the capacity of the indigenous population of mini-contamination. The micro-organisms are used to perform the bio-therapeutic function.
Hydrocarbon exploration and production activities by oil and gas companies have resulted to the pollution of the environment. The defilement of the natural environment basically the soil environment, Hence the need to seek for alternative means of mitigate or remediate the affected soil cannot be over emphasized.

Hydrocarbon impact the land and the ground water which dissolves the nutrients and minerals in the soil and washes them away before trees and plants can be absorb them out of the ground for use. Hydrocarbon also releases toxic substances such as Benzene, Toluene and Xylene into the soil which in very small amounts are very harmful to trees and plant generally. After this occurs, the leaves cannot perform photosynthesis and the trees are left unhealthy, weak, and usually die from disease or from insect attacks.
Other major problems associated with Hydrocarbon polluted soil environment includes the reduced rate of growth crops, abnormal cell development root system damage, reduced regeneration, premature loss of leaves and needled, leaching of leaf nutrients, bacterial activities inhibited reduced soil fertility. This study is therefore aimed at developing and evaluating the performance of *vernonia* plant species in bioremediation of hydrocarbon polluted soil.

The objective of this study will be to perform a small scale laboratory test of the performance of bitter leaf in oil contaminated soil, it will also tend to evaluate the effectiveness of different bitter-leaf species basically Vernonia galamensis and venonia amygdalina in the remediation activity. The study will also seek to describe a relationship between the concentration of the bitter leaf and its remediation effect in different soils types and finally establish a statistical model of the bio-remediation process.

II. Materials and Methods

2.1 Description of Study

The study was conducted at Petroleum testing lab of Rivers State University, Port Harcourt, Rivers State, Nigeria. The materials used for the experimental work include;
Clay, and Sandy/Loam soil, Crude oil (Bonny light), two samples of bitter leat (*vernonia galamensis and vernioa Amygdalina),* Melter weighing balance, beakers, pH meter for measurement of the decomposed sample, measuring cylinders, retort stands.
50 ml of crude oil was introduced into each 1kg soil pollute it, the soil samples were thoroughly mixed and stirred to attain uniform concentration. The *vernionia* extracts were then added into the reactors in varying grams starting from 10g-40g to ascertain its effects on the soil from 1 - 40days. The readings of the soil were taken before and after the application of the pollution reagent (Bonny light). This is to ascertain the remediation effect of the leaves on the various soil types by applying the sun dry leaf extract, room dry extract and wet blended vernonia extracts. The obtained data via the experiment was recorded accordingly. This included the dependent variables of metals, hydrocarbon contents, pH, method of applications, soil types and different weights of the leaf extracts. Analyzing the data for the

remediation effect required adopting the fundamental remediation model.

$$Q_e = \left(\frac{C_o - C_e}{m}\right) \quad (1)$$

Where;
Q_e = Remediation effect
C_o = initial concentration before remediation
C_e is the Concentration after remediation
M = mass of the remediant
V = volume of the pollutant (Crude oil)
Response factors to consider:
Hydrocarbon content, HC, Lead (Pb), Zinc (Zn), Chromium (Cr)

Performing a multiple regression analysis, the use of the least square method will be employed using the Minitab software.
Table 1 below shows values of variables for experimental process before and after contamination of the sandy soil.

Table 1- Initial and Final Readings of the Response Factors for Sandy Soil

pH and HC Readings for Samples before Contamination					
Initial Content sample	pH	HC	Pb (ug/ml)	Zn (ug/ml)	Cr (ug/ml)
Sandy Loam Soil, SLSi	6.76	2.59	0.018	0.022	0.015
pH and HC Readings for Samples after Contamination					
Final Content sample	pH	HC	Pb (ug/ml)	Zn (ug/ml)	Cr (ug/ml)
Sandy Loam Soil, SLSf	6.75	4.67	1.22	0.923	1.103

2.2 pH Analysis

The pH stability of the soil sample was determined by cross examining the differences in the pH as more *vernonia* species are being added into the contaminated soil. The examination will cut across all the methods of the *vernonia* specie preparation, the different mass addition and the time taken for the experiment to be observed.

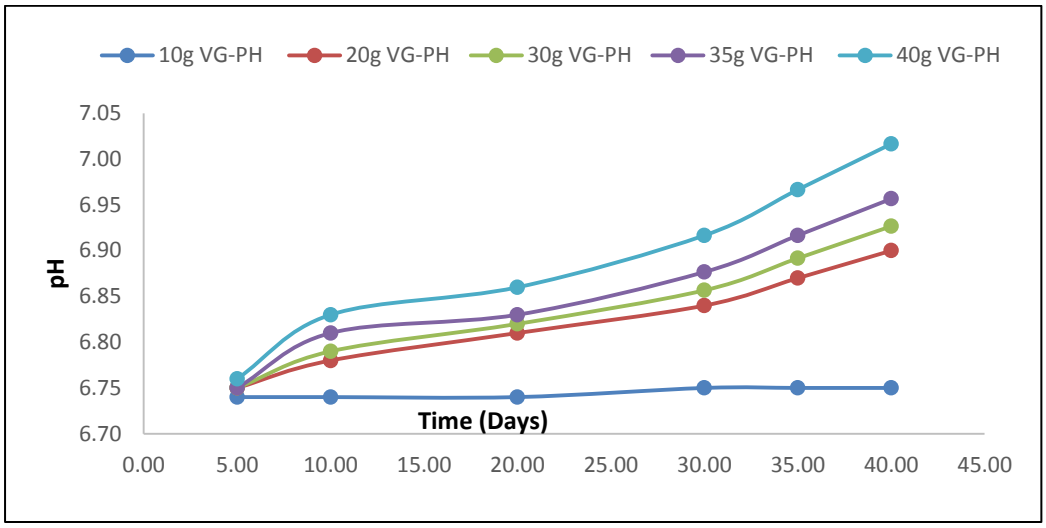

Figure 3: Variation in pH with Time for *Vernonia Galamensis* (Room Dried)

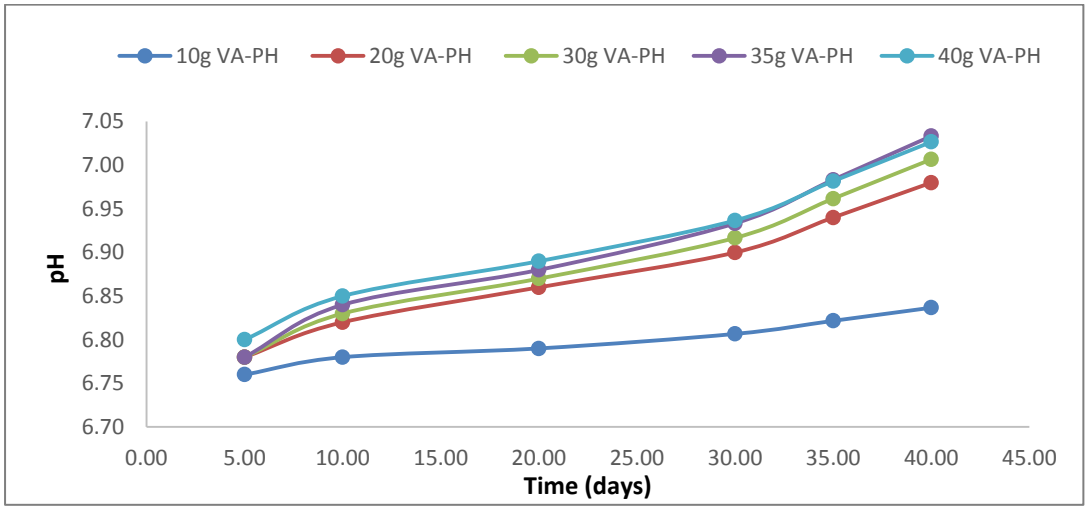

Figure 2: Variation in pH with Time for *Vernonia Amygdalina* (Room Dried)

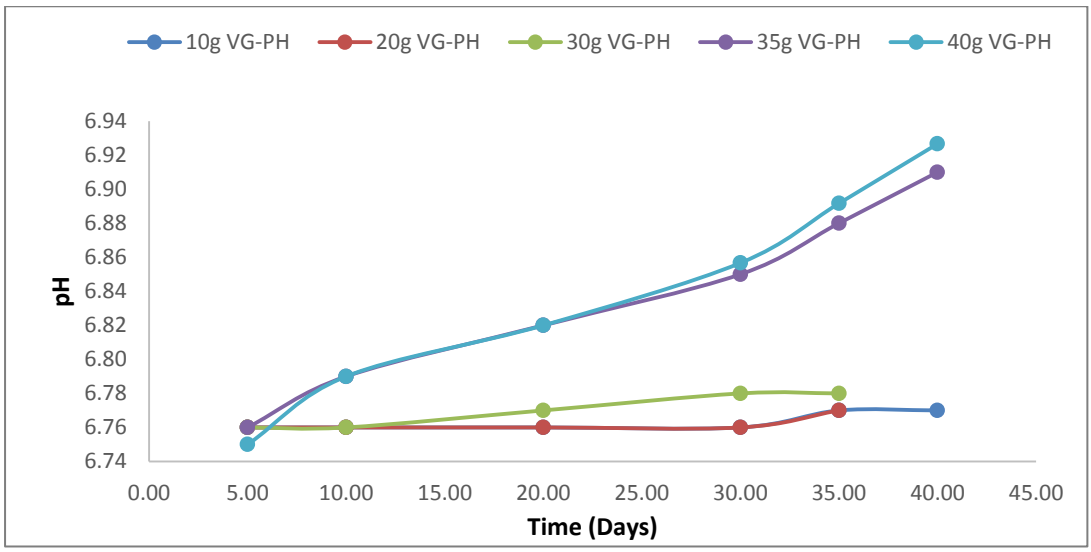

Figure 3: Variation in pH with Time for *Vernonia Galamensis* (Sun Dry)

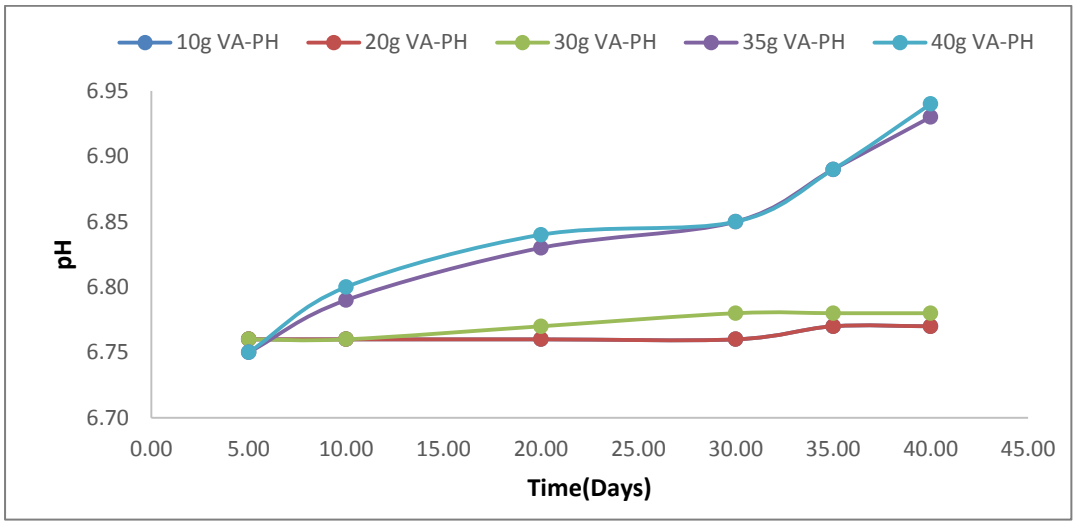

Figure 4: Variation in pH with time for *Vernonia Amygdalina* (Sun Dry)

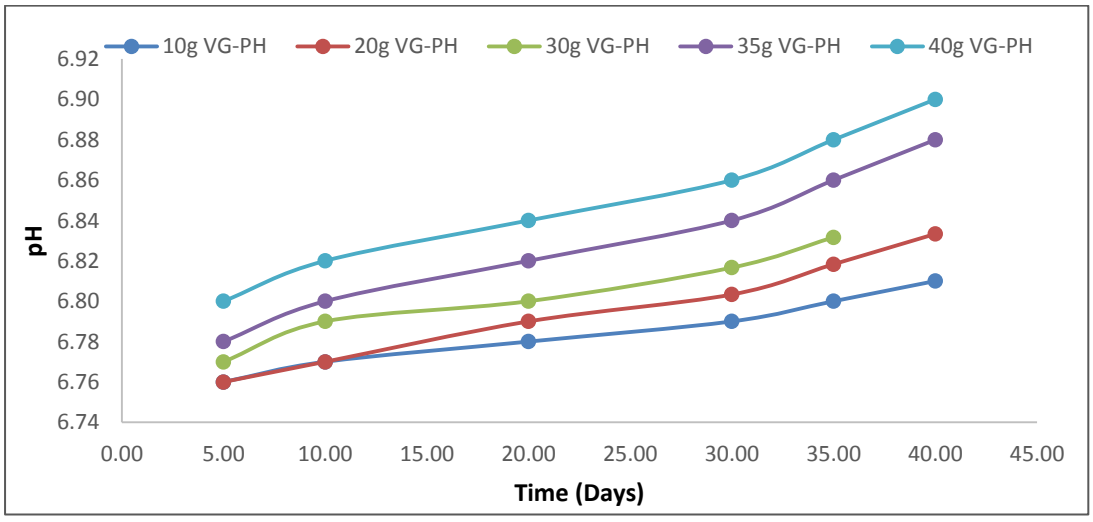

Figure 5: Variation in pH with Time for *Vernonia Galamensis* (Wet Blended)

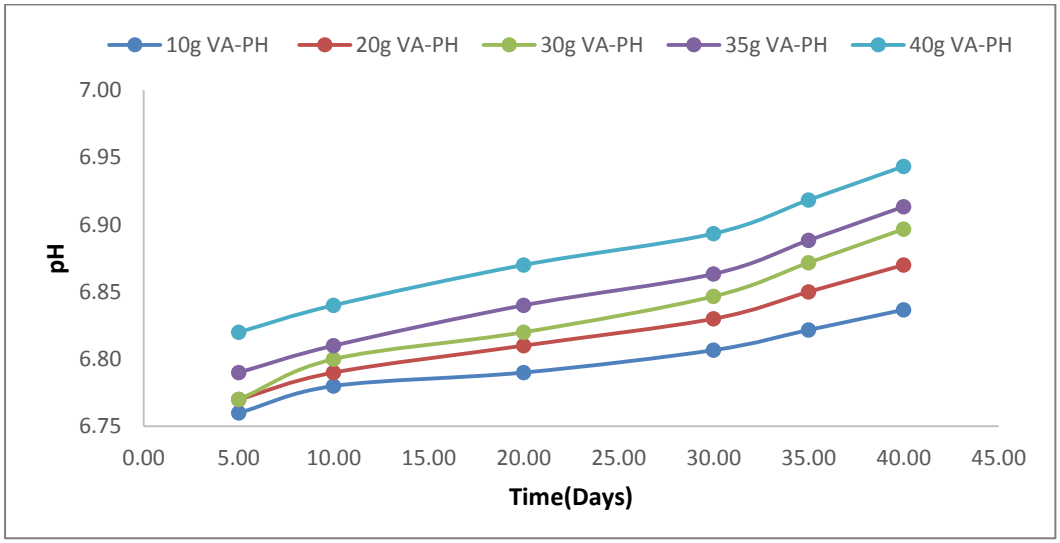

Figure 6: Variation in pH with Time for *Vernonia Amygdalina* (Wet Blended)

From figures 1 through 6, we can observe the trend of pH levels in the soil as the *vernonia* extracts are introduced in the soil. Only the sun dried prepared at 10g and 20g gave a stable pH whereas all other treatment changed the nature of the pH, increasing it towards a neutral indication. This phenomenon was traceable to the reduction in metals in the soil.

2.3 HC Analysis

The hydrocarbon content analysis showed that more hydro carbons were remediated using the *vernonia Amygdalina* leaf extract which was dried in room condition. The performance of the *Vernonia Amygdalina* was about more than twice the effect of *Vernonia Galamensis*. At 40g of extracts for 40 days 0.90ug/ml and 1.80ug/ml of hydrocarbon were remediated for the *Vernonia Galamensis* and *Vernonia Amygdalina* respectively.

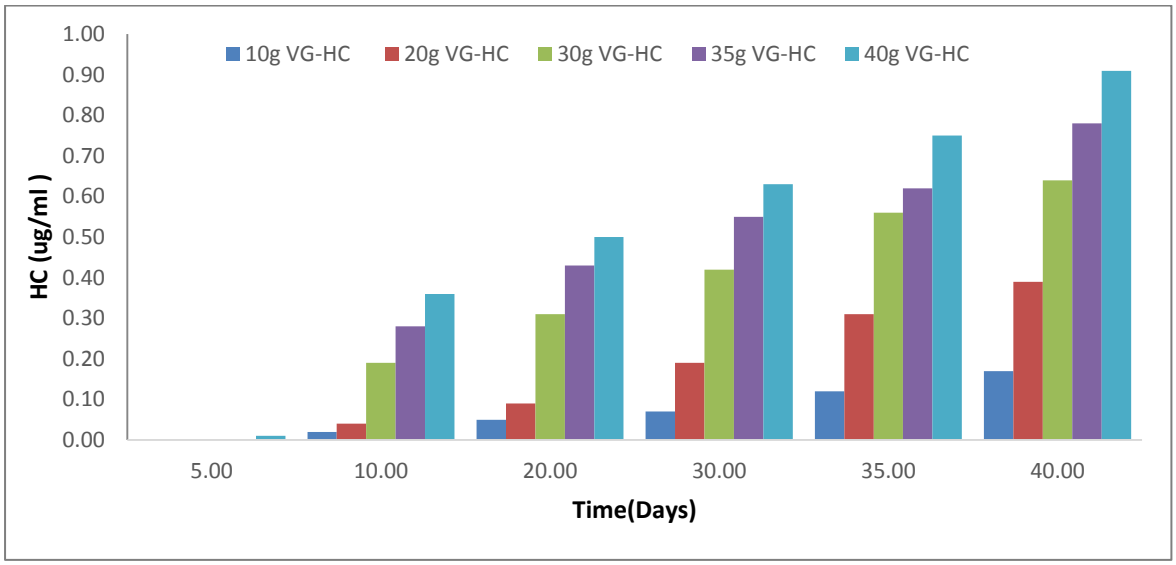

Figure 7: Hydrocarbon Content Remediation using *Vernonia Galamensis* Extract in varying Masses for Different Days in Sandy-Loamy Soil

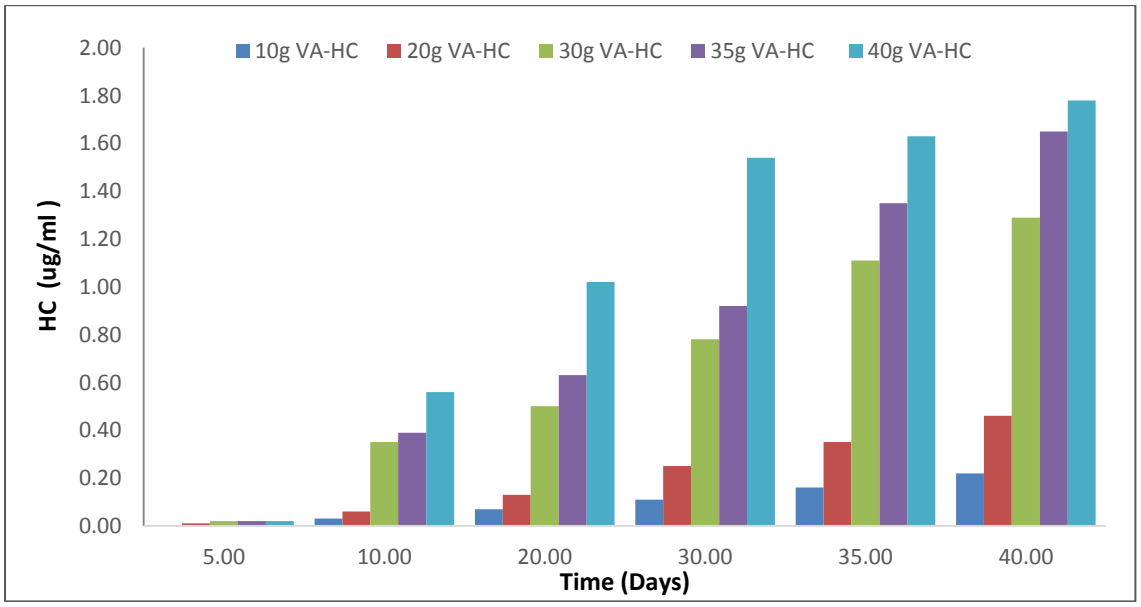

Figure 8: Hydrocarbon Content Remediation using *Vernonia Amygdalina* Extract in Varying Masses for Different Days in Sandy-Loamy Soil

Thus far, we can compare the efficiency of the methods of preparation for the remediating processes. 40g of extracts will be tested using the room dry, sun dry and wet blended for both species. Form this analysis, it is observed in Figure 9 and Figure 10that the wet blended extracts remediate more hydrocarbon contents of the polluted soil. We can see that 2.11ug/ml and 2.40ug/ml were remediated from the wet blended *vernonia Galamensis* and *vernonia Amygdalina* respectively. The remediation effect of the room dried extracts was more effective when considering *vernonia Amygdalina* than *vernonia Galamensis* yielding values of 1.78ug/ml and 0.90ug/ml respectively.

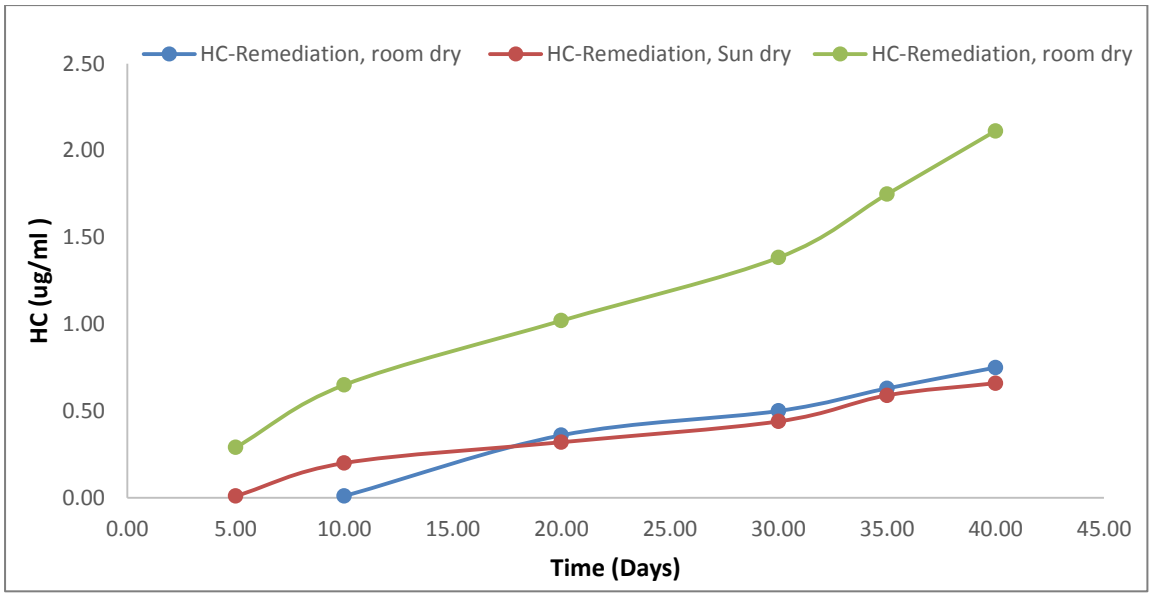

Figure 9: Hydrocarbon Remediation using *Vernonia Galamensis* Extract for Room Dry, Sun Dry, and Wet Blended.

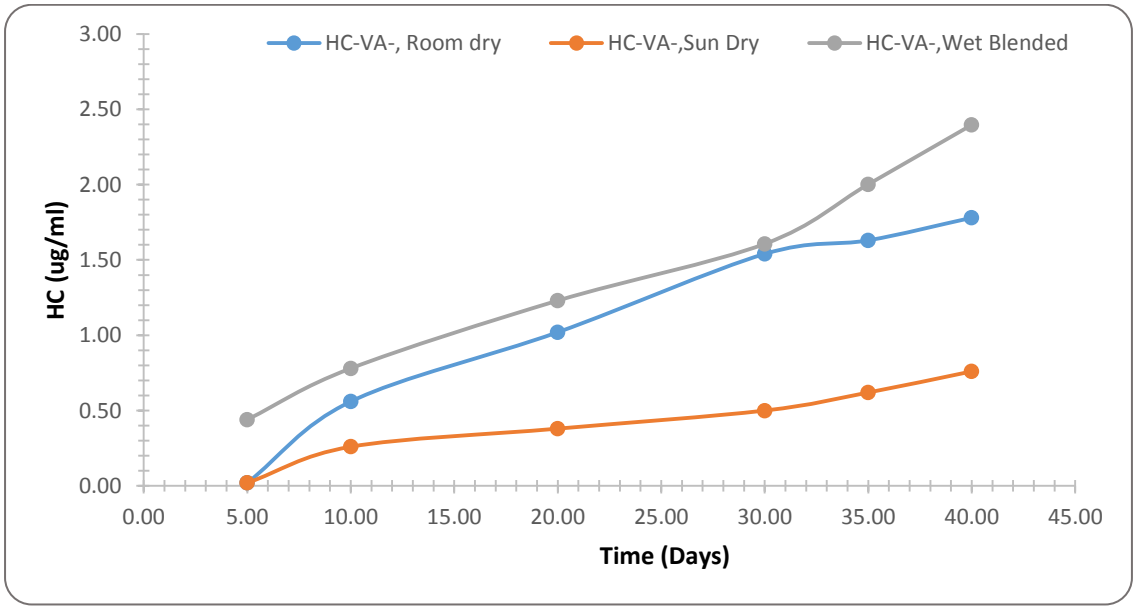

Figure 10: Hydrocarbon Remediation using *Vernonia Amygdalina* Extract for Room Dry, Sun Dry, and Wet Blended.

2.4 Metal Analysis

As the remediation process continued, metals were reduced in the contaminated soil which can be observed form the shift in pH ranges towards a neutral pH indication. Hence, a cross examination of the metal drop in the contaminated soil was conducted to ascertain the level of the potentiality of the mass of vernonia extracts.

2.4.1 Pb Remediating Response for Sandy-Loamy Soil

As the masses of the vernonia species were introduced into the polluted soil, the concentration of Pb in the soil reduced as shown in figure 11 and 12.

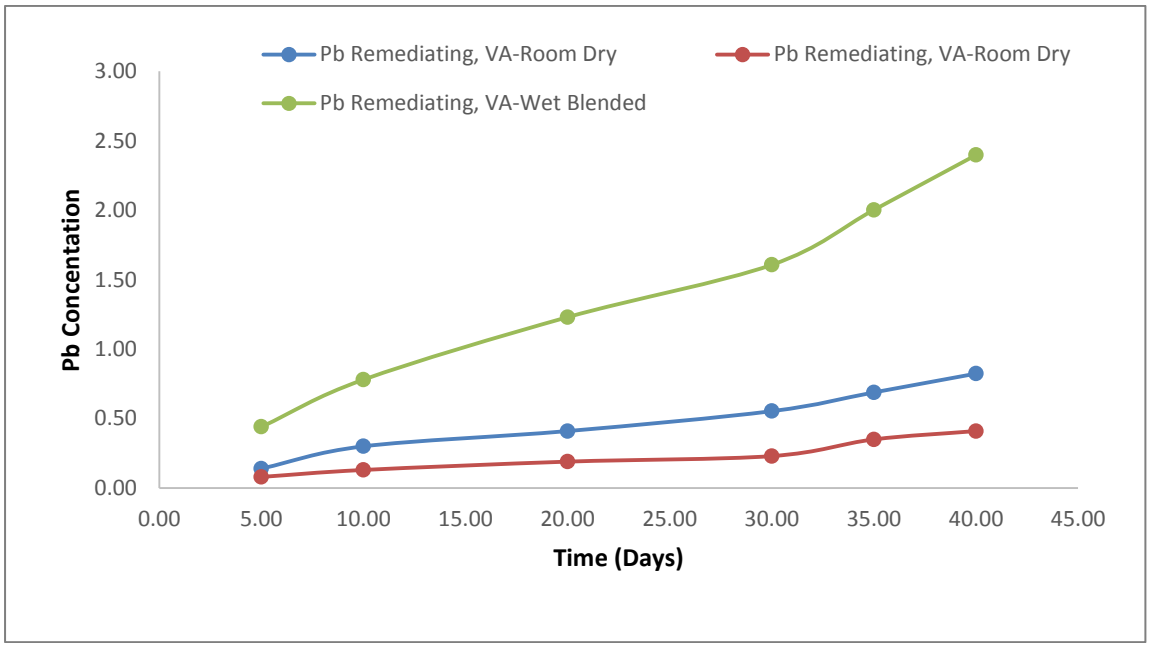

Figure 11: Pb Remediation using *Vernonia Amygdalina* Extract for Room Dry, Sun Dry, and Wet Blended.

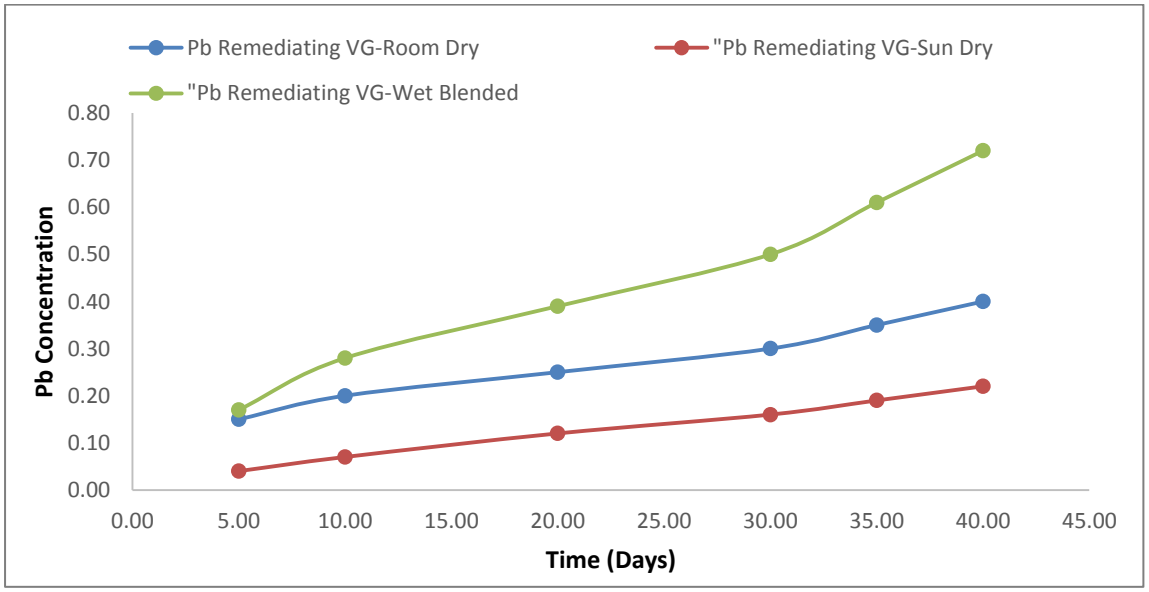

Figure 12: Pb Remediation using *Vernonia Galamensis* Extract for Room Dry, Sun Dry, and Wet Blended.

Figures 11 and 12 shows that the wet blended vernonia species perform best in the Pb remediation activity. The sun dry vernonia species had a poor Pb remediating effects as compared to the others. This can be attributed to the inactivity of the micro-organisms and the phytochemicals responsible for Pb remediation in that condition. Both species of vernonia leaf achieved about 0.72ug/ml of Pb remediation for *Vernonia Galamensis* and 0.99ug/ml for *Vernonia Amygdalina*.

2.4.2 Zn Remediating Response

On evaluation, it was also observed that Zn traces present in the sandy-loamy soil were also remediated. from figure 13 and 14, the room dry and sun-dry *vernonia Galamensis* gave closely related results leaving the wet blended extract to give about 0.51 ug/ml remediating effect for the *vernonia Amygdalina* extract, the room dry, sun dry and wet blended gives an approximate remediating values of 0.31ug/ml, 0.52ug/ml, 0.71ug/ml respectively. ultimately, the wet blended gives the best Zn remediating effect.

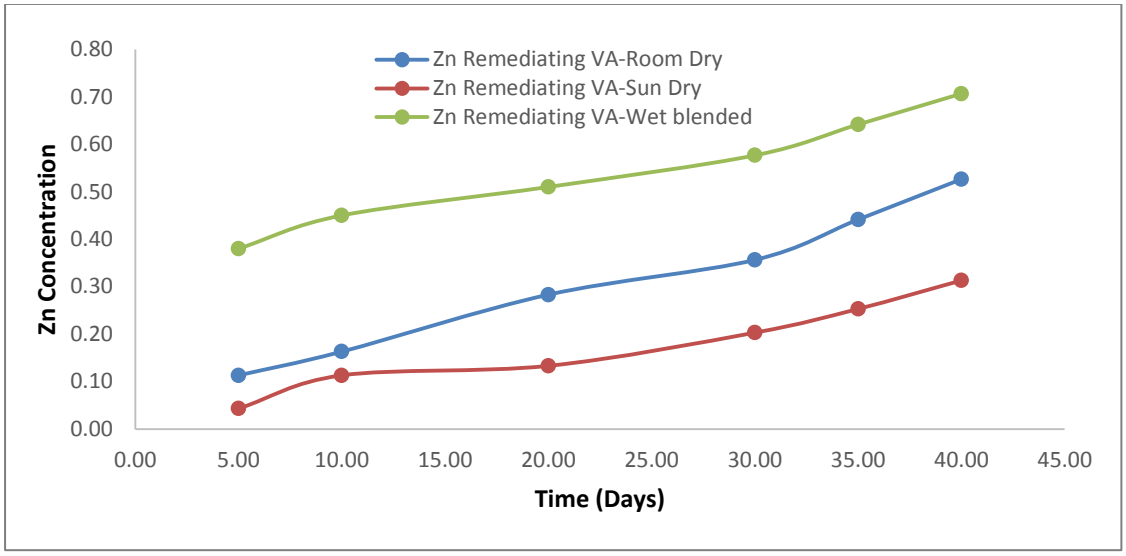

Figure 13: Zn Remediation using *Vernonia Amygdalina* Extract for Room Dry, Sun Dry, and Wet Blended.

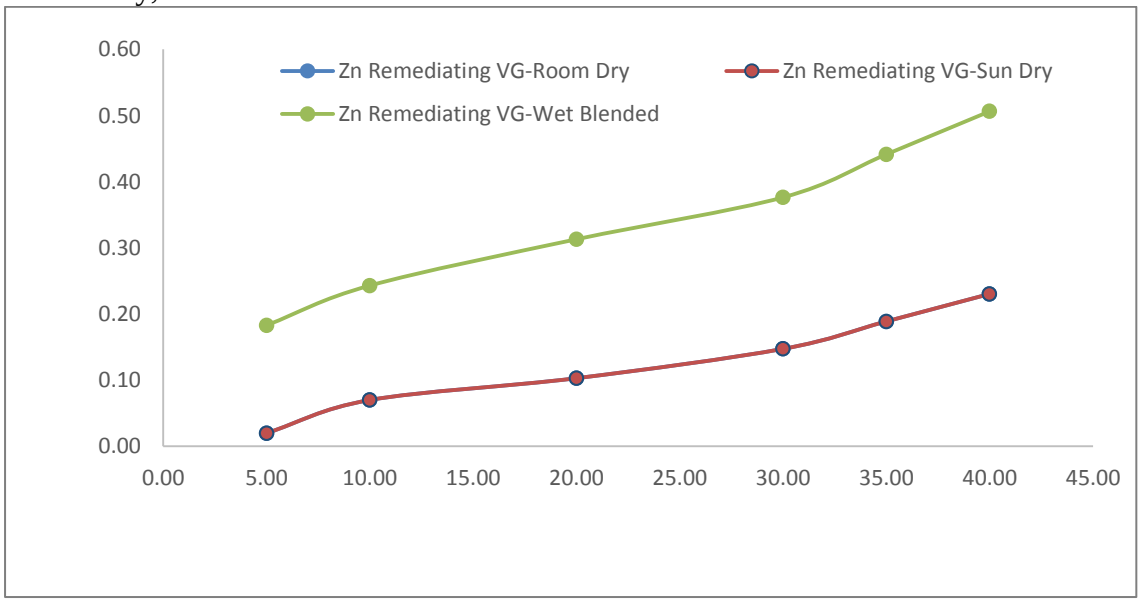

Figure 14: Zn Remediation using *Vernonia Galamensis* Extract for Room Dry, Sun Dry, and Wet Blended.

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_k x_{ik} + u_i \qquad \text{for } i = 1, \ldots, n.$$

In matrix form, we can rewrite this model as

$$\begin{bmatrix} y_1 \\ y_2 \\ \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & x_{12} & \ldots & x_{1k} \\ 1 & x_{21} & x_{22} & \ldots & x_{2k} \\ & & & & \\ 1 & x_{n1} & x_{n2} & \ldots & x_{nk} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \\ \beta_k \end{bmatrix} + \begin{bmatrix} u_1 \\ u_2 \\ \\ u_n \end{bmatrix}$$

n x 1 n x (k+1) (k+1) x 1 n x 1

$$Y = X\beta + u$$

We want to estimate β.

III. Results and Discussion

3.1 Sandy-Loamy Soil Bio Remedial Analysis

The sandy-loamy soil samples were also collected and mixed with the bonny light crude to simulate a similar condition obtainable in the ogoni land. The variation in the pH, Hydrocarbon contents and metals traces were measured considerably by considering the initial and final readings before and after crude contamination.

3.2 Model for Sandy-Loamy Soil

The least square method will be employed to determine the model governing the various factors. This was conducted for both species of bitter leaf.

3.2.1 *Vernonia Galamensis* Modelling

a. Regression Analysis: HC versus Time, Mass, pH

The regression equation is

HC = - 126 - 0.0207 Time - 0.0157 Mass + 18.6 pH

Predictor	Coef	SE Coef	T	P
Constant	-125.51	38.99	-3.22	0.003
Time	-0.02073	0.01316	-1.58	0.127
Mass	-0.01570	0.01327	-1.18	0.247
pH	18.619	5.812	3.20	0.004

S = 0.328763 R-Sq = 62.3% R-Sq(adj) = 57.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	4.6396	1.5465	14.31	0.000
Residual Error	26	2.8102	0.1081		
Total	29	7.4498			

b. Regression Analysis: Pb versus Time, Mass, pH

The regression equation is

Pb = - 37.7 - 0.00290 Time + 0.00340 Mass + 5.56 pH

Predictor	Coef	SE Coef	T	P
Constant	-37.681	6.988	-5.39	0.000
Time	-0.002902	0.002358	-1.23	0.229
Mass	0.003397	0.002378	1.43	0.165
pH	5.562	1.042	5.34	0.000

S = 0.0589214 R-Sq = 93.3% R-Sq(adj) = 92.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	1.25980	0.41993	120.96	0.000
Residual Error	26	0.09026	0.00347		
Total	29	1.35007			

c. Regression Analysis: Zn versus Time, Mass, pH

The regression equation is

Zn = - 23.3 - 0.00135 Time + 0.00122 Mass + 3.45 pH

Predictor	Coef	SE Coef	T	P
Constant	-23.321	1.407	-16.57	0.000
Time	-0.0013451	0.0004749	-2.83	0.009
Mass	0.0012178	0.0004789	2.54	0.017
pH	3.4524	0.2098	16.46	0.000

S = 0.0118667 R-Sq = 99.2% R-Sq(adj) = 99.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.45649	0.15216	1080.55	0.000
Residual Error	2	6	0.00366	0.00014	
Total	29	0.46015			

d. Regression Analysis: Cr versus Time, Mass, pH

The regression equation is

Cr = - 18.6 + 0.00396 Time + 0.00195 Mass + 2.76 pH

Predictor	Coef	SE Coef	T	P
Constant	-18.648	1.941	-9.61	0.000
Time	0.0039635	0.0006550	6.05	0.000
Mass	0.0019489	0.0006605	2.95	0.007
pH	2.7577	0.2893	9.53	0.000

S = 0.0163666 R-Sq = 99.0% R-Sq(adj) = 98.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.70052	0.23351	871.73	0.000
Residual Error	26	0.00696	0.00027		
Total	29	0.70749			

3.2.2 *Vernonia Amygdalina* Modelling

a. Regression Analysis: HC_1 versus Time_1, Mass_1, pH_1

The regression equation is

HC_1 = - 145 - 0.0395 Time_1 - 0.0217 Mass_1 + 21.5 pH_1

Predictor	Coef	SE Coef	T	`P
Constant	-144.86	22.10	-6.55	0.000
Time_1	-0.03950	0.01004	-3.93	0.001
Mass_1	-0.021669	0.008885	-2.44	0.022
pH_1	21.498	3.297	6.52	0.000

S = 0.208392 R-Sq = 87.5% R-Sq(adj) = 86.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	7.9029	2.6343	60.66	0.000

Residual Error	26	1.1291	0.0434
Total	29	9.0320	

b. Regression Analysis: Pb_1 versus Time_1, Mass_1, pH_1

The regression equation is

Pb_1 = - 23.7 + 0.00058 Time_1 + 0.0130 Mass_1 + 3.48 pH_1

Predictor	Coef	SE Coef	T	P
Constant	-23.74	10.01	-2.37	0.025
Time_1	0.000577	0.004546	0.13	0.900
Mass_1	0.012987	0.004024	3.23	0.003
pH_1	3.484	1.493	2.33	0.028

S = 0.0943675 R-Sq = 90.6% R-Sq(adj) = 89.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	2.23640	0.74547	83.71	0.000
Residual Error	26	0.23154	0.00891		
Total	29	2.46794			

c. Regression Analysis: Zn_1 versus Time_1, Mass_1, pH_1

The regression equation is

Zn_1 = - 4.66 + 0.00585 Time_1 + 0.0124 Mass_1 + 0.669 pH_1

Predictor	Coef	SE Coef	T	P
Constant	-4.662	3.611	-1.29	0.208
Time_1	0.005848	0.001641	3.56	0.001
Mass_1	0.012441	0.001452	8.57	0.000
pH_1	0.6695	0.5388	1.24	0.225

S = 0.0340525 R-Sq = 97.1% R-Sq(adj) = 96.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.99199	0.33066	285.16	0.000
Residual Error	26	0.03015	0.00116		
Total	29	1.02214			

d. Regression Analysis: Cr versus Time_1, Mass_1, pH_1

The regression equation is

Cr = - 16.8 + 0.00258 Time_1 + 0.00153 Mass_1 + 2.48 pH_1

Predictor	Coef	SE Coef	T	P
Constant	-16.768	1.707	-9.82	0.000
Time_1	0.0025843	0.0007755	3.33	0.003
Mass_1	0.0015306	0.0006863	2.23	0.035
pH_1	2.4798	0.2547	9.74	0.000

S = 0.0160963 R-Sq = 99.0% R-Sq(adj) = 98.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.70075	0.23358	901.55	0.000
Residual Error	26	0.00674	0.00026		
Total	29	0.70749			

From the different models obtained, of a particular interest is the p-value which is the probability value and the R^2 value which is the co-efficient of determination. Statistically, a

model is said to be significantly accepted if the overall p-values of the model is less than 0.05. The R^2 shows the relationship between the variables, the higher the value closer to 100% the better.

Conclusion

From this study, the remediation of contaminated soils consisting of sandy-loamy soils has been established using 2 species of bitter leaf; *vernonia Galamensis* and *vernonia Amygdalina*. The contamination involved an addition of hydrocarbons and metals to the soil in which the micro-organisms (P. *aeruginosa*, S. *aureus* and E. *coli*) and phytochemicals present in the leaf extracts were responsible for the degradation of the metals as wells as the hydrocarbon contents in the soil. It was also that using about 40g of both vernonia extracts, more than 50% of the contaminants concentration were reduced after 40 days of investigation. Hence, both vernonia extracts were good for using as bio-remediating agents in any polluted soils. It is however recommended that for the maximization of the remediating effect of these extracts and depending on the vernonia extracts available, it should be applied wet and blended into the contaminated soils. This is because the micro-organisms present to perform the bio-remediating activity is still very much active in the leaf. Applying the room dry may also prove effective in some areas as it helps the remediation of areas high Pb content in clay soils than using the wet blended extracts.

References

Aldrett, S., Bonner, J.S., McDonalds, T.J., Mills, M.A., Autenrieth, R.L. (1997) Degradation of Crude Oil Enhanced by Commercial Microbial Cultures. Proceedings of 1997 International Oil Spill Conference. American Petroleum Institute, Washington DC, 995-996.

Atlas, R. M., (1988). Biodegradation of Hydrocarbons in the Environment, In: Environmental Biotechnology Omenn, GS Ed. Plenum Press, NewYork

Cheesbrough, M., 2002. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, UK., pp: 135-162.

Environmental Inquiry - Bioremediation. , retrieved (http://ei.cornell.edu/biodeg/bioremed/) 6th March, 2016.

Forsyth, J.V., Tsao, Y.M., Blem, R.D. (1995) Bioremediation: when is Augmentation Needed? In Hinchee, R.E. et al. (eds) Bioaugmentation for Site Remediation. Battelle Press, Columbus, OH, pp1 14.

Gray, M. R., Banerjee, D. K., Fedora, K. P. M., Habhimoto, A., Maslryah,J. H., Pickard, M.A., (1994). Biological Remediation of Anthracene-Contaminated soil in Rotating bioreactors. Appl. Microbial Biotechnol., 40, 933-944

Huan Jing Ke Xue (February 2007). "Chemical Fixation of Metals in Soil Using Bone Char and Assessment of the soil Genotoxicity". Huan Jing Ke Xue. 28: 232–7. PMID 17489175.

Kris S. Freeman (2012). Remediating Soil Lead with Fishbones. Environmental Health Perspectives. 120: A20–1.

Le Floch, S., Merlin, F.X., Guillerme, M., Dalmazzone, C., and Le Corre, P. (1999) A field Experimentation on Bioremediation: Bioren. Environmental Technology, 20, 897-907.

Lovley, D. R., (2003). Cleaning Up with Genomics: Applying Molecular Biology to Bioremediation. Nature Reviews/Microbiology, 1, 35-44. Available at: (www.nature.com/reviews/micro).

Mohan, R.R., Byrd, G.H., Nixon, J., and Bucker, E.R. (1975) Use of Microorganisms to disperse and Degrade oil spills. US Patent 3,871,957

Olson, B. H., Tsai, Y., (1992). Molecular Approaches to environmental management. In: Environmental Microbiology ed Ralph Mitchell. John Wiley and sons Inc. Pub. New York,239-263.

O'Loughlin, E. J; Traina, S. J. & Sims, G. K. (2000). Effects of sorption on the biodegradation of 2 methylpyridine in aqueous suspensions of reference clay minerals. *Environment Toxicol And* Chemical 19: 2168–2174.

Onwurah, I . N. E., (2000). A Perspective of Industrial and Environmental Biotechnology. Snaap Press / Publishers Enugu, Nigeria, 148

Prince, P. C., (1992). Bioremediation of oil Spills with Particular Reference to the Spill from Exxon Valdez. Microbial Control of Pollution. (Eds. Fry Jc, Gadd GM Herbert RA, Jones CW and Watson-Craik 1A.) Society for General Microbiology symposium 48.

Sims, G.K. (2006). "Nitrogen Starvation Promotes Biodegr adation of N-Heterocyclic Compounds in Soil". Soil Biology & Biochemistry. 38: 2478–2480. doi:10.1016/j.soilbio.2006.01.006.