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COMPARATIVE PHYTOREMEDIATION CHARACTERISTICS OF WATER LEAF, BITTER LEAF AND VETIVER GRASS IN THE CLEAN UP OF CRUDE OIL CONTAMINATED WATER SAMPLES

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ABSTRACT

Phytoremediation characteristics of water leaf (Talinum Triangulaire), bitter leaf (Vernonia Amygdalina) and vetiver grass (Vetiveria Zizaniodes) where determined through their ability to clean up crude oil contaminated water samples caused by oil spillage. The leaves of these plants were dried, ground and sieved to a mesh size of 30µm before use. The Total Petroleum Hydrocarbon (TPH) of the crude oil sample before and after spillage were obtained using Gas Chromatographic analyses. Results obtained showed that the TPH of the crude before and after spillage were 267598.7834 and 46059.4399 mg/L respectively, while the TPH of the contaminated water samples after phytoremediation with water leaf, bitter leaf and vetiver grass were 24.8243, 53.5559 and 25.7114 mg/L respectively. Light ends of the crude such as octane (C_8), nonane (C_9) and decane (C_{10}) were completely eliminated after remediation.

Key Words: Adsorbtion, contamination, hydrocarbons, phytoaccumulation, phytoextraction, spillage.

INTRODUCTION

Crude oil is one of the most important and profitable natural resource in the world as suchthere has been an increase in its exploration, production and distribution in different parts of the universe. There is a high tendency of spillagewhen oil is explored, transported, stored and its derivatives used for different purposes. This spillage may happen accidentally, intentionally or from everyday human activities as suchthe causes and sources of oil spills may vary [1, 2]. Crude oil spillage is the release of petroleum hydrocarbons into the environment due to human activities. It is predominant within the marine ecosystem. However, spills may also occur on lands. The toxic compounds in crude oil consist predominantly of hydrocarbons collectively known as Total Petroleum Hydrocarbons [3]. Contamination of water samples by crude oil is primarily caused by oil spillage. Crude oil also contains nitrogen-oxygen compounds, sulphur compounds and heavy metals which have the potential to cause acute and chronic effects on humans, ecosystem and on the environment at large [4]. Total petroleum hydrocarbon refers to hydrocarbon mixture found in oil. It is an aggregation of extractable petroleum hydrocarbons and volatile petroleum hydrocarbons[5]. There is an offset in theentire physicochemical properties of an area once it iscontaminated with crude oil owing to the presence of some toxins in the crude. In addition, oil iseasily adsorbedto anything it encounters (e.g., land, beach, rocks, feathers of a duck or a bather's hair), thereby making it difficult to remove [6]. Pollution by crude oil affects marine life, land, economy, tourism and leisure activities due to the adverse effects of crudehence effective treatment and clean-up are necessary after an oil spill for the protection of the environment and human health [7]. Thermal, mechanical, and chemical methods applied for cleanup process are expensive, energy consuming and disruptive to the environment. Phytoremediation therefore has been developed to be an ecofriendly and cost-effective clean-up technique. It uses the ability of plants to extract, degrade, stabilize, and volatilize the crude oil contaminants on land and aquatic environments after a spill has occurred [8, 9]. Phyto means 'plant' while remediation means 'restoring balance' hence 'Phytoremediation' refers to the use of plants to clean up or treat soil, air, and water contaminated with hazardous contaminants. It is defined as the use of green plants and the associated microorganisms, together with proper agronomic techniques to either contain, remove or render toxic environmental contaminants harmless [10]. Phytoremediation just like most remediation techniques is based on adsorption. Adsorption denotes physicochemical processes during which adsorbents remove adsorbates from any fluid system through physical attraction onto its surface using intermolecular forces. In the case of phytoremediation of crude oil spillage, the adsorbent is the plant while the adsorbate is the crude oil [11]. Various plants have been identified for their potential to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons. In most studies, grasses and legumes have been singled out for their ability in this regard [12]. Grasses have extensive, fibrous root systems, which favour a vast community of micro-organisms, there inherent genetic diversity gives them a competitive advantage in under unfavorable soil condition [13]. Legumes are thought to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen. Legumes do not have to compete with micro-organisms and other plants for limited supplies of available soil nitrogen at oil contaminated sites. Examples of legumes are alfalfa, clover, reed canary grass, peas, etc [14, 15].

The aim of this study is to study the phytoremediation characteristics of plants other than the known and widely used legumes and grasses in the phytoremediation of oil spillage. This study focuses on water leaf, bitter leaf and vetiver grass in phytoremediation. The choice of these plants for this study is because they are common, readily available and accessible especially in the southern part of Nigeria which coincidentally is the hub of oil exploration in Nigeria. Pictures of water leaf, bitter leaf and vetiver grass are shown in Figures 1, 2 and 3.

Fig. 1: Water Leaf (*Talinum Triangulare*)

Fig. 2: Bitter Leaf (*Vernonia Amygdalina*)

Fig. 3: Vertiver Grass (*Vetiveria Zizanioides*)

MATERIALS AND METHODS

Sample Collection and Preparation.

Freshly obtained water leaf, bitter leaf and vertiver grass were properly washed with distilled water and oven dried at 35 ^{0}C at a well monitored and controlled laboratory temperature for 72 hours, the dried leaves were thereafter crushed using electrical grinder machine and subjected to shaking using magnetic shaker into fine particles of 30 µm.

Sample Simulation

Oil spillage sites were simulated using three thermoplastic containers of 20 liter capacity labelled A, B, and C. Each of the containers represents sites to be remediated with water leaf, bitter leaf and vertiver grass. Fifteen liters of tap water was added to each of these containers respectively and 200 ml of crude oil samples with similar characteristics were thereafter added to each container. The water-oil mixture were allowed to equilibrate for five hours. 1 litre of the water-oil mixture was used for remediation.

Gas Chromatographic (GC) Analysis

Gas chromatograph (Model no.: Agilent 7890B) was utilized for total petroleum hydrocarbon (TPH) determination of water-oil mixtures of A, B and C after phytoremediation with water leaf, bitter leaf and vertiver grass respectively. The Gas Chromatograph was equipped with column of fused silica capillary (0.25 mm x 30 m x 0.25 mm), Helium gas (carrier gas) with a flow rate of 1.9 mL/min. Column temperature was formally set to periodically increase from 64 ^{o}C to 191 ^{o}C at 4.0 ^{o}C/min and also to 282 ^{o}C at 6.0 ^{o}C/min. It was held for 20 minutes at 274 ^{o}C. The samples L each) were properly injected in split less modes. The quadrupole, ion exchanges and interface temperatures were held constant at 162 ^{o}C, 153 ^{o}C and 276 ^{o}C respectively. Ionization was carried out in electrons impact mode at 67 eV and data were thereafter obtained by selected ion monitoring mode. TPHs identification were based on selected ions, retention time comparison between sample and standard solutions. TPHs quantification was carried out using relative response factor of the target or specific TPH to internal standard. This was achieved using quantification ions and confirmation ions[16].

Remediation Processes

The remediation process was practically carried out using solid – phase technique of extraction (SPTE). The Environmental protection agency (EPA) remediation method (EPA – 33535) was adopted for contaminated sample remediation. Measured amount of 1Litre water- oil mixture (contaminated sample) was set up in a laboratory cartridge (cleaved on retort stand) containing ten gram of powdered water leaf sample of 30μm particle size. The set up was uninterruptedly allowed to stand for duration of 10 days as the remediation time. Thereafter, the liquid was allowed to elute from the laboratory cartridge and the eluted liquid was subjected to gas chromatographic analysis. Same procedure was adopted for 30 μmpowdered bitter leaf and water leaf samples to clean up 1 Litre of each contaminated sample. The total petroleum hydrocarbons (TPHs) in the contaminated samplesafter the remediation processusing 30 μm particle sized water leaf, bitter leaf and vertiver grass were analyzed and determined using Gas Chromatography respectively[17]. Gas chromatographic analyses were also carried out for the crude oil before and after spill.

RESULTS AND DISCUSSION

Table -1,Characteristics of Crude oil Before / After Simulated Spillage

Hydrocarbon Group	C_o (ppm)	C_e (ppm)
C8	3673.78519	686.14732
C9	1276.29236	250.83553
C10	1425.13502	80.65155
C11	2341.3753	905.54489
C12	2272.41101	1525.70584
C13	4357.74496	1.77E+03
C14	14567.7	263.11129
C15	2.21E+03	192.14356
C16	1.47E+04	2.17E+03
C17	1.39E+04	2.30E+03
Pristane	7788.83915	1.71E+03
C18	1.35E+04	3.01E+03
Phytane	7.64E+03	3.24E+03
C19	1.48E+04	1.25E+03
C20	1.07E+04	2.05E+03
C21	1.35E+04	3.00E+03
C22	4.23E+03	3.25E+03
C23	3.22E+03	3.21E+03
C24	7.57E+03	2.17E+03
C25	3.52E+03	2.18E+03
C26	6.42E+03	6.65E+02
C27	5.68E+03	3.01E+03
C28	1.00E+04	9.28E+02
C29	7.28E+04	3.73E+03

C30	2.76E+03	8.34E+01
C31	2.02E+03	4.07E+02
C32	8.77E+03	6.56E+01
C33	6.86E+03	4.24E+01
C34	2.17E+03	4.93E+01
C35	5.77E+02	2.24E+01
C36	4.13E+02	1.55E+01
C37	1.85E+01	1.77E+03
C38	7.14E+02	1.30E+01
C39	5.98E+02	2.99E+01
C40	6.05E+02	1.38E+01
TPH	267598.7834	46059.4399

Where: Co = Mass Concentration of crude oil before spillage
Ce = Mass Concentration of crude oil after spillage

Table -2, Characteristics of Crude Oil ContaminatedWater Samples after Phytoremediation with Water Leaf, Bitter Leaf and Vetiver Grass

Hydrocarbon Group	$C_{water\ leaf}$(ppm)	$C_{bitter\ leaf}$ (ppm)	$C_{vetiver\ grass}$ (ppm)
C8	-	-	-
C9	-	-	-
C10	-	-	-
C11	-	9.00E-01	7.65E-01
C12	2.72E-01	6.20E-01	6.00E-01
C13	0.2063	1.9182	6.59E-01
C14	2.22E-01	3.31E+00	1.04887
C15	3.37E-01	4.78E+00	1.73351
C16	3.39E-01	2.53E+00	6.35E-01
C17	5.73E-01	1.62E+00	1.04727
Pristane	3.39E-01	8.06E-01	4.45E-01
C18	3.69E-01	1.75E+00	1.2806
Phytane	2.93E-01	5.39E-01	1.23E-01
C19	4.53E-01	1.64E+00	9.98E-01
C20	2.25E-01	1.23E+00	8.26E-01
C21	2.07E-01	1.33E+00	7.90E-01
C22	1.47E-01	8.98E-01	5.05E-01
C23	2.41E-01	5.66E-01	2.63E-01
C24	2.55E-01	4.52E-01	3.26E-01
C25	2.61E-01	3.81E-01	3.14E-01
C26	1.11E-01	2.96E-01	3.25E-01
C27	1.14E-01	2.28E-01	2.36E-01
C28	6.99E-01	3.48E-01	3.86E-01

C29	4.99E-01	6.50E-01	2.26E-01
C30	8.26E-01	1.08E+00	2.48E-01
C31	1.27E+00	2.05E+00	1.25662
C32	2.04E+00	2.72E+00	9.36E-01
C33	2.70E+00	4.24E+00	2.67082
C34	2.04E+00	3.33E+00	7.00E-01
C35	1.59E+00	3.78E+00	6.08E-01
C36	4.15E+00	4.47E+00	1.12922
C37	1.46E+00	1.46E+00	5.03E-01
C38	1.30E+00	1.98E+00	1.92652
C39	9.52E-01	7.95E-01	1.44556
C40	3.34E-01	8.48E-01	7.56E-01
TPH	24.8243	53.5559	25.7114

Where:
$C_{water\ leaf}$ = Mass Concentration after phytoremediation with water leaf
$C_{bitter\ leaf}$ = Mass Concentration after phytoremediation with bitter leaf
$C_{vetiver\ grass}$ = Mass Concentration after phytoremediation with vetiver grass

Table -3,Percentage Reduction (%) of (TPH) for Contaminated Samples before / after Phytoremediation

Parameter	Result (%)
% Reduction of TPH after simulated spillage	58.3206
% Reduction of TPH after phytoremediation with water leaf	99.9888
% Reduction of TPH after phytoremediation with bitter leaf	99.9758
% Reduction of TPH after phytoremediation with vetiver grass	99.9884

Percentage reduction of TPH is obtained using Equation 1,

$\frac{C_0 - C_e}{C_0} \times 100$……………………………………………(1)
where:

C_0 = TPH before remediation (Initial Concentration)

C_e = TPH after remediation (Equilibrium Concentration)

Total Petroleum Hydrocarbon refers to a mixture of hydrocarbons found in crude oil. There are several of these compounds. It is of great importance to measure the total amount (TPH) at a site. TPHs are one of the most common groups of persistent organic contaminants, It is also worthy to note that not all the petroleum hydrocarbons occur in one sample. The TPH of a sample depends on the origin of the sample,it is therefore the sum of the volatile petroleum hydrocarbons, including poly aromatic hydrocarbons (PAH) and extractable petroleum hydrocarbon present in the sample[18].Table 1 shows the TPH of the crude oil sample before and after spillage. From the table it can be deduced that there is a reduction in the concentration of each of the hydrocarbon components of the crude after spillage which was due to the volatility of the components hence the reduction of the Total petroleum hydrocarbon after spillage. It is worthy to note that some portions of the petroleum hydrocarbon are released to the atmosphere resulting in air pollution which in turn reduces the air quality [6]. Released petroleum hydrocarbons in form of volatile organic compounds (VOCs) have a lot of adverse health implications to humans and animals. Some health defects arising from exposure to VOCs include damage to liver, kidney, central nervous system, cancer in humans and animals, loss of coordination, nausea etc [19]. The reduced TPH of the crude after spillage may not also be unconnected to the high water repelling characteristics of petroleum hydrocarbons which reduces their accumulation in water bodies compared to sediments and soils [9].Table 2 shows the characteristics of the crude contaminated samples after phytoremediation with water leaf, bitter leaf and vetiver grass. Considering the fact that crude oil is in liquid form, the leaves of the plants were used in their dried forminstead of the fresh form in other to increase their capacity to adsorb liquid. The leaves were ground and sieved to a smaller particle size compared to their natural form because adsorption increases with increase in surface area and the smaller the particle size, the larger the surface area [20]. Results show that there is a complete cleanup of the lighter ends of the crude samples (octane (C_8), nonane (C_9), decane (C_{10})) after phytoremediation with water leaf, bitter leaf and vetiver grass respectively. Phytoremediation with water leaf was able to totally clean the contaminated water samples up to undecane (C_{11}). The degradation of stubborn petroleum hydrocarbons such as the heavier ends (C_{30} to C_{40}) by phytoremediation is made possible due to the harmonious correlation between the powdered samples obtained from the plant leaves and petroleum hydrocarbons in the water body [21]. Table 2 also shows TPH after phytoremediation with water leaf, bitter leaf and vetiver grass. From the results it can be deduced that the TPH of the oil contaminated water sample after phytoremediation with water leaf was the least, making it the best plant for phytoremediation amongst the three plants. Results from Table 3 shows that the three plants under study (water leaf, bitter leaf and vetiver grass) were effective in the phytoremediation of crude oil contaminated samples with a TPH percentage reduction of over 99%. This affirms that the plants are quite tolerant to petroleum hydrocarbon contaminated environment. Phytoremediation of petroleum hydrocarbon-contaminated water just like most other remediation techniques is mainly based on biodegradation, Plants can indirectly influence degradation by causing an alteration in the physical / chemical conditions in the water body for instance by increasing aeration through the provision of oxygen for the degradation of petroleum hydrocarbon contaminants [22]. The most common form of phytoremediation is phytoextraction. The principle of phytoextraction is based on the absorption of metals, explosives, solvents, and petroleum hydrocarbons etc by the roots of plants the same way water and other nutrients are being absorbed.The ability of the plants to clean up a contaminated environment after phytoextraction is dependent on its ability to accumulate the absorbed substances in its leaves and stems [21].

For this study, the cleanup of the contaminated samples is achieved by the leaves of the plants and it is by adsorption considering the fact that the situation is simulated using plants without roots. Adsorption is the adhesion of atoms, ions or molecules of a gas, liquid or dissolved solid to a surface. It is different from absorption in which the ions, molecules do not just adhere to the surface but is imbibed into the internal structure of the absorbent [20]. Plants cleanup pollutants through its roots by absorption (phytoextraction) while the pollutants are being accumulated in the leaves by adsorption and it is in the leaves that biodegradation takes place [21]. It is however worthy to note that though plants have the inherent ability to detoxify hydrocarbon contaminants (TPH), they lack the catabolic pathway to completely degrade these compounds compared to microorganisms. For this purpose, researchers have tried to engineer plants with genes that can bestow superior degradation abilities on the plants [23].

CONCLUSION

One of the basic factors that determines the cleanliness and environmentally friendliness of a crude oil contaminated site after remediation is the Total Petroleum Hydrocarbon. The lower the TPH after remediation the cleaner the remediated site. There are different remediation techniques for both marine/water and soil crude oil spills. However the most suitable remediation is determined by the physical and chemical properties of oil and the location of the spill. Phytoremediation is the best environmentallyfriendly remediation technique for crude oil spills. The phytoremediation ability of water leaf, bitter leaf and vetiver grass was based on their respective abilities to considerably reduce the Total Petroleum Hydrocarbon from a water body after crude oil spillage. The percentage reduction of TPH for the crude oil contaminated sample after remediation with water leaf was the highest compared to bitter leaf and vetiver grass thereby making water leaf the best phytoremediation agent compared to the other two plants. Bitter leaf had the least phytoremediation ability because the TPH of the crude oil contaminated sample was highest after phytoremediation with the plant.

REFERENCES

1. Ebuehi, O. A. T., Abibo, I. B., Shekwolo, P. D., Sigismund, K. I., Adoki, A., & Okoro, I. C. (2005). Remediation of crude oilcontaminated soil by enhanced natural attenuation technique.*Journal of Applied Sciences & Environmental Management*,103–110.
2. Frick, C. M., Farrell R. E. & Germida, J. J. (1999). Assessment of Phytoremediation as an In-Situ Technique for Cleaning Oil-Contaminated Sites. *Petroleum Technology Alliance of Canada (PTAC) Calgary, AB*, 110-121.
3. Gunther, T., Dornberger, U. &Fritsche, W. (1996). Effects of ryegrass on biodegradation of hydrocarbons in soil. *Chemosphere*, 33: 203-216
4. Mendelssohn, I. A., Andersen, G. L., Baltz, D. M., Caffey, R. H., Carman, K. R., Fleeger, J. W., & Rozas, L. P. (2012). Oilimpacts on coastal wetlands: implications for the MississippiRiver Delta ecosystem after the Deepwater Horizon oil spill.*Biosciences*, 62(6), 562–574.
5. Demirbas, E., Kobya, M. & Sulak, M. (2008). Adsorption Kinetics of A Basic Dye From Aqueous Solution Onto Apricot Stone Activated Carbon.*Bio resources Technology*, 5368- 5373.
6. Kawasaki, A., Watson, E. R. & Kertesz, M. A. (2011). Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizosphere. *Plant soil*, 1-14.
7. Yang, S. Z., Jin, H. J., Wei, Z., He, R. X., Ji, Y. J., Li, X. M., & Yu, S. P. (2009).Bioremediation of oil spills in cold environments: a review. *Pedosphere*, 19(3), pp 371–381.
8. Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation.Annual Review of Plant Biology, 49 (1), 643–668

9. Gerhardt, K. E., Huang, X. D., Glick, B. R., & Greenberg, B. M., (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science*,176(1), 20–3.
10. Peer, W. A., Baxter, I. R., Richards, E. L., Freeman, J. L. & Murphy, A. S. (2006). Phytoremediation and Hyperaccumulator Plants in Molecular Biology of Metal Homeostasis and Detoxification. *Springer, Berlin*, 299-340.
11. Aurangzeb, N., Nisa, S., Bibi, Y., Javed, F. &Hussain, F.(2014).Phytoremediation potential of aquatic herbs from steel foundry effluent.*Brazillian Journal of Chemical Engineering*, 31 (04), pp. 881-886
12. Kamath, R., Rentz, J. A., Schnoor, J. L. & Alvarez P. J. J. (2007). Phytoremediation of hydrocarbon-contaminated soils: principles and applications, Department of Civil and Environmental Engineering, Seamans Centre, University of Iowa, Iowa City, Iowa, U.S.A. – 52242.
13. Chiapusio, G., Kacelli, B. & Huggern, T.(2007). Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (Lolium perenne L. and Trifolium pratense L.) in three spiked soils. *Plant Soil*, 294: 103–112.
14. Suominen, L., Jussila, M. M., Mäkeläinen, K., Romantschuk, M. & Lindström, K. (2000). Evaluation of the Galega-Rhizobium galegae system for the bioremediation of oil contaminated soil. *Environmental pollution*, 107:239-244
15. Miller A. J. & Cramer M. D. (2004). Root nitrogen acquisition and assimilation. *Plant and Soil*, 274: 1–36.
16. Cortes, J. E., Suspes, A., Roa, S., Gonzalez, C. & Castro, H. E. (2012). Total Petroleum Hydrocarbons by Gas Chromatography in Colombian Waters and Soils. *American Journal of Environmental science*, 8(4), 396 - 402.
17. Dave, D. & Ghaly, A.E. (2011). Remediation Technologies for Marine Oil Spills: A Critical Review and Comparative Analysis. *American Journal of Environmental Science*,7, 423 – 440.
18. Daniel, I. E. & Nna, P. J. (2016). Total Petroleum Hydrocarbon Concentration in Surface of Water of Cross River Estuary, Niger Delta, Nigeria.*Asian Journal of Environment & Ecology*, 1(2), 1 - 7.

19. Huffman, M.A.(2003). Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proceedings of the Nutrition Society*, 62(2): 371–381.

20. Merkl, N., R. Schultze-Kraft, R. &Infante C. (2005). Phytoremediation in the tropics—influence of heavy crude oil on root morphological characteristics of graminoids. *Environmental Pollution,* vol. 138, no. 1, 86–91.

21. Abhilash, P.C, Jamil, S. & Singh, N. (2009). Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics.*Biotechnology Advances*, (4): 88-474

22. Atagana, H. I. (2011). Bioremediation of co-contamination ofcrude oil and heavy metals in soil by phytoremediation usingChromolaena Odorata. *Water, Air, & Soil Pollution*, 215(1–4), 261–27

23. Agwaramgbo, L., Thomas, C., Grays, C., Small, J., & Young, T. (2012). An Evaluation of Edible Plant Extracts for the Phytoremediation of Lead Contaminated Water. *Journal of Environmental Protection,* Vol. 3 No. 8, 722-730.