



## PHYTOREMEDIATION FOR CRUDE OIL-CONTAMINATED SOIL USING ORGANIC WASTES

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### Abstract

This paper investigated the effectiveness of using organic waste in plant remediation of soil contaminated with a certain percentage of crude oil. Clean soil was polluted with 10% crude oil then treated with 50% weight of decomposed organic wastes. The results were compared with a control sample (without wastes), each sample prepared under conditions (70% water content, 25 - 28 °C, Alfalfa plant in the standard germination chamber). The effect of organic waste on improving growth of plant and its role in degradation oil through microbial effect was specified. The results showed that 70.34% of crude oil was degraded during 133 days while the control sample was 16.44% during the same period. Organic waste (Refuse) has played a many of roles in the successful treatment of contaminated soil, increasing soil porosity, allowing oxygen to enter the soil structure, increasing the soil's ability to keeping water, and certainly providing nutrients, (N, K, O, P) and organic matters to the soil and encouraging microbial communities to augmentation and stimulate which have an important role in hydrocarbons degradation that have long chains and degraded to carbon dioxide and water and enhanced the ability of Alfalfa plant to resistance the pollution and take up heavy elements, this is reflected in the project results.

**Keywords:** phytoremediation, Organic waste, Alfalfa plant

### Introduction

The increasing demand fuel and energy due to technological and industrial development led to the increase in the extraction of crude oil and thus increased errors and accidents in the extraction, transport and storage (Shirazi *et al.*, 2015). According to previous studies, researchers have been interested in methods of treatment and soil recovery due to toxicity of total petroleum hydrocarbons (TPHs) and soil loss of biological structure (Moubasher *et al.*, 2015). Studies were based on physical and chemical methods. Despite the effectiveness of traditional methods, these techniques are very expensive and uneconomical. Environmental Protection Agency (EPA) has classified methods of removing pollutants from oil-polluted soil into three categories: (1) chemical-physical treatments such as soil vapor extraction, soil washing, freezing processes and stability. (2) Biological methods such as bio-venting, phytoremediation, and bioremediation. (3) Thermal methods like steam injection and extraction, using radio frequency heat and electrical resistance heating (Ndimele 2010; Oyem 2014; Baruah, *et al.* 2016). Efforts have therefore been directed towards biotechnologies that are less expensive and more effective. "phytoremediation," which involves the interaction of plant roots and associated rhizospheric microorganisms for the remediation of soil contaminated with high levels of metals, pesticides, solvents, radionuclides, explosives, crude oil, organic compounds and various other contaminants (Ansari *et al.* 2016). This approach is based on the decomposition of contaminants by microorganisms and plants which are important sources of carbon in metabolic activity (Wilde *et al.*, 2005; Alarcon *et al.*, 2008; Ogbo, 2009). Studies have demonstrated the effect of phytoremediation on organic and inorganic contaminants (Sarkar *et al.*, 2005; Gkorezis *et al.*, 2016). The degradation of hydrocarbons occurs through the effect of the rhizosphere. Plant roots exude organic compounds that help to increase the density, diversity, and activity of microorganisms in the zone of the rhizosphere which in turn breaks down hydrocarbon contaminants (Sessitsch *et al.*, 2006, Fatima and Afzal *et al.*, 2015). The

disadvantages of biotechnology are the length of treatment that can take months depending on natural attenuation, as well as poor biological activity and nutrients (Delille and Pelletier 2002). The aims of this study, access to best results of the phytoremediation with using refuse (organic wastes) that works as an improved matter of oil contaminated soil in terms of structural modification, bacterial stimulation and augmentation because it is rich in organic matters and nutrients and obtain degradation in a shorter period, exceeding the long-term problem by promoting organic wastes.

### Materials and Methods

General methodology for determining the efficiency of phytoremediation by choosing the percentage of added of (refuse) under the crude oil pollution concentrations (10%w/w) investigated in this study consists of the following major steps; waste analysis, crude oil contaminated soil analysis, seeding Alfalfa plant, oil extraction experiments.

#### 1. Preparation of glassware

All glassware was cleaned by detergent, washed thoroughly and rinsed with deionized water three times. The glassware was then dried in an oven before use. All plastic pots were cleaned by deionized water and dried.

#### 2. Preparation of soil

Clean soil samples were air-dried in the laboratory and sieved through 2 mm screens. Then, the soil was contaminated with 10% (w/w) of crude oil and air-dried for 4 days and thoroughly mixed until homogeneous. Plastic pots were used for planted and prepared plant seeds of Alfalfa after sterilized.

Mass fraction of refuses samples (wastes)

1. 50% refuse: 750g ,10% petroleum-contaminated soil (dry weight) plus 750 g refuse (dry weight) plus alfalfa; 1pot

2. Control :750g ,10% petroleum- contaminated soil (dry weight) plus alfalfa; 1 pot

Each treatment was sowed 200 seeds, moisture: 70% of water holding capacity (WHC), Treatments was incubated in a Walk-In Environmental Chamber at 25 with plant growth light table 1.

**Table 1:** Samples of refuse at 25°C

Refuse(w)%	Plants	Moisture%	Oil%
50%	<i>Alfalfa</i>	70% WHC	10%
Zero	<i>Alfalfa</i>	70% WHC	10%

Soil sampling of plant seedlings pots

Soil samples were taken from all treatment plants pots to assess the initial contaminant concentration in varying times among (14 -133) days. It has taken the final results after the total period of growth 19 weeks of seedlings of Alfalfa. Growth parameters in the chamber room experiment included; temperature of 25-28°C during the day, and night and 16 hours' light/8 hours' dark. Plants were watered with equal volumes of water as required by the plants. Samples of soils are taken 70 g in every time, dried in the oven at the temperature of 30 C for 4 days, screened by a 2-mm sieve, kept in sacks parameter, and the experiments are done on it

## Petroleum Hydrocarbons (PH) analysis

Petroleum Hydrocarbons (PH-C<sub>8</sub>-C<sub>40</sub>) were quantified by GC-FID and GC-MS. Initially, 5 g air-dried soil samples were mixed with 5 g anhydrous sodium sulfate. The samples were then extracted using a Soxhlet extraction method with 80 ml dichloromethane for 16 h. Soil extracts were concentrated to dryness under a steady flow of nitrogen gas, re dissolved in 5 ml of hexane, filtered through 0.45 ml Teflon syringe filters into 2 ml GC vials prior to analysis.

GC-FID analysis was undertaken using Kromat KB<sup>-1</sup> capillary column (50 m× 0.25 mm× 0.25 μm) using nitrogen as carrier gasses. The column oven temperature was set at 50°C for 2 min, 50–250 °C programmed at 10 °C min<sup>-1</sup> and held at 250 °C for 0.5 min, followed by a linear increase to 320 °C at 20 °C min<sup>-1</sup>, finally maintained for 20 min at 320 °C. Injector and detector temperatures were both maintained at 320 °C. Hydrocarbon concentrations were reported per g air-dried soil.

## Results and Discussion

### 1. Results of oil-contaminated soil and refuse properties

General characteristics of the oil-contaminated soil and aged refuse are presented in table 2

**Table 2:** General characteristics of the oil-contaminated soil and refuse

Properties	Value	
	Oil-contaminated soil	refuse
Sand (%)	11.39	—
Silt (%)	21.86	—
Clay (%)	66.75	—
Texture	Sandy loam	—
Bulk density (g·cm <sup>-3</sup> )	1.3	0.59
Total porosity (%)	50.78	77.56
Maximum water holding capacity(%)	45.94	63.18
pH (1:5 deionized water)	8.35	7.64
Electrical conductivity (μS cm <sup>-1</sup> )	116	1561.3
Organic C(%)	4.28	6.67
Total-P, (mg·kg <sup>-1</sup> )	681.71	1511.8
Total-N (mg·kg <sup>-1</sup> )	973.9	3602.8
Ava. P (mg·kg <sup>-1</sup> )	9.36	79.18
Ava. N (mg·kg <sup>-1</sup> )	30.28	273.01
Total K (g·kg <sup>-1</sup> )	21.72	14.58
Total Pb (mg·kg <sup>-1</sup> )	31.97	121.6
Total Cd (mg·kg <sup>-1</sup> )	ND	ND
Total Cr (mg·kg <sup>-1</sup> )	159.66	260.2
Total Cu (mg·kg <sup>-1</sup> )	35.27	224.71
Total Ni (mg·kg <sup>-1</sup> )	31.79	67.86
Total Mn (mg·kg <sup>-1</sup> )	989.77	692.88
Total Fe (mg·kg <sup>-1</sup> )	44971.43	54241.1

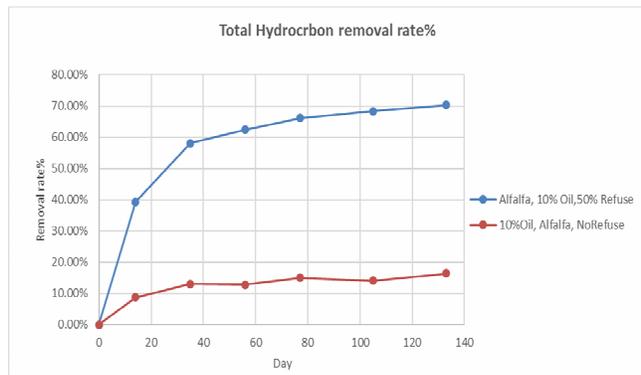
By testing, the properties of each of oil-contaminated soil and organic wastes (refuse) were different. It had noticed the oil- contaminated soil were poor in terms of: (1) physical properties where the density was high due to oil viscosity, porosity is low, and field capacity to hold water is also low. (2) Chemical properties of high acidity (pH), weak organic matter, availability (N, K, P) weak. While the properties of the refuse were better than the soil contaminated with oil: (1) Low density, high porosity, high water holding capacity (2) High availability of organic matter, moderate acidity (PH)

and high availability of nutrient elements. When comparing the characteristics of each of the sources, it is possible to observe the large difference, which provides a greater opportunity to modify and repair contaminated soil when mixed with refuse. This helps to achieve a high degree of degradation of oil waste. However, the refuse was of higher heavy metal levels than soil, and when refuse addition rate more than 50% wt., the heavy metal concentrations in the soil will exceed the standard.

**Results of total oil degradation**

Results of total oil degradation of Alfalfa plant tests

The supply of organic waste to the oil-contaminated soil has enhanced soil ability to remove and break up the oil contaminants. Furthermore, the presence of the alfalfa plant used has increased the success of the degradation of the oil contaminants. The dramatic increase in the removal of oil contaminants has been observed through oil recovery experiments (Soxhlet extractor) from oil-contaminated soils, where the results were monitored after 133 days, 70.34% compared with the control sample (without organic waste) that was 16.44%, with the best percentage of organic waste 50%wt. In the presence of Alfalfa plant Fig. 1.

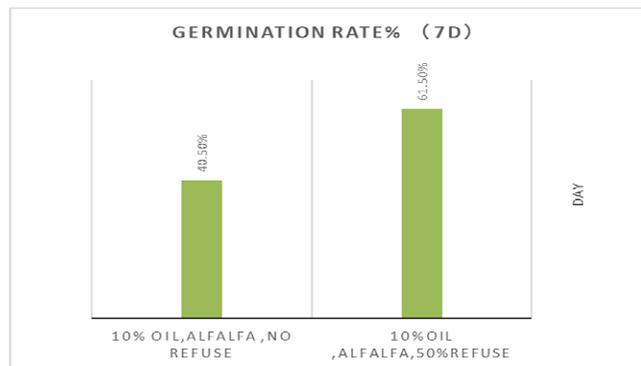


**Fig. 1:** Total degradation of petroleum hydrocarbons rates with 50% organic waste and compared with control samples under conditions (10% oil, 70% WHC, Alfalfa plant)

**Germination and growth of plants**

For Alfalfa plant, 50 seeds were planted, most of the seeds were able to germinate in contaminated soil and a few seeds were spoiled due to the toxicity of the oil contaminants. The growth of the seedlings was monitored at varying intervals and the height was recorded with 50%waste (refuse) so, that percentage of waste have worked as enhanced and fertilized agent.

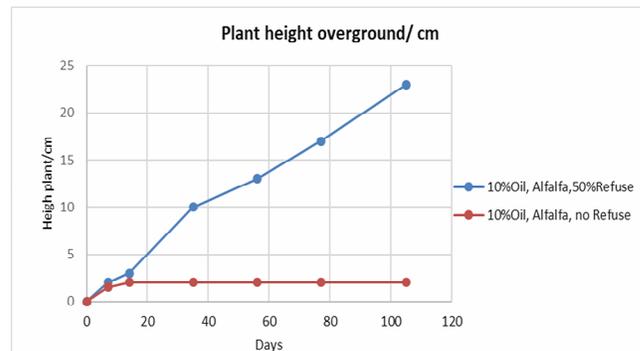
High concentrations of Oil Pollutants didn't prevent good growth with height and increased of plants shoot. Certainly, the control sample (without refuse) they suffered from the weakness of germination and growth is very slow and some plant species died after only 14 days.



**Fig. 2:** the germination rates through 7 days under conditions (70%WHC, 10% oil with 50%Refuse, Alfalfa plant) and compared with control

It has been recorded height of Alfalfa plant under conditions (10% oil, 70% WHC, 50% refuse) as 23cm, while no growth or height was observed in alfalfa plant in control

sample during the same period. The leaves that appeared were weak and soon yellow color was obtained because the toxicity of contaminants Fig. 3.



**Fig. 3:** The height values of Alfalfa plant samples with 10%Oil, 70% WHC, 50% refuse and compared with height of control samples

After extracting the plant from the soil and cleaning it from the clay, it was noticed that the roots were weak and shortened, as they could not penetrate in soil because of the high concentration and privation of enhanced waste Fig. 4.



**Fig.4:** The aboveground biomass and roots of Alfalfa plant with 50% Refuse and control samples 10%Oil, 70% of WHC

**Conclusion**

The results of the research can be recorded very briefly by noting the large difference between Alfalfa soils that treated by organic waste and without organic waste in a period of time up to 133 days. Where the first recorded a total degradation of hydrocarbons 70.34%, while the last 16.44%, and this demonstrated the ability of organic waste to reduce the period of treatment and the end of pollution. Analysis results of organic waste showed that they were rich in nutrients (K, P, O, N) and organic matter, creating an environment rich in microbial communities that contribute with the plant the degradation of organic. Plant growth can also be observed in the presence of waste, where germination rate was only 7 days, 61% and plant height 23 cm.

**References**

- Alarcon, A.; Davies, F.T.; Autenrieth, R.L. and Zuberer, D.A. (2008). Arbuscular Mycorrhiza and Petroleum-Degrading Microorganisms Enhance Phytoremediation of Petroleum-Contaminated Soil, *International J. of Phytoremediation*, 10: 251-263.
- Ansari, A.A.; Gill, S.S.; Gill, R.; Lanza, G.R. and Newman, L. (2016). *Phytoremediation: Management of Environmental Contaminants*, Volume 3, Cham, Springer International Publishing : Springer e-books.
- Baruah, P.; Deka, S. and Baruah, P.P. (2016). "Phytoremediation of crude oil-contaminated soil employing *Crotalaria pallida* Aiton, *Environ Sci Pollut Res Environmental Science and Pollution Research*, 23: 10595-10603.
- Delille, D. and Pelletier, E. (2002). Natural attenuation of diesel-oil contamination in a subantarctic soil (Crozet Island), *Springer-Verlag*, 25: 682-687.
- Fatima, K.; Afzal, M.; Imran, A. and Khan, Q.M. (2015). Bacterial Rhizosphere and Endosphere Populations Associated with Grasses and Trees to be Used for Phytoremediation of Crude Oil Contaminated Soil, *Bulletion of Environmental Comination and Toxicology*, 94: 314-320.
- Gkorezis, P.; Daghigho, M.; Franzetti, A.; Van Hamme, J.D.; Sillen, W. and Vangronsveld, J. (2016). The Interaction between Plants and Bacteria in the Remediation of Petroleum Hydrocarbons: An Environmental Perspective. *Frontiers in microbiology*, 7: 1-27
- Moubasher, H.A.; Hegazy, A.K.; Mohamed, N.H.; Moustafa, Y.M.; Kabiell, H.F. and Hamad, A.A. (2015). Phytoremediation of soils polluted with crude petroleum oil using *Bassia scoparia* and its associated rhizosphere microorganisms, *INBI International Biodeterioration & Biodegradation*, 98: 113-120.
- Ndimele, P.E. (2010). A review on the phytoremediation of petroleum hydrocarbon, *Pak. J. Biol. Sci.*, 13: 715-722.
- Ogbo, E.M. (2009). Effects of diesel fuel contamination on seed germination of four crop plants-*Arachis hypogaea*, *Vigna unguiculata*, *Sorghum bicolor* and *Zea mays*, *Afr. J. Biotechnol*, 8: 250-253.
- Oyem (2013). Effects of Crude Oil Spillage on Soil Physico-Chemical Properties in Ugborodo Community, *International J. of Modern Eng. Research (IJMER)*, 3: 3336-3342.
- Sarkar, D.; Ferguson, M.; Datta, R. and Birnbaum, S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation, *Environmental Pollution Environmental Pollution*, 136: 187-195.
- shirazi, A.R.; Karimi, L.; Baneshi, M.M.; Rezaei, S.; Zadeh, A.M. and Jamshidi, A. (2015). Phytoremediation of Petroleum Contaminated Soil Using *Matricaria Chamomilla*, *Acta medica Mediterranea*, 31: 1387-1392.
- Wilde, E.W.; Brigmon, R.L.; Dunn, D.L.; Heitkamp, M.A. and Dagnan, D.C. (2005). Phytoextraction of lead from firing range soil by *Vetiver grass*, *CHEM Chemosphere*, 61: 1451-1457.