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OPTIMUM CONDITIONS OF PHYTOREMEDIATION FOR PETROLEUM-CONTAMINATED SOIL USING AGED REFUSE

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Abstract

In this study, the optimum conditions of phytoremediation for oil contaminated soil using aged refuse have demonstrated. It has used four aged refuse ratios (10%, 25%, 50%, 75% wt.) and one control, four plant species (Alfalfa, Ryegrass, Tall Fescue, Suaeda Heteroptera) one control, three moisture content ratios were set as 50%, 60%, 70% of water holding capacity (WHC), all these were selected to enhance remediation of 5% wt. crude oil contaminated soil in the chamber room and keeping the temperature at 25-28°C. First condition has selected was ages refuse ratio. The best one was 50% according results show, it recorded 92,32% of total oil degradation under conditions (Alfalfa plant, 70%WHC, 5% crude oil) after 133 days. The best plant species results showed Alfalfa plant. It's were 91.07%, under conditions (50% aged refuse, 70%WHC, 5% crude oil) after 133 days. Suaeda Heteroptera treatments failed because Suaeda Heteroptera could not grow under these conditions. The best moisture has selected was 70% among (50%,60%,70%). the total oil degradation rate 89.43%, 91.07% ,92.55% respectively under conditions (Alfalfa plant, 50%aged refuse,5% crude oil) after 133 days. In general, the optimum conditions and plants that most appropriate to achieve a success phytoremediation in this study were using Alfalfa, the best-aged refuse percentage is 50% and best moisture 70%.

***Keywords*:** Phytoremediation; Oil-contaminated soil; Plant species; Aged refuse.

Introduction

Define phytoremediation as a new approach to cleaning up contaminated sites, involves the use of plants or plants associated with microorganisms in a symbiotic relationship Glick, (2003), Wilde *et al.* (2005), Chekol *et al.* (2004), Alarcon *et al.* (2008), Seniyat (2014), Gkorezis *et al.* (2016), Sarkar *et al.* (2005), Mohammad *et al.* (2014), Tang *et al.* (2010), Wang *et al.* (2013), Isama *et al.* (2014), and Tang *et al*. (2012). One of the studies described phytoremediation is ecological engineering that exploits the natural symbiotic relationship between plants and microbes Germida *et al.* (2002). Phytoremediation includes various processes such as detoxification, insulation, and biodegradable, install various environmental pollutants and have the ability to be sustainable waste management technology Glick (2003). While the expression "phytoremediation" resulting from "phyto"-"plant" and "remediation" to "correct evil" was invoked in the 1980s, the remediation of radionuclide-contaminated soils with plants was achieved as early as 1950 Gerhardt *et al.* (2009). The basic Information of that was taken from various research and studies involving the constructed wetlands, oil spills, the accumulation of heavy metals in agricultural crops (UEPA) Agency, U.S.E.P. (2000). On close watching of the pathway used by plants to metabolize pesticides, in one study was proposed the "green liver concept" referring to the detoxification methods which involves transformation, conjugation, and sequestration in plants is highly similar to that of mammalian liver Sandermann (1994). This model shows how to drain and the fate of organic compounds in plants Singh and Jain (2003). Some conventional plants of phytoremediation that have proved their ability (such as herbs, sunflower, corn, flax, cotton, alfalfa, hemp, willow, poplar, Indian mustard, tobacco, wheat and legumes Macek *et al.* (2009). In recent decades more explanations of phytoremediation appeared, the elucidation of molecular and biochemical plant metabolism with respect to various chemical compounds Morikawa *et al.* (2003), Gerhardt *et al.* (2009), Sandermann (1994), Singh and Jain (2004), Gao *et al.* (2007), and James *et al.* (2009). There are a number of utilities of phytoremediation compared to the physicochemical treatments. It is a natural solar-driven clean-up process with lower carbon footprint and is less effort intensive. The in situ nature of the process means minimal environmental confusion preserves the biological activity of the soils and the rhizosphere subscribes to the microbial diversity of the soil. Phytoremediation is also a cost-dynamic approach typically 60-80% or even less costly than the traditional physicochemical methods of treating contaminated soils and groundwater Reichenauer *et al.* (2008) and Zhang *et al.* (2010). For example, cleaning up of petroleum contaminated sites by phytoremediation costs nearly \$162/m^3 compared to \$810/m^3 for excavation and incineration Cunningham *et al.* (1996). Other features as eco-friendly and accepted by the public as an attractive green biotechnology as well as being therapeutic strategy for organic and inorganic pollutants that persistent in soil, water and air through a series of different processes Morikawa and Erkin (2003), Morikawa (2003), Zhuang *et al.* (2007), Wenzel (2008), and Schnoor *et al.* (1995). Besides the ease of site restoration, erosion control and production of bio-fuels. Disadvantages of this technique are concentrations of pollutants and toxicology of vegetation and accumulation of pollutants in leaves and plant tissue allows her return back to the environment Macek *et al.* (2000), and Van Aken *et al.* (2010). As a result, phytoremediation increasing solubility contaminants may have negative effects on the environment as it is a slow process compared to other techniques Morikawa (2003), and Van Aken *et al.* (2010). In developing countries where resources are insufficient, this technique is one way economically and as a result, various studies focused on the optimization of the process. For the advantages that owned this technique, phytoremediation program investments have increased from \$50 million in 1999 to \$3000 million in 2007 Hosokawa *et al.* (2009). All research studies have addressed modify conditions for supporting biotechnology process by improving the soil with fertilizers, nutrition supplements, microbial inoculation, soil aeration, water content and plant selection or modification of genetic engineering for both bacteria and plant Schnoor *et al.* (1995), Vazquez *et al.* (2009), and Kuiper *et al.* (2004). Careful selection of plants consisted of choosing plants with fast growth as well as an adaptable plant with a deep root system. Some researchers took the approach of transgenic plants with bacterial or mammalian genes, and the use of genetically modified bacteria with more efficient decomposition capabilities for restructuring Hosokawa *et al.* (2009). Have been using plants with the desired characteristics and contaminated environments, such as, the ability to thrive in soils contaminated with oil, fibrous root system configuration that creates a large area in rhizoplane to be colonized by microorganisms living in soil and ability to configure high biomass Adam (2002). Grasses and legumes have the ability to creating the dense roots with a large surface area and great biomass produced proliferation and degradation of petroleum hydrocarbons. Legumes are able to stimulate diversity of metabolic activity of microbes in addition to nitrogen, which is critical in the contaminated soil, which usually suffers from a lack of nitrogen Tang *et al.* (2010). According to another research the order of effectiveness for rhizo-remediation of TPH is Tall fescue> Ryegrass> Alfalfa> Cotton Agamuthu *et al.* (2010).

Hall *et al.* (2011), has been a used non-edible plant such as *Jatropha curcas*, that submits an environmentally friendly and cost-effective method for recovery the soil from lubricating oil pollutants. And application fertilization from organic wastes [Banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)]. Jatropha root did not accumulate hydrocarbons from the soil, but the number of hydrocarbons utilizing bacteria was great in the rhizosphere of the Jatropha plant, thus refer to the procedure of the oil degradation was by rhizo-degradation. These studies have demonstrated that *J. curcas* with organic amendment has a possibility of reclaiming hydrocarbon-contaminated soil. Liu *et al.* (2012), tested Fabaceae species (legumes) to remediate hydrocarbons in soils has been conducted, but these plants are commonly condoned due to slower recorded rates of degradation compared with many grass species. Evidence in the studies proposes that in some cases Fabaceae species may raise total degradation of hydrocarbons and promoting the degradative capacity of the soil microbial community, particularly for contaminants which are normally more recalcitrant to decomposition. Leewis *et al.* (2013), has been showed Gaillardia aristata, Echinacea purpurea, Fawn (*Festuca arundinacea* Schreb), Fire Phoenix (a combined *F. arundinacea*), and *Medicago sativa* L. could effectively increase TPHs, Further, the total biomass did not significantly decrease for all plants that tested. These results suggested that the model ornamental species including *G. aristata*, *E. purpurea*, Fawn, Fire Phoenix, and *M. sativa* can be adopted in phytoremediation of oil-contaminated soil. Tara *et al.* (2014), A study was done to determine how role of plants (*Festuca rubra*, *Lolium multiflorum*), nutrients (fertilizer), or their combination would affect degradation of petroleum hydrocarbon (TPH) contaminated soils (crude oil or diesel) over time. A study suggests that primary treatment with native tree species in combination with grasses could be an effective means for phytoremediation petroleum contaminated soils and promote ecological recovery in cold regions. Margesin and Schinner (2001), Carpet grass (*Axonopus affinis*) was planted in soil grow with diesel and inoculated with different bacterial strains to determine possessing alkane-degradation and 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity, plant growth and phytoremediation activity with promoting by different bacterial strains led to revealed that the combined use of different bacterial strains, resulting different beneficial traits, is a highly effective strategy to improve plant growth and phytoremediation activity 47. In 2015, a research showed the potential of *Bassia scoparia* (L.) A.J. Scott to remediate petroleum-contaminated arid land sandy soil that was studied with natural and sterilized soils, and with supplemental nutrients and water. Highest degradation of PHs for both saturated and poly-aromatic fractions happened when plants were present. Changes in saturated and aromatic fractions were showed and assessed using gas chromatography and high-performance liquid chromatography. Moderate concentrations of PHs activated for oil-degrading microorganisms which in turn enhanced the efficiency of phytoremediation. Polluted soils planted with *B. scoparia* also showed a significant reduction in sulfur levels. The ability demonstrated for breaking down of petroleum hydrocarbons and sulfur by *B. scoparia* that refers it may be a useful tool for recovery of arid land soils contaminated with crude oil. Phytoremediation is increasingly used to remediate contaminated soils. However, in order to provide technically efficient phytoremediation of contaminated sites the plant and pollutants uptake have to be enhanced dramatically, thus should find the appropriate combination of plant species and fertilizers capable of improving germination of plant and ability on the degradation of pollutants. For example, in all studies, fertilizers were only supplemented into the contaminated soil during the primary stage of the remediation treatment. Therefore, degradation was promoted for only a limited period of time after which fertilizer became depleted and degradation slowed down. This could have happened before the complete removal of the contaminant was achieved since fertilizers supplemental period was short. Therefore, the purpose is to investigate whether fertilizers addition to a contaminated soil at periodic intervals can grant stimulate the process of petrol degradation. In 2010, in this study have been using animal manure as agents of bio-stimulation. A combination of treatments consisting of the application of poultry manure, piggery manure, goat manure, and chemical fertilizer was evaluated in oil contaminated soil. After 4 wk. The results showed that poultry manure, piggery manure, goat manure, and NPK (nitrogen, phosphorous, and potash [potassium]) fertilizer exhibited 73%, 63%, 50%, and 39% total petroleum hydrocarbon degradation, respectively, Thus, all the bio-stimulate process methods showed the ability to enhance petroleum hydrocarbon microbial degradation. In 2011, one of researches was used three fertilizers (urea, horse manure, and "ispolin") for increasing ability of Wheat *Triticum vulgare* to improving yields and stimulate a transfer of pollutants to be more available to the plant's forms through enhance the mobility of pollutants in the rhizosphere the soils. Known to bacteria in rhizosphere plays an essential role in decomposition of hydrocarbon contaminants and transformation, biodegradation in contaminated soils need stimulation and augmentation. Harsh soil conditions due to pollution and nutrients deficiency led to slow bacterial activity. Bio-stimulation is a kind of natural remediation in which conditions are manipulated and optimized in order to increase degradation of contaminants by indigenous bacteria that produce the catabolic enzymes that enhance biodegradation Basumatary *et al.* (2012). Has been tested plant *Cyperus brevifolius* (Rottb) with and without fertilization after planting in soil contaminated with crude oil by 8% (w/w). Plant capacity to enhance biodegradation was examined after 60 days crude oil contaminated content in soil, *C. brevifolius* could decrease up to 86.2% in (crude oil-contaminated soil with fertilizer) and 61.2% in (oil-contaminated soil without fertilizer) Cartmill *et al.* (2014), observed in the study had been conducted to test the effect of the application of controlled release fertilizer (CRF). Plant adaptation, growth, photosynthesis, and chlorophyll content were enhanced by the application of CRF in contaminated soil. Proline content showed limited use as a physiological indicator of petroleum induced stress in plants. Bacterial and filamentous fungi populations were stimulated by the petroleum concentrations. Bacterial populations were stimulated by CRF application. At low petroleum contamination, CRF did not enhance TPH degradation. However, petroleum degradation in the rhizosphere was enhanced by the application of medium rates of CRF, especially when plants were exposed to intermediate and high petroleum contamination. Application of CRF allowed plants to overcome the growth impairment induced by the presence of petroleum hydrocarbons in soils. Different forms and types of fertilizers to promoting degradation process of oil pollutants. Generally, the least expensive fertilizers can be used with high organic carbon content such as animal fertilizers, municipal solid wastes, and agricultural crop residues. Etc.

Method and Materials

General methodology for determining the efficiency of phytoremediation by choosing the optimal conditions (moisture, plants species, the percentage of added of aged refuse) under the crude oil pollution concentration (5%) demonstrated in this study consists of the following main steps; seeding of selected plant species, moisture experiment, oil extraction experiments. 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 and dried. Clean soil samples were collected from the top 20 cm of an agricultural field**,** used crude oil**,** the aged refuse was excavated from the landfill which has been buried 8 years The aged refuse samples were sifted to remove particles >10 mm in diameter and stored in polyethylene bags before use. Clean soil samples were air-dried in the laboratory and sieved through 2 mm screens. Then, the soil was contaminated with 5 %(w/w) of crude oil and air-dried for 4 days and thoroughly mixed until homogeneous.

1. Preparations of pots and plants species

Plastic pots were used for planted and prepared the following plant seeds after sterilized: Alfalfa, Ryegrass, Tall Fescue and Suaeda Heteroptera. 1.750g 5% petroleum-contaminated soil (dry weight) plus 750g aged refuse (dry weight) plus each plant separately Alfalfa, Ryegrass, and Tall fescue and Suaeda Heteroptera. Every plant has 3 pots.

Control:

1. 750g 5% petroleum-contaminated soil (dry weight) plus 750g aged refuse (dry weight); have1 pot.
2. 750g 5% petroleum-contaminated soil (dry weight) plus Alfalfa have 1 pot, Ryegrass have 1pot, Tall fescue have1pot, Suaeda Heteroptera have1pot.

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 as table 1

Table1: Samples of plants species at 25-28 ° C

Plants	Oil %	Moisture%	Aged refuse%
Alfalfa	5%	70% WHC	50%
Ryegrass	5%	70% WHC	50%
Tall fescue	5%	70% WHC	50%
Sueada H	5%	70% WHC	50%
No plant	5%	70% WHC	50%

2. Moistures samples

Three treatments: 750g 5% petroleum-contaminated soil (dry weight) plus 750g 50% aged refuse (dry weight) plus alfalfa; the soil moisture content was adjusted to 50%, 60%, 70% of water holding capacity (WHC) respectively; 3pots. Each treatment was sowed 200 seeds; Treatments were incubated in a Walk-In Environmental Chamber at 25 with plant growth light as table2

Table 2: Samples of moisture at25-28^{o}C.

Moisture%	Plants	Aged refuse%	Oil%
50% WHC	alfalfa	50%	5%
60% WHC	alfalfa	50%	5%
70% WHC	alfalfa	50%	5%

3. Mass fraction of aged refuses samples

1. **10% aged refuse:** 1350g 5% petroleum-contaminated soil (dry weight) plus 150g aged refuse (dry weight) plus alfalfa; have1 pot
2. **25% aged refuse:** 1125g 5% petroleum-contaminated soil (dry weight) plus 375g aged refuse (dry weight) plus alfalfa; have 1 pot
3. **50% aged refuse:** 750g 5% petroleum-contaminated soil (dry weight) plus 750g aged refuse (dry weight) plus alfalfa; have 1pot
4. **75% aged refuse:** 375g 5% petroleum-contaminated soil (dry weight) plus 1125g aged refuse (dry weight) plus alfalfa; have1pot. 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 as table 3.

Table 3: Samples of aged refuse at25-28^{o}C

Aged refuse(w)%	Plants	Moisture%	Oil%
10%	alfalfa	70% WHC	5%
25%	alfalfa	70% WHC	5%
50%	alfalfa	70% WHC	5%
75%	alfalfa	70% WHC	5%
no w	alfalfa	70% WHC	5%

Soil sampling after chamber experiment

Samples are taken from the soil of plants cultivated in 70 g in different period, dried in the oven at the temperature of 30 C for 4 days, it is screened by a 2-mm sieve, kept in sacks parameter, and the following experiments are done:

1 Petroleum Hydrocarbons (PH) analysis

Petroleum Hydrocarbons (PH-C_8-C_{40}) 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.

Solved 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^{-1} capillary column (50 m× 0.25 mm× 0.25 µm) using nitrogen as carrier gasses. The column oven temperature was set at 50℃ for 2 min, 50–250 ℃ programmed at 10 ℃ min^{-1} and held at 250 ℃ for 0.5 min, followed by a linear increase to 320 ℃ at 20 ℃ min^{-1}, finally maintained for 20 min at 320 ℃. Injector and detector temperatures were both maintained at 320 ℃. Hydrocarbon concentrations were reported per g air-dried soil.

Results and Discussion

1. Results of total oil degradation of plant species tests

The supply of aged refuse to the oil-contaminated soil has enhanced its ability to remove and break up the oil contaminants. Furthermore, the presence of the plant species 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 from oil-contaminated soils, where the results were monitored after 133 days, 91.07% when the oil concentration was %5 with the Alfalfa plant, where it is the best if compared with other plants, in addition to that, the enhanced degradation of aged refuse as compared with control samples without aged refuse that have suffered from high toxicity of pollutants to plants and low bioactivity where the degradation was very slow fig 1.

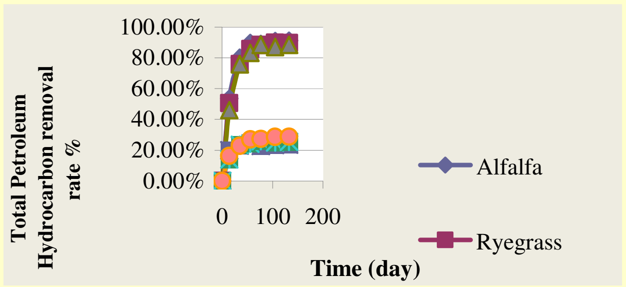

Fig. 1 : Total degradation with species plants of oil contaminated soil 50% aged refuse and compared with control samples without aged refuse under conditions (70%WHC, 5%oil)

2. Results of total oil degradation of moisture tests

The moisture relationship has already been clarified with the degradation of organic pollutants by supplying the soil with the oxygen necessary to sustain the biological activity. In addition to its role in photosynthesis of plants, it is the carrier's medium of nutrients, biological and chemical processes. According to the results obtained in the inspection of the recovery or extraction of the remaining oil in the soil, the water contents of 50%, 60%, 70% of water holding capacity (WHC) were given as a result total degraded petroleum hydrocarbons 89.43%, 91.7%, 92.55% respectively, in the presence of Alfalfa plant and 50% of organic waste. All results are considered successful due to the high rate of degradation during a period of 133 days and continuing to increase as shown in the Fig. 2.

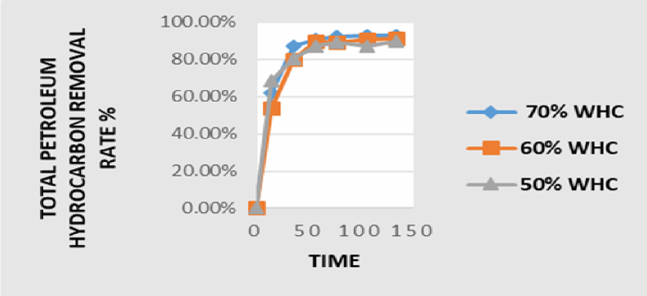

Fig. 2 : Total degradation oil of oil contaminated soil with different WHC rates under conditions (50%aged refuse, 5% oil, Alfalfa plant)

3. Results of total oil degradation of mass fraction of aged refuse tests

The percentage of aged refuse was 50% as the best added ratio as shown in Fig. 3 due to the good abundance of organic matters and bacterial activity in addition to the availability of other conditions (70%WHC, Alfalfa plant, 5% crude oil). Although 70% of the waste is the largest of most organic and biological content, but it has a negative impact on the soil because of its metal contaminated, so it was chosen 50% because it is a state of medium and gave high results degradation.

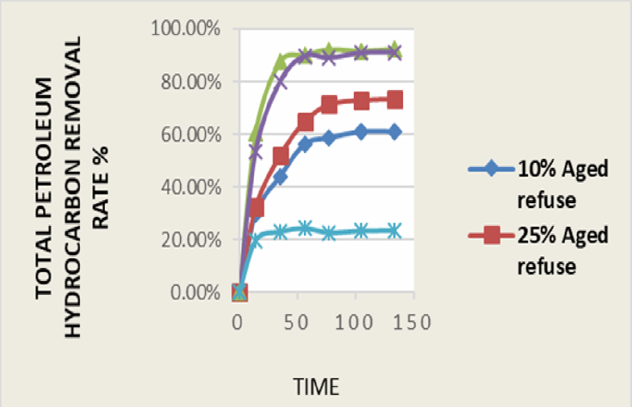

Fig. 3 : Total degradation of petroleum hydrocarbons with different aged refuse rates of oil contaminated soil and compared with control samples under conditions (5% oil, 70% WHC, Alfalfa plant)

Conclusion

The impact of the existence of aged refuse was evident after the use of different ratios of it and compared with control samples without (aged refuse). Augmentation and stimulation are the basic principles of waste work. The total removal of petroleum pollutants was 92.32% with 50%, solid waste (aged refuse), after 133 days under conditions (Alfalfa plant, 5% crude oil, 70%WHC, 25-28°C) Compared with control samples (without aged refuse) which showed 23.56% results after the same period and under the same conditions. In general, the process of removing the oil pollutants was successful within only 133 days under the terms of those identified through experimentation. The results of the extraction of the remaining oil in the soil after degradation have shown great compatibility with those conditions that can be scheduled as optimum conditions that can be adopted for the removal of oil pollution.

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