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Bioremediation Protocols of Hydrocarbon Contamination: A critical Appraisal of a Case Study of Soil Contamination

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Abstract

Soil contamination by petroleum hydrocarbons is a widespread and global environmental contamination concern that needs to be carefully treated and controlled. This research investigates, compare, and analyse the viability of bioremediation technologies for the *ex situ* remediation of hydrocarbon polluted soils. It also outlines the most appropriate bioremediation technique. Results showed one of the important advantages of necrophytoremediation as a remediation protocol. The degradation amount of oil in different treatments follows this sequence: pea straw (PS) > combination of pea straw and Bacillus consortium (BAPS) > Bacillus consortium (BA) > natural attenuation (NA). The same amount of "bacteria" was added into treatment BAPS and BA. Necrophytoremediation using pea straw has a positive effect on the degradation of TPH by 96% during 12 weeks of treatment; the same pattern was followed for the combination of necrophytoremediation and bioaugmentation (pea straw and Bacillus consortium) with 95% total petroleum hydrocarbons (TPH) reduction. Natural attenuation and bioaugmentation microcosms modified with Bacillus were the least practical with TPH reduction of 79% and 76% respectively. The findings from this study recommend researching the possibility of relying on *in situ* necrophytoremediation as valuable, economical and invulnerable method for enhancing the bioremediation efficiency of oil contaminated soils.

Keywords: Soil contamination; Bioremediation technique; Hydrocarbon contaminated soil; Necrophytoremediation; Bioaugmentation; Ex situ remediation; Pea straw; Bacillus consortium; Libya

1. Introduction

According to the Environmental Agency (2004), the main reason why preserving the environment is beneficial is that tomorrow's generation needs to inherit a world that is healthy and clean. Every country has the responsibility of tackling pollution and flooding which will lead to a reduction in environmental impact. Cleaning up rivers, clearing coastal waters and working on contaminated land is vital if soil pollution is to be curbed. Some of the most important factors that are investigated when analyzing soil composition include organic matter, type, moisture content, and pH levels. Soil contamination has been classified as part of land degradation due to xenobiotic chemicals or developments which alter the natural composition of soil.

Soil contamination is typically caused by agricultural chemicals, poor waste disposal, and industrial activity (Krishna and Govil, 2007). The commonest form of soil pollution is via petroleum hydrocarbons, aromatic hydrocarbons, pesticides, heavy metals, solvents, and lead. The level of contamination is linked to the extent to which industrialization has flourished unregulated in a region. It is both expensive and difficult to map areas that have been contaminated with chemicals on land. To undertake the task professionally, one requires heavy investments in hydrology, geology, hydrology, GIS, and computer modelling. The United States and Europe have been subjected to these tests over time. However, developing countries in Asia and South America have not been explored as much. A country such as Kingdom of Saudi Arabia (KSA) has not been exposed to these tests despite the decades of oil-related industrial activities.

Before remediation, contaminated soil takes various forms depending on the nature of industrialization in the area. Soil may be contaminated due to oil spills around extraction points and in transit via pipelines. Oil spills can be costly when the area impacted is close to a water mass such as a sea, lake or dam. Oil spills on land are also damaging since they choke off life in plants (Margesin and Schinner, 2001). Unlike oil spills on water, it is difficult to determine how much soil is impacted. Moreover, correcting the damage made on land is more difficult than that made on water masses. Severe spills may sometimes take years to clear. During this time, plant life is impacted negatively with young shrubs being more vulnerable. One of the most important elements of soil is oxygen. Oil spills prevent oxygen from being replenished below the surface by creating an impermeable film. If oil patches remain stagnant for a prolonged period, the oil could seep into the ground and make its way into the water table (Margesin and Schinner, 2001). Heavy industries and mining also contaminate land by exposing chemical substances to agricultural land (Imperato et al., 2003). In most cases, mining activities leave the land in a poorer state than before. This is because the soil profile and catena are destroyed by open quarrying. Filling the mines with the debris does not take soil profile into account. Corrosion of underground tanks has also been noted to contribute to land degradation (Sowards and Mansfield, 2014).

Industrial accidents and road debris also contribute to soil contamination. The accidents usually entail the spillage of chemical substances from their containers which is considered to be an environmental issue. Liquids settle on the lowest surface. In most cases, such spilled liquids will flow to lowest lying plains before seeping into the ground. Road debris consists of waste materials following the construction of roads and related infrastructure (Environment Agency, 2004). Waste disposal is another common source of soil contamination.

Most industries produce a lot of effluents which is usually directed toward treatment plants or landfills. In countries where regulation is done poorly, plants may direct their waste disposal toward land and water masses. Some of the sources of waste disposal include fuel dumping, direct discharge, sewerage discharge, coal ash, illegal dumping, and electronic waste. This research aims to investigate and analyze the feasibility of bioremediation methods for the *ex situ* reclamation of hydrocarbon polluted soil.

Soil remediation is also referred to as soil washing since it refers to the process of restoring a soil's natural health (Cheng et al., 2016). It may also be applied to infertile or physically-depraved soil. Remediation covers all the processes that soil undergoes in order to remove contaminants such as heavy metals, hydrocarbons, cyanides, volatiles, creosotes, pesticides, and hydrocarbons. It makes it possible to maintain quality and standard in soil making it ready for use such as cultivation or the growth of non-domestic flora and fauna. There are various kinds of remediation whose application depends on the nature of contamination.

2. Biological Remediation

Bioremediation is applied with the aim of treating soils which have been spoiled using biological means. Engineers use both aerobic and anaerobic bacteria to target pollutants (Truu et al., 2015). The bacteria can consume hydrocarbons breaking them down into simple elements that do not harm living organisms in soil (Cueva et al., 2016). One major merit associated with this approach is that the bacteria usually die after they have completed their treatment function.

There are two categories of treatments that are done, *ex situ*: which is done by extracting the contaminated soil or water and remediating it outside the area, and *in situ*: which treatment takes place within the contaminated source. The approach fails in that it cannot be applied universally to all geographical locations. The soil must maintain at least 21^0C and adequate moisture for the bacteria to thrive and complete the breakdown. The only way to apply this approach is by covering the soils in order to maintain the temperatures needed for the process to work. This practical obstacle means that the approach cannot be used on extensive ground. It takes the bacteria longer to complete the digestion of hydrocarbons in colder temperatures (Vidali, 2001).

2.1 Bioaugmentation Strategy

Bioaugmentation is the addition of endogenous or genetically engineered microorganisms (GEM) with the potential of pollution biodegradation (Vogel, 1996; Limbergenet al., 1998; Zhang et al., 2000). In this case the addition of hydrocarbon reduces (mostly bacteria and to a lesser extent fungi) which are typically separated or enriched in the laboratory from samples taken from contaminated sites (Perelo, 2010; Sarkar et al., 2005). This method is chosen because these microorganisms have more capability for adaptation to particular contaminant than non-endogenous microorganisms (Silva et al., 2009). Furthermore; Bioaugmentation is considered as an important method to improve the bioremediation in removal of polycyclic aromatic hydrocarbons (PAHs) in polluted soils (Nasseri et al., 2010).

2.2 Necrophytoremediation Strategy

Necrophytoremediation can be defined as the use of dead plant biomass (e.g. hay and straw) for the reclamation of contaminated land (Esmaeil et al., 2015). Necrophytoremediation have numerous advantages over the phytoremediation techniques. For instance, necrophytoremediation is toxic independent and can be useful to any type of contamination, as there is no necessity to study the length of the growing season, rainfall, and temperature rates. Moreover, petroleum-contaminated soil is generally contaminated with high concentrations of soluble salts and other metal toxicities which may hinder the benefit of phytoremediation (Hutchinson et al., 2004); on the other hand, necrophytoremediation may help to not only degrade the hydrocarbon, nevertheless; it can improve the desalination of contaminated soils (Zhang et al., 2008).

2.3 Natural attenuation Strategy

Natural attenuation is the most effortless bioremediation method; the only requirement is to observe the natural degradation process. This strategy can be applied in certain circumstances; for instance, it can be used for inaccessible areas or when levels of contamination are fairly low (Pilon-Smits, 2005). It is predicted that nearly 25 % of all hydrocarbon-contaminated soil has been remediated using natural attenuation (Stroud et al., 2007).

3. Research Design and Methodology

3.1 Case study

Secondary data on oil-contaminated soil samples obtained from (Koshlaf et al., 2016) was assessed and analysed to investigate the feasibility of bioremediation technologies for the *ex situ* remediation of petroleum contaminated Libyan soil. Oil-contaminated soil samples were collected from the top layer (0-15 cm; 20 kg) of pipeline leak from the main oil reservoirs in Libya before they were referred to RMIT University in Australia for testing. The soil properties were investigated to determine the level of contamination (Table 1). The concentration of contamination, in terms of TPH was 18,966 mg/kg, while the percentages of organic content, nitrogen, available hydrogen, moisture content, and organic carbon matter content were found to be 0.8%, 0.03%, 0.08%, 12.5%, and 8%, respectively. The pH of the sample was 7.5 to be moderately alkaline according to The Food and Agriculture Organization of The United Nation with 7 being neutral, below 7 acidic and above 7 alkaline (FAO, 2019). The concentration of primary nutrients such as Calcium (Ca), Potassium (K), Magnesium (Mg) Iron (Fe), Phosphorus (P), Sulfur (S) and Zinc (Zn) in the samples were identified using, x-ray fluorescence spectrometry following the associated procedures. Heavy metal substances values were also obtained using the advanced technique of inductively coupled plasma mass spectrometry (ICP-MS) in Table 1.

Table 1. Chemical and physical properties of the contaminated soil (Koshlaf et al., 2016).

Soil properties	**Values**
Organic carbon content (%)	0.8
Nitrogen (%)	0.03
Available H (%)	0.08
Soil pH	7.5
Moisture content (%)	12.5
Water holding capacity (%)	51
Organic matter content (%)	8
Soil texture	Sandy loam
Initial TPH contamination (mg/kg)	18,966
Ca (mg/kg)	91,100
K (mg/kg)	49,200
Fe (mg/kg)	31,800
P (mg/kg)	18,300
Mg (mg/kg)	4500
S (mg/kg)	2900
Zn (mg/kg)	230
Heavy metals (mg/kg)	All less than 7

3.2 Critical appraisal of various bioremediation methods

Bioremediation methods can be useful either *in situ* or *ex situ* based on the nature of contaminant, distributions, and properties of the site (Mallavarapu and Ravi, 2017). Although the analyzed study (Koshlaf et al., 2016) considered the *ex situ* approach, i*n situ* treatment is more viable and cost effective as it appears less disruptive and does not implicate excavation and transport of contaminated soils. The generally used *in situ* approaches include natural attenuation, and bioaugmentation which both were reflected in the study.

On the contrary, the *ex situ* approaches involve excavation and removal of contaminated soil for treatment either on the site or transportation to a suitable place before treatment (Mallavarapu and Ravi, 2017). The proposed criteria relied on the various applications of bioremediation in which reduction of concertation of TPH in contaminated soil and time of decay are considered to be the main factors of assessment. The authors selected four different treatment technologies to be investigated during 12 weeks of soil reclamation, the treatment technologies used in the study were: Necrophytoremediation, bioaugmentation,

combination of necrophytoremediation and bioaugmentation, and natural attenuation. In this regard**,** the combination of both necrophytoremediation and bioaugmentation as an environmental treatment method was applied for the first time to help with the degradation of diesel contaminated soil.

One of the essential advantages of the bioremediation treatment is the faster decay rates in order to achieve the target level of pollutant concentration. Number of studies on bioremediation of hydrocarbons were done on circa 35 weeks, such as (Jorgensen, et al., 2000), this study however was made in only 12 weeks of treatment. In addition, all of these elements (Table 2) were also taken into consideration when selecting and applying the previously-mentioned bioremediation methods. The three generic technologies which are outlined in Table 2 were the most appropriate to be used to degrade hydrocarbon-contaminated soils (Esmaeil, et al. 2015.

Table 2. A comparison between necrophytoremediation, bioaugmentation, and natural attenuation (Esmaeil et al., 2015).

Technology	**Key point**	**Advantages**	**Disadvantages**
Necrophytoremediation	Addition of dead plant biomass and their associated microorganisms.	Toxic independent. Can be applied to any level of contamination. Enhance the desalination of contaminated soils. Cheap as plant residues are also waste Rapid progress	Some plants residues (e.g. willow plants) may not work.
Bioaugmentation	Addition of hydrocarbon-degrading microorganisms.	Using high biomass of hydrocarbonoclastic microorganisms.	Changes the natural microbial structure. Poor adaptation of hydrocarbonoclastic microorganisms to the contaminated site.
Natural attenuation	Using indigenous microorganisms and natural condition.	Cheapest technology.	Requires extensive long-term monitoring. Not always successful.

3.3 Comparative analysis

3.3.1 Time frame influence

This study examined four different treatment techniques of TPH in four time slots. Time of decay plays a significant role in determining the efficacy of the proposed treatment methods (Esmaeil et al., 2015). It is also noteworthy that the degradation of each treatment method was analyzed throughout the entire treatment (Table 3) at time points of 0, 4, 8, and 12 weeks.

Table 3. Reduction of TPH concentration after applying four different treatment methods in 12 weeks.

Treatment methods	**TPH reduction in mg/kg**		
	4 week	**8 week**	**12 week**
Pea straw (PS)	10,431.3	13,276.2	18,240.0
Bacillus consortium (BA)	9,957.1	7,965.7	14,991.0
Combination of pea straw and Bacillus consortium (BAPS)	9,957.1	11,379.6	17,991.0
Natural attenuation (NA)	5,879.5	7,965.7	14,444.0

3.3.2 TPH reduction

This study emphasized the degradation of diesel contamination in soil with the aid of four distinctive remediation methods. Each remediation method was measured in correlation to time in mg/kg. Furthermore, the TPH decomposition percentage of each remediation method was measured at the designated time frames.

3.3.3 Residual analysis

One of the major assessment tools in this study is to provide a residual analysis of the contamination in the soil sample. The initial TPH concentration which was found to be 18,966 mg/kg subtracted the amount of decomposition of each treatment method to provide the net mass of the untreated soil in each time slot.

3.4 Statistical analysis

The Microsoft Excel tool was used to conduct statistical analysis to present numerical information of the concentration and percentage of each bioremediation method in distinct time frames.

4. Results and discussion

4.1 Total petroleum hydrocarbon degradation

Most of the previous studies presented including (Koshlaf et al., 2016) were concerned with industrial soil contamination by total petroleum hydrocarbons substantiate the success of bioremediation strategies. The four treatment methods led to significant degradation in the concentration of contamination in 12 weeks. As a result of these compelling data in Figure 1, necrophytoremediation using pea straw had a positive effect on the degradation of TPH by 96% during 12 weeks of treatment, followed by the same pattern for the combination of necrophytoremediation and bioaugmentation (pea straw and Bacillus consortium) with 95% TPH reduction. Natural attenuation and bioaugmentation microcosms modified with Bacillus were the least practical with TPH loss of 79% and 76%, respectively.

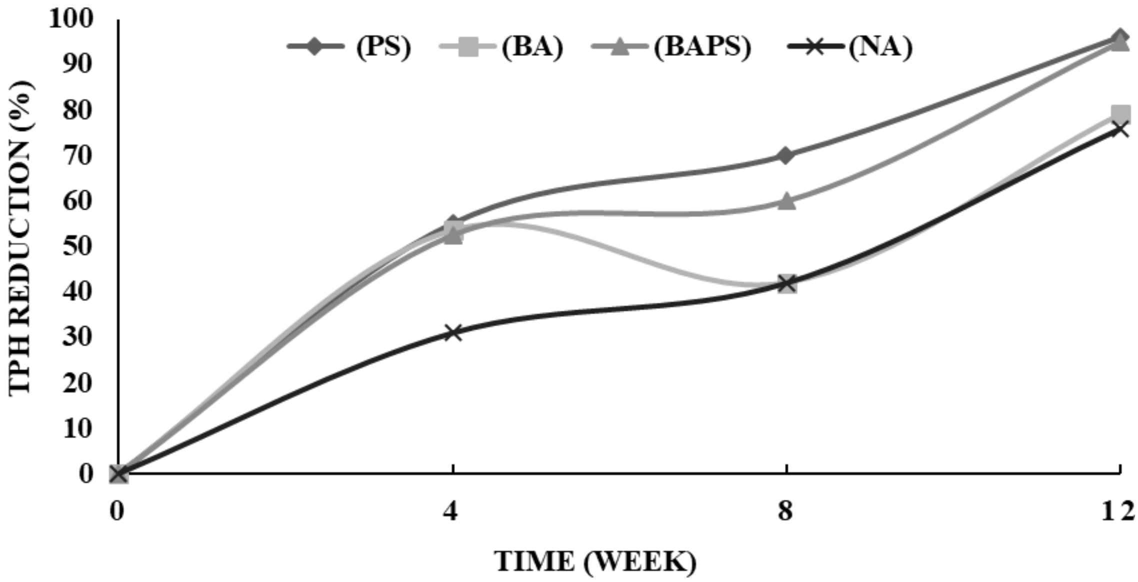

Figure 1. Reduction of TPH (%) in ex situ remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) over 12 weeks of incubation (Koshlaf et al., 2016).

4.2 Critical appraisal of various bioremediation methods

Bioremediation strategies to the reclamation of contaminated sites exhibit the metabolic diversity and adaptability of microorganisms to decompose a broad range of organic and inorganic pollutants. The field experiment done by (Koshlaf et al., 2016) elucidates the remarkable reduction of concertation of hydrocarbons during 12 weeks treatment period (Figure 2). Among the selected bioremediation methods, the pea straw was the most effective to stimulate hydrocarbon degradation in a contaminated site throughout the treatment period. Significantly, the highest TPH degradation was found to be 18,240 mg/kg followed by microcosms modified with pea straw combined with Bacillus (17,991 mg/kg). Nevertheless, natural attenuation and microcosms amended with the Bacillus were the least effective with an average TPH reduction of 14,444 mg/kg and 14,991 mg/kg respectively.

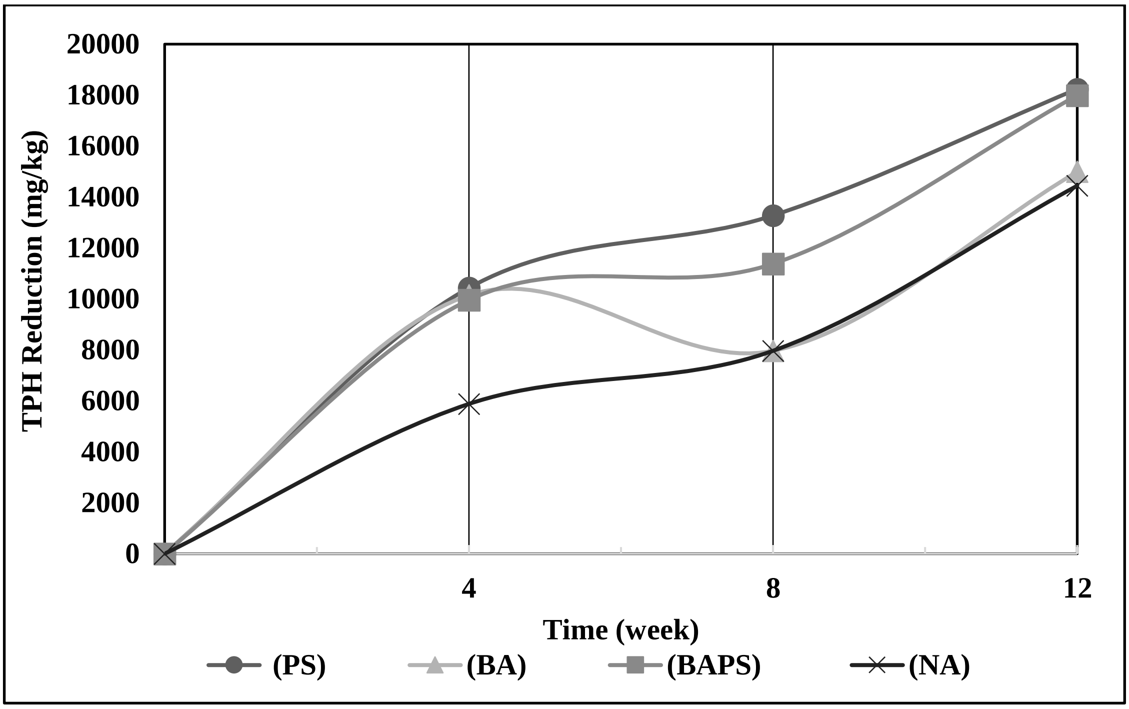

Figure 2. Reduction of TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) over 12 weeks of incubation.

4.3 Comparative analysis

4.3.1 Time frame influence

This study was able to analyze four different treatment strategies of TPH in four time slots. Time of decay plays a significant role in determining the efficacy of the proposed treatment methods. It is also noteworthy to mention that the degradation of each treatment method was analyzed throughout the treatment (Table 3) at time scale of 0, 4, 8, and 12 weeks.

4.3.1.1 Four weeks of incubation

During the first four weeks of incubation, the results appeared similar for most of the treatment methods as pea straw had degraded diesel contamination by 55% out of the initial 18,966 mg/kg TPH (Figure 3). While Bacillus consortium degrade 53.5% slightly higher than the incorporation of pea straw and Bacillus consortium (52.5%), the natural attenuation however had the least contamination reduction of 31% in the first month of bioremediation.

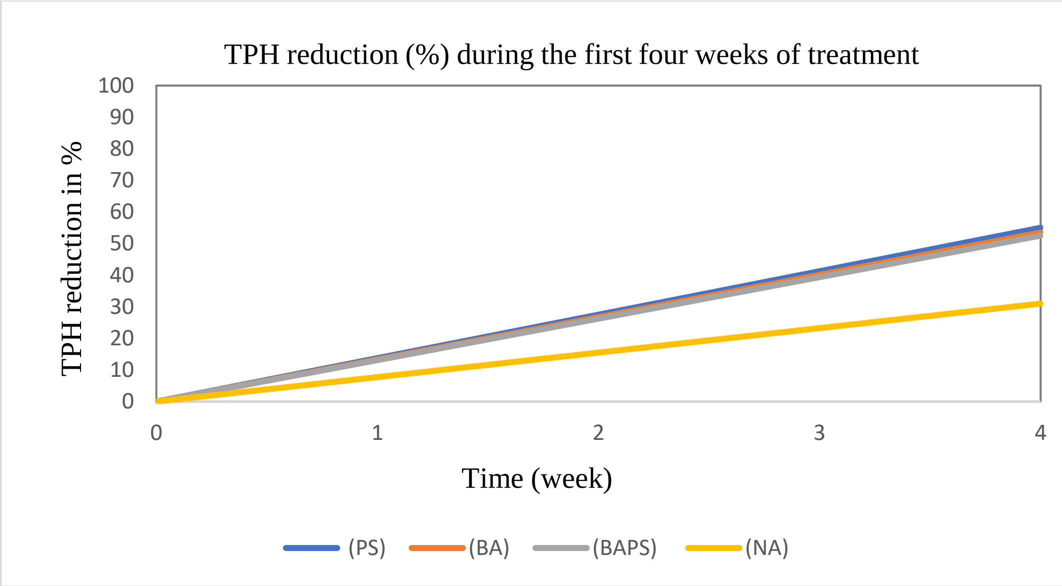

Figure 3. Reduction of TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) during the first 4 weeks.

4.3.1.2 Eight weeks of incubation

After eight weeks of treatment, pea straw had degraded diesel contamination by 70%, followed by combination of pea straw and Bacillus consortium (60%). Despite the high reduction in the previous treatment period, the Bacillus consortia exhibited a comparatively weak response in terms of improving degradation. The Bacillus consortium was reduced TPH by only 42%, this suggests that the activity of the injected bacteria in the soil was limited as it confirms that the quality of Bacilli bacteria was low in treatments modified with the Bacillus consortia, in addition to the poor adaptation of hydrocarbonoclastic microorganisms to the contaminated (Esmaeil et al., 2015; Koshlaf et al., 2016). Natural attenuation also succeeded in maintaining a reduction of 40 % (Figure 4).

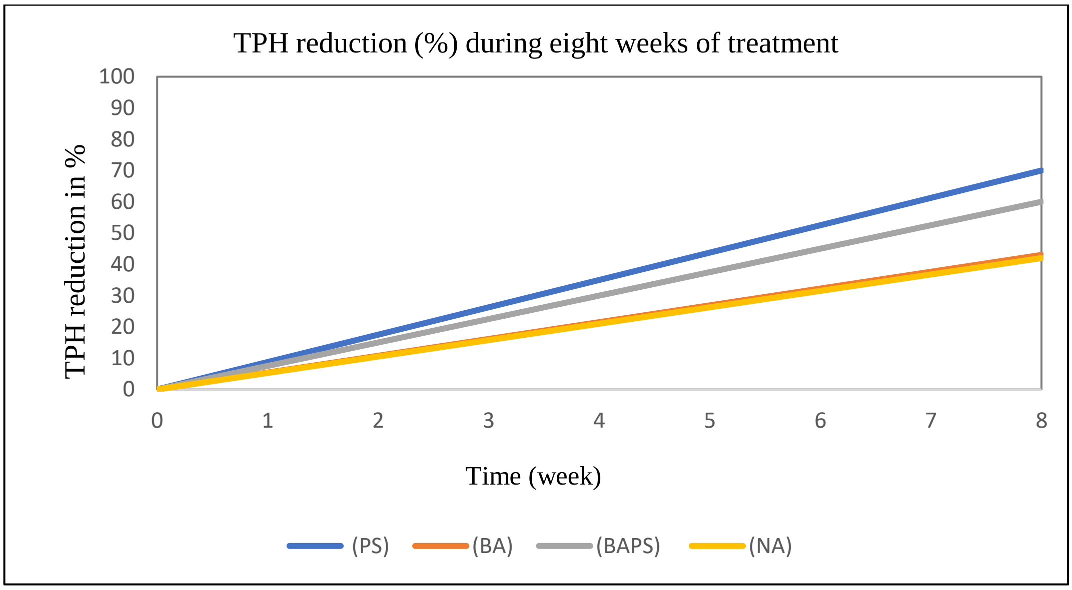

Figure 4. Reduction of TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium(BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) during 8 weeks of treatment.

4.3.1.3 Twelve weeks of incubation

At the end of the experiment, the presence of pea straw sustained the TPH degradation by total of 96 %, the combination of pea straw and Bacillus consortium also degraded 95%. While the Bacillus consortium and natural attenuation reduced TPH by 79 % and 76 % respectively (Figure 5).

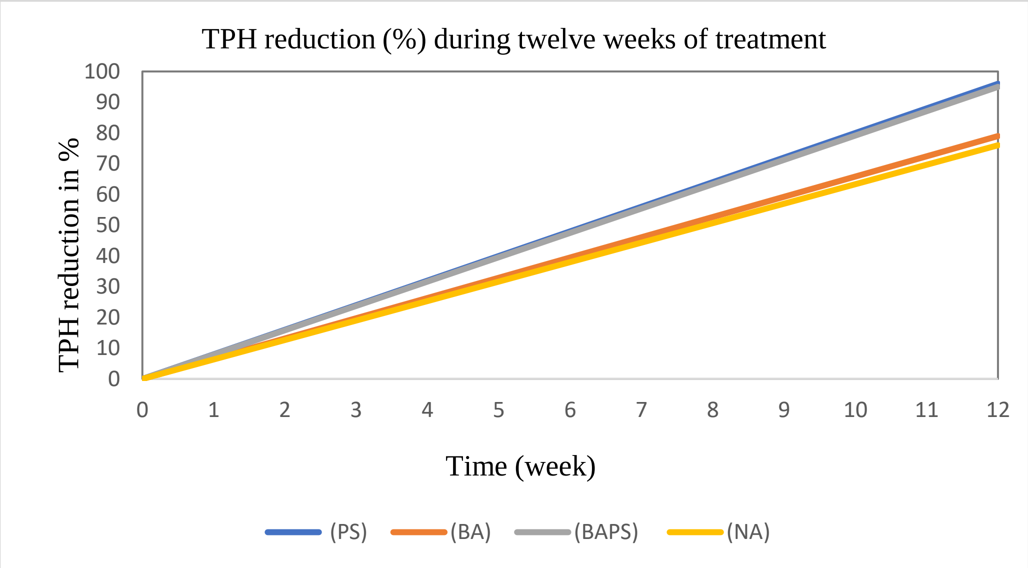

Figure 5. Reduction of TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) during 12 weeks of treatment.

4.4.1 Residual analysis

Table 4 demonstrates the residues of TPH in the soil samples during treatment after applying different treatments. Necrophytoremediation using pea straws effectively removed most of the contamination with 726 mg/kg remains left of TPH at the end of the experiment. TPH-degrading microbial communities were greater in BAPS soil than in BA soil, and TPH degradation residuals were 975 mg/kg and 3,975 mg/kg respectively, where using indigenous microorganisms and natural condition for the natural attenuation could not decrease the remains of TPH contaminants more than 4,522 mg/kg.

Table 4. The net mass of the untreated soil in of TPH during treatments during 12 weeks of incubation

Treatment method	**TPH residues in mg/kg**		
	4 week	**8 week**	**12 week**
Pea straw (PS)	8,534	5,689	726
Bacillus consortium (BA)	9,008	11,000	3,975
Combination of pea straw and Bacillus consortium (BAPS)	9,008	7,586	975
Natural attenuation (NA)	13,086	11,000	4,522

4.4.2 Statistical analysis

The same degradation pattern for the hydrocarbons in the diesel-contaminated soils continues in (Table 5) as pea straw degraded circa 2845 mg/kg. That is 50% more reduction than that of the combination of pea straw and Bacillus consortium in week four to eight of treatment (Figure 6). Meanwhile the natural mitigation method degraded 2086 mg/kg (11%). In contrast, the Bacillus consortium unexpectedly has increased the TPH concentration by 11.5 %. The presence of long length aliphatic hydrocarbons (i.e. diesel) forms the production of oil films and slicks which hinder nutrient and oxygen interaction in the soil (Wasmund et al. 2009), leasing to a significant drop in soil qualities and crucial changes in microbial bacteria (Militon et al., 2010; Esmaeil et al., 2015). By the time the samples were in 8 to 12 weeks of treatment, the pea straw degraded 26 % of TPH, Bacillus consortium (37%), the combination of pea straw and Bacillus consortium (35%), and natural attenuation method was able to degrade 34%. (Figure 7). By week 4 to 12, pea straw degraded 41 % of TPH, Bacillus consortium (26%), the combination of pea straw and Bacillus consortium (43%), and natural attenuation method degraded the degrade 45% in eight weeks of incubation (Figure 8).

Table 5. TPH reduction with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS), and natural attenuation (NA) treatments during different sets of time frames

Treatment method	**TPH Reduction in mg kg-1**		
	During week 4 to 8	**During week 8 to 12**	**During week 4 to 12**
Pea straw (PS)	2844.9	4964	7809
Bacillus consortium (BA)	-2180.09	7025	4845
Combination of pea straw and Bacillus consortium (BAPS)	1422.45	6611	8034
Natural attenuation (NA)	2086.26	6478	8565

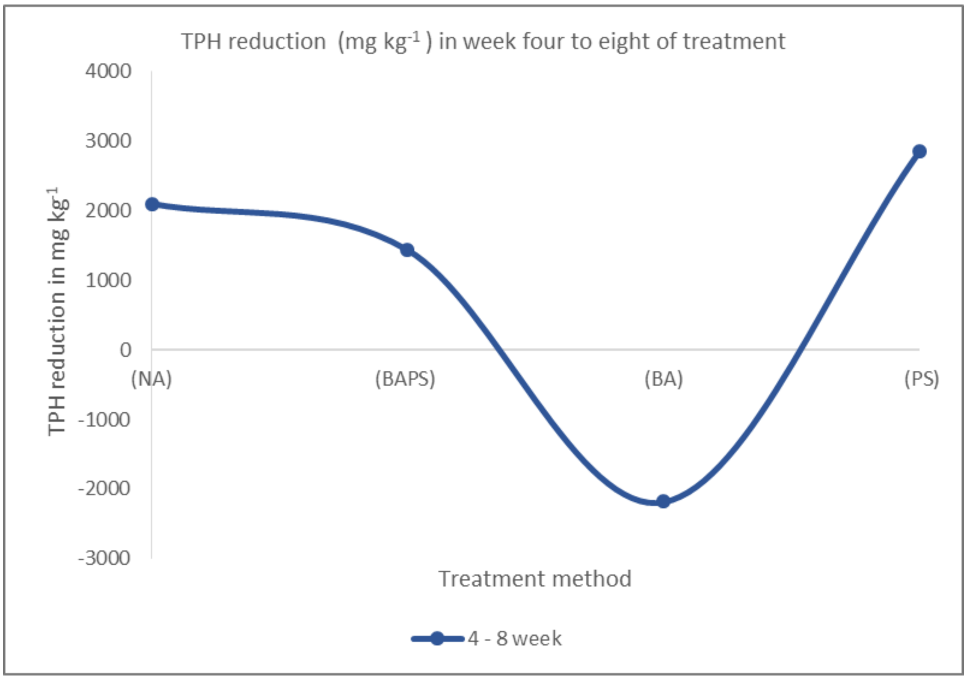

Figure 6. TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) in week four to eight of treatment

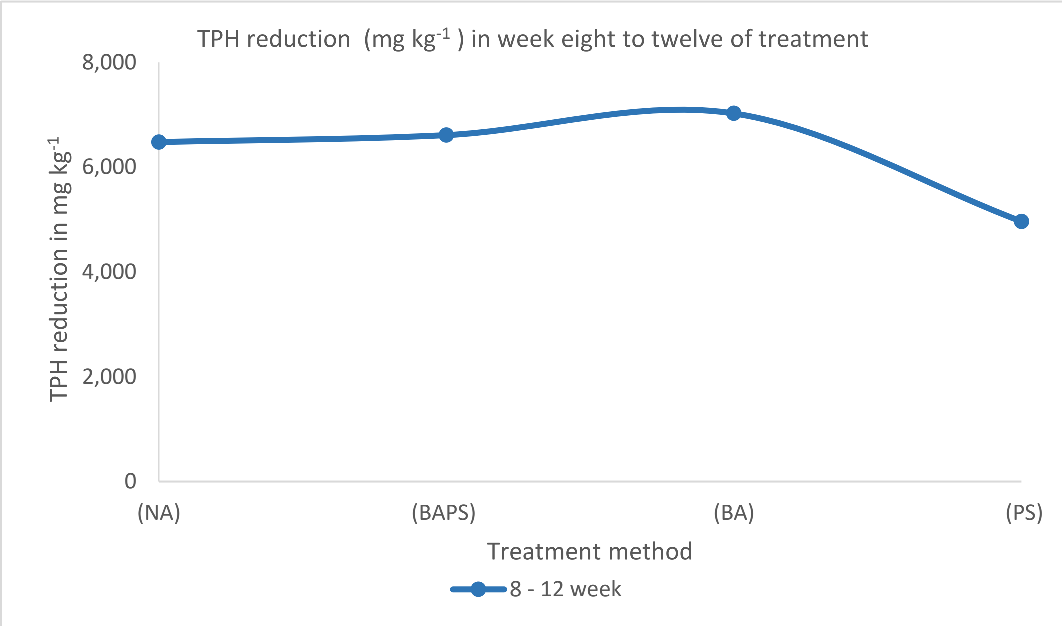

Figure 7. TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) in week eight to twelve of treatment.

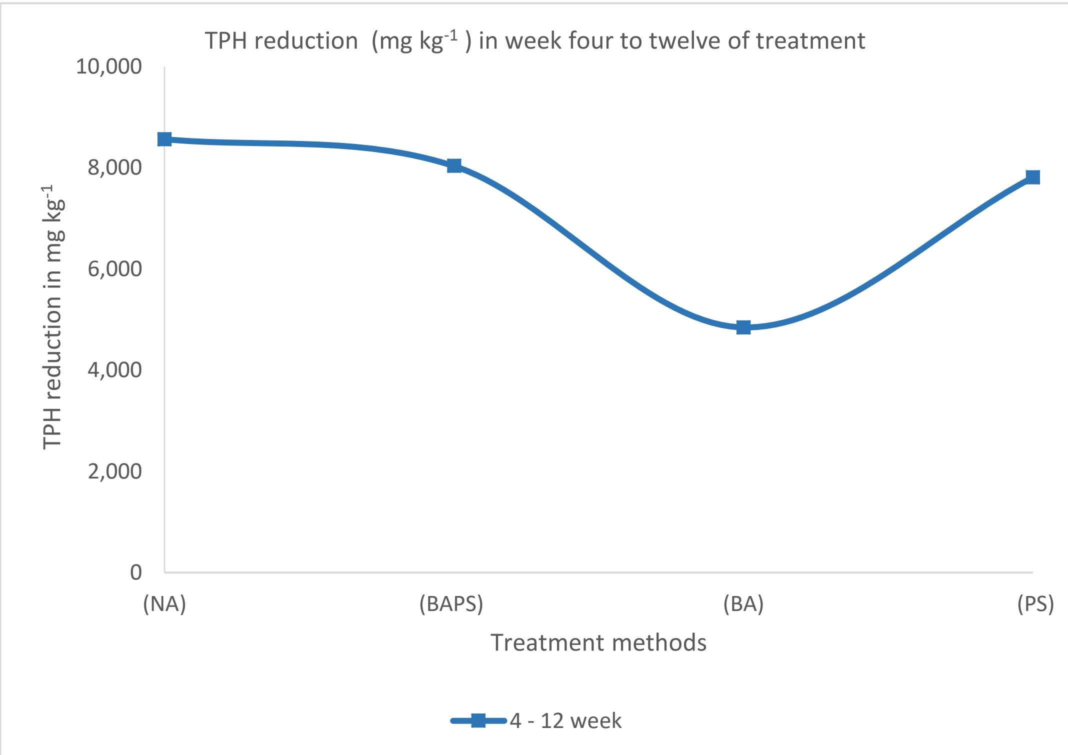

Figure 8. TPH concentration in *ex situ* remediation with pea straw (PS), a Bacillus consortium (BA), pea straw & Bacillus consortium (BAPS) and natural attenuation (NA) in week four to twelve of treatment.

5. Conclusions and Recommendations

5.1 Conclusions

Results showed that the investigated remediation methods were substantial. Bioremediation is a feasible technique for the reclamation of TPH contaminated soils. According to the presented data, more than 96% of TPH can be extracted from soil in 12 weeks (Figure 1). The initial TPH content was 18966 mg/kg. Furthermore; necrophytoremediation using pea straw degraded 18240 mg/kg (Table 3). It also contributes to increased rates of degradation by increasing the microbial activities when combined with bioaugmentation (addition of bacterial consortium including several Bacillus) to decompose a comparable level of contamination (95%). As well as these positive effects associated with the use of bioaugmentation which is a technology of injecting exogenous microorganisms into the soil to decompose contamination (Wu et al., 2013), it reduced hydrocarbons alongside with natural attenuation where both methods gave comparable results to the bioremediation of contaminated soil (79 % and 76 % respectively). As shown in Table 6, necrophytoremediation can degrade contamination effectively under a variety of different conditions. Applying necrophytoremediation may help to not only decompose the contamination but also enhance the desalination of effected soils (Zhang et al., 2008).

Table 6. The advantages of necrophytoremediation used for hydrocarbon-contaminated environments

Condition	**Necrophytoremediation**
Toxicity	Independent
Climate	Less dependent
Soil conditions (e.g. pH, aeration, and structure)	Do not require consideration
Soil salinity	Does not limit application
Hydrocarbons levels	Not limited to hydrocarbon levels
Plant husbandry	Not required
Screening stage	Not required
Usage in biopile	Can be used
Price	Cheaper than bioaugmentation as plant residues are also waste

5.2 Recommendations

Nowadays, one of the main environmental dilemmas is to control and remediate soil contamination in its all forms. Therefore; the study recommends the environmental organizations to further invest in researching the possibility of relying on *in situ* necrophytoremediation as valuable, economical and invulnerable method for enhancing the bioremediation efficiency of oil contaminated soils.

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